Development and Optimization of Integrated Microwave Enhanced Extraction as a Sample Preparation Technique: Environmental, Clinical and Green Chemistry Applications

Sejal Iyer

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Development and Optimization of Integrated Microwave-Enhanced Extraction as a Sample Preparation Technique: Environmental, Clinical and Green Chemistry Applications

A Dissertation Presented to the Bayer School of Natural and Environmental Sciences of Duquesne University

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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“If we stopped to think more, we’d stop to thank more”…D. Evans

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“Give me six hours to chop down a tree, and I will spend the first four sharpening my axe”...Abraham Lincoln

1.1. Background

Attempting a recap of roughly six years is a daunting task in itself. This chapter attempts to confer some sort of a sketch for the rest of the dissertation, so that the dissertation flows more logically.

The dissertation is made up of three parts, viz., environmental, clinical and green chemistry applications of a sample preparation technique. The technique that runs like a common thread through all these sections is Integrated Microwave Enhanced Extraction, binding all the three aspects into a common goal of improved extraction efficiencies giving better accuracies and tighter precision values across the board.

Thus, the overall project was based on the principles of microwave-enhanced chemistry. I joined Dr. Skip Kingston’s Research Group in Fall of 1999. It started out to be an application of solvent extraction of compounds of biological significance like morphine using microwave energy. Dr. Marlene Franke had begun this project, and I inherited a different section of this project to help transition me from my pharmacy degree into analytical chemistry. As luck and graduate school would have it, while this was the first project I started, it also was the last project I finished. The clinical project...
was the extraction of biologically significant compounds like drugs of abuse. Morphine was chosen as a representative of its class of narcotic analgesics. The matrices chosen were human serum as well as bovine serum. The technique was, of course, Microwave Enhanced Extraction and the platform for comparison was Liquid/Liquid Extraction (LLE) by virtue of its being the default technique used for analysis of morphine by our collaborative laboratory, the Pittsburgh Criminalistics Labs. This project was then extended to Solid Phase Extraction (SPE) as a natural platform for comparison for MAE. This project was also eventually (at a much later stage) extended into a green chemistry application, viz., microwave extraction of morphine and codeine using ionic liquids as the extracting solvent. (SPE part cross-referenced to David Lineman’s dissertation, ionic liquids cross-referenced to Pallavi’s thesis).

The project was then extended to compounds of environmental interests like Polycyclic Aromatic Hydrocarbons (PAHs) and pesticides. I would like to acknowledge Dr. Robert Richter for introducing me to microwave chemistry. We started working with PAHs, and performed a lot of trial and error experiments to finally arrive at a compatible method for the extraction of PAHs into different solvents without destroying either the analytes or the microwave.

This then further led to the development and optimization of other parameters of extraction that influence recoveries, namely, temperature, pressure, matrix effects, equipment integration, analyte chemistry, sample size and time. While this list was not exhaustive, it did incorporate significant parameters that influence extraction. The temperature-study extended into theoretical modeling in collaboration with Zhigang Zhou and Jeff Madura.

In Fall of 1999, Rob, George Lusnak and I began work on the ACS-EPA project, for which our initial work on PAHs helped tremendously in predefining the parameters needed for efficient extractions. While this project began as a check for feasibility for the acceptance of Performance Based Methods for compliance monitoring versus
Prescriptive Methods, it also extended into other more fundamental studies of comparison of methods, extractants, and other parameters.

There were other applications that we worked on along the way. Extraction of polymer additives was published in 2000. We also worked on extraction of organochlorine pesticides from soil, extraction of lipophilic material from food products, PAHs and phthalates from food products (cross-referenced with David Lineman’s work).

Eventually the project graduated to green chemistry, and the contribution of microwaves towards green chemistry. After PAHs, pesticides and other environmental analytes, green chemistry was a natural progression for an original research proposal. This proposal was then converted into a laboratory project in itself, and it wraps up my dissertation. Pallavi continues with a part of the project. Thus, our research project and, therefore, this dissertation have three facets to it: Environmental, Clinical and Green Chemistry Applications.

Over the last couple of decades, ultra-trace analysis and shorter sample processing time for higher sample throughput are fast becoming imperative factors. Microwave Enhanced Chemistry (MEC) plays a significant role in achieving this goal. Microwaves have been used for digestions and extensively for other sample preparation of inorganics. Elemental analysis of nearly every matrix requires dissolution of the sample before instrumental analyses. MEC is a fast, efficient and reproducible sample preparation method. Combination of clean chemistry with MEC has made detection at sub-picogram levels feasible. MEC also makes it possible to reduce sample preparation time from days to minutes. Standardization and automation has enabled an increase in accuracy and precision. For decades, analysts have used some form of an open-vessel digestion or a Carius tube closed-vessel digestion. In 1975, microwaves were first used for the rapid heating source for wet, open-vessel digestions. An initial search revealed the increasing interest in extraction of organics using microwave energy as evidenced by Figure 1.
1.2. Content

The dissertation comprises of the following chapters:

**Chapter 1:** Introduction to Sample Prep: This chapter describes the background about the history of sample preparation as well as introduces the reader to the current state of the art in this field. It also introduces the concepts of integration and the development of fundamentals related to the automation of traditional microwave extraction that are the focus of this project.

**Chapter 2:** Extraction: Since the dissertation is based on extraction techniques, it only seemed appropriate to discuss the theory that characterizes extraction. This chapter also describes the extraction theory in context of microwave heating, and the hypothesis of microwave effect.

**Chapter 3:** Microwave Assisted Extraction: This chapter focuses on the intricacies of microwave extraction and the theory that delineates this method of extraction. The second part of this chapter focuses on Integrated Microwave Extraction.

**Chapter 4:** Development & Optimization of Fundamental Parameters Affecting Microwave Extraction: A variety of factors were evaluated to examine their possible contribution to either improvement or adverse effects of these factors on the extraction recoveries of analytes of interest. This chapter will discuss the evaluation and results obtained from the observation of these influences. Theoretical Modeling of Temperature Dependence of Extraction: In collaboration with Dr. Jeff Madura and Zhigang Zhou, a theoretical model will be presented that predicts the temperature dependence of extraction efficiencies.

**Chapter 5:** Environmental Phase: The ACS/EPA Study: This chapter focuses on the possibility of switching to Performance Based Methods for compliance monitoring as opposed to the currently used Prescriptive Methods as a way for improvement in compliance monitoring as well as to provide encouragement for technical innovation. The chapter also discusses other effects that influence these methods like sample size and the presence (or absence) of moisture. This project incorporated comparison with Soxhlet as our Prescriptive Method, and included a cost effectiveness study.

**Chapter 6:** Clinical Phase: The Drugs of Abuse Study: This chapter discusses the possibility of using microwave enhanced extractions for narcotic analgesics like
morphine and codeine. Caffeine was also evaluated (as a part of Chapter 4). This is the only chapter that included two platforms of comparison (LLE and SPE) as well as an extension into Green Chemistry.

**Chapter 7:** Applications of IME: This chapter included the following sections:
- Part 1: Equipment Integration and Application to pesticides and PAHs
- Part 2: Polymer Extraction
- Part 3: Extraction of environmental contaminants from food products
- Part 4: Lipid Extraction

**Chapter 8:** Green Chemistry Phase: The Ionic Liquid Study: This chapter started out as my original research proposal and extended into actual laboratory experimentation to include the following sections:
- Part 1: Proposal
- Part 2: Synthesis of IL
- Part 3: Preliminary results with PAHs
- Part 4: Extraction of acetaminophen and caffeine

**Chapter 9:** Conclusions: This will be the wrap-up chapter discussing the conclusions and summarizing the dissertation.
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Chapter 1

1. Sample Preparation

1.1. Introduction

The proper choice of a measurement technique is only one step in the development of a successful application. All of the steps leading up to the analyte measurement are equally important. The sampling and sample preparation process begins at the point of collection and extends to the measurement step\(^1\textsuperscript{-}\textsuperscript{5}\). The proper collection of sample during the sampling process (called primary sampling), the transport of this representative sample from the point of collection to the analytical laboratory, the proper selection of the laboratory sample itself (called secondary sampling), and the sample preparation method used to convert the sample into a form suitable for the measurement step can have a greater effect on the overall accuracy and reliability of the results than the measurement itself\(^6\textsuperscript{,}7\).

1.1.1. The Analytical Process

The major stages of an analytical process are depicted in Figure 1\(^1\).

![Figure 2. The analytical process](image)

Although many of the chromatographic instrumental techniques have matured and automation is commonplace, sample preparation still is considered to be slow, labor-intensive, and even a bottleneck in laboratory processes. Advances in analytical chemistry have led to the development of instruments with detection limits as low as one part per billion\(^3\). Sample preparation techniques, however, have lagged behind in
development. These antiquated techniques may take hours to days to complete and are greatly dependent on the skills of the operator. It is important to note here that sample preparation contributes as much as, if not more, towards the final results as analytical techniques. Some high-throughput laboratories, particularly in the pharmaceutical industry, take advantage of the latest automation equipment to process hundreds and sometimes thousands of samples a day, but many laboratories use techniques based on age-old methodologies with some degree of miniaturization or low levels of automation. Some of the processes involved in a typical sample preparation in a laboratory are depicted in Figure 2. The analytical process depicted in Figure 1 is described briefly in Section 1.1.2.

1.1.2. Sample Processing Sequence

1.1.2.1 Sampling

Primary sampling is the process of selecting and collecting the sample to be analyzed. The objective of sampling is a mass or volume reduction from the parent batch, which itself can be homogeneous or heterogeneous. If collected incorrectly, then all of the further stages in the analysis are meaningless and the resulting data are worthless. Sampling thus forms a very important start to the entire process.

1.1.2.2 Sample Transport and Storage

Once the primary sample is taken, it must be transported to the analytical laboratory without a physical or chemical change in its characteristics. When the system under investigation is a dynamic entity, such as samples containing volatile, unstable or reactive
materials, the act of transportation can present a challenge, especially if the laboratory is a long distance from the point of collection. Often, prepared laboratory standards, surrogate samples, and blanks are carried through the entire preservation, transport and storage processes to ensure that sample integrity is maintained. Physical, chemical and/or microbiological degradation are minimized by proper preservation techniques. Appropriate sampling containers, addition of chemical stabilizers such as antioxidants and antibacterial agents, freezing the sample to avoid degradation, etc. are examples of preservation techniques. Once the sample has been brought into the laboratory, storage conditions are equally important to maintain sample integrity before analysis. Often, prepared laboratory standards, surrogate samples, and blanks are carried through the entire preservation, transport, and storage processes to ensure that sample integrity is maintained.

1.1.2.3 Secondary Sampling

Once the sample has made it to the laboratory, a representative sub-sample must be taken. This process is called secondary sampling. The size or in-homogeneity of the sample may be a problem in secondary sampling. Statistically appropriate sampling procedures are applied to avoid discrimination, which can further degrade analytical data.

1.1.2.4 Sample Preparation

The next stage of the sampling process is the preparation of the chosen secondary sample. Sample preparation is seen as the last bottleneck in the analytical process, as evident from Figure 3 adapted from Majors. Over the past decades, considerable time has been devoted to improving analysis speed, resolution, and automation of analytical measurement techniques and developing and improving data handling and report generation software. In contrast, sample preparation, particularly its automation, has been neglected. Many analytical chemists use time-consuming manual methods that have been around for
decades. A Gas Chromatograph (GC) separation and measurement can require a few minutes; however, preparation of the sample itself can take one or two orders of magnitude longer. Clearly, speeding up or automating the sample preparation will reduce the analysis time and improve sample throughput.

Every step in the analytical process plays a vital role. Error generation at each step has to be considered for the final product. This is so since the combination of errors is the square root of the sum of the squares of the standard deviation of each error of each of the components that contributes towards the final measurement. This leads to propagation of error when the measurement is a function of input quantities where the function can be defined as: \( x \times y = z \). The propagation of error for the uncertainty \( s_z \) of product is given by the following equation,

\[
s_z = z \times \sqrt{\left(\frac{s_x}{x}\right)^2 + \left(\frac{s_y}{y}\right)^2}
\]

…where \( s_x \) is the standard deviation for \( x \) and \( s_y \) is the standard deviation for \( y \). Another case is when the instrument is at a known uncertainty, the blank, the sampling and the extraction uncertainties are known, since some extractions are not efficient or is there is degradation involved of the analyte due to processing parameters, both change the actual measurement and make the instrumental error irrelevant when the extraction error is taken into consideration. For the purposes of this dissertation, accuracy is defined as the closeness of agreement between a measured value and a true value. True value is defined as the value consistent with the definition of a given particular quantity approached by averaging an increasing number of measurements. Precision (used interchangeably in this dissertation with error) is defined as the degree of consistency and agreement among independent measurements of a quantity under the same conditions; a measure of how well the result has been determined, and the reproducibility or reliability of the result\(^{18,19}\). *(The author wishes to thank Dr. Skip Kingston, Dr. Mike Tobin, and Dr. Mitch Johnson for their input on this section).*
1.1.3. Brief History of Sample Preparation

The art of sample preparation dates back to ancient Greece and Egypt, to the era of alchemists who developed different methods for the pretreatment of samples\(^8\). The elimination of undesired interferences has been the major goal of most sample preparation methods. Some of the sample preparation methods that we use today were developed between 1800s-1900s. For instance, Kjeldahl method for the determination of nitrogen content of proteins was published in 1883. For decades, analysts have used some form of an open-vessel digestion and/or a Carius tube closed-vessel digestion. The Soxhlet method for extraction of fat from biological material has been in use for over 150 years\(^3, 4, 9\).

With automation and computerization of analytical instruments, the onus for precision and accuracy lies on sample preparation now more than ever before. Being a part of an analytical method, any variances resulting from sample...
preparation methods contribute to the total variance of the analytical method. According to a study conducted by LC-GC, sample processing and operator errors account for a significant portion of overall error and sample loss or modification. 4 (Figure 4)

With reference to the same study, some of the most frequently encountered problems in sample preparation are time, cost and lack of reproducibility (Figure 3). An often-overlooked aspect of sample preparation is its effect on error generation. Each sample transfer and each stage in the analytical process represents a potential source of error due to sample loss or modification. Sample preparation accounts for almost one-third of the error generated during the performance of an analytical method; operator error is responsible for another 20%. Thus, improving and automating sample preparation can decrease error in a typical analytical method by as much as 50%1 (Figure 5). It is important that a clear sample preparation strategy be outlined to minimize the number of steps10. Optimization is extremely important as well. This is also linked to a history of theory which has not been optimized for these new capabilities. Chapter 2 will describe these traditional observations where these new capabilities may be improving the abilities of microwaves to accomplish this optimization.

1.1.4. Goals and Objectives of Sample Preparation
Successful sample preparation has a threefold objective: to provide the analyte in solution, to free the analyte from interfering matrix elements, and to obtain the analyte at a concentration appropriate for detection and measurement. A sound sample pretreatment procedure provides quantitative recovery in minimum number of steps.

1.1.4.1 Analyte Quantitation
There are three basic approaches in measuring an analyte in the presence of interfering species found in the sample matrix:

- A selective analytical technique that can measure the analyte in the matrix without the need for sample isolation.
- Conversion of analyte in situ into another chemical species. This approach includes derivatization, digestion, complexation, etc.
- Removal of analyte from the sample matrix by a separation or extraction process. This is the most commonly used approach.

### 1.1.4.2 Evaporation/Sample Preconcentration

Often, when analysis involves the measurement of trace amounts of a substance, it is desirable to increase the concentration of the analyte to a level where it can be measured more easily. Concentration of an analyte can be accomplished by transferring it from a large volume of phase to a smaller volume of phase. This preconcentration is often performed in series or combined with the sample preparation step.

### 1.1.5. Significance of Extraction

As discussed above, most of our research is aimed at tackling and reducing some of the above-mentioned challenges with the help of a comparatively new and rapidly developing technique, Microwave Assisted Extraction.

Extraction techniques are the most widely used of all sample preparation techniques and are extremely useful for both rapid and “clean” separations of both organic and inorganic substances. For many years, laboratory workers were content to use traditional methods extraction. These methods, however, had inherent drawbacks. Most of these methods, e.g., Soxhlet, are time-consuming. The role of any extraction method is to speed up the process whereby analytes are removed from their solid matrix effectively and efficiently. The demand for increased productivity, faster assays and more automation required newer techniques to meet some of these needs. Some of these new techniques are Supercritical Fluid Extraction and Microwave Assisted Extraction. Whichever technique the analyst chooses to use, extraction of the analyte from its matrix remains an integral part of sample preparation. The theoretical basis of extraction as an equilibrium process will be discussed in Chapter 2.

### 1.1.6. Traditional Methods of Extraction

The extraction of analytes from sample matrices requires selection of the right combination of solvent and technique. Table 1 lists popular traditional methods for the
sample preparation of solid samples. Most of these methods (such as Soxhlet extraction and leaching) have been around for over 100 years and are time-tested and provide results that are accepted by most scientists. Regulatory agencies such as the United States Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) and their equivalents in other countries recognize these classical methods as being appropriate for the extraction of solid samples. For the most part these methods use organic solvents, often in copious amounts, although there has been a trend in recent years to miniaturize these systems to minimize sample and solvent requirements.

<table>
<thead>
<tr>
<th>Method of Sample Prep</th>
<th>Principles of Technique</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-liquid extraction</td>
<td>Sample placed in a stoppered container; solvent added; solution separated from solids by filtration</td>
<td>Solvent is usually boiled or refluxed; sample size reduced.</td>
</tr>
<tr>
<td>Soxhlet extraction</td>
<td>Sample placed in thimble; constant reflux of solvent</td>
<td>Extraction occurs in pure solvent; sample must be stable at boiling point of solvent.</td>
</tr>
<tr>
<td>Homogenization</td>
<td>Sample placed in a blender, solvent added, sample homogenized; solvent removed for further workup</td>
<td>Used for plant, animal tissue, food and environmental samples</td>
</tr>
<tr>
<td>Sonication</td>
<td>Sample in ultrasonic bath with solvent and subjected to ultrasonic radiation</td>
<td>Sample size reduction necessary, heat can be added for additional extraction.</td>
</tr>
<tr>
<td>Dissolution</td>
<td>Sample taken into direct solution with or without chemical change</td>
<td>Heat required in many cases; inorganics may also need acids.</td>
</tr>
</tbody>
</table>

1.1.7. Modern Technologies for the Extraction of Solids

For many years analysts have been content to perform sample preparation using traditional methods. However, as the need for increased productivity, faster assays, and more automation arose, newer extraction techniques were developed to meet these requirements. Table 2 lists some of these methods. Some of these methods are automated versions of the traditional methods and are easier to use. Other methods were developed that used new technology. For the most part, these newer approaches, especially those that are automated, are more expensive in terms of the initial purchase price but may cost less on a per-sample basis.

<table>
<thead>
<tr>
<th>Method of Sample Prep</th>
<th>Principles of Technique</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerated Solvent Extraction (ASE)</td>
<td>Sample placed in sealed container; heated to above its boiling point, causing pressure to rise, extracted sample is automatically transferred</td>
<td>Greatly increases speed of liquid-solid extraction and is automated. Vessel must withstand high-pressure; safety provisions</td>
</tr>
<tr>
<td>Extraction Method</td>
<td>Description</td>
<td>Additional Info</td>
</tr>
<tr>
<td>------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Automated Soxhlet Extraction</td>
<td>A combination of hot leaching and Soxhlet; sample in thimble first immersed in boiling solvent then raised for traditional Soxhlet.</td>
<td>Solvent could potentially be recovered for re-use.</td>
</tr>
<tr>
<td>Supercritical Fluid Extraction (SFE)</td>
<td>Sample in flow-through container; supercritical fluid (CO₂) passed; depressurized, extracted analyte trapped on sorbent followed by desorption with solvent.</td>
<td>To affect polarity of supercritical fluid, density can be varied and solvent modifiers can be added. Matrix has an effect on the extraction process.</td>
</tr>
<tr>
<td>Microwave Assisted Solvent Extraction (MASE)</td>
<td>Sample placed in an open or closed container and heated by microwave energy.</td>
<td>In case of open vessel, solvent(s) or azeotropes can be refluxed at boiling point, mimicking solid-liquid extraction; for closed vessels, extraction can be carried out at temperatures higher than the boiling point of the solvent.</td>
</tr>
</tbody>
</table>

1.1.8. Relevant Methods of Extraction: Traditional

1.1.8.1 Hot plate

Heating using a hot plate was the most commonly employed technique for the extraction of selected analytes. The matrix from which the analyte is sought is placed in a beaker with an appropriate amount of a chemically similar solvent (using the rule of thumb: "like dissolves like"). This beaker is then placed on a hot plate and allowed to heat for a specific time. Not only is the extraction governed by the solubility of the analyte in the chosen solvent but also on the boiling point of the solvent as most commonly this will be the temperature where extraction is carried out. A most common drawback of this method is that there is no uniform temperature control on the surface and as illustrated by Figure 6, beakers placed in different positions attain different temperatures.
1.1.8.2 Soxhlet:
The objective of Soxhlet is to extract semi-volatile organic compounds, pesticides and PCBs from solid matrices such as soil, sediments, sludge and solid waste for GC/MS analysis. This technique is by far the most widely used method for solid-sample pretreatment. In this method, the solid sample is placed in a Soxhlet thimble, which is a disposable, porous container, made of stiffened filter paper. The thimble is placed in a Soxhlet apparatus, in which the refluxing extraction solvent condenses into the thimble and the soluble components leach out. The Soxhlet apparatus is designed to siphon the solvent into the extracted components after the inner chamber holding the thimble is filled to a specific volume with solution. The siphoned solution containing the dissolved analytes then is returned to the boiling flask, and the process is repeated until a maximum amount of analyte is successfully removed from the solid sample. A major drawback is that Soxhlet extractions are usually slow—often requiring 24 hours or more. Samples can only be extracted one at a time for each apparatus. It uses hundreds of milliliters of very pure solvent, which is expensive. Disposal of these solvents as hazardous waste is expensive. Because the dissolved analyte is allowed to accumulate in the flask, the sample must be stable at the boiling point of the solvent. The extraction methods require some method development. Solvent extractions are concentrated by evaporations during most soil extractions. Excess solvent is usually evaporated in a hood and vented to the atmosphere, potentially leading to environmental concerns. This method is usually applicable only to solid samples (Figure 7).

![Figure 7. Schematic of Soxhlet Extractors](image)

*Figure 7. Schematic of Soxhlet Extractors*
1.1.9. Relevant Methods of Extraction: Modern

1.1.9.1 Sonication
Ultrasonic agitation is another method used for the extraction of nonvolatile and semivolatile organic compounds from soils. In this method, a portion of the sample is mixed with anhydrous sodium sulfate to form a free-flowing powder. To this is added a chemically similar solvent followed by placing it in a “booth” in a sonicator. This is extracted repeatedly using sonication. The extraction solvent is then filtered through a plug of sodium sulfate. This is then concentrated. Ultrasonic agitation allows more intimate solid-liquid contact and the gentle heating generated during sonication can aid the extraction process. A drawback is that matrix interferences may be co-extracted from the sample. Pre-concentration requires evaporation and necessitates the venting of solvent to atmosphere creating environmental concerns similar to Soxhlet.

1.1.9.2 Solid Phase Extraction (SPE)\textsuperscript{13}
Liquid-liquid extractions have certain limitations. The extracting solvents are limited to those that are water immiscible. Emulsions form when solvents are agitated and relatively large volumes of solvents are used which generate substantial waste disposal problem. The operations are usually manually performed, and may require a back extraction.

Solid phase extraction (SPE) is an increasingly useful sample preparation technique Figure 8. With SPE, many of the problems associated with liquid/liquid extraction can be prevented, such as incomplete phase separations, low recoveries, use of expensive, breakable specialty glassware, and disposal of large quantities of organic solvents. SPE is usually more efficient than liquid/liquid extraction, yields near quantitative extractions, is easy and rapid, and can be automated. Solvent use and lab time are reduced.
SPE is used most often to prepare liquid samples and extract semivolatile or nonvolatile analytes, but also can be used with solids that are pre-extracted into solvents. SPE products are excellent for sample extraction, concentration, and cleanup. They are available in a wide variety of chemistries, adsorbents, and sizes. Selecting the most suitable product for each application and sample is important.

In this technique, hydrophobic functional groups are chemically bonded to solid surface e.g. powdered silica. A common example is the bonding of C₁₈ chains on silica. These groups interact with hydrophobic organic functional compounds by Vander Waal’s forces, dipolar attraction, hydrogen bonding and electrostatic attraction and extract them from an aqueous sample in contact with the solid surface. The powdered phase is generally placed in a small cartridge. Sample is placed in the cartridge and forced through. Trace organic molecules are extracted, preconcentrated on the column and separated away from the sample matrix. Then they can be eluted with a solvent such as methanol and then analyzed.

The following are the type of interactions involved in this technique of extraction:¹⁴

- Reversed Phase (polar liquid phase, non-polar modified solid phase)
  Hydrophobic interactions, nonpolar-nonpolar interactions, Van der Waal’s dispersion forces
- Normal Phase (non-polar liquid phase, polar modified solid phase)
  Hydrophilic interactions, polar-polar interactions, hydrogen bonding, pi-pi interactions, dipole-dipole interactions, dipole-induced dipole interactions
- Ion Exchange
Electrostatic attraction of charged group on compound to a charged group on the sorbent’s surface

- Adsorption (interactions of compounds with unmodified materials)
  Hydrophobic and hydrophilic interactions may apply (Depends on which solid phase is used).

1.1.9.3 Supercritical Fluid Extraction (SFE)\textsuperscript{15-18}

![Schematic of Supercritical Fluid Extraction](image)

SFE is a technology, which uses a solvent with properties between that of a gas and a liquid to more efficiently extract contaminants from solid matrices such as wastes, sludges and soils. The solvent, or supercritical fluid, most commonly consists of pure, non-toxic carbon dioxide or CO\textsubscript{2} that contains small amounts of modifiers like methanol or acetonitrile to enhance extraction of some compounds. In the SFE process, a fluid is passed through a pump and raised to its supercritical temperature and pressure. This fluid enters a high-pressure stainless-steel extraction cell containing the solid matrix, e.g., soil, co-mixed with a drying agent such as sodium sulfate. Organic contaminants sorbed to the soil rapidly dissolve in the fluid while water in the soil (which can adversely affect contamination extraction and recovery) is retained by the sodium sulfate. The fluid containing the dissolved contaminants exits the extraction cell and passes through a restrictor into a collection vessel containing a small amount of organic solvent. As the fluid passes through the restrictor, it cools and expands to a gas at atmospheric pressure. The extract in the collection vessel is further concentrated under nitrogen gas and then may be subjected to a variety of possible chromatographic, spectroscopic measurements. Drawback of SFE is that it releases significant levels of hazardous chemicals to the atmosphere during its time of operation. Also, while SFE extraction efficiency from aged soils was demonstrated to
be high and comparable to Soxhlet, recovery of the analytes by SFE was low due to poor solvent trapping efficiency (Figure 10).

1.1.9.4 Accelerated Solvent Extraction (ASE):

ASE is a technique that combines elevated temperatures and pressures with liquid solvents to achieve fast and efficient removal of analytes from various matrices. It is, in principle, a liquid-solid extraction process performed at elevated temperature (50-200° C) and pressures (1500-2000 psi); thus, all of the principles inherent to that technique apply to this as well. As the temperature is increased, the viscosity of the solvent is decreased, thereby increasing its ability to wet the matrix and solubilize the target analytes. The added thermal energy also assists in breaking the analyte-matrix bonds and encourages analyte diffusion to the matrix surface. The effect of pressure is to maintain the solvents as liquids while above their atmospheric boiling points and to rapidly move the fluids through the system. An advantage is that the system is automated and typical extraction times vary from 10-20 min per sample. Another advantage is less use of solvent. Drawbacks are that the rigorous conditions sometimes used in extractions may remove more substances from a solid sample. The extracted sample is dissolved in a slightly greater volume of solvent, hence it has to be concentrated involving additional manual steps (Figure 11).
1.1.9.5 Microwave Assisted Extraction:

Microwave Extraction method is the process of heating solid sample-solvent mixtures in a sealed (closed) vessel with microwave energy under temperature-controlled conditions. Although used less frequently, the extraction can also be performed in an open vessel at atmospheric pressure. The closed system provides significant temperature elevation above the atmospheric boiling point of the solvent, accelerates the extraction process, and yields performance comparable to the Soxhlet method. Samples are processed in batches of as many as 12 per run (this figure depends on the make of the instrument used). The microwave energy provides very rapid heating of the sample batch to the elevated temperatures, which shortens the extraction time to 10-12 minutes per batch. Solvent consumption is only 25-30 ml per sample. After the heating cycle is complete, the samples are cooled and the sample is filtered to separate the sample from the extract for the analytical step. This technique is further discussed in detail in Chapter 3.

Microwave Extraction is fast gaining acceptance and it is the latest technique to be included in SW-846. Draft Update IVB, which was recently issued by the EPA's Office of Solid Waste and contains methods which are being considered for inclusion in SW-846. One of the methods that is included is Method 3546. Some of the standards methods that either focused on, or based on microwave extraction and/or digestion are included in Table 3.

<table>
<thead>
<tr>
<th>Standard Method</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA 3015</td>
<td>Microwave Assisted Acid Digestion of Aqueous Samples and Extracts</td>
</tr>
<tr>
<td>EPA 3051</td>
<td>Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils</td>
</tr>
<tr>
<td>EPA 3052</td>
<td>Microwave Assisted Acid Digestion of Siliceous and Organically Based Matrices</td>
</tr>
<tr>
<td>EPA 3050B</td>
<td>Acid Digestion of Sediments, Sludges, and Soils</td>
</tr>
<tr>
<td>ASTM D 6010</td>
<td>Standard Practice for Closed Vessel Microwave Solvent Extraction of Organic Compounds from Solid Matrices</td>
</tr>
<tr>
<td>EPA 3546</td>
<td>Microwave Extraction</td>
</tr>
<tr>
<td>GP28-A</td>
<td>Microwave Device Use in the Histology Laboratory; Approved Guideline (Vol. 25, No. 7—CLSI document index of NCCLS Standards) (Feb 2005)</td>
</tr>
</tbody>
</table>

Integration of the above mentioned steps that are involved in microwave extraction leads a step further towards automation, and is the focus of this dissertation. The classical

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*CLSI: Clinical and Laboratory Standards Institute  
NCCLS: National Committee for Clinical Laboratory Standards
theory of extraction and its relation to microwave extraction will be explained in Chapters 2 and 3.

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1.3. References:


(14) Supelco; Sigma-Aldrich Co.: Bellefonte, 1998, pp 1-12.


Chapter 2 Overview

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Chapter 2

2. Extraction

2.1. Extraction

2.1.1. Introduction

By and large, extraction techniques are the most widely used of all sample preparation methods and are extremely useful for both rapid and clean separations of both organic and inorganic substances. More than 50% of the respondents in a survey by Majors said that they used sample preparation procedures for solubilizing some or all of a sample matrix through contact with liquids or supercritical fluids\textsuperscript{1-3}. (Figure 1)

For many years, laboratory workers were content in using the traditional methods. Most of these methods, e.g., Soxhlet, are time tested and provide results that are readily acceptable to most scientists. These methods are also accepted by USEPA as well as other regulatory agencies like the Food and Drug Administration (FDA). These methods, however, had inherent drawbacks. These techniques are time-consuming and use copious amounts of solvents, usually hazardous, thus proving to be not so viable economically as well as environmentally. An attractive extraction method speeds up the process whereby analytes are removed from their solid matrix effectively and efficiently. The demand for increased productivity, faster assays and more automation required newer techniques to meet some of these needs. Some of
these new techniques are Supercritical Fluid Extraction and Microwave Assisted Extraction. Whichever technique the analyst chooses to use, it remains that extraction of the analyte from its matrix is an integral part of sample preparation.

2.1.2. Classic Extraction Technology

Before extraction, solid samples must be changed into a physical state that provides the extracting medium with a greater surface area per unit mass. Samples that are finely divided can be extracted more rapidly than samples with larger surface area. There are many methods available to reduce particle sample size, namely, chopping, cutting, blending, grinding, homogenizing, macerating, pulverizing and sieving. Furthermore, before solid samples can be injected into gas or liquid chromatographs they must be converted into a liquid state. Thus, solid samples must be treated so that the components of interest are put into solution either by dissolving the entire sample matrix or by leaching the analytes from the solid matrix using a suitable solvent. No single solvent or extraction technique can be used for all the organic or inorganic compounds from all possible sample matrices.

The extraction of analytes from sample matrices requires the right combination of solvent and technique. Table 1 (Chapter 2) lists popular traditional methods for the sample preparation of solid samples. Most of these methods (such as Soxhlet extraction and leaching) have been around for over 100 years and are time-tested and provide results that are accepted by most scientists. Regulatory agencies such as the United States Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) and their equivalents in other countries accept these classical methods for the extraction of solid samples. For the most part these methods use organic solvents, often in copious amounts, although there has been a trend in recent years to miniaturize these systems to minimize sample and solvent requirements. Some other techniques considered to be traditional methods of extraction include sonication, homogenization, shake-filter methods, etc. Table 2(Chapter 1) lists some of the popular modern methods for sample preparation in context of extraction. Some of these methods include Accelerated Solvent Extraction (ASE), Microwave Assisted Solvent Extraction (MASE), Supercritical Fluid
Extraction (SFE), Solid Phase Extraction (SPE), etc. These methods have been discussed in relevant detail in Chapter 1.

2.1.3. Modern Techniques versus older technologies: Are the comparisons always valid?

Solid-liquid extraction takes many forms. The shake-flask method merely involves the addition of a solvent (for example, organic solvent for organic compounds and dilute acid or base for inorganic compounds) to the sample and by agitation allows the analytes to dissolve into the surrounding liquid until they are removed as completely as possible. This method works well when the analyte is very soluble in the extracting solvent and the sample is quite porous. To get more effective solid-liquid contact, samples must first be brought into a finely divided state thereby increasing surface area. Heating or refluxing the sample in hot solvent may be used to speed up the extraction process. The shake-flask method can be performed in batches, which increases overall sample throughput. Once the analytes are removed (determined during method development by making analyte measurements as a function of time), the insoluble substances are removed by filtration or centrifugation.

Sonication can be used to get faster and more complete extraction. The ultrasonic agitation allows more intimate solid-liquid contact and the gentle heating that results during sonication can aid the extraction process. Sonication is also a recommended procedure for the pretreatment of solid environmental samples. For example, EPA Method 3550 for extracting nonvolatile and semivolatile organic compounds from solids such as soils, sludges, and wastes specifies sonication extraction. In this method, different extraction solvents and sonication conditions are recommended depending on the type of pollutants and their concentration in the solid matrix. Homogenization in the presence of solvent is also an effective way to maximize extraction yield.

By far the most widely used method for the sample pretreatment of solids is Soxhlet extraction. The thimble is placed in the Soxhlet apparatus, where refluxing extraction solvent condenses into the thimble and leaches out the soluble components. The Soxhlet
apparatus is designed to siphon the solvent with extracted components once the inner chamber holding the thimble fills up with solution to a certain volume. The siphoned solution containing the dissolved analytes then returns to the boiling flask and the process is repeated over and over again until the analyte is successfully removed from the solid sample. Soxhlet extractions are usually slow, often approaching 18 to 24 hr. However, the process takes place unattended, so once the sample is loaded and refluxing begins, there is little operator involvement until the conclusion of the extraction. Each sample requires a dedicated apparatus. Thus, one often sees rows of Soxhlet extractors in the fume hood in laboratories that use this technique. Soxhlet extraction is less expensive than some of the more modern extraction techniques. Glassware itself is rather inexpensive. However, the most common extractors use hundreds of milliliters of high purity solvent. Small-volume Soxhlet extractors and thimbles are available for small amounts of sample, down to milligram sizes. In the Soxhlet process, fresh extraction solvent is always presented to the sample. Because the dissolved analyte is allowed to accumulate in the boiling flask, it must be stable at the boiling point of the extraction solvent. Method development in Soxhlet extraction involves finding a solvent or solvent mixture that has a high affinity for the analyte and a low affinity for the solid sample matrix. The solvent should have a high volatility because it must be removed at the conclusion of the extraction in order to concentrate the analyte of interest. Usually, all the newer modern techniques use Soxhlet as a comparison platform for the validation of this newer technique (and have been occasionally compared in the context of this dissertation). However, these mechanisms of extraction, especially matrix effects, may be different and such comparisons are not always relevant, and other validation parameters must be ratified for the technique to be convincing and justifiable. However, because this form of extraction is one of the oldest methods, it is the de facto standard and many newer extraction technologies, such as SFE, accelerated solvent extraction, and microwave-assisted extraction will continue to be compared to Soxhlet extraction.

2.1.4. Theory of Extraction
The general importance of the separation method to the chemist needs little elaboration, as discussed in the earlier part of this chapter. Separation is essential in many analytical
schemes, in the purification of synthetic products, and also in the isolation of natural products from plant, animal or mineral sources. Most laboratory separations involve one or more solvents that play an essential role in the separation process. Solvent selection therefore falls within the scope of this chapter and will be discussed further in Section 2.1.9.

2.1.5. Factors Affecting Solubility and Separation

In many separation processes the ability of the solvent to selectively dissolve certain sample components directly affects the resulting separation. In solvent extraction, for example, one sample component $x$ may be extracted to a greater extent than $y$, thus effecting the separation of these two compounds. Similarly, in liquid-liquid column chromatography, compound $x$ may be more soluble in the mobile phase, while compound $y$ may be more soluble in the stationary phase. Compound $x$ will then move through the chromatographic column more rapidly than $y$, again resulting in the separation of $x$ from $y$. Since solubility of a compound in a given phase is significant, it becomes necessary to know the factors that govern the relative solubility of that compound in that phase.

Considering two solvents A and B, what makes A a better solvent than B for a given solute $x$? For convenience, let us assume that A and B are immiscible. Thus, if $x$ is more soluble in A than B, its concentration in phase A will increase, and will be greater than that in B, once the mixture of solvents and solute has equilibrated. This equilibrium is further discussed in Section 2.1.6. The concentrations or the mole fractions of $x$ in the two phases will be given as:

\[
\frac{C_{x,a}}{C_{x,b}} = e^{-\Delta GRT}
\]

Equation 1

Where $C_{x,a}$ and $C_{x,b}$ are the concentrations of $x$ in phases A and B respectively, $R$ is the gas constant, $T$ is the temperature (K) and $\Delta G$ is the free energy transfer for one mole of compound $x$ from phase B to phase A. Solution theory commonly ignore entropy effects, since these are usually subordinate to heat effects; thus, Equation 1 can be re-written as
Here, $\Delta H$ is the enthalpy change for the transfer of one mole of compound $x$ from phase B to A. If $\Delta H$ is positive (interactions of $x$ with solvent B are stronger), the quantity on the right will be less than 1, and $x$ will prefer phase B ($C_{x,b} > C_{x,a}$).

This transfer of a molecule $x$ from solvent B to solvent A, which corresponds to the quantity $\Delta H$ and therefore determines the relative solvency of B versus A for $x$ can be visualized as depicted in Figure 2. Figure 2a portrays the interactions of a part of a molecule $x$ ($x_i$) with surrounding molecules of solvent B; $x_i$ might correspond to a specific functional group $i$ in $x$. The interactions between $x_i$ and surrounding molecules B are shown in Figure 2 by indicated arrows.

In Figure 2b, $x$ is removed from phase B, leaving a cavity that subsequently collapses (Figure 2c) when the original interactions between molecules of B and $x_i$ are replaced by interactions between adjacent molecules of B. In figures 2d-2f, the group $x_i$ is added to solvent A, the reverse of the process shown in Figures 2a to Figure 2c: bond breaking between adjacent molecules of A with cavity formation ($d, e$) and insertion of $x_i$ into the

$$
\frac{C_{x,a}}{C_{x,b}} \approx e^{-\Delta H/RT}
$$

Equation 2
cavity (Figure 2f). The overall process (Figures 2a-2f) corresponds to the transfer of the group $x_i$ from the solvent B to the solvent A and gives us some insight into the factors that determine $\Delta H$ and the relative solubility of $x_i$ in B versus A.

In steps $a$ and $b$ of Figure 2, bonds (or interactions) between B and $x_i$ must be broken, requiring addition of heat to the system. The stronger these interactions, the greater the preference of $x_i$ for solvent B, and the greater is solubility of $x_i$ in B. In step $c$, interactions between like molecules B are formed, which releases heat from the system. The stronger these interactions, the less the preference of $x_i$ for solvent B. In steps $d$ and $e$, interactions between like molecules A are broken, requiring addition of heat. The stronger these interactions, the less the preference of $x_i$ for A. In step $f$, interactions between A and $x_i$ are formed, releasing heat from the system. The stronger this bond, the greater is the preference of $x_i$ for solvent A.

Thus, it is clear that the value of $\Delta H$ for transfer of $x_i$ from solvent B to A depends on the interactions between molecules of A (A-A), molecules of B (B-B), and between molecules of A or B and the group $x_i$ (A-$x$, B-$x$). The nature and the magnitude of these interactions are discussed in Section 2.1.7.

### 2.1.6. The Polarity of Solvents and Solutes

Whether the solvent under consideration is “better” will be determined by the relative magnitude of the above-mentioned different interactions between molecules of A, B, and $x_i$. However, this involves a large number of individual contributions to solvency, particularly if (as is usually the case) more than one type of interaction exists, and if several groups, $x_i$ are present in the solute molecule. Polarity is the relative ability of a molecule to engage in strong interactions with other “polar” molecules (not specifically the presence in a molecule of a large dipole moment). Thus water is commonly regarded as one of the most polar compounds. Yet, water has a relatively small dipole moment compared to less “polar” compounds like ketones and nitriles. Polarity, then, represents the ability of a molecule to enter into interactions of all kinds—dispersion, dipole, hydrogen bonding, and ionic. “Relative polarity” is the sum of all possible interactions.
Thus, if all the individual interactions between A, B and $x_i$ are lumped together, we can define one single “polar” interaction. Thus, as previously identified, there are four bond-breaking or bond-making steps in the transfer of $x_i$ from solvent B to A, and a contribution $H$ to the total enthalpy change $\Delta H$ can be defined for each step as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Bonds Affected</th>
<th>Contribution $H$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a, b$</td>
<td>(B)-(xi) bonds broken</td>
<td>$2H_{x,b}$</td>
</tr>
<tr>
<td>$c$</td>
<td>(B)-(B) bonds formed</td>
<td>$-H_{b,b}$</td>
</tr>
<tr>
<td>$d, e$</td>
<td>(A)-(A) bonds broken</td>
<td>$H_{a,a}$</td>
</tr>
<tr>
<td>$f$</td>
<td>(A)-(xi) bonds formed</td>
<td>$-2H_{x,a}$</td>
</tr>
</tbody>
</table>

Note that half as many bonds or interactions are involved during cavity collapse or formation (steps $c$ and $d, e$) as in the removal or addition of $x_i$ to a solvent; this accounts for the factor 2 shown in the $H$ values for steps involving $x_i$. $H$ is positive (energy required to be added to the system) when bonds are broken and negative when bonds are formed. The $H$ terms above are added to give an overall value of $\Delta H$ for the transfer of group $x_i$ from solvent B to A:

$$\Delta H = \left(H_{a,a} - H_{b,b}\right) + 2\left(H_{x,b} - H_{x,a}\right)$$

Equation 3

Solvent and solute polarities have thus been defined in terms of the strength of total interactions between adjacent molecules (i.e. the $H$ values above). Thus, a polar molecule $i$ interacting with a polar molecule $j$ should give a large value of $H$. Theoretical expressions for a specific interaction (e.g., dispersion) confirm this$^4,5$ and suggest that $H$ can be related to the “polarity” $P_i$ and $P_j$ of molecules $i$ and $j$ as

$$H_{i,j} = P_i P_j$$

Equation 4

Equation 4 is referred to as the “geometric mean approximation.”

If we define the polarities of A, B, and xi as $P_a$, $P_b$ and $P_x$, substitution of these values into Equation 4 gives
\[ \Delta H = \left( P^2_a - P^2_b \right) + 2P_x \left( P_b - P_a \right) \]

Equation 5

Thus, from Equation 5, \( \Delta H \) and the relative solvency of A and B for \( x_i \) depend on the polarities of A, B and \( x_i \). If \( x_i \) is exactly intermediate between A and B in terms of polarity (i.e., \( P_x \) is the average of \( P_a \) and \( P_b \)), Equation 5 becomes zero, and \( x_i \) is distributed equally between the two solvents A and B at equilibrium. This means that the solvencies of A and B for \( x_i \) are exactly equal. If the polarity \( P_x \) of the solute is closer to that of the solvent A \( \Delta H \) becomes negative, and at equilibrium \( x_i \) will concentrate into solvent A (i.e. \( x_i \) is now more soluble in A than in B). Thus, Equation 5 provides a quantitative statement of the rule of thumb, “like dissolves like”. A corollary to this rule and Equation 5 is that solute solubility in a given solvent is greatest when the polarities of solvent and solute are equal. For polarity of solvent mixtures however, the interaction heats \( H_i \) will be averaged for the two solvents \( i \) and \( j \) of the mixture, according to their volume fractions and in the solvent mixture. Thus, for a mixture of solvents \( i \) and \( j \), the polarity \( P_{i,j} \) of the mixture is given as

\[ P_{i,j} = \phi_i P_i + \phi_j P_j \]

Equation 6

2.1.7. Intermolecular Interactions

Intermolecular interactions exist in several different varieties, and these are important in affecting relative solvency and separation:

- Dispersion interactions
- Dipole interactions
- Hydrogen bonding
- Covalent bonding

2.1.7.1 Dispersion Interactions

Dispersion or London forces exist between every pair of adjacent molecules, and these interactions normally account for the major part of the interaction energy that holds the molecules together in the liquid phase. Consider two unlike molecules (e.g. X and S) immediately adjacent to each other. The electrons associated with each molecule are in
constant, random motion, and at any instant in time the electrons of molecule X will have a certain configuration. In general, this specific configuration is not symmetrical about the atomic nuclei, and an instantaneous dipole moment results for molecule X (Figure 3a). This instantaneous dipole in X then induces an interactive dipole in molecule S as in Figure 3b. Because the resulting dipoles are aligned for electrostatic interaction (attraction of opposite charges), a net attractive interaction between molecules X and S results. These dispersion interactions are independent of the interactions of permanent molecular dipoles discussed later, and they occur in case of both polar and non-polar molecules.

The relative strength of this dispersion interaction between two molecules depends on the number of electrons per unit volume of pure liquids X and S and on their polarizability. As illustrated in Figure 3, the larger the induced dipole formed in the molecule S, more electrons there are in S and the easier it is to displace or polarize each of these electrons. Since the overall tendency of compounds to interact by dispersion forces is closely related to the refractive index values of the compounds in question, the stronger the dispersion interactions, the greater the refractive index values of compounds X and S.

2.1.7.2 Dipole Interactions

When a molecule possesses a permanent dipole (as opposed to a transient dipole as discussed in Section 2.1.7.1), two additional interactions with adjacent molecules are possible. 

*Dipole induction* is the same type of interaction illustrated in Figure 3b, except that the transient dipole of molecule X is replaced with a permanent molecular dipole. This
permanent dipole in X then induces a dipole in S, just as occurs in dispersion interactions. The net effect is an increase in the total interaction between X and S, due to permanent dipole originally present in molecule X.

_Dipole orientation_ involves the alignment of two adjacent molecules, each one possessing a permanent dipole moment, for maximum electrostatic attraction. For example, if X and S each refer to a molecule of acetonitrile, the molecules will line up as denoted in the following equation for maximum attraction between unlike charges.

\[
\begin{array}{c}
\text{H}_3\text{C} & \text{C} \equiv \text{N} & \text{CH}_3 \\
\text{CH}_3 & \text{N} \equiv \text{C} & \text{CH}_3
\end{array}
\]

Equation 8

Because dipole interactions are short range, dipole interactions are determined by the sum of group dipoles within the molecule, and not by the overall molecular dipole moment of the molecule. Thus, dipole interactions often play an important role in affecting solubility and separation.

### 2.1.7.3 Hydrogen Bonding

The hydrogen bonding interactions between a proton-donor molecule A and a proton-acceptor molecule B play a dominant role in affecting solubility and separation. The donor and the acceptor molecules will align themselves to permit a hydrogen atom of the donor to interact with an electron pair of the acceptor. These interactions by hydrogen bonding can be quite strong, with interaction increasing for more acidic donors and more basic acceptors. The donor properties have often been ascribed to weakly acidic compounds such as sulfoxides, nitro compounds, ketones and esters; even hydrocarbons have been postulated as having donor properties that can yield significant hydrogen bonding interactions. However, it now appears that these later, weakly acidic substances are very rarely significant as proton donors.
2.1.7.4 Covalent Bonding

Certain covalent interactions are often used in separation processes, mainly those that are readily reversible, allowing recovery of original ample components after separation. Compounds that are either acids or bases can be made ionic or nonionic, depending on solvent pH. Usually, there is a large change in the relative solubility of the compound as a result of ionization.

2.1.7.5 Other interactions

Hydrophobic interactions are usually mentioned when discussing aqueous solutions. This is not treated as an additional type of interaction; rather, it is the consequence of interactions already discussed. Hydrophobic interactions are said to be associated with non-polar solutes in polar solvents, i.e., the distribution of a non-polar solute $x$ between a polar solvent and a non-polar solvent, B and A respectively. The value of $P_x$ will be small enough to be negligible,$^4$ (This is never actually the case, but the authors have made this supposition to emphasize the point). Now, the $\Delta H$ value for transfer of $x$ from solvent B to solvent A is given by Equation 5 as $(P^2_a - P^2_b)$. If B is the polar solvent, $P_b >> P_a$, and $\Delta H$ is seen to be negative; that is, $x$ will concentrate into the non-polar solvent A. The driving force is seen from Equation 5 to consist not of non-polar interactions between $x$ and the non-polar solvent A, but rather comes from the term $(H_{a,a} - H_{b,b})$ of Equation 4, which describes the heat required to form a cavity into which molecule $x$ is then placed. In effect, the polar solvent “squeezes” out the non-polar solute $x$ because the interactions of $x$ with B are much weaker than the interactions of molecules B with themselves.

Where the polar solvent is water, and the non-polar solutes are being thus “squeezed out” by hydrophobic interactions, the polarity of the water phase can be increased by addition of various salts (“salting out”). The effectiveness of different salts in increasing these hydrophobic interactions varies widely, leading to the use of the so-called lyotropic series of salts for the salting out of proteins from aqueous solutions.
2.1.8. Solvent Selectivity

If there were only one type of intermolecular interaction (e.g., dispersion forces), Equation 5 would be a reasonably reliable relationship. It would be possible to arrange all solvents in order of their polarity values $P_i$, and the solubility of a given solute would change regularly as $P_i$ is changed (being a maximum when the polarities of the solvent and solute are the same). Thus, relative solvent polarity can be used to estimate a rough solubility (or relative solubility value). Then specific intermolecular interactions between solvent and solute can be considered.

The failure of Equation 5 because of different intermolecular interactions is in fact a practical advantage. If only a single parameter $P_i$ determined the solute polarity, Equation 5 suggests that solutes of similar polarity could not be separated by distribution between two solvents A and B. However, differences in specific interactions between solute and solvent lead to corresponding differences in solubility, and these can be exploited to achieve separation. Such differences in solubility for solvents of similar polarity are collectively referred to as solvent selectivity, meaning the ability of the solvent to discriminate or preferentially dissolve different solutes of similar polarity.

2.1.9. Solvent Selection

Most laboratory separations involve one or more solvents that play a basic role in the separation process. Although in some cases a solvent is already a part of the starting sample, more often the solvent(s) must be added during the separation process. The selection of a specific solvent or solvent mixture for use in a given separation is one of the more complex and less understood operations required of the analyst. Many factors and solvent properties should be considered, apart from those bearing directly on the ability of the solvent to affect an efficient separation. Usually, the analyst relies mainly on chemical “intuition” making use of simple acid-base or complexation equilibriums, a rough understanding of the properties of “polar” (hydrophilic) versus “non-polar” (hydrophobic) molecule, or such qualitative concepts as hydrogen bonding. Emphasis should be put not only on solvent properties that directly affect the separation of the
sample, but also on the peripheral considerations such as safety, economics and compatibility of the solvent with operations that precede or follow separation.

2.1.9.1 “Peripheral” Properties of the Solvent

The peripheral properties of pure solvents include those that are of interest in choosing an appropriate solvent but often do not directly affect separation. Often only the boiling point or density of the solvent is of interest, and this information is available in most general handbooks. Table 2 provides an abbreviated listing of such solvent properties for a number of common solvents (arranged roughly according to solvent polarity).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>n&lt;sup&gt;a&lt;/sup&gt;</th>
<th>η&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ε&lt;sup&gt;c&lt;/sup&gt;</th>
<th>d&lt;sup&gt;d&lt;/sup&gt;</th>
<th>UV Cutoff nm</th>
<th>Solubility (weight %)</th>
<th>Solubility S/W</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Pentane</td>
<td>36</td>
<td>1.355</td>
<td>0.22</td>
<td>1.84</td>
<td>0.61</td>
<td>190</td>
<td>0.010</td>
<td>0.0038</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>69</td>
<td>1.372</td>
<td>0.30</td>
<td>1.88</td>
<td>0.65</td>
<td>190</td>
<td>0.010</td>
<td>0.0009</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>98</td>
<td>1.385</td>
<td>0.40</td>
<td>1.92</td>
<td>0.68</td>
<td>190</td>
<td>0.010</td>
<td>0.0003</td>
</tr>
<tr>
<td>n-Octane</td>
<td>126</td>
<td>1.395</td>
<td>0.52</td>
<td>1.95</td>
<td>0.70</td>
<td>190</td>
<td>0.010</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>81</td>
<td>1.423</td>
<td>0.90</td>
<td>2.02</td>
<td>0.77</td>
<td>190</td>
<td>0.012</td>
<td>0.0055</td>
</tr>
<tr>
<td>Toluene</td>
<td>110</td>
<td>1.494</td>
<td>0.55</td>
<td>2.4</td>
<td>0.86</td>
<td>285</td>
<td>0.046</td>
<td>0.054</td>
</tr>
<tr>
<td>Methylene Chloride</td>
<td>40</td>
<td>1.421</td>
<td>0.41</td>
<td>8.9</td>
<td>1.32</td>
<td>233</td>
<td>0.17</td>
<td>1.32</td>
</tr>
<tr>
<td>Acetone</td>
<td>56</td>
<td>1.356</td>
<td>0.30</td>
<td>20.7</td>
<td>0.78</td>
<td>330</td>
<td>Miscible</td>
<td>Miscible</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>82</td>
<td>1.341</td>
<td>0.34</td>
<td>37.5</td>
<td>0.78</td>
<td>200</td>
<td>Miscible</td>
<td>Miscible</td>
</tr>
<tr>
<td>N,N-DMF</td>
<td>153</td>
<td>1.428</td>
<td>0.80</td>
<td>36.7</td>
<td></td>
<td>270</td>
<td>Miscible</td>
<td>Miscible</td>
</tr>
<tr>
<td>Methanol</td>
<td>65</td>
<td>1.326</td>
<td>0.54</td>
<td>32.7</td>
<td>0.79</td>
<td>190</td>
<td>Miscible</td>
<td>Miscible</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>1.333</td>
<td>0.89</td>
<td>80.0</td>
<td>1.00</td>
<td>190</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a= Refractive Index, 25°C  
b=Viscosity (cP), 25°C  
c= Dielectric Constant, 20°C  
d= Density, 25°C  
W/S: Water in Solvent; S/W: Solvent in Water

There are usually many solvents available that have acceptable peripheral properties for a given application. One approach is to select from this large group of solvents the best solvents from the standpoint of separation. Since binary or ternary solvent mixtures can often be employed in place of pure solvents, an enormous choice of solvents and solvent mixtures is available.
2.1.9.1.1. Boiling Point

Normally we require a solvent whose boiling point is above the temperature of the separation process. In separations where temperature varies during the separation (e.g. Soxhlet extraction), a solvent is needed whose boiling point falls at some accessible higher temperature. (Refer to Table 2 for the boiling points of some common solvents).

We often want to remove the solvent from the separated sample fractions on completion of the separation process. Or, following the solvent extraction of a solid sample, the solvent must be removed from a recovered fraction. The easiest technique for removal of solvent from nonvolatile samples is simple solvent evaporation, which means that solvents boiling 10°C to 50°C above the temperature of separation are preferable to higher-boiling solvents. In the case of volatile samples, fractional distillation can be used to separate solvent from final sample fractions. Again, the boiling points of solvent and sample can be used to select appropriate sample-solvent combinations.

2.1.9.1.2. Viscosity

Low-viscosity solvents are preferable for their ease of use. This is particularly true in liquid chromatography, where more viscous mobile phases mean poorer separations. Generally speaking, solvents with low viscosities also have low boiling points, as indicated by Table 3.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Viscosity (cP at 20°C)</th>
<th>Boiling Point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Pentane</td>
<td>0.23</td>
<td>36</td>
</tr>
<tr>
<td>n-Octane</td>
<td>0.55</td>
<td>126</td>
</tr>
<tr>
<td>n-Dodecane</td>
<td>1.51</td>
<td>216</td>
</tr>
<tr>
<td>n-Hexadecane</td>
<td>3.34</td>
<td>287</td>
</tr>
</tbody>
</table>

These data typically show a regular increase in solvent viscosity with boiling point. Exceptions are very polar solvents (e.g. alcohols) and compact molecules (e.g. cyclohexanes, aromatics, CCl₄), which generally have higher viscosities than predicted. The viscosity of a solvent mixture is normally intermediate between the viscosities of the
pure solvents composing the mixture. For a binary mixture of pure solvents A and B, the viscosity $\eta$, of the mixture is given approximately by the following relationship:

$$\eta = (\eta_a)^{x_a} (\eta_b)^{x_b}$$

Equation 9

Where $\eta_a$ and $\eta_b$ refer to the viscosities of pure A and B respectively, and $x_a$ and $x_b$ refer to the mole fractions of A and B in the mixture. The primary practical significance of this relationship is that dilute solutions of a viscous solvent B in a non-viscous solvent A will have viscosities close to that of solvent A. Thus in applications where solvent viscosity must be as low as possible, it is nevertheless possible to use solutions of a relatively viscous solvent.

2.1.9.1.3. Solvent Properties Affecting Detection

In some cases it is of interest to assay for one or more separated sample compounds in a solvent phase resulting from the separation (i.e., without separation of solvent from sample). This is true, for example, in liquid chromatography, where separated compounds in the mobile phase (solvent) go directly to a photometric, refractive index or other detector. Thus, a solvent that absorbs strongly at a given wavelength cannot be used for analysis at that wavelength. Alternatively, (e.g., with refractive index detection), one may wish to maximize the difference in sample versus solvent refractive index values, for maximum detection sensitivity.

2.1.9.1.4. Other Properties

Solvent density is an important parameter in phase separations based on “gravity”. Solvent mixtures have densities close to the arithmetic average of the pure solvent components; for example, for binary solvent mixtures,

$$d \approx d_a \phi_a + d_b \phi_b$$

Equation 10

Here, $d$, $d_a$, and $d_b$ refer respectively to densities of the mixture, of solvent A, and of solvent B; $\phi_a$ and $\phi_b$ refer to volume fractions in the mixture of solvents A and B respectively.
Solvent toxicity is an important consideration. It should be noted that several solvents formerly regarded as being relatively innocuous are now considered to be dangerous for long-term exposure. It is therefore essential to check the relative toxicity of any solvent before designing an experiment with that solvent.

Solvent flammability is of general interest in selecting solvents for some practical applications, and of particular interest for microwave assisted extraction using volatile organic solvents. Low boiling solvents tend to be the most flammable. Hydrocarbons boiling below 100°C generally have flash points less than 30°C, whereas oxygenated solvents such as alcohols, acids, and esters have somewhat higher flashpoints relative to hydrocarbons of similar boiling point. Halogenated solvents such as methylene chloride do not even have flash points and are therefore less flammable (but more toxic).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Phenol</th>
<th>Methanol</th>
<th>Ethanol</th>
<th>Acetonitrile</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>Im.</td>
<td>Im.</td>
<td>Mis</td>
<td>Im.</td>
<td>Im.</td>
</tr>
<tr>
<td>n-Heptane</td>
<td>Im.</td>
<td>Im.</td>
<td>Mis</td>
<td>Im.</td>
<td>Im.</td>
</tr>
<tr>
<td>Benzene</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Im.</td>
</tr>
<tr>
<td>Toluene</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Im.</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Im.</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>Im.</td>
<td>Mis</td>
<td>Mis</td>
<td>Mis</td>
<td>Im.</td>
</tr>
<tr>
<td>Ethyl Ether</td>
<td>-</td>
<td>Mis</td>
<td>Mis</td>
<td>-</td>
<td>Im.</td>
</tr>
</tbody>
</table>

Key: Mis, miscible; Im., immiscible.

2.1.9.1.5. Solvent chemical reactivity

This is often an important consideration, since solvents that may react with the sample are generally undesirable. For this reason, aldehydes are seldom used as solvents and ketones are unsuitable in some applications while esters are known to form peroxides that can then react with a sample and therefore avoided if possible.

2.1.9.1.6. Solvent miscibility
Miscibility with other solvents is of obvious interest in some applications (e.g., liquid-liquid extractions). Table 3 summarizes solubility for some of the most common solvents. Other solvent properties such as surface tension and freezing point can also play a role in special situations.

2.1.10. Solvent Classification Schemes

Hydrogen bonding can play a potential role in solvent selectivity. In the past, such solvent properties as dielectric constant and the solubility of water in the solvent were used as quantitative indices of solvent polarity.

2.1.10.1 Solvent and Solute Polarity Scales

a. The Hildebrand Solubility Parameter:

The solubility parameter $\delta$ is currently the most widely applied index of solvent or solute polarity, and in principle it can be used to make quantitative calculations of solubility and separation. It is defined as:

$$\delta = \left( \frac{-\Delta E_v}{V} \right)^{1/2}$$

Equation 13

Where $\Delta E_v$ is the vaporization energy per mole of the compound in question and $V$ is its molar volume; $\Delta E_v$, in turn, is equal to $\Delta H_v$-RT where $\Delta H$ is the heat of vaporization, and can be estimated from the compound boiling point $T_b$ (K at 760mm) from the Hildebrand rule:

$$\Delta H_v (298 K) = 2950 + 23.7T_b \times 0.02T_b^2$$

Equation 14

Thus, the values of $\delta$ are easily calculated for any compound whose boiling point is known.
If we consider the simple vaporization of a molecule B from pure B, the species B, within the cavity of Figure 3a, now represents some fraction of a molecule of B, such that 1 mole of these B1 groups equal 1 ml. As before, the enthalpy changes can be listed,

<table>
<thead>
<tr>
<th>Step</th>
<th>Bonds Affected</th>
<th>Contribution of H</th>
</tr>
</thead>
<tbody>
<tr>
<td>b</td>
<td>(B)-(B) bonds broken</td>
<td>2H_{b,b}</td>
</tr>
<tr>
<td>c</td>
<td>(B)-(B) bonds formed</td>
<td>-H_{b,b}</td>
</tr>
</tbody>
</table>

The net enthalpy change $\Delta H$ is then equal to the sum of these two H values: $H_{b,b}$. But the value of $\Delta H$ is also the heat of vaporization of 1 ml of B, equal to $\Delta H_v/V \approx \delta^2$ (the term $RT$ is small and tends to be negligible in many solution processes). We have previously defined $H_{b,b} = P_b^2$, and $H_{b,b} \approx \delta^2$, so we see that $\delta$ is essentially the polarity parameter $P$ defined in Equation 5.

To calculate $\Delta H$ from the transfer of the molecule $x$ from solvent B to A, Equation 5 (expression on the right hand side) is multiplied by $V_x$, the molar volume of $x$ and replace all $P_i$ values are replaced by the corresponding $\delta$ values:

$$\Delta H = V_x \left( \delta_a^2 - \delta_b^2 \right) + 2\delta_x \left( \delta_b - \delta_a \right)$$

Equation 15

The main significance of Equation 13 as opposed to Equation 5 is that the molecular size $V_x$ of the solute $x$, affects its relative solubility; the larger the $V_x$, the more affected will be the solubility of $x$ by a change in solvent polarity. Values of $\delta$ for pure compounds can be estimated from the above discussion by noting that the homologs of polar compounds tend to have similar $\delta$ but slightly lower values as compound molecular weight increases. The solubility parameter decreases slightly with temperature.

### 2.2. Extraction as an equilibrium process

Extraction is essentially a separation process which is governed by the distribution of a solute between two immiscible phases. This partitioning of a solute between the two phases is an equilibrium phenomenon governed by the distribution law given by the expression:
\[ [S]_{aq} \leftrightarrow [S]_{org} \]

**Equation 16**

where subscripts refer to aqueous and organic phases respectively.

Ideally, the ratio of activities for solute in the two phases will be constant and independent of the total quantity of solute, i.e., at any given temperature,

\[ K_d = \frac{[S]_{org}}{[S]_{aq}} \]

**Equation 16**

This equilibrium constant, called the distribution coefficient is an expression of the ratio of the concentrations of the solute in the two phases. Distribution coefficients are useful because they provide guidance as to the most efficient way to perform an extraction and/or separation.

In some cases, a solute is partially ionized in aqueous phase. For such solutes, it is more meaningful to describe a different term, the distribution ratio, \( D \), which is the ratio of the concentrations of all the species of the solute in each phase. From the expression acidity constant \( K_a \) for the solute ionization of the solute and the distribution coefficient described previously, the equation for distribution ratio can be derived and written as

\[ D = \frac{K_d}{1 + \frac{K_a}{(H^+)}} \]

**Equation 17**

Of paramount practical importance is the percent of solute extracted into the organic phase.

\[ \%E = \frac{(100 \times D)}{D + \left( \frac{V_{aq}}{V_{org}} \right)} \]

**Equation 18**

where \( \% E = \% \) Extraction Recovery
D= Distribution Ratio

Thus, extraction efficiency is independent of the original concentration of the solute. This is the most salient future of extractive separation, since it can be applied to both trace concentrations and large quantities alike, so long as the solubility of the solute in one of the phases is not exceeded and there are no side reactions such as dimerization of the extracted solute. In cases where the solute ionizes, the extraction efficiency will be influenced by pH.

2.2.1. Multiple extractions

Consider \(a_0\) mmol as the concentration of solute S in \(V_{aq}\) ml of aqueous solution extracted with \(V_{org}\) ml of an immiscible organic solvent. At equilibrium \(a_1\) mmol of solute remains in aqueous layer and \((a_0 - a_1)\) mmol has been extracted into the organic layer. The concentration of solute in the two phases are then written as follows:

\[
\left[ S_{aq} \right] = \frac{a_1}{V_{aq}}
\]

Equation 16

And,

\[
\left[ S_{org} \right] = \frac{(a_0 - a_1)}{V_{org}}
\]

Equation 17

Substitution into Equation 15 and rearrangement gives:

\[
a_i = \frac{V_{aq}}{V_{org}K_d + V_{aq}} a_0
\]

Equation 20

For second extraction:

\[
a_2 = \left[ \frac{a_i}{V_{org}K_d + V_{aq}} \right] a_0
\]

Equation 21

Substituting (20) in (21),
Thus after $n$ extractions,

$$a_0 = \left[ \frac{V_{aq}}{V_{org} K_d + V_{aq}} \right]^n a_0$$

Equation 23

Substituting (20) in (23),

$$\left[ S_{aq} \right]_n = \left[ \frac{V_{aq}}{V_{org} K_d + V_{aq}} \right]^n \left[ S_{aq} \right]_0$$

Equation 24

Equation 24 illustrates that several extractions using small volumes provide a more efficient extraction than does a single extraction using large volume.

2.3. Microwave Heating and Reaction Rates

Energy must be supplied to molecules in order for them to react. In a typical reaction coordinate (Figure 4), reactants have a certain energy level. When the molecules of the reactant collide in the correct geometrical orientation, there is an increase in energy. When the energy within the molecules is equal to or exceeds the activation energy of the system (which is constant for a given system) the molecules will react to completion. For exothermic reaction, the energy of the products is lower than the energy of reactants, the excess energy being lost to the surroundings.

At any given instant, molecules are distributed in energy over a wide range. Figure 5 shows the distribution
of energies at two different temperatures, comparing them with minimum energy needed for the reaction, $E_a$. At higher temperatures, two phenomena take place independent of each other. Energy of system increases and more molecules will have the increased energy as depicted in Figure 5 (red curve). At higher temperature, a much greater fraction of the molecules has kinetic energy greater than $E_a$, which leads to a much higher rate of reaction.

Fraction of molecules that has energy equal to or greater than $E_a$ is given as:

$$ f = e^{-\frac{E_a}{RT}} $$

where $R =$ gas constant (8.314 J/molK)

$T =$ is absolute temperature

$E_a =$ activation energy

Hence, if $E_a = 100$ kJ/mole and $T = 300$ K (room temperature), $f = 3.9 \times 10^{-18}$ and at 310 K, $f = 1.4 \times 10^{-14}$. This is a 3.6 fold increase in the fraction of molecules at $E_a$.

According to the Arrhenius equation, the increase in the rate of reaction with increase in temperature is non-linear. The rate obeyed an equation based on three factors:

1. Frequency of collisions,
2. Probability of molecules in correct orientation
3. Fraction of molecules at energy $E_a$ or greater.

These factors are incorporated in the Arrhenius equation.

$$ k = Ae^{\frac{E_a}{RT}} $$

where, $k =$ reaction rate

$A =$ constant accounting for frequency of collisions at correct orientation,

$e^{-E_a/RT} =$ fraction of molecules with energy activation energy barrier

As $E_a$ increases, reaction rate decreases because the fraction of molecules that have the required energy is smaller.
2.4. **Hypothesis: Microwave heating**

Conventional heating takes place by conduction and convection. The vessel walls are first heated with conduction followed by setting up of convective currents within. In this case, extraction will be governed by the average bulk temperature, $T_B$ of the system. On the other hand, microwave heating involves transfer of microwave energy rapidly and directly to the solution without having to heat the vessel walls\textsuperscript{10}. Some content and pictures used with permission from Dr. Skip Kingston and Dr. Mike Collins, President, CEM Corp, Matthews, NC).

One of the most important aspects of microwave energy is the rate at which it heats. Microwaves will transfer energy in $10^{-9}$ seconds with each cycle of electromagnetic energy. The kinetic molecular relaxation from this energy is approximately $10^{-5}$ seconds. This indicates that the energy transfers faster than the molecules can relax, which results in non-equilibrium conditions and high instantaneous temperatures $T_i$ that affect the kinetics of the system. The high $T_i$ temperatures activate a larger fraction of molecules above the activation energy. The higher fraction of molecules based on Arrhenius equation will increase the rate constant making the transfer of heat faster. In addition most of the intermediates are highly polar species and many of them are even ionic in character, making them good candidates for microwave energy transfer\textsuperscript{10, 11}.

If reaction rate increases with increase in temperature, it is essential to consider that $T_B = T_i$ for conductive heating that translates to slower energy transfer, while for microwave heating kinetics will be controlled by $T_i$ where $T_i \gg T_B$. (Figure 6)
This is applicable to traditional microwave extraction. For IME, a combination of both heating mechanisms comes into play. For a polar solvent, the kinetics are controlled by $T_i$. Mechanisms of convection and conduction also start at this point because of the carbon-fluoropolymer. (This is Teflon impregnated with carbon to make it microwave absorbing). The difference though, is that $T_B$ will be achieved rapidly and $T_i$ is approximately equal to $T_B$, moving a higher fraction of molecules to energy greater than or equal to $E_a$.

With non-polar solvents, the kinetics will be driven by $T_B$, which in turn will be dependent upon the $T_i$ of the carbon-fluoropolymer. The heating is therefore still rapid as compared to conventional heating, but a little less rapidly than the heating by polar solvents. This has been supported by practical observation where 10 ml hexane (boiling point 69°C) takes slightly longer to reach the desired temperature as compared to 10 ml methanol (boiling point 65°C).

2.5. Conclusions

Theory of extraction has been discussed with elaboration on the factors affecting solubility and separation followed by a discussion on the polarity of solutes and solvents. Intermolecular interactions described are dispersion, hydrogen, covalent and dipole. The section on solvent selectivity covers the peripheral properties of a solvent that play an important role in extractions. Some of the properties discussed include: boiling points, viscosity, chemical reactivity, solvent miscibility, solvent polarity and Hildebrand solubility parameter. Extraction is discussed as an equilibrium process covering multiple extractions, followed by a section devoted to the hypothesis for microwave heating.

Based on experimental data from works published over the last few years, chemists have found that reaction rates can be faster than those of conventional heating methods by as much as 1000-fold\textsuperscript{10, 11}. The temperature enhancements needed to increase the energy levels can be provided by microwave energy instantly. These instantaneous temperatures are very consistent with the temperatures that would be expected in a microwave system and are directly responsible for the reaction rate and yield enhancements. The activation
energy parameter expresses the temperature dependence of the rate constant. A small $E_a$ corresponds to a rate constant that does not increase rapidly with temperature, whereas a system with strong temperature dependence has a large $E_a$. With the elevated molecular energy generated by the transfer of microwave energy, extractions that required many hours to complete have been accomplished in minutes. It is also possible to use non-polar solvents to actually reduce bulk heating and directly energize the molecule (the solvent can act as a heat sink to pull thermal energy away from reactants). Thus, microwave heating greatly expands the options for extraction in a variety of fields including environmental, clinical, pharmaceutical, and food industries. Some of these applications are discussed in this dissertation in the following chapters. This is under investigation by many researchers including this laboratory. Microwave energy is a unique form of heating. These observations will not alter the mechanisms but will aid their explanation and depth of understanding once they are completely developed and documented.

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(10) Kingston, H. M.; Collins, M., 2005. Personal communication from Dr. Skip Kingston

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CHAPTER 3

3. Microwave Extraction

3.1. Introduction

Environmental analysis often involves analytes in a wide variety of matrices, ranging from air to waste water to polluted soil samples. The matrix can be aqueous /non-aqueous, solid or air. The analytes are characterized as either non- or semi-volatile organic compounds. Samples analyzed for nonvolatile or semi-volatile organic compounds require a solvent extraction step, with the exception of non-aqueous solvent-soluble samples. The solvent-soluble samples use a simple solvent dilution step, a so-called dilute-and-shoot method.

Over the last couple of decades, ultra-trace analysis and shorter sample processing time for higher sample throughput have become imperative factors. Microwave Enhanced Chemistry (MEC) has played a significant role in achieving this goal.

Microwaves have been used for digestions and extensively for other sample preparation of inorganics. Elemental analysis of nearly every matrix requires dissolution of the sample before instrumental analyses. MEC is a fast, efficient and reproducible sample preparation method. Combination of clean chemistry with MEC has made detection at sub-picogram levels feasible. MEC also makes it possible to reduce sample preparation time from days to minutes. Standardization and automation has enabled an increase in accuracy and precision. For decades, analysts have used some form of an open-vessel digestion or a Carius tube closed-vessel digestion. In 1975, microwaves were first used for the rapid heating source for wet, open-vessel digestions. An initial search revealed...
the increasing interest in extraction of organics using microwave energy as evidenced by
Figure 1. The applications range from solid to liquid matrices as well as a variety of solid
matrices.\textsuperscript{7-20}

Microwave extraction is the latest technique to be included in \textit{SW-846}.\textsuperscript{3} (SW-846 is the Resource Conservation and Recovery Act’s (RCRA)
congressionally mandated methods manual. Draft Update IVB, which was recently issued by the
EPA’s Office of Solid Waste and contains methods which are being considered for inclusion
in SW-846, which includes the microwave extraction method, EPA 3546. The microwave
extraction method is the process of heating solid sample-solvent mixtures in a sealed (closed)
vessel with microwave energy under temperature-controlled conditions. Although used less
frequently, the extraction also can be performed in an open vessel at atmospheric pressure. Figure 2 depicts a typical microwave extraction cell used in a closed extraction system.

This closed system provides significant temperature elevation above the atmospheric boiling point of the solvent, accelerates the extraction process, and yields performance comparable to the standard Soxhlet method. Samples are processed in batches of as many as 14 samples per run. The microwave energy provides very rapid heating of the sample batch to the elevated temperatures, which shortens the extraction time to 10–20 min per batch\textsuperscript{3}. Solvent consumption is only 25–50 ml per sample. After the heating cycle is complete, the samples are cooled and the sample is filtered to separate the sample from the extract for the analytical step.
The use of microwave-enhanced chemistry, offers many advantages over traditional heating methods. As discussed above, closed-vessel microwave extraction allows extraction solvents to be rapidly heated to 2-3 times higher than their atmospheric boiling points resulting in shorter extraction times (10-30 minutes). The amount of solvent consumed is considerably less (20-30 ml). As any new method, for the purpose of acceptance, some comparison platform is needed. IME by nature has been compared to Soxhlet. Table 1 gives a comparison of Soxhlet vs. IME. As indicated in the table, the operating costs of IME come to about 18% of Soxhlet. Time required for the processing of samples is about 2% of the amount required of Soxhlet. Total solvent consumption is about 4% of Soxhlet, translating to disposal costs being around 4% of Soxhlet. Stirring is possible which makes the extraction conditions more homogenous, promotes interaction with the solvent, and assists in releasing the analyte from the matrix. However, MASE has some inherent drawbacks that preclude its widespread use. A chemical compound will absorb microwave energy roughly in proportion to its dielectric constant, i.e., the higher the value of the constant the higher the amount of energy absorbed. Because organic extractions typically involve non-polar solvents with very small, if any, dielectric constants, a polar co-solvent often had to be used to assist in heating the solution. Use of a polar co-solvent led to the extraction of a broader spectrum of compounds in addition to the analytes of interest, creating potential interference problems during analysis. MASE also does not overcome the traditional processing steps of filtration and evaporation. This chapter describes the development of a technique called Integrated Microwave Extraction (IME) designed to specifically overcome these deficiencies. IME integrates the processes of extraction, filtration, evaporation and solvent recovery through the use of integrated hardware to overcome some traditional limitations. Utilization of a microwave absorbing component makes possible the use of non-polar solvents for microwave extraction.

<table>
<thead>
<tr>
<th></th>
<th>Soxhlet</th>
<th>IME</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cost ($)</strong></td>
<td>20,666</td>
<td>3783</td>
</tr>
<tr>
<td><strong>Total Time (Hours)</strong></td>
<td>13,167</td>
<td>257</td>
</tr>
<tr>
<td><strong>Total Solvent (L)</strong></td>
<td>450</td>
<td>15.96</td>
</tr>
<tr>
<td><strong>Solvent Disposal ($)</strong></td>
<td>1261</td>
<td>45</td>
</tr>
</tbody>
</table>
3.2. Instrumentation\textsuperscript{1,2,5}

The two most common types of laboratory microwave units are the multimode cavity and waveguide or focused-type. In the traditional multimode cavity system, the magnetron produces microwaves that radiate from an antenna into a waveguide (a metallic rectangular channel). The reflective walls of the waveguide direct the microwaves into the oven cavity. Then the microwaves are homogenized using a mode stirrer and by rotating the samples on a turntable through the microwave field. The walls of the cavity are made of a reflective material that prevents microwave leakage and increases the cavity’s efficiency. To prevent magnetron damage, non-absorbed radiation is reflected into a load or a secondary waveguide, where the excess energy is dissipated. These types of systems are primarily used for closed-vessel MEC, but they can also be used for open-vessel MEC using a special rotor that evacuates reaction gases and byproducts.\textsuperscript{1}

Multimode cavity laboratory microwave ovens differ from their kitchen counterparts in that they are designed with additional safety features. These units are equipped with explosion-resistant doors, corrosion-resistant cavity walls, and safety interlocks. Laboratory microwave ovens are computer-controlled and equipped with pressure and temperature feedback control mechanisms, which are used to control reaction conditions. All multimode cavity laboratory microwave systems have multiple venting systems for safe operation. To safely remove the gases from a venting or leaking vessel and aid in the external cooling of the vessels, the microwave cavity empties into a fume hood or exhaust system. The control electronics are air-cooled and isolated from the microwave cavity, which prevents them from being damaged by corrosive fumes. Many laboratory microwaves are now also equipped with NO\textsubscript{x} and organic solvent detectors that shut down the ovens when a leak is detected.\textsuperscript{1}

The typical microwave system used for heating analytical samples consists of six major components: the microwave generator (magnetron), the wave guide, the microwave cavity, the mode stirrer, a circulator and a turntable. Microwave energy is produced by the magnetron, propagated down the wave guide, and injected directly into the
microwave cavity where the mode stirrer distributes the incoming energy in various directions.

3.2.1. The magnetron
The magnetron is a cylindrical diode with an anode and cathode. Superimposed on the diode is a magnetic field that is aligned with the cathode. The electrons under the influence of the magnetic field resonate and the magnetron oscillates. The oscillating electrons surrender energy to the microwave field that radiates from an antenna enclosed in the vacuum envelope of a tube.

3.2.2. Power output of the magnetron
The microwave energy of the magnetron is generally measured in watts and is typically 600-1200 W in most microwave systems (the microwave system used for research for the purpose of this dissertation had a power output of 900 W). The power output can be indirectly determined by measuring the temperature rise of a quantity of water large enough to absorb essentially all of the energy delivered to the microwave cavity. The apparent power output is determined by measuring the rise in temperature, in degrees centigrade, of 1L of water heated at full power for 2 minutes, as defined by the following general relationship:

\[
P = \frac{C_p K \Delta T m}{t}
\]

Equation 821

where \( P \) is the apparent power (in Watts), \( K \) is the conversion factor from thermal chemical calories to watts; \( C_p \) is the heat capacity (or thermal capacity in calories per degree, \( \Delta T \) is the change in temperature; \( m \) is the mass in grams and \( t \) is the time in seconds. Because the dielectric dissipation factor and radiant losses are a function of temperature, the same initial temperature and approximate \( \Delta T \) are used.
3.2.3. The Wave Guide:
The microwaves generated by the magnetron are channeled to the microwave cavity by the wave guide. Wave guides are constructed of a reflective material such as sheet metal, and are designed to direct microwaves to the cavity without a mismatch.

3.2.4. The Mode Stirrer
This is a fan-shaped blade that is used to reflect and mix the energy entering the microwave cavity from the wave guide. The function of a mode stirrer is to distribute the incoming energy so that the heating of the sample will be more independent of position.

3.2.5. The Microwave Cavity
The sample applicator into which microwaves are propagated is the microwave cavity. Simply stated, the microwaves entering the cavity are repeatedly reflected from wall to wall. The pathways of the microwaves are well-defined into recognizable patterns. The microwaves entering the cavity are intercepted by absorptive samples placed inside the microwave cavity, and lose energy with each interaction until no energy remains in a given wave. When a sample has a low dissipation factor the microwaves continue to be reflected and have a greater chance of finding their way back to the magnetron.

3.3. Part I: Theory
Microwaves are electromagnetic energy. Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles, but does not cause changes in the structure. Microwave energy has a frequency range from 300 to 300,000 MHz. Four frequencies are used for industrial and scientific microwave heating, extraction and drying: 915 ± 25, 2450 ± 13, 5800 ± 75 and 22,125 ± 125 MHz\(^2\). These frequencies were established for industrial, scientific and medical use by the Federal Communications Commission and conform to the International Radio Regulations adopted at Geneva in 1959. Of these frequencies, 2450MHz is the most commonly used and is the frequency used in all home microwave units. The typical energy output in a microwave unit is 600-700W. Thus, within 5 minutes, approximately 43,000 cal is supplied to the microwave cavity for sample heating.
3.3.1. Dielectric Loss

The heating pattern of a sample that is heating with microwave energy will depend, in part, upon the dissipation factor of the sample (\( \tan \delta \)). The dissipation factor is a ratio of the sample’s dielectric loss or “loss” factor (\( \varepsilon'' \)) to its dielectric constant (\( \varepsilon' \)), defined by the following relationship:

\[
\tan \delta = \frac{\varepsilon''}{\varepsilon'}
\]

Equation 9

The dielectric constant is a measure of a sample’s ability to obstruct the microwave energy as it passes through, and the loss factor measures the sample’s ability to dissipate that energy. The word “loss” is used to indicate the amount of input microwave energy that is lost to the sample by being dissipated as heat.

When microwave energy penetrates a sample, the energy is absorbed by the sample at a rate dependent upon its dissipation factor. Penetration is considered infinite in materials that are transparent to microwave energy and is considered zero in reflective materials such as metals. The dissipation factor is a finite amount for absorptive samples. Because the energy is quickly absorbed and dissipated as microwaves pass into the sample, the greater the dissipation factor of a sample, the less the penetration of the microwave energy at a given frequency. A useful way to characterize penetration is by the half-power depth for a given sample at a given frequency. The half-power depth is defined as that distance from the surface of a sample at which the power density is reduced to one-half that at the surface. The half-power depth varies with the dielectric properties of the sample and approximately with the inverse of the square root of the frequency.

Typically, microwave energy is lost to the sample by two mechanisms: ionic conduction and dipole rotation. In many practical applications of microwave heating, ionic conduction and dipole rotation take place simultaneously. Microwave is an integrating device that adds all the dielectric mass simultaneously in the microwave unit for total absorption.
3.3.1.1 Ionic Conduction

Ionic Conduction is the conductive (i.e., electrophoretic) migration of dissolved ions in the applied electromagnetic field. This ionic migration is the flow of the current that results in $I^2R$ losses (heat production) due to resistance to ion flow. All ions in a solution contribute to the conduction process, but the fraction of current carried by any given species is determined by its relative concentration and its inherent mobility in the medium. Therefore, the losses due to ionic conduction depend on the size, charge and conductivity of the dissolved ions and are subject to the effects of ion interaction with the solvent molecules.

The parameters affecting ionic conduction are ion concentration, ion mobility, and solution temperature. Every ionic solution will have at least two ionic species (e.g. Na$^+$ and Cl$^-$ ions) and each species will conduct current according to its concentration and mobility. Table 2 shows that an increase in the ion concentration will increase the dissipation factor. The contribution of ionic conductance to microwave heating is illustrated Table 2 by the large increase in the dissipation factor when NaCl is added to water. The dissipation factor of an ionic solution will change with temperature because temperature affects ion mobility and concentration.

Table 2. Effect of increasing ionic concentration on the dissipation factor (3000MHz, 25°C)

<table>
<thead>
<tr>
<th>Molal Concentration (water)</th>
<th>$\tan \delta \times 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1570</td>
</tr>
<tr>
<td>0.1</td>
<td>2400</td>
</tr>
<tr>
<td>0.3</td>
<td>4350</td>
</tr>
<tr>
<td>0.5</td>
<td>6250</td>
</tr>
</tbody>
</table>

3.3.1.2 Dipole Rotation:

Dipole rotation refers to the alignment, due to the electric field of the molecules in the sample that have permanent or induced dipole moments. Dipole rotation is illustrated in Figure 3a. As the electric field of the microwave energy increases, it aligns the polarized molecules. As the field decreases, thermally induced disorder is restored. The applied microwave field causes the molecules, on average, to temporarily spend very slightly more time pointing in one direction rather than in other directions. Associated with that
tiny bit of preferred orientation there is a tiny bit of molecular order imposed and therefore a tiny bit of energy. When the field is removed, thermal agitation returns the molecules to disorder, in relaxation time $t$, and thermal energy is released. At 2450 MHz, the alignment of the molecules followed by their return to disorder occurs $4.9 \times 10^9$ times per second, and results in very rapid heating. However, the efficacy of heating by dipole rotation depends upon the sample’s characteristic dielectric relaxation time, which in turn depends upon temperature and the viscosity of the sample.

Thus, the electric field oscillates, forcing the dipole molecules to move, and the resulting friction heats the solution. At 2.45 GHz, the frequency of most laboratory microwave ovens, the dipoles align and then randomize 5 billion times a second. In the ionic conduction mechanism, ionic species migrate in one direction or the other according to the polarity of the electromagnetic field. Heating is the natural consequence when the accelerated ions meet resistance to their flow. These two unique mechanisms heat solutions much faster than conduction and convection. The heating is so fast that, in open vessels, vaporization alone cannot dissipate the excess energy. This results in solutions “superheating” above their normal boiling points by as much as 5 °C for water to 26 °C for acetonitrile.
3.3.2. Effect of Dielectric Relaxation Time on Dipole Rotation

The dielectric relaxation time is the time that it takes for the molecules in the sample to achieve 63% of their return to disorder. The maximum energy conversion per cycle by many materials (dielectric loss due to dipole rotation) will occur when

$$\omega = \frac{1}{\tau}$$

Equation 10

where $\omega$ is the angular frequency of the microwave energy in radians per second and $\tau$ is the dielectric relaxation time of the sample. A non-ionic polar sample with a $1/\tau$ close the angular frequency of the input microwave energy will have a high dissipation factor. In contrast, when $1/\tau$ of the sample is considerably different from the microwave angular frequency, the dissipation factor of the sample will be low. Figure 4 illustrates the relationship between input microwave frequency and dielectric relaxation time on microwave penetration. The half power depth for water is about 4 inches for 915 MHz and about 1 inch for 2450 MHz.

As the sample is heated, the dielectric relaxation time will change as will the dissipation factor, and therefore, the penetration depth. As the temperature of water is raised, the dissipation factor decreases. This decrease occurs because the $1/\tau$ of water increases, as the water temperature increases, and therefore the rotational frequency of water is further out of coincidence with the input microwave angular frequency, and absorption decreases.
3.3.3. **Effect of Sample Viscosity on Dipole Rotation**

A sample’s viscosity affects its ability to absorb microwave energy (dissipation factor) because it affects molecular rotation. The higher the viscosity, the lesser is the ability of the molecule to rotate. When frozen, water molecules become locked in a crystal lattice. This locking greatly restricts the molecular mobility and makes it difficult for the molecules to align with the microwave field. Thus, the dielectric dissipation factor is low, $2.7 \times 10^{-4}$ at 2450 MHz. When the temperature of the water is increased to 27°C, the viscosity has decreased, and the dissipation factor is 12.2, which is much higher.

3.3.4. **Relative Contributions of Dipole Rotation and Ionic Conduction**

To a great extent, temperature determines the relative contributions of each of the two energy conversion mechanisms (dipole rotation or ionic conduction). For small molecules, such as water and other solvents, the dielectric loss to a sample due to the contribution of dipole rotation decreases as the sample temperature increases. In contrast, dielectric loss due to ionic conduction increase as the sample temperature increases. The percent contribution of these two mechanisms of heating depends upon the mobility and concentration of the sample ions and the relaxation time of the sample. If the ion mobility and concentration of the sample ions are low, then sample heating will be entirely dominated by dipole rotation. If however, the mobility and concentration of the sample ions increases, the heating will be dominated by ionic conduction and the heating time will be independent of the relaxation time of the solution. As the ionic concentration increases, the dissipation factor will increase and the heating time will decrease. Heating time also depends on the microwave system design as well as the sample size.

3.3.5. **Sample Size**

The input microwave frequency also affects the penetration depth of the microwave energy. In large samples with high dissipation factors, the heating that occurs beyond the penetration depth of the microwave energy is due to thermal conductance through molecular collisions. Therefore, temperatures at or near the surface will be higher. Because boiling and other agitation increases the rate of thermal conductance, surface
heating is not a problem (unless the penetration is low). In that case, heat loss through the vessel walls can become significant and an increase in sample heating time will occur.

3.3.5.1 Predicting Conditions

In laboratories that process a large number of samples routinely, a one-sample-at-a-time is not a pragmatic solution. However, when using multiple samples, the total mass inside the microwave cavity will increase, which will increase the amount of power absorbed. This absorption of power can be predicted by the following equation that has been calculated from experimental data for acids and water$^{21}$:

\[
\ln(\text{absorbedPower}) = A + B \times \ln(\text{mass}) + C \times (\ln(\text{mass}))^2 + D \times (\ln(\text{mass}))^3 + E \times (\ln(\text{mass}))^4
\]

Equation 11

Equation 4 is a natural logarithm based quartic model, and the actual coefficients A through E used in these generalized equations are given in the ACS Reference book on Microwave Sample Preparation by Kingston and Jassie$^{21}$. This fourth order equation represents data with greater accuracy than a linear model for the same data.

3.3.6. Microwave Heating

The difference between normal (e.g. hotplate) heating and microwave heating is due to the sample heating mechanism. “Normal” heating uses conduction and convection; the conventional heating mechanisms. Because vessels used in conductive heating are usually poor conductors of heat, it takes time to heat the vessel and transfer that heat to the solution. Also because vaporization at the surface of the liquid occurs a thermal gradient is established by convection currents, and only a small portion of the fluid is at the temperature of the heat applied to the outside of the vessel. Therefore, when conductively heating, only a small portion of the fluid is above the boiling point temperature of the solution. On the other hand, microwave heating takes place by direct molecular induction. Microwaves heat all of the sample fluid simultaneously without heating the vessel. Therefore, when heating using microwave energy, the solution reaches its boiling point very rapidly.
3.3.7. **Polarity**

The magnitude of the solvent-dipole moment is the main factor that correlates with the microwave heating characteristics of the organic solvent. The larger the dipole moment, the more rigorously the solvent molecules will oscillate in the microwave field. Polar solvents such as alcohols, ketones and esters strongly couple microwave energy. Benzenes, xylene and straight chains aliphatic hydrocarbons are non-polar and do not interact with the microwave field and as a result do not heat. Acetone with a dipole moment of 2.69 or acetonitrile with a dipole moment of 3.44 will rotate easily when exposed to an alternating electric field of microwave energy. This oscillation produces collisions with surrounding molecules and energy is transferred with subsequent heating. For microwave solvent extraction to be effective, the solutions or the sample must heat when exposed to microwave energy.

3.3.8. **Dielectric Compatibility:**

Dissipation or dielectric loss coefficient $\varepsilon''$ is the physical parameter that describes the ability of a material to heat when placed in a microwave field. The larger the loss factor or coefficient the more optimal the heating. Dielectric loss coefficient is the measure of the ability of the material to transform the electromagnetic (EM) energy to heat through internal mechanical motion and is wavelength dependent. Short wavelengths heat intensely and at surfaces, whereas longer wavelengths heat less intensely over long distances. The dielectric constant $\varepsilon'$ is the ability of the material to slow the velocity of EM radiation. When MAE is conducted in closed vessels, the temperatures achieved will be greater than the atmospheric boiling points of the solvents. The elevated temperatures of the solvent increase the solubility of analytes of interest in the extraction solvent and also increases the desorption kinetics of the analyte from the matrix being extracted. All mass transport phenomena are sped up at elevated temperatures and therefore influence the rate of microwave heated extractions. The major benefit of microwave heating is the speed and efficiency of the delivery of energy to the organic solvent. The ability to work in a closed container at elevated pressures and temperatures is also advantageous because volatile analytes are retained.
3.4. Development of a Microwave Assisted Extraction Method\textsuperscript{3,6,22-30}
Optimization of MAE conditions has been reported for the extraction of phenols, PAHs, triazines, methylmercury and organotin compounds. Factorial, central composite and orthogonal array designs have been generally used. The parameters studied most of the time are pressure or temperature (for closed vessel systems), extraction time, microwave power, solvent nature, and volume.

3.4.1. Nature of the solvent
It is common to perform MAE with the same solvent as is prescribed for the traditional extraction. The solvent should generally be capable of absorbing the microwave energy (though with the secondary absorbing technique, this factor is no longer critical). As microwave absorption occurs owing to the reorientation of permanent dipoles by the electromagnetic field, the amount of energy absorbed is proportional to the dielectric constant of the solvent. Generally speaking, absorption is also proportional to the solvent polarity. Apart from absorbing the energy, the solvent must be able to convert this energy into heat, so the efficiency of the conversion process is dependent on the dielectric factor loss. In some cases, the solvent volume may be important for efficient extractions.

3.4.2. Temperature
Temperature is of prime importance in ensuring efficient extraction, as elevated values usually enhance the extraction, as a result of an increased diffusivity of the solvent into the internal parts of the matrix under high temperatures, as well as an enhanced desorption of the components from the active sites of the matrix. In closed systems, pressure is also an important variable; however, this is directly dependent on the temperature. In some cases, increasing the temperature may be detrimental to the extraction, due to the degradation of the selected components. The optimum temperature may depend on the matrix to be extracted.

3.4.3. Power
In closed vessel systems, the chosen power setting depends on the number of samples to be extracted during one extraction run, as up to 12 vessels can be treated in a single run.
The power must be chosen correctly to avoid excessive temperatures, which could lead to solute degradation and overpressure inside the vessels. Ethos 900 and 1600 (the two units which were used for research purposes for this dissertation) utilize a PID algorithm, with a feedback wherein the software controls the power input into the microwave based on the desired system temperature and given time frame. PID algorithm is a Proportional, Derivative, Integral algorithm; depicted schematically in Supplemental Figure 1. In a closed-loop system, the variable (e) represents the tracking error, the difference between the desired input value (R) and the actual output (Y). This error signal (e) will be sent to the PID controller, and the controller computes both the derivative and the integral of this error signal. The signal (u) just past the controller (calculated by both the derivative and integral components) will be sent to the microwave, and the new output (Y) will be obtained. This new output (Y) will be sent back to the sensor again to find the new error signal (e). The controller takes this new error signal and computes its derivative and its integral again. This process goes on and on. Using this feedback mechanism, the unit has complete control on the power input inside the microwave cavity based on the two factors of temperature desired and time given. This, therefore, increased the safety of the microwave procedure.

3.4.4. Extraction time

As in other extraction techniques, time is another parameter whose influence needs to be taken into account. With thermolabile compounds, long extraction times may result in degradation. This parameter will be further discussed in Chapter 4.

3.4.5. Nature of the matrix

The water content of the matrix is of great importance, as water molecules have a high dipole moment, and so absorb microwave energy strongly, leading to efficient heating of the sample. As a consequence, obtaining reproducible results requires control of the matrix water content. In addition, MAE may be subject to interferences from the presence of microwave energy-absorbing mater in the sample that can cause arcing. Also, the
organic carbon content of the matrix is known to hinder the extraction, owing to strong analyte-matrix interactions that are difficult to disrupt. For the same reason, spiked compounds are readily extractable, while native solutes are much more difficult to extract under the same conditions.

3.4.6. Pressure

Unique temperature and pressure relationships are involved in closed-vessel MEC\(^1\). The gas pressure inside a microwave-closed vessel is not determined by the liquid-phase temperature. Instead, it depends on the vessel volume, gas-phase temperature, and vessel composition. For example, when water is placed in a high-pressure steel-jacketed Teflon bomb and heated in a convection oven, an equilibrium vapor pressure is established. This vapor pressure depends on the water vapor’s rate of evaporation and condensation. When the temperature rises, the evaporation rate increases, and the condensation rate decreases, because the vessel walls heat both the solution and gas phases. The decrease in condensation rate leaves more water in the vapor phase, increasing the internal pressure.

In contrast, when water is heated to the same temperature in a microwave-closed vessel, the internal pressure is significantly lower because of the heating mechanism and the vessel materials. The microwave-closed vessel’s liner and outer casing are microwave-transparent and have minor insulating capacity. Thus, they remain cool relative to the solution during the heating process. The less insulating the vessel system, the more efficient they will be at removing water molecules from the vapor phase. The increased condensation rate results in lower internal pressures at higher temperatures. This microwave reflux action is illustrated in Figure 5. The microwave-closed vessel’s liner
and outer casing remain relatively cool during the heating process, because they are microwave-transparent and have only a small insulating capacity. The cooler the vessel walls, the more efficient they will be at removing water molecules from the vapor phase. The increased condensation rate results in lower internal pressures at higher temperatures. The higher temperatures reached in the closed system give microwave digestion a kinetic advantage over hot plate digestion, as described by the Arrhenius equation, which, when integrated, gives

$$\ln \frac{k_1}{k_2} = \frac{E_a}{2.303R} \left( \frac{1}{T_1} - \frac{1}{T_2} \right)$$

Equation 12

where $k_1$ and $k_2$ are rate constants for the reaction of interest at temperatures $T_1$ and $T_2$, respectively; $E_a$ is the activation energy; and $R$ is the ideal gas constant. This equation shows that the reaction rate increases exponentially with increasing temperature, which translates into ~100-fold decrease in the time required to carry out a digestion at 175 °C when compared with a digestion at 95 °C.

3.5. Part II: Integrated Microwave Extraction

Traditional microwave extraction had some inherent drawbacks. It is not enough to use a new energy source but it is also required to integrate the process of extraction, microwave energy with solvent heating, refreshing, stirring and filtration into the new apparatus. As described in Chapter 1, sample handling and operator errors account for a significant portion of overall error and sample loss or modification. All these steps are now combined into one single non-transfer step that decreases the sample handling and potential loss of the analyte. The use of microwave-enhanced chemistry in itself offers many advantages over traditional heating methods. Closed-vessel microwave extraction allows extraction solvents to be rapidly heated to 2-3 times higher than their atmospheric boiling points resulting in shorter extraction times (10-30 minutes). The amount of solvent consumed is considerably less (20-30 ml). Stirring is possible which makes the extraction conditions more homogenous, promotes interaction with the solvent, and assists in releasing the analyte from the matrix. However, MASE has some inherent
drawbacks that had to be overcome to allow for its widespread use. A chemical compound will absorb microwave energy roughly in proportion to its dielectric constant, i.e., the higher the value of the constant; the higher the amount of energy absorbed. Because organic extractions typically involve non-polar solvents with very small, if any, dielectric constants, a polar co-solvent often had to be used to assist in heating the solution. Use of a polar co-solvent led to the extraction of a broader spectrum of compounds in addition to the analytes of interest, creating potential interference problems during analysis. MASE also does not overcome the traditional processing steps of filtration and evaporation. This section describes the development of a technique called Integrated Microwave Extraction (IME) designed to specifically overcome these deficiencies. IME integrates the processes of extraction, filtration, evaporation and solvent recovery through the use of integrated hardware to overcome some traditional limitations. Utilization of a microwave absorbing component (Figure 6) makes possible the use of non-polar solvents for microwave extraction.

Hexane is a non-polar solvent with a negligible dielectric constant, and as such possesses poor microwave coupling ability, as denoted by Figure 7. The heating profile for pure
HPLC grade hexane as it comes from the bottle and dried over molecular sieves. The plot shows constant heating throughout most of the cycle until the temperature almost reaches the boiling point. The temperature never goes over the boiling point shown by the flat line of the pressure curve. A commonly used solution for this drawback was the use of a polar co-solvent, i.e., mixing of a solvent miscible with hexane that also absorbs microwave energy, thereby transferring the heat to the entire solvent mixture. A major problem with this scenario however, as depicted in Figure 7b is the development of pressure which leads to vessel venting and leaking. Another significant problem with using a co-solvent is the loss of selectivity; i.e., compounds other than the analyte of interest may also be extracted by the co-solvent, making the extraction defined by solvent chemistry rather than by analyte chemistry. This difference in extraction is denoted by the difference in the chromatograms in Figure 8a and b.
However, when the same solvent is heated using Weflon™, a carbon-impregnated Teflon polymer, an appreciable difference in both time to reach the boiling point of the solvent as well as pressure is observed as illustrated by Figure 9. Weflon™, as described before, is a chemically inert polymer that can absorb microwave radiation and convert it to heat, thereby heating up the surrounding (non-) polar solvent. This presents a dual advantage. Firstly, the lower power setting can be lowered; 550 W vs. 850 W. The curve comes close to the program time of 150°C at 5 minutes. Secondly, the pressure has been reduced to a range of 40-100 psi. So by using the Weflon™ for heating the temperature has increased to 150°C and at the same time the pressure has been reduced to at least 100 psi.

3.6. Microwave Extraction and Evaporation System Integration
The microwave-assisted extraction system used for this work was the Ethos SEL (Milestone Inc., Monroe, CT) which is an integrated microwave solvent extraction system. This system consisted of an Ethos laboratory microwave unit with a built-in magnetic stirrer, a fiber optic temperature sensor, and a solvent sensor, which terminates the heating program in the event of a vessel leak or over-pressurization. The sample rotor used was the basic 12-position extraction rotor consisting of 100 ml, fluoropolymer lined, TFM vessels that have a maximum operating temperature and
pressure of 220°C and 30 bar (500 psi) respectively. The need for a polar co-solvent is eliminated because of the incorporation of a secondary microwave absorber (Weflon™), a chemically inert, microwave-absorbing fluoropolymer. Post-extraction filtration and evaporation was done using Milestone FiltEX™ (Figure 10) and EvapEX™ (Figure 11) systems respectively without transferring the extracts. The evaporated solvent was collected and recycled using the EvapEX™ in conjunction with the Solvent Recovery System. EasyWAVE™ control software was used to monitor and control the microwave system. The user can change the microwave parameters during the run, which allows real time optimization during method development. The software uses PID (Proportional Integrating Derivative) algorithms for precise temperature and process control that delivers the minimum power required to sustain the set temperature.⁴

3.7. Summary and Conclusions
Even though the use of microwave energy to enhance the extraction of organic compounds is rather recent, numerous applications have been reported, with special emphasis on environmental matrices. Hence, several classes of compounds (such as PAHs, PCBs, pesticides, phenols, dioxins, and organometallic compounds) have been extracted efficiently from a variety of matrices (mainly soils, sediments, animal and
botanical tissues), either spiked or containing native compounds. All the reported applications have shown that microwave-assisted extraction is a viable alternative to conventional techniques for such matrices.

Comparable efficiencies have been reported along with acceptable reproducibility. In addition, MAE offers a great reduction in time and solvent consumption, as well as the opportunity to perform multiple extractions. The emergence of commercial systems, using diffused or focused microwaves, affords a high level of safety. Evidence has also been presented that MAE may compete favorably with recent techniques, namely supercritical fluid extraction and accelerated solvent extraction. In particular, optimization of MAE conditions is rather easy, owing to the low number of parameters (i.e., matrix moisture, nature of solvent, time, power, and temperature in closed vessels) as compared to SFE. On the other hand, less selectivity may be achieved using MAE, so a cleanup procedure may be required before chromatographic analysis.

3.7.1. Final Remarks
Integrated Microwave Extraction, IME, as presented, shows promise to be a time saving method with the added advantages of being economical, safe and environmentally friendly process. The data that will be presented subsequently indicate equivalent recoveries for both classes of solvents (polar as well as non-polar) within a 95% confidence interval. Comparable accuracy with increased precision and enabling of a greener environmental extraction process will promote acceptance for IME.

The principles governing microwave heating did not permit the use of chemically specific solvents (e.g. non-polar solvents) which made a total conversion of traditional methods to microwave impossible. Use of co-solvents to aid in energy absorption was necessary. Also, the number of sample manipulation steps needed to be streamlined in an effort to decrease error due to potential sample loss. IME addresses these drawbacks. Occasionally, the recoveries are higher than the values reported on the CRM, with better precision. CRM values reported were on the basis of Soxhlet extraction. This could because of the integration theme. The integration has made possible lesser number of
steps/ sample process, which makes it a convenient, less time-consuming and more economical option. Also, because of decreased sample loss (evidently), the precision of the technique is very high, especially when compared to conventional methods. This, coupled with the fact that one can achieve temperatures higher than the boiling points in the sealed vessels possibly explains the reason why we have seen comparable recoveries as conventional methods. This technique is further optimized and the parameters that influence the recoveries have been studied as explained in the following chapter. The optimized technique was then applied to a variety of applications, which will be covered in Chapter 7. Some of the applications attempted include the following: Equipment Integration and Validation/ Application to pesticides and PAHs, polymer additives extraction, extraction of environmental contaminants from food products and extraction of lipoidal material from solid matrices.

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Chapter 4 Overview
Optimization of Parameters Influencing Microwave Extraction; Theoretical Model and Experimental Verification of Temperature Dependence of Extraction Efficiencies

4. OPTIMIZATION OF PARAMETERS INFLUENCING MICROWAVE EXTRACTION; THEORETICAL MODEL AND EXPERIMENTAL VERIFICATION OF TEMPERATURE DEPENDENCE OF EXTRACTION EFFICIENCIES

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CHAPTER 4

4. Optimization of Parameters Influencing Microwave Extraction; Theoretical Model and Experimental Verification of Temperature Dependence of Extraction Efficiencies

4.1. Abstract
In this chapter, we have initiated the experimental verification of some of the theory discussed in Chapter 3. Factors affecting microwave extraction like nature of solvent, analyte chemistry, time, sample size, nature of matrix and the effect of moisture on the efficiency of extraction are studied in detail. Microwave extraction and various evaporation systems were examined, and the optimizations of parameters influencing microwave extraction were elucidated. A theoretical model for the temperature dependence of extraction was postulated, and the experimental verification of temperature dependence of recovery of MAE from solid matrices was given.

4.2. Part 1: Optimization of Parameters
4.2.1. Introduction
Reliable trace-level analysis begins with the quantitative extraction of the analytes from the sample matrix in a manner which is compatible with the rest of the analytical procedure. The most widely used liquid/solid extraction technique is still Soxhlet extraction, which requires 6–48 h, consumes a large volume of organic solvents and is laborious. Microwave extraction has been reported as an alternative sample preparation technique for various solid samples and is one of the techniques developed in the past decade to reduce the volume of solvents required, improve the precision of analyte recoveries, reduce extraction time and decrease the costs. A number of applications have reported the use of microwave energy in assisting extraction of environmental organic pollutants, and the number of publications is steadily rising.

It is customary to aim for the most efficient extraction in order to make the sample preparation process, and consequently, the analysis to be as accurate as possible. An
effective microwave extraction (or solvent extraction) is a function of a number of different parameters. For the extraction to give the most effective results, it is necessary to study, and if possible, to optimize the factors that influence the extraction process either directly or indirectly. Each analyte will have its own unique pattern during the extraction process. Thus, optimization of the parameters is necessarily related to the individual analyte of interest. However, there are some parameters that influence the extraction and its outcome regardless of the analyte. The study of these independent factors forms the basis of this chapter. Previously, some studies have been carried out for the optimization using both open and closed vessel microwave extractions\textsuperscript{9,17}; the current study was focused on the outcome of the extraction using an integration of different equipment. This integration gives rise to heating by two effects simultaneously, namely, heating due to microwave effect as well as heating due to conduction effect. The influence of this integration will also be discussed in this chapter.

4.2.2. Literature Survey

Some factors that have been previously researched include the influence of power, final temperature of extraction, time of exposure, amount of solvent needed, and the moisture content of the matrix.\textsuperscript{2,4,6,18-23}

Experimental design has been used to either streamline the experiments needed to study the parameters or to determine the statistically significant factors\textsuperscript{9,18,23-26}. Some of the experimental design models used were fractional factorial (most frequently used), screening of extraction factors. In the study of the role of water in the microwave extraction of PAHs from soil in dichloromethane–acetone system, it was found that the way the water was introduced into the system (before or after the addition of solvent) affects the extraction efficiency\textsuperscript{27,28}. In the study of the effects and interactions of five parameters (microwave power, extraction time, solvent volume, nature of solvent, and moisture content of the sample), it was found that the effect of power and nature of solvent depends on the water content of marine sediments\textsuperscript{29}. Most of the reported results revealed that the extraction efficiencies for organic pollutants by microwave-assisted systems are comparable to the conventional techniques such as Soxhlet and sonication.
methods. In this study, the effect of these conventional factors (such as solvent, moisture content, temperature, and time) and comparison of the microwave extraction efficiency were evaluated. Additionally, some other parameters unique to microwave extraction were also evaluated (e.g., matrix effects, solute-solvent ratios, sample size study, analyte chemistry, and equipment integration). While the influence of some of these parameters on traditional microwave extraction was similar to Soxhlet, this study attempted to verify and either validate or disprove the similarity of the parameter influence trend of Integrated Microwave Extraction with traditional microwave extraction as well as Soxhlet extractions. The influence of temperature on extraction efficiency has been studied separately in Part II with theoretical modeling.

4.3. Flow chart of optimization procedure

The optimization procedure was planned and implemented as represented by Figure 1.

Figure 7. Schematic of Parameter Optimization
4.3.1. Extractant (Solvent)
A variety of solvents were selected based on their physical properties, following the guidelines given in Chapter 2, Solvent Selection. The goal of the experiment was to determine a “range” of solvents that would give optimal extraction efficiencies for the given analytes, viz., Polycyclic Aromatic Hydrocarbons (PAHs). These compounds were selected as analytes mainly because of the environmental concern that they have evoked. PAHs are widely distributed extensive group of compounds, and are serious and ubiquitous environmental contaminants. Because of their high mutagenicity and carcinogenicity, the existent level of PAHs in a wide range of environmental samples has brought high interest among analytical chemists. Also, PAHs are relatively non-polar compounds, and as such a solvent of choice would be hexane. The non-polar property of hexane made this an ideal system to evaluate the secondary absorbing technique of the microwave.

4.3.1.1 Experimental:

4.3.1.1.1. Standards, Solvents and Reagents
The following solvents were evaluated:

- Polar solvents: Acetone, Acetonitrile, and Methanol.
- Non-polar solvents: n-Hexanes and Toluene

The solvents selected were obtained from Fisher Scientific, Fairlawn, NJ. All solvents were Optima grade and used as received.

The standard used in this study were a Certified Reference Material (CRM); CRM 104-100 (Sediment-BNAs) and CRM 105-100 (Sandy Loam- PAHs/Pesticides); obtained from RTC, Laramie, Wyoming. Individual PAHs for preliminary studies were obtained from Aldrich Chemicals, Sigma-Aldrich, St. Louis, MO.

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT.

4.3.1.1.2. Preparation
As depicted in Figure 2, the extraction assembly consisted of:
• Glass extraction vessel with Teflon outlet
• Weflon base
• Filter/ Glass wool
• Stopper discs
• Teflon lid with vent aperture
• Teflon/ PTFE liner
• TFM sleeve
• Teflon cap
• Pressure plate
• Spring
• Teflon sleeve
• Magnetic stir bar

4.3.1.1.3. Extraction

The glass wool was attached to the Weflon base by the Teflon outlet. Then, this Teflon outlet was first blocked with a filter/ glass wool. This filter was held in place with a stopper disc. This arrangement made it possible for the vessel to be inserted into the filtration apparatus for direct filtration post-extraction. The matrix/ extraction chamber is the volume of space above the stopper disc. The matrix is placed in this chamber along with a stirbar and appropriate amount of solvent. This lidded extraction assembly was then placed into the liner which contains the same solvent as inside the extraction chamber. This liner was then inserted into the sleeve, which was further capped. The pressure plate and spring were secured in place with the Teflon sleeve. This assembly was then inserted into its segment, twelve of which form a rotor. Thus, up to 12 extractions can be performed simultaneously. Each vessel has a heating capacity of up to 220°C and can withstand pressure of up to 30 bars. Vessels were placed in a sample rotor.
and secured with a calibrated torque wrench for uniform pressure. If the operating pressure exceeded the vessel limits, a patented spring device allowed the vessel to open and close instantaneously, bringing the internal pressure down to a containable level. This “vent and reseal” design releases only the excess pressure, allowing valuable sample materials (including volatile elements) to remain in the vessel.

The monitor vessel differs from the remaining eleven vessels in that it contains a ceramic thermal well that holds the fiber optic sensor in place during extraction. It is important to note here that the monitor vessel is the only vessel that gives direct feedback to the software, thus it is essential that the solvent in the monitor vessels be representative of the rest of the vessels in both content as well as quantity. Only then will the assumption hold true that the “unmonitored” vessels are following the same heating profile as depicted by the software on the controlling computer. This software, EasyWAVE™, (Figure 3) allows the user to draw a temperature profile and press “Start.” Using process control algorithms, the Ethos SEL will precisely follow the profile by continuously modulating the microwave power for precise and repeatable sample extraction (±1°C). Method parameters can be changed in real time during a sample run, even after a vessel has vented, so that a reaction can be brought under control. The software also plots the heating profile in real time. The ATC-400FO Automatic Fiber Optic Temperature Control system allows continuous monitoring and control (± 1°C) of internal temperature within a standard reference/monitor vessel. The QPS-3000 Solvent Sensor actively monitors and responds to the concentration of solvent vapors inside the cavity and reduces the applied microwave power until the vapors have been cleared from

Figure 9. EasyWAVE™ software panel snapshot
the cavity by the exhaust module and if necessary, shuts down power input into the cavity for the safety of the operator in event of a solvent leak.

4.3.1.1.4. Procedure

Extraction

After some trial and error runs (to optimize a balance between high temperatures for increasing extraction efficiency but decreasing the possibility of analyte degradation), the following protocol was used for the extractions described in this section:

- **Step 1**: Ramp T1 (primary monitoring temperature) to 110°C in 5 minutes (occasionally altered in real time to accommodate high boiling solvents)
- **Step 2**: Hold T1 at 110°C for 20 minutes

Evaporation

Vessels are allowed to cool to room temperature (preferably to at least 10°C below boiling point of solvent) to avoid venting/flashing when opening the vessel. The opened vessel is directly placed into the filtration equipment (FiltEX™) (discussed in chapter 3) and upon completion of setting all vessels; vacuum is applied from a central position of the equipment. The filtered solvent was collected into extraction vials. Since the concentration of the analytes were at a lower level, further processing became necessary. Evaporation was therefore carried out. The matrix was rinsed and the rinsed extracts were collected in the pre-weighed evaporation vial. The vials were weighed again to calculate the solvent recovery. The filtration lid was replaced with evaporation lid. This evaporation system, EvapEX™, (discussed in chapter 3) was placed in microwave cavity. Vacuum was applied at central position and evaporated solvent was collected in the recovery vessel. The path to this vessel was cooled by the attached chiller (usually cold water was sufficient for the organic solvents that were evaluated). The evaporation lid also has another inlet to which we applied argon to ensure an inert atmosphere for the evaporation. This helped prevent unwanted oxidation and/or degradation of the analytes. Pulsed microwave power was applied to further control the temperature and discourage analyte degradation. The evaporation program used was

- **Step 1**: 500 W 1 min
• Step 2: 0 W 1 min
• Step 3: 500 W 30 sec
• Step 4: 0 W 30 sec
• Step 5: 250 W 30 sec
• Step 6: 0 W 30 sec

...and so on until the desired extract volume was reached (the final time depended on the boiling point of the solvent— the higher boiling the solvent, the more time it needed to evaporate to specified volume). This layered program allowed for an equal cooling time.

The evaporation vials were then measured to calculate the final volume of the extracting solvent (densities of the solvents were calculated at the same temperature on the same day as the extractions). A 1-mL aliquot was introduced into a GC/MS vial, capped and ready for analysis.

**GCMS Analysis**

The capped vials were then analyzed using GC/MS. Saturn GCMS/ Varian 3410 high-temperature gas chromatograph coupled to a Varian Saturn II ion trap mass spectrometer and an autosampler was used for this analysis. Data collection and processing was done using Saturn and SaturnView software. A 1-µl aliquot was introduced into the Varian 3410 Gas Chromatograph (using autosampler).

### 4.3.1.2 Results and discussion

The analytes chosen were: pyrene, fluoranthene, anthracene, phenanthrene, fluorene, and

![Figure 10. Polycyclic Aromatic Hydrocarbons](image-url)
acenaphthene, ranging from molecular weight of 154 to 202. Some selected PAHs are displayed in Figure 4. The solvents selected for evaluation had physical properties as given in Table 1.

Table 1. Physical properties of the solvents selected\textsuperscript{30,31}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Dielectric Constant</th>
<th>Density g/ml (25°C)</th>
<th>Hildebrand Solubility Parameterδ</th>
<th>Polarity Index (Snyder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>69</td>
<td>1.88</td>
<td>0.65</td>
<td>7.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>110</td>
<td>2.4</td>
<td>0.86</td>
<td>8.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Acetone</td>
<td>56</td>
<td>20.7</td>
<td>0.78</td>
<td>9.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>82</td>
<td>37.5</td>
<td>0.78</td>
<td>11.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Methanol</td>
<td>65</td>
<td>32.7</td>
<td>0.79</td>
<td>13.7</td>
<td>6.6</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>80.0</td>
<td>1.00</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

The extractions were each run in four replicates. The experimental design is represented in Table 2.

Table 2. Extraction Sample Design

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Replicates</th>
<th>Blank</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>2g CRM</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Toluene</td>
<td>2g CRM</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acetone</td>
<td>2g CRM</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>2g CRM</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Methanol</td>
<td>2g CRM</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

The typical solvents used for PAHs encountered in classical solvent extractions have been evaluated. Because of the nature of PAHs, non-polar solvents are usually preferred. However, traditional microwave extraction precludes the use of non-polar solvents unless they are used in mixtures (with co-solvents). Since IME features the use of
secondary absorbing techniques, this phase of the study focused only on the pure solvents (even non-polar solvents) as opposed to traditionally used mixtures.

The results for the extractions of analytes from CRM are depicted in Figure 5 for polar solvents and Figure 6 for non-polar solvents. The results were also plotted against the values reported with the Certified Reference Material. It is essential to note here the values reported with the CRM were obtained using classical Soxhlet extraction. Tabulated results are included in the appendix at the end of the chapter (Tables 3-5).

As is evident from the plots, IME extraction efficiency is equal to, or higher than the CRM values for both types of solvents. For polar solvents, the extraction was typically higher than those of CRM within 95% confidence intervals. Within polar solvents, extraction efficiencies were best for acetonitrile and acetone (both comparable with each other). No particular differing trend was seen between these two solvents. Methanol, the most polar solvent, seemed to fall in efficiency, and, in case of fluorene, the absolute accuracy value was lower than that of CRM (the efficiency was however, comparable within intervals). Precision seemed to be consistent with acetone, but had more variance with acetonitrile. All error values, unless otherwise stated, are expressed as 95% Confidence Limits with n=4.
For the non-polar solvents, hexane and toluene were evaluated. These were used without any co-solvents to aid with the microwave absorption as is evident from Figure 6, hexane consistently performs equal to or better than, the certified values with precision limits.

The precision obtained with hexane is tighter than with most polar solvents. Toluene, however, in many cases like fluorene and acenaphthene fails to meet the extraction efficiency of the CRM values. This could be attributed to the fact that extractions were performed at 110°C, which for hexane is nearly twice its boiling point. However, this extraction temperature is the exact boiling point of toluene and hence the conditions were emulating Soxhlet and classical solvent extraction techniques.

When comparing all solvents simultaneously, as presented in Figure 7, hexane performance is equivalent to the polar solvents acetone and acetonitrile. However, an important difference in the performance is the precision values. Hexane precision values are better than either of the polar solvents making it the most viable choice for the extraction of PAHs. Hexane also seems to be better at specificity and this observation can be substantiated by a visual comparison of the solvents, post-extraction (Figure 8). In Figure 8, Act stands for acetone, Tol for toluene, Met for methanol, Acn for acetonitrile, and Hex for hexane. As is evident from the results discussed earlier, hexane extracts do not suffer from analyte loss. Hexane extracts are also the cleanest extracts for chromatographic analysis.
The solvents displayed different colors upon completion of extraction, and roughly, the darker the color, the less specific the solvent was towards the analyte being evaluated. Following the rule of thumb, “like dissolves like” (Chapter 2), aliphatic hexane would most definitely be a solvent of choice as it is chemically more similar to the non-polar PAHs as compared to the highly polar methanol. The less specific nature of a solvent like acetone makes the analysis of these extracts more challenging and less accurate.

4.3.1.3 Conclusions

Different solvents typically encountered for the extraction of PAHs have been tested. When considering microwave-assisted extraction, due to the mechanisms involved in microwave heating, the choice of the solvent hitherto depended on its ability to absorb microwaves, defined by its dielectric constant. Since apolar solvents such as aliphatic hydrocarbons do not meet this requirement they were typically not used as pure solvents in traditional microwave extraction despite the fact that they are known to be good solvents for PAHs. There is no significant difference in the recoveries obtained with the polar solvents versus the non-polar solvents, and as such, the influence of the solvent lies primarily in its specificity for the analytes to be studied as well as the solute-solvent chemistry as described in the next section. Non-polar solvents seem to be more specific in their extractions, thereby making analyses more accurate and sensitive. The precision values are also better with hexane than most non-polar solvents. Thus, classical solvents used for Soxhlet and/or classical solvent extraction can also now be used for microwave extraction."
4.3.2. Analyte Chemistry
The chemistry between the solute and solvents is of paramount importance when
designing a microwave experiment (as well as other extraction experiments). As
discussed in Section 4.3.1, hexane, an aliphatic solvent, is the optimal choice for the
extraction of relatively non-polar PAHs. Extending the same logic to other compounds, it
could be safely assumed that “like dissolves like” would apply to polar systems as well.
A polar solvent would give better extraction efficiencies for polar analytes as compared
to apolar solvents. To test this theory, a Certified Reference Material that contained a
mixture of both polar and non-polar analytes was selected as samples to evaluate
extraction with polar and non-polar solvents.

4.3.2.1 Experimental

a. Samples, Reagents and Standards
The following solvents were evaluated:

- Polar solvents: A mixture of 1:1 Hexane: Acetone
- Non-polar solvents: n-Hexanes

The solvents:
All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

The sediment sample:
The sediment matrix used for this study was a sample randomly selected from the
samples that were sent for the ACS/EPA study as described in Chapter 5. The sediment
sample that was chosen was MC2427.

The Standards and Reagents:
- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene
  chloride) from Absolute Standards, Inc., Hamden, CT
- EPA Method 620 Diphenylamine 70314 (nominal concentration of 1000 µg/ml in
  methanol) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of
  1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles GC/MS Tuning Standard GCM-150 (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

Certified Reference Material:
Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

Microwave Instrument and Apparatus
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave system used for this study. Ethos labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

GC/MS Determination
GC/MS analysis was carried out on Agilent (HP) 5972 equipped with an autosampler (courtesy of Dr. F. Fochtman, Mylan School of Pharmacy, Duquesne University). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 series II GC which was equipped with a DB-5ms capillary column (30 m × 0.25 mm I.D. × 0.5 mm. ((5%-Phenyl)-methylpolysiloxane). The GC oven program started at 40°C for 5 minutes, ramped from 40-290°C at 12°C/minutes, held at 290°C for 6 minutes, ramped from 290-325°C at 20°C/minutes, and finally held at 325°C for 5 minutes. A splitless injector was used at 250°C. A Hewlett Packard 5972 MSD was with a source temperature at 325°C to monitor PAHs in the Selected Ion Monitoring (SIM) mode. The instrument was tuned daily with decafluorotriphenylphosphine (DFTPP) at a concentration of 50ng/µl introduced. The DFTPP mass intensity criteria as given in Table 3, EPA Method 8270 C,
page 36 were used as tuning acceptance criteria\textsuperscript{32}. The calibration relationship established during the initial calibration was verified at periodic intervals. As a general rule, the initial calibration must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed. If the response (or calculated concentration) for an analyte is within ±15% of the response obtained during the initial calibration, then the initial calibration is considered to remain valid\textsuperscript{32,33}. In any case, a one-point calibration (with a standard at 5.00 ng/µl) was performed daily for quantitative analysis. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve.

4.3.2.1.1. Preparation

The preparation for this experiment is the same as that described in the previous section for Extractant. The glass wool was replaced with a filter plug.

4.3.2.1.2. Extraction Procedure

A precisely weighed 2.00g of the sample was placed in a prepared extraction vessel as per the description given above. 1.00g of Na\textsubscript{2}SO\textsubscript{4} was introduced along with the sample. Surrogate/ Internal Standards were introduced into the extraction vessel as per the procedure given by EPA Method 8270C (“Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”. 10 ml of hexane/acetone or pure hexane was introduced in the extraction chamber. 15 ml of the same solvent was placed in the extraction liner. The chamber was capped and inserted into the liner and the assembly was sealed by placing it into the rotor segment. One method blank sample was run with each extraction. The extraction protocol was as follows:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (1:1Hex: Act)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td></td>
<td>5 minutes (Hexane)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>100°C to 100°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>100°C to RT</td>
</tr>
</tbody>
</table>
Once the samples cooled down to room temperature, they were opened filtered into evaporation vials. Post filtration, EvapEX™ lid was inserted and the samples evaporated using the pulsing evaporation protocol given in the previous section (Extractant).

Post-evaporation, the extracts were weighed and the final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. Internal Standard (EPA Method 8270 C) was introduced and the sample placed in an appropriate vial for GC/MS analysis.

4.3.2.2 Results and Discussion

![Diagram of PAHs and Phenols](image_url)

Figure 15. Mixtures of PAHs and Phenols

The analytes chosen were: phenol, 2-chlorophenol, 2-chloronaphthalene, Isophorone, anthracene, benzo(b)-(k) fluoranthene, and benzo(a)pyrene ranging from molecular weight of 94 to 252. (Figure 9).

The solvents selected for evaluation had physical properties as given in Table 4.
Hexane and acetone are miscible with each other (Chapter 2), and at a 1:1 proportion form an azeotropic mixture that boils at 49°C (determined experimentally).

From Chapter 2, for a mixture of solvents $i$ and $j$, the polarity $P_{i,j}$ of the mixture is given as 

$$P_{i,j} = \phi_i P_i + \phi_j P_j$$

Equation 13

where $\phi_i$ and $\phi_j$ are mole fractions of solvents $i$ and $j$.

Thus, for a 1:1 mixture of hexane and acetone in a 100 ml total volume, the mole fraction of hexane is 0.362 and the mole fraction of acetone is 0.638. Based on Snyder scale, polarities of hexane and acetone are 0.0 and 5.4 respectively. Thus, substituting these values in Equation 1, the polarity of the mixture is 3.45 (Snyder scale).

For solvent mixtures, from Chapter 2, density of the resulting solvent mixture is given by the equation:

$$d \approx d_i \phi_i + d_j \phi_j$$

Equation 14

Using the same mole fractions as above, for the same solution, the density was calculated to be 0.73 g/ml (experimental measurements give the density of this mixture to be 0.72 g/ml).

The extractions were each run in four replicates. The experimental design is represented in Table 5.
From the results obtained by running these extracts on the GC/MS, it was evident that analyte chemistry plays an important role in the extraction. In Figure 10a, the samples were run with two different extraction methods, Soxhlet and IME on the same day. The solvent platform was kept constant, i.e., both methods used 1:1 hexane/acetone as the extracting medium. The solute to solvent ratio was different for the two methods, however. Soxhlet used a 1:35 matrix: solvent ratio while for IME 1:5 ratio was used (as will be discussed in the next section). This ratio however did not deter the method from giving a good performance in terms of extraction efficiency as well as better precision than the classical extraction method. For all the analytes studied here, IME performed equal to or better than Soxhlet. Of note, however, is the much better precision values obtained using IME. This was especially true of the earlier eluting analytes like isophorone (although this precision trend was not true for 2-chlorophenol). This system was then subjected to extraction using hexane, this time the variable varied was the solvent (to verify the solute-solvent chemistry). Secondary heating mechanism was used to heat up the hexane to 100°C (the same temperature as for IME for 1:1 hexane/acetone, however, Soxhlet was carried out at the boiling point of the solvent mixture). The results obtained from this extraction gave results that were directly related to the structure of the analyte. In Figure 10b, the blue bars represent numbers already obtained from IME.
hexane/acetone extraction (represented by the red bar in Figure 10a). These results were interesting in that there was a clear demarcation regarding which solvent was preferred by which analyte. All the polar compounds like phenols and isophorone preferred hexane/acetone as the solvent possibly due to the chemically similar environment, as evident from the loss when the analytes were extracted using hexane. Also, the hexane extracts for these compounds had lower precision as compared to hexane/acetone (which is the traditional solvent mixture used for the extraction of these type of compounds). On the other hand, all the non-polar compounds, the PAHs showed preferential extraction in pure hexane. From this it can be concluded that analytes prefer chemically similar environment for their extraction to be the most efficient.

4.3.2.3 Conclusions
Two different classes of analytes were chosen, viz., polar analytes; e.g. phenol, 2-chlorophenols and isophorone and non-polar analytes; e.g. PAHs. The two classes showed remarkable difference in the preference for an optimal solvent of extraction. As could be predicted, chemically similar environments gave the most efficient extractions. Thus, non-polar solvents (hexane) gave better results for non-polar analytes like PAHs, while a polar solvent mixture proved to be better for polar analytes. Thus, the final solvent of choice will be determined by the solute-solvent chemistry. In case of a mixture of analytes, the optimum solvent will frequently involve a compromise depending on the target analyte. Professional judgment on the part of the analyst will be needed. Thus, the possibility of using secondary absorbing mechanism is very important if the analyst wants to carry out an extraction based on the solute-solvent chemistry rather than the microwave absorbing capacity of the solvent.

4.3.3. Sample Size
During the process of analyzing samples for the ACS/EPA study (Chapter 5), two types of extractions were set up simultaneously: Soxhlet and IME. Both extractions were done simultaneously so as to reduce the influence of other factors like atmospheric, environmental and/or instrumental. While these extractions were being performed, there was a very practical consideration: Soxhlet used 10g of sample matrix that was to be
processed with 350 ml of the solvent as per the EPA Method. With IME, the maximum volume of solvent that could be held in the glass vessel was 15 ml. Thus, there was a real potential of saturating the solvent if 10g of the sample matrix was used. But on the other hand, there also existed the possibility of losing analytes if the sample matrix selected was too low especially due to non-homogeneity of the sample matrix. It was therefore decided to carry out a solute/solvent ratio influence on extraction recovery. With a view to test this theory, a Certified Reference Material that contained a mixture of PAHs was selected. Different quantities of the CRM were extracted with 10 ml of the solvent and the extraction efficiencies were evaluated. The influence of solute/solvent ratio on precision was also determined.

4.3.3.1 Experimental

b. Samples, Reagents and Standards
The solvent selected for the optimization of the sample-solvent ratio was 1:1 hexane/acetone, chiefly to maintain consistency with the Soxhlet extractions (and also so as not to change more than one variable at a time).

- Polar solvents: A mixture of 1:1 Hexane: Acetone

The solvents:
All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

The sediment sample:
The sediment matrix used for this study was a sample randomly selected from the samples that were sent for the ACS/EPA study as described in Chapter 5. The sediment sample that was chosen was MC2427.

The Standards and Reagents:
- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- EPA Method 620 Diphenylamine 70314 (nominal concentration of 1000 µg/ml in methanol) from Absolute Standards, Inc., Hamden, CT
• Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles GC/MS Tuning Standard GCM-150 (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

Certified Reference Material:
Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

Microwave Instrument and Apparatus
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

GC/MS Determination
GC/MS analysis was carried out on Agilent (HP) 5972 equipped with an autosampler (courtesy: Dr. F. Fochtman, Mylan School of Pharmacy, Duquesne University). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 series II GC which was equipped with a DB-5ms capillary column (30 m × 0.25 mm I.D. ×0.5 mm. ((5%-Phenyl)-methylpolysiloxane). The GC oven program started at 40°C for 5 minutes, 40-290°C at 12°C/minutes, 290°C for 6 minutes, 290-325°C at 20°C/minutes, 325°C for 5 minutes. Injector: Splitless, 250°C. A Hewlett Packard 5972 MSD was with a source temperature at 325°C to monitor PAHs in the Selected Ion Monitoring (SIM) mode. The instrument was tuned daily with decafluorotriphenylphosphine (DFTPP) at a
concentration of 50ng/µl introduced. The DFTPP mass intensity criteria as given in Table 3, EPA Method 8270 C, page 36 were used as tuning acceptance criteria. The calibration relationship established during the initial calibration was verified at periodic intervals. As a general rule, the initial calibration must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed. If the response (or calculated concentration) for an analyte is within ±15% of the response obtained during the initial calibration, then the initial calibration is considered still valid. In any case, a one-point calibration (with a standard at 5.00 ng/µl) was performed daily for quantitative analysis. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve.

4.3.3.1.1. Preparation
The preparation for this experiment is the same as that described in the section for Extractant. Glass wool was used for the filtration process.

4.3.3.1.2. Extraction Procedure
Four different sample sizes were selected, viz., 10g, 5g, 2g and 1g. The CRM that was chosen for the matrix was relatively homogeneous, hence that variable was not considered. A precisely and appropriately weighed amount of the sample was placed in a prepared extraction vessel as per the description given in Section 4.3.1.1.1. 1.00g of Na₂SO₄ was introduced along with the sample. Surrogate/ Internal Standards were introduced into the extraction vessel as per the procedure given by EPA Method 8270C (“Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”. 10 ml of 1:1 mixture of hexane/acetone was introduced in the extraction chamber. 15 ml of the same solvent was placed in the extraction liner. The chamber was capped and inserted into the liner and the assembly was sealed by placing it into the rotor segment. One method blank sample was run with each extraction. The extraction protocol was as follows:
Table 9. Extraction protocol for sample size study

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (1:1Hex: Act)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>100°C to 100°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>100°C to RT</td>
</tr>
</tbody>
</table>

Once the samples cooled down to room temperature, they were opened filtered into evaporation vials. Post filtration, EvapEX™ lid was inserted and the samples evaporated using the pulsing evaporation protocol given in the previous section (Extractant).

Post-evaporation, the extracts were weighed and the final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. Internal Standard (EPA Method 8270 C) was introduced and the sample placed in an appropriate vial for GC/MS analysis.

![Figure 17. PAHs selected from CRM 104-100 for Sample Size Study](image-url)

4.3.3.2 Results and Discussion

The analytes chosen were: Acenaphthylene, acenaphthene, anthracene, benzo(a)anthracene and benzo(a)pyrene ranging from molecular weight of 152 to 228. (Figure 11).

The solvents selected for evaluation had physical properties as given in Table 7.
Table 10. Physical properties of the solvents selected\textsuperscript{30,31}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Dielectric Constant</th>
<th>Density g/ml (25°C)</th>
<th>Hildebrand Solubility Parameter</th>
<th>Polarity Index (Snyder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane:Acetone</td>
<td>49</td>
<td>ND</td>
<td>0.72</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>80.0</td>
<td>1.00</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

Hexane and acetone are miscible with each other (Chapter 2), and at a 1:1 proportion form an azeotropic mixture that boils at 49°C (determined experimentally). Based on the calculations described in the section on analyte chemistry, the density of the solvent mixture was found to be close to the theoretical density of 0.72 g/ml, and the polarity was found to be the same as calculated above, 3.45 on the Snyder scale. The extractions were each run in four replicates (with the exception of the 5.00 g point), thus the experimental design is shown in Table 8.

Table 11. Extraction Sample Design for Sample Size

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Replicates</th>
<th>Method Blank</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

When the first attempt was made to extract 10 g of the solid matrix with 10 ml of the solvent, we encountered a unique problem: the volume of solvent was not sufficient to wet the entire matrix bed. An additional 5 ml of solvent was added, and extraction performed as given. From the results obtained by running these extracts on the GC/MS, it was evident that the sample size does not play a predictable role in the extraction.

Table 12. Sample Size Study

<table>
<thead>
<tr>
<th>Compound</th>
<th>10g</th>
<th>5g</th>
<th>2g</th>
<th>1g</th>
<th>Certified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthylene</td>
<td>0.99</td>
<td>1.54</td>
<td>1.37</td>
<td>1.45</td>
<td>1.21</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.74</td>
<td>1.64</td>
<td>1.88</td>
<td>2.01</td>
<td>1.44</td>
</tr>
<tr>
<td>Benzo(a) Anthracene</td>
<td>ND</td>
<td>7.11</td>
<td>6.18</td>
<td>7.03</td>
<td>7.98</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.61</td>
<td>0.55</td>
<td>0.57</td>
<td>0.56</td>
<td>0.77</td>
</tr>
<tr>
<td>Benzo(a) Pyrene</td>
<td>4.77</td>
<td>6.11</td>
<td>7.17</td>
<td>5.99</td>
<td>5.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound (Error)</th>
<th>10g</th>
<th>5g</th>
<th>2g</th>
<th>1g</th>
<th>Certified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthylene</td>
<td>0.37</td>
<td>ND</td>
<td>0.11</td>
<td>0.2</td>
<td>0.77</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.78</td>
<td>ND</td>
<td>0.12</td>
<td>0.14</td>
<td>0.87</td>
</tr>
<tr>
<td>Benzo(a) Anthracene</td>
<td>ND</td>
<td>ND</td>
<td>1.08</td>
<td>0.35</td>
<td>2.56</td>
</tr>
</tbody>
</table>
Table 9 presents the results obtained by running the samples prepared above. The solvent platform was kept constant, i.e., all samples used 1:1 hexane/acetone as the extracting medium. Soxhlet used a 1:35 matrix: solvent ratio while for IME the different ratios evaluated were 1:1 (10 g) to 1:10 (1 g). As can be seen from the above table and a representative plot given in Figure 12, it is clear that the sample size does not play any significant role in the extraction. However, this holds true for homogeneous solids. Extensive sampling studies are required to assess trends for non-homogeneous matrices.

The problem encountered for the 10-g sample of incomplete matrix-wetting could possibly have led to channel formation, and can explain the reason for the large values on the 95%CL error bars for 10 g as evident from the representative plot (Figure 12). This was
especially true of the late eluting molecule, Benzo(a)pyrene. (This particular PAH however, had peak tailing problems on the chromatograph, and precision for the extraction of this molecule was affected across the board).

Precision values for the other compounds were typically better than those of CRM. In most cases, there was an appreciable decrease in the error of the extraction efficiencies, (and hence an increase in the precision values. Figure 13 indicates the improvement in precision in percent terms over the numbers reported on the Certificate of Analysis supplied with the CRM. For example, for the 2-g sample the improvement in precision was 86% for anthracene as well as acenaphthylene. The 10-g samples suffer from poor precision as well as accuracy, but it can be assumed that these were not typical results. The precision values for 1-g tend to be lower than 2-g. It is a possibility that the 2-g sample may be the optimal solvent-solute ratio in interest of both precision and accuracy.

**4.3.3.3 Conclusions**

A range of five different PAHs were chosen to carry out the sample size (and solute-solvent ratio) study. A 1:1 hexane/acetone solvent mixture was used as the extractant. For a homogeneous matrix like a CRM, the sample size did not seem to affect the extraction efficiencies. IME results appear to be more consistent compared to the CRM values as evidenced by a decrease in the error values. The error values seem to be smallest for the 2-g sample. However, further study is required prior to making an absolute conclusion on whether error increases as sample size decreases as well as for the influence of the solute-solvent ratio on the recoveries if the matrix is not homogeneous. From the results obtained, 2-g sample size in 10 ml of solvent seems to be the optimal solute-solvent ratio. Since the recoveries were comparable to the Soxhlet recoveries (from a 1:35 solute-solvent ratio), multiple extractions were not required.

**4.3.4. Time**

Since extractions in general, and microwave extraction in particular are a function of temperature, an extensive temperature study was done to determine its influence on the recoveries. However, during the temperature study, a different problem was encountered.
At certain temperatures and certain holding times, the recoveries were less than predictable. These were the cases when the extractions were performed for 10 minutes. Thus, this led to the estimation of time of exposure on the extraction efficiency.

Since the analytes were semi-volatile, the amount of time the compounds were held at a given temperature would possibly degrade them. Thus, time the compounds were kept at any temperature and the subsequent effect on the compounds was evaluated.

4.3.4.1 Experimental
c. Samples, Reagents and Standards

The solvents:
The solvent selected for the evaluation of the influence of time of exposure was a non-polar solvent since the analytes were semi-volatile PAHs.

- Non-polar solvents: n-hexanes

All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

The sediment matrix:
The sediment matrix used for this study was blank sediment that was pre-extracted using Soxhlet. This blank sediment was then baked at 300°C for a period of four days to remove any further traces of the analytes of interest. These were then also subjected to method blank during extraction to ensure the absence of any compounds of interest.

The Standards and Reagents:

- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, 31024 (nominal concentration of 1000 µg/ml in methylene chloride) from Restek Corporation, Bellefonte, PA.
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
**Microwave Instrument and Apparatus**

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

**GC/MS Determination**

GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. The GC oven program started at 40°C for 5 minutes, 40-290°C at 12°C/minutes, 290°C for 6 minutes, 290-325°C at 20°C/minutes, 325°C for 5 minutes. Injector: Splitless, 250°C. A Hewlett Packard 5970B MSD was with a source temperature at 325°C to monitor PAHs. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve.

**4.3.4.1.1. Preparation**

The preparation for this experiment is the same as that described in the section for Extractant. Glass wool was used for the filtration process.

**4.3.4.1.2. Extraction Procedure**

Four different time points were selected in an increment of 15 minutes, viz., 15, 30, 45 and 60 minutes. These time points indicate the amount of time that the extraction assembly would be held at 100°C. A precisely and appropriately weighed amount of the sample was placed in a prepared extraction vessel as per the description given in Section 4.3.1.1.1. Surrogate/ Internal Standards were introduced into the extraction vessel as per the procedure given by EPA Method 8270C (“Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”. 10 ml of hexane was introduced in the extraction chamber (by weight). 15 ml of the same solvent was placed in the extraction
liner. The chamber was capped and inserted into the liner and the assembly was sealed by placing it into the rotor segment. One method blank for solvent and one method blank for sediment sample was run with each extraction. The extraction protocol was as follows:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 minutes (Hex)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>15/30/45/60 minutes</td>
<td>100°C to 100°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>100°C to RT</td>
</tr>
</tbody>
</table>

Once the samples cooled down to room temperature, they were opened and filtered into evaporation vials. Post filtration, EvapEX™ lid was inserted and the samples evaporated using the pulsing evaporation protocol given in the previous section (Extractant).

Post-evaporation, the extracts were weighed to determine the final weight of the extracts. Final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. Internal Standard (EPA Method 8270 C) was introduced and the sample placed in an appropriate vial for GC/MS analysis.

4.3.4.2 Results and Discussion

The analytes chosen were: Naphthalene, acenaphthene, anthracene and fluoranthene ranging from molecular weight of 128 to 202. (Figure 14).

The solvent selected for evaluation had physical properties as given in Table 11.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Dielectric Constant</th>
<th>Density g/ml (25°C)</th>
<th>Hildebrand Solubility Parameter</th>
<th>Polarity Index (Snyder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>69</td>
<td>1.88</td>
<td>0.65</td>
<td>7.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>80.0</td>
<td>1.00</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>
The samples were each run in three replicates, thus the experimental design could be represented as:

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Time Point</th>
<th>Replicates</th>
<th>Blanks Solvent</th>
<th>Blanks Sediment</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>15 minutes</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>30 minutes</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>45 minutes</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>60 minutes</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

This study was based on a similar study carried out by Lopez-Avila and coworkers\(^1\). The compounds have shown a varied response when exposed to 100°C for different amounts of time. Lopez-Avila and coworkers evaluated time of exposure for some PAHs\(^1\), and the results obtained have been analyzed in Figure 15 (Figure 15 was plotted for percent recoveries at 115°C as it was the closest reference point for temperature for the present study which was carried out at 100°C). While the study was focused on temperature influence, time exposure was also studied. However, it remains inconclusive in that no tangible relationship can be determined between the PAH and the influence of time of exposure.

Some compounds like naphthalene and pentachlorophenol (denoted in the plot as PCP) showed an increase in extraction efficiency with increasing time. Compounds like anthracene and pyrene did not show any significant trend with increasing time. Other compounds are depicted in Figure 15 in Appendix. With the
exception of naphthalene, all compounds exhibit same trend for 5 minutes as for 20 minutes. The conclusion derived was that 5 minutes was sufficient for the extraction of PAHs under the given conditions.

However, the results we obtained from the procedure described earlier, did indicate a relationship between time and recovery. The study is summarized in Figure 16 (The results are given in micrograms/gram; error expressed as one Standard Deviation for \( n=3 \)). The study was done using closed vessel extraction using a 1:5 sample-solvent ratio (the optimal ratio) with pure hexane as the extractant (the optimal solvent). As is evident from Figure 16, the compounds show a general trend of lower efficiencies for 15 minutes and the efficiency increases for 30-minute extractions. 45-minute extractions do not show any improvement over the 30-minute figures. 60-minute extractions however show a noticeable decrease in recovery. This could possibly be due to the degradation of compounds when they are being held at 100°C for an hour. Precision values are generally best for the 15-minute extractions, but do not show a trend for the other extraction time points.

4.3.4.3 Conclusions
A range of four different PAHs were chosen to carry out the time study. Pure n-hexane was used as the extractant. A pre-extracted blank sediment was used as a matrix where the PAHs were spiked onto the matrix and allowed to equilibrate. 2.00 g of sample was used per vessel, and the extractions were performed in replicates of three at each time point. 10-ml of solvent was added to obtain an optimal solute-solvent ratio. Internal standard was added. Temperature selected was 100°C for the extractions. Though the results obtained from literature indicate that 5 minutes is sufficient time for the extraction of the PAHs, we found that a time range between 15-30 minutes is the most optimal range. It should be noted that the literature article has used hexane-acetone mixture as the extractant, and the influence of the solvent cannot be ruled out. For the purpose of this study, however, there exists a trend. The efficiencies seem to be maximal for 30 to 45 minutes, while at 15 minutes not all compounds seem to be extracted and at 60 minutes the compounds apparently are undergoing degradation resulting in poorer recoveries.
Since 30 minutes and 45 minutes do not show statistically significant differences, it can be concluded that the extraction is completed within 30 minutes.

4.3.5. **Moisture Content**

On a general basis, extractions are performed on dry matrices. In fact, a lot of sediment and soil matrices dried and stored (freeze-dried or otherwise) and subsequently processed in a dry state. This drying allows a convenient storage of the samples as it prevents bacterial degradation. It also makes the matrices more homogeneous. Moreover, most of the reported environmental studies provide data based on the dry mass of the samples. In the case of the extraction itself, drying improves the efficiency of the conventional extraction processes such as the Soxhlet extraction\textsuperscript{34}. Soxhlet often uses hydrophobic extractants which are not miscible with water and the removal of water from the matrix prevents the formation of emulsions. However, the surface tension of a solvent in the pores of a dry matrix can be sufficient to prevent the diffusion of the liquid into the (micro) cavities of the matrix. It can be useful in such a case to humidify the matrix. The water demonstrates a swelling effect\textsuperscript{35, 36}. Moreover in the environment, especially in the aquatic matrices, the natural samples that are collected are wet and show various amounts of water, from 20\% for sandy samples to more than 40\% for sludges. Thus, the influence of moisture on the extraction recovery was an important factor that needed to be studied.

4.3.5.1 **Experimental**

d. **Samples, Reagents and Standards**

*The solvents:*  
The solvent selected for the evaluation of the influence of moisture was a mixture of solvents to provide a combination of polar and non-polar solvent systems. Acetone was added mainly to avoid the emulsion formation that would otherwise take place at the interface between hexane and the moisture in the sample matrix.

- Solvent mixture: 1:1 hexane/acaceton

All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.
The sediment matrix:
The sediment matrix used for this study was blank sediment that was pre-extracted using Soxhlet. This blank sediment was then baked at 300°C for a period of four days to remove any further traces of the analytes of interest. These were then also subjected to method blank during extraction to ensure the absence of any compounds of interest. The sediment was then spiked with appropriate amount of water, followed by thorough mixing. The samples were then spiked with analytes of interest and allowed to equilibrate.

The Standards and Reagents:
- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, 31024 (nominal concentration of 1000 µg/ml in methylene chloride) from Restek Corporation, Bellefonte, PA.
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

Microwave Instrument and Apparatus
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

GC/MS Determination
GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. The GC oven program started at 40°C for 5 minutes, 40-290°C at 12°C/minutes, 290°C for 6 minutes, 290-325°C at 20°C/minutes, 325°C for
5 minutes. Injector: Splitless, 250°C. A Hewlett Packard 5970B MSD was with a source temperature at 325°C to monitor PAHs. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve.

4.3.5.1.1. Preparation
The preparation for this experiment is the same as that described in the section for Extractant. Glass wool was used for the filtration process.

4.3.5.1.2. Extraction Procedure
Six different moisture points were selected in an increment of 10 percent w/w, viz., 0% (dry), 10, 20, 30, 40, and 50 %. An appropriately weighed amount of the sample was placed in a prepared extraction vessel as per the description given in Section 4.3.1.1.1. Surrogate/ Internal Standards were introduced into the extraction vessel as per the procedure given by EPA Method 8270C (“Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (Hex/Act)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>100°C to 100°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>100°C to RT</td>
</tr>
</tbody>
</table>

Once the samples cooled down to room temperature, they were opened filtered into evaporation vials. Post filtration, EvapEX™ lid was inserted and the samples evaporated using the pulsing evaporation protocol given in the previous section (Extractant).
Post-evaporation, the extracts were weighed to determine the final weight of the extracts. Final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. Internal Standard (EPA Method 8270 C) was introduced and the sample placed in an appropriate vial for GC/MS analysis.

### 4.3.5.2 Results and Discussion

The analytes chosen were: Naphthalene, acenaphthene, anthracene and fluoranthene ranging from molecular weight of 128 to 202. (Figure 14 in Section 4.3.4, Time Study).

The solvent selected for evaluation had physical properties as given in Table 7 (Section 4.3.3; Sample Size Study). The samples were each run in three replicates, thus the experimental design could be represented as:

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Moisture Content (%w/w)</th>
<th>Replicates</th>
<th>Blanks</th>
<th>Solvent</th>
<th>Sediment</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>40</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Moisture content of the matrix is bound to have some effect on the final recoveries. Depending on the method of extraction chosen, the effect can be either detrimental or advantageous. For microwave extraction, we obtained results which indicate a direct proportionality between the recoveries and the amount of moisture present. This trend for the moisture study can be visually interpreted from a snap-shot of the

![Figure 17. Moisture study. Conc. in µg/g, Error as one SD, n=3](image-url)
extracts. Post-extraction, these samples were collected in centrifuge vials. The volume was kept similar, so as to enable an unbiased visual interpretation. As can be seen from the Figure 18, the higher the moisture, the darker the extracts. The darker extracts translated to higher percent recoveries as summarized in the plot in Figure 17. (The results are given in µg/g; error expressed as one Standard Deviation for \( n=3 \)). The study was done using closed vessel extraction using a 1:5 sample-solvent ratio with 1:1 hexane/acetone as the extractant. As is apparent from Figure 19, the trend is discernible. The compounds show a general trend of lower efficiencies for 0% moisture while the efficiency keeps increasing and is maximal for 50% moisture. For 0 to 20%, within confidence intervals, the recoveries are comparable. However, 30-50% show marked improvement in efficiency. This could possibly be due to the fact that water absorbs microwave energy and can set up its own heating independent of the solvent by conduction and convection. The water present in the matrix can allow local heating which could favor the expansion of the pores and “liberate” the molecules in the solvent, possibly accelerating the extraction. It has however been reported in literature\(^{36}\) that if the amount of water in the matrix gets too significant, there could be problems of miscibility with the organic solvent used for extraction. The water acts as a barrier and hinders the transfer of analytes from the matrix to the solvent. This is especially evident from related moisture study done by other group members (David Lineman\(^{37}\)). Precision values on the other hand are generally best for the 20% extractions, but do not show a trend for the other extraction moisture points.

![Figure 18. Visual comparison of the moisture study extracts](image)
4.3.5.3 Conclusions

Four different PAHs were chosen to carry out the moisture study. Six moisture levels were selected for the study ranging from 0-50% with constant increments of 10%. 1:1 hexane/acetone was used as the extractant. A pre-extracted blank sediment was used as a matrix where the PAHs were spiked onto the matrix and allowed to equilibrate. 2.00 g of sample was used per vessel, and the extractions were performed in replicates of three at each time point. 10-ml of solvent was added to obtain an optimal solute-solvent ratio. Deuterated internal standard was added. Temperature selected was 100°C for the extractions. Results indicate that the higher the moisture content, the higher the recoveries. We found that 0-20% show equivalent recoveries while any moisture point higher than 20% showed steadily increasing recoveries. The poorer recoveries for the drier samples could be due to channel formation. At high moisture values, water contributes to the heating effect of the microwaves by local heating and therefore aids in the extraction process.

4.3.6. Equipment Integration

The most salient feature of the microwave system being evaluated was the integration of a number of components. This integration along with other components was aimed at addressing the inherent drawbacks of traditional Microwave Assisted Solvent Extraction (MASE). The use of microwave-enhanced chemistry, the theory of which has been extensively discussed (Chapter 3), offers many advantages over traditional heating methods. Closed-vessel microwave extraction allows extraction solvents to be rapidly heated to temperatures that are 2-3 times higher than their atmospheric boiling points resulting in shorter extraction times (10-30 minutes in most cases). The amount of solvent consumed is considerably less (20-30 ml in most cases). However, the inability to use non-polar solvents for organic extractions (or the need to couple polar and non-polar solvents) as well as the number of transfer steps during the processing of the samples were some of the shortcomings. Integrated Microwave Extraction (IME) designed to specifically overcome these deficiencies. IME integrates the processes of extraction, filtration, evaporation and solvent recovery through the use of integrated hardware. This
study was undertaken to study the influence of this integration on the extraction recoveries and subsequently to verify the validity and robustness of the instrument.

**4.3.6.1 Experimental**

e. *Samples, Reagents and Standards*

**The solvents:**
The solvent selected for the evaluation of the influence of equipment integration was a mixture of solvents to give a combination of polar and non-polar solvents.
- Solvent mixture: 1:1 hexane/acetone
- Pure solvent: Hexane
- Pure solvent: Acetonitrile (analysis)

All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

**The Standards and Reagents:**
- Base/Neutrals Surrogate Standard Mixture, 31024 (nominal concentration of 1000 µg/ml in methylene chloride) from Restek Corporation, Bellefonte, PA.
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI.
- Individual PAHs for preliminary studies were obtained from Aldrich Chemicals, Sigma-Aldrich, St. Louis. MO.

**Certified Reference Material:**
- Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY
- Natural Matrix Certified Reference Material, Organochlorine Pesticides on Soil, CRM 805-050 (Sandy Loam, pH 7.78) from Resource Technology Corporation (RTC), Laramie, WY

**Microwave Instrument and Apparatus**
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel. The solvent sensor terminates the heating program in the event of a vessel leak or over-pressurization. The sample rotor used was the basic 12-position extraction rotor consisting of 100 ml, fluoropolymer lined, TFM vessels that have a maximum operating temperature and pressure of 220°C and 30 bar (500 psi) respectively. Secondary microwave absorber (Weflon™), a chemically inert, microwave-absorbing fluoropolymer was used. Post-extraction filtration and evaporation was done using Milestone FiltEX™ and EvapEX™ systems respectively without transferring the extracts. The evaporated solvent was collected and recycled using the EvapEX™ in conjunction with the Solvent Recovery System. EasyWAVE™ control software was used to monitor and control the microwave system which uses a PID algorithm for precise temperature and process control that delivers the minimum power required to sustain the set temperature.

Analysis
The extracts were analyzed using a Saturn GCMS/ Varian 3410 high-temperature gas chromatograph coupled to a Varian Saturn II ion trap mass spectrometer (Varian Inc., Walnut Creek, CA) and an autosampler was used for this analysis. Data collection and processing was done using Saturn and SaturnView software. A 1-µl aliquot was introduced into the Varian 3410 Gas Chromatograph (using autosampler). Pesticide analysis was done on a GC equipped with a flame ionization detector (Shimadzu Scientific Instruments, Kyoto, Japan) as well as by HPLC. Waters 600 quaternary gradient system with manual injector equipped with He sparge degassing and a Waters 2487 dual wavelength detector was used (Waters, Inc. Milford, MA). The linear dynamic range was established by 5-point calibration curve.
4.3.6.1.1. Preparation

The preparation for this experiment was the same as that described in the section for Extractant. Glass wool was used for the filtration process.

4.3.6.1.2. Procedure

Microwave Extraction

For 100 ml extraction chamber, the sample was prepared in the following manner: the soil sample (range: 1-5 g)/ CRM was introduced into the extraction chamber with the solvent (range: 10-15 ml). The extraction chamber contains the same solvent as the extractant, enough in volume to immerse the secondary absorber base and part of the vessel (~20ml). This solvent can be recycled for subsequent runs. The vessel was capped with a Teflon lid for separation of inner and outer solvents. Glass coated magnetic stir bars were added. Stirring was set to 40% of maximum. The closed extraction chambers were sealed into the individual rotor segments. The soil samples were extracted using the following temperature program: a 5-minute ramp to 100°C and a 15-minute hold at 100°C. After cooling to 25°C, the extraction chambers were opened and vessels were removed. The secondary absorber base was snapped off, and the vessel was then directly fitted into the slot in the filtration system lid. Samples were vacuum filtered into vials in which evaporation was subsequently carried out. The Teflon cap can be removed for additional washings if necessary. After the completion of filtration, only the closure from the filtration system was replaced with the evaporation closure.

Microwave Assisted Evaporation

A large batch of appropriate solvent was used. The solvent was spiked with the PAH solution. This spiked solvent was used for evaporation studies. Evaporation was carried out under argon (connected at the central position, Fig. 2B) using alternate heating and cooling steps of 700 W for 2 minutes and 0 W for 30 seconds. A cooling step was incorporated to avoid possible overheating of analytes, which could potentially cause thermal degradation. This cycle was repeated 4 to 5 times depending on the solvent used. The see-through microwave door provides easy real-time visual monitoring. Processing of 12 samples simultaneously can be accommodated in one rotor assembly for 25ml
(approximate) extraction vial size using this current instrument configuration. The instrument also enabled an integrated solvent recovery system to permit recycling of the solvents permitting a minimization of fresh solvent usage. Evaporation was carried out to decrease the solvent from 15 ml to 5 ml (arbitrarily). Later however, tests were done to evaluate the influence of the final volume of the solvent on the recoveries.

4.3.6.2 Results and Discussion

The effect of extraction solvent on recovery has already been discussed in the Section on Extractant. Pesticides were used as test analytes in addition to PAH results reported earlier. Therefore, extraction of pesticides (Figure 20) was done using 1:1 hexane/acetone mixture to simulate the Soxhlet procedure closely. Results illustrate good agreement between IME and certified values while using only about 1/50th of the amount of time needed by Soxhlet. Twelve samples were extracted simultaneously in 15 minutes.

As depicted in Figure 19, the recoveries exhibit no loss of analytes due to the integration process. Within 95% CL, the efficiencies of IME were comparable to those reported on the CRM 805-050. Thus, the equipment integration shows no influence (no detrimental effects) on the extraction recoveries. The evaporation study was divided into polar and non-polar solvents. Tabulated results are presented in the appendix. Representative plots (Methanol for polar and Hexane for non-polar) are included here. (Figures 20 a & b). The observed values are in close agreement with the expected values.

The solvents that were recovered by the Solvent Recovery System were evaluated for any loss in analytes, and were confirmed to be clean. Solvent recovery varied for the different
4.3.6.3 Conclusions

Extractions are proven to give comparable results to existing platforms of processing. A range of analytes from PAHs to organochlorine pesticides was employed to verify extraction capability for both types of analytes, viz., non-polar as well as polar. The use of the microwave for evaporation allowed good control over the evaporation conditions. The microwave power output is varied to produce slow heating, even at small solvent volumes (<2ml). Results from evaporation recovery of PAHs in hexane verify complete recovery of the analytes where the analyte concentration ranged from 10-30 µg/ml each. The recovered solvent when subjected to GC/MS analysis showed no analyte loss, thereby making it possible for the solvent to be recycled. These recovered solvents can be recycled and reused as they were chromatographically confirmed to be clean and devoid of any analytes of interest.

4.3.7. Effect of Stirring (report)

This parameter was not studied by itself in the lab. However, since stirring was found to have a certain degree of impact on the extraction recoveries, it is presented here as a report.
The Ethos is a rugged, system specifically designed for laboratory studies. The chassis of the Ethos oven is made of corrosion-resistant stainless steel, and interior cavity and the inside of the door are plasma coated with 5 layers of PTFE applied at 350 °C to protect the interior of the unit. Ethos Labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

The ASM-400 Magnetic Stirring Module can be built in to the bottom of the microwave cavity. The stirring module is a complete stirring system, like the ones used in conventional stirplates. The independently rotated magnet produces consistent stirring of solutions in all vessels, independent of their position within the cavity. Stir bars were supplied to us in PTFE, Weflon, and glass. (Manufacturer makes quartz stirbars as well).

Stirring in a microwave unit ensures:

- Faster reactions via increased surface area contact between sample/solvent
- Accelerated extraction of even difficult samples
- No charring of samples at the bottom of the container
- More homogeneous temperature distribution

According to a report presented by Milestone, Inc. in 1997, stirring has a positive influence on extraction efficiencies as indicated by the following results.

| Table 18 Effect of Stirring on pesticides |
|-----------------|---|---|---|---|
|                | Lindane | Aldrin |
| **Sample**     | **Without** | **With** | **Without** | **With** |
| **Soil**       | 91.9   | 92.78  | 90.13  | 102.1    |
| **Sand**       | 76.34  | 89.61  | 86.46  | 102.3    |

<table>
<thead>
<tr>
<th>Table 19. Effect of stirring on PCBs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clay Sample 100 ng/g PCB</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Araclor 1016</td>
</tr>
<tr>
<td>Araclor 1260</td>
</tr>
</tbody>
</table>
4.3.8. Matrix Effects

In routine analysis for either environmental laboratories or for the pharmaceutical industry, a decisive factor for the choice of method processing is the environment from which the analyte is to be taken out of. Almost all extraction processes are governed to some degree by this “background” factor which can be defined as the matrix. According to IUPAC\textsuperscript{38}, the combined effect of all components of the sample other than the analyte on the measurement of the quantity, where the matrix is defined as a component of the sample other than the analyte. If a specific component can be identified as causing an effect then this is referred to as interference. Matrix effects could be physical or chemical. Physical matrix effects in the context of this study are those that focus on the physical influences of the matrices, e.g. barrier effects, or the actual hindering of the analyte/solvent by the matrix particles. Chemical matrix effects have more to do with the changes in the chemical composition of the solid which affect the responses or quantitation of analyte. With microwave extraction, temperatures play a major role in the background effects. At high temperatures, reactions can take place that would not at room temperature, or there could be solvent-matrix interactions that could be set in motion, or occasionally, the physical state of the matrix could change.

Earlier, Lopez-Avila and co-workers reported that the average recoveries and the 95% confidence intervals are a function of matrix\textsuperscript{1}. These data indicate that, just as with other extraction methods, method performance was a function of the matrix. It could not be confirmed however, whether the recovery was independent of the amount of analyte present in the matrix prior to the extraction.

This study aims to evaluate some of these matrix effects. The analyte selected for this study was caffeine. There were many reasons for this choice. Caffeine is a widely researched molecule, and has a well-established analytical profile which allows for straightforward analytical comparisons. Since caffeine is easily available in a variety of matrices, ranging from solids to semi-solids to liquids. Many of the caffeine products are available over the counter, so the analyte and matrices were easy to procure.
4.3.8.1 Experimental

a. Standards, Solvents and Reagents

- The following solvents were utilized for this study: Acetonitrile, Acetone. Formic acid was used for making the mobile phase. The solvents selected were obtained from Fisher Scientific, Fairlawn, NJ. All solvents were Optima grade.
- The standard used in this study was caffeine anhydrous (Fluka 27600) and was purchased from Fluka Lab Chemicals (Sigma-Aldrich), St. Louis. MO.
- Caffeine Products were obtained from the following manufacturers: Bristol-Myers Squibb Co. (New York, NY), SmithKline Beecham Consumer HealthCare (Morristown, NJ), Bayer Corporation (Pittsburgh, PA), Goody's Pharmaceuticals (Memphis, TN).

Filters and Accessories:

- 0.2 µm, 47mm Polycarbonate Membrane filters for the HPLC were procured from Osmonics (Poretics09-732-35) from Sigma-Aldrich, St. Louis, MO.
- Millipore Glass Fiber Filters, 25mm, 1.0 µm (PFB02500) were obtained from Sigma-Aldrich, St. Louis, MO.
- Acrodisc® GHP Syringe Filters, PP, 13 mm, 0.45 µm, mini spiking fitting, (Z26,036-30) were obtained from Sigma-Aldrich, St. Louis, MO.
- PP/PE Syringe, 1.0 ml, All PP/PE, Sterilized (Z23,072-3) were obtained from Sigma-Aldrich, St. Louis, MO.

Microwave Instrument and Apparatus

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.
Analysis
GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-μl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. A Hewlett Packard 5970B MSD used to monitor PAHs. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve ranging from 2 μg/ml to 10μg/ml. The preliminary work was carried out using HPLC. Waters HPLC (Waters, Milford, MA) was used for this purpose equipped with a Waters 600 quaternary gradient system with manual injector, helium sparge degassing, and a Waters 2487 dual wavelength detector.

Preparation
The preparation for this experiment is the same as that described in the section for Extractant. Glass wool was used for the filtration process. Syringe filters were used post-extraction for some of the matrices.

Extraction Procedure
Six different products were selected for this study. Products with differing concentrations of caffeine were selected. All products were obtained over the counter.
The products and their contents are summarized in Table 17.

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Company</th>
<th>Matrix</th>
<th>Abbreviation</th>
<th>Tab wt(mg)</th>
<th>Caffeine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Doz</td>
<td>BMS</td>
<td>Caplets</td>
<td>ND</td>
<td>450</td>
<td>200</td>
</tr>
<tr>
<td>Vivarin</td>
<td>SB</td>
<td>Coated Tablets</td>
<td>VN</td>
<td>625</td>
<td>200</td>
</tr>
<tr>
<td>Excedrin Migraine</td>
<td>BMS</td>
<td>Coated Tablets</td>
<td>EC</td>
<td>675</td>
<td>65</td>
</tr>
<tr>
<td>Excedrin Migraine</td>
<td>BMS</td>
<td>Geltabs</td>
<td>EG</td>
<td>780</td>
<td>65</td>
</tr>
<tr>
<td>Midol Menstrual</td>
<td>Bayer</td>
<td>Geltabs</td>
<td>MD</td>
<td>800</td>
<td>60</td>
</tr>
<tr>
<td>Goody's Headache Powder</td>
<td>Goody's</td>
<td>Powder</td>
<td>GD</td>
<td>1000</td>
<td>32.5</td>
</tr>
</tbody>
</table>

Key: BMS= Bristol-Myers Squibb, SB= SmithKline Beecham Healthcare.

Four replicates were run for each brand. Ten tablets (or other unit doses) were weighed together to get an average weight of the tablets (or unit doses). Twice this weight was
used per extraction vessel (to account for the binders). In some cases, where the caffeine concentration was very high (ND and VN) only one unit dose was necessary to be introduced (since the weights of the caplet/tablet were in the higher range). For the powder, 10 unit doses were mixed together to create a sample pool. Amount equal to the average weight was introduced in each vessel. In case of the other unit doses, (tablets and caplets), 10 unit doses were crushed together using a mortar-pestle to create a sample pool. Gelcaps were introduced into the extraction vessels without any processing (whole unit doses were introduced). These tablet sampling procedures were followed as per the guidelines established in the United States Pharmacopoeia\textsuperscript{39} monographs. A precisely and appropriately weighed amount of the sample was placed in a prepared extraction vessel as per the description given in Section 4.3.1.1.1. Internal standard (benzoic acid was used as internal standard for HPLC analysis, anthracene was used for the GC/MS analysis) was introduced into the extraction vessel as per the procedure given by United States Pharmacopoeia. 10 ml of acetonitrile was introduced in the extraction chamber (by weight). 15 ml of the same solvent was placed in the extraction liner. The chamber was capped and inserted into the liner and the assembly was sealed by placing it into the rotor segment. One method blank was run with each extraction. The extraction protocol was as follows:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes</td>
<td>RT to 110°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>110°C to 110°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>110°C to RT</td>
</tr>
</tbody>
</table>

Once the samples cooled down to room temperature, they were opened filtered into evaporation vials. Evaporation vials were pre-weighed and the solvent volumes were calculated based on weights. Final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. No evaporation was needed for these samples as the concentrations were in the higher range and needed to be diluted further before analysis.
4.3.8.2 Results and Discussion

Caffeine was chosen as the analyte, Molecular weight of 194.

The solvent selected for evaluation had physical properties as given in Table 11.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling Point (°C)</th>
<th>Dielectric Constant</th>
<th>Density g/ml (25°C)</th>
<th>Hildebrand Solubility Parameterδ</th>
<th>Polarity Index (Snyder)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile</td>
<td>82</td>
<td>37.5</td>
<td>0.78</td>
<td>11.7</td>
<td>6.2</td>
</tr>
<tr>
<td>Water</td>
<td>100</td>
<td>80.0</td>
<td>1.00</td>
<td>21</td>
<td>9</td>
</tr>
</tbody>
</table>

The samples were each run in three replicates, thus the experimental design could be represented as: (Acn= Acetonitrile)

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Replicates</th>
<th>Blank</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acn</td>
<td>ND</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acn</td>
<td>VN</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acn</td>
<td>EC</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acn</td>
<td>EG</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acn</td>
<td>MD</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Acn</td>
<td>GD</td>
<td>One unit dose</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

The analysis was started using GC/MS; eventually however, a shift was made to using HPLC for analysis. The following mobile phases were tried, before settling on one mobile phase.

<table>
<thead>
<tr>
<th>Mobile Phase</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Acen, 90% (2%Amm Acetate; 2.5%Ace Acid)</td>
<td>254</td>
</tr>
<tr>
<td>Acetic Acid/ Na-acetate: Methanol</td>
<td>214</td>
</tr>
<tr>
<td>Different combinations of Water: Acen: formic acid</td>
<td>275</td>
</tr>
<tr>
<td>Acen: THF: Conc. Ace Acid: Water (20:20:5:95.5)</td>
<td>273</td>
</tr>
</tbody>
</table>
The mobile phase used was a gradient of Acetonitrile and water with 2.5 ml formic acid/100 ml water.

The results presented have been obtained with the Waters HPLC. The column used for this purpose was obtained from Waters (Milford, MA), C\textsubscript{18}, Particle Size: 5\textmu m Dimensions: 3.9 x 150mm (Waters WAT046980).

The matrix in which the analytes of interest are contained clearly has an important influence on the efficiency of extraction. Extraction of analytes from complex matrices such as soils and sediments is strongly dependent on the nature of the medium. Soil matrices with weak analyte/matrix interactions, such as sand, release analytes easily\textsuperscript{40}. Soils with a highly adsorptive nature, composed of large amounts of clay, organic carbon and other strongly adsorbing species tend to show strong analyte/matrix interactions\textsuperscript{41}.

Generally speaking, heating the sample may help to release analytes to the headspace, or other factors like the addition of a small amount of water or other surface active compounds can also improve the release of analytes from the matrix\textsuperscript{40}. Change of pH also influences the release of the analyte from the matrix to the solvent.

From the results obtained from the caffeine study undertaken, there is no evidence of any detrimental effect of the different matrices on the extraction recoveries. From the plot...
illustrated in Figure 18, the extract concentrations are in agreement with the amounts labeled on the product packages with confidence intervals.

However, it needs to be noted here that the extractions had to be accommodated to the matrix. As can be predicted, Goody’s powder was the easiest matrix to be extracted. It mimicked the physical conditions of soils. The extraction involved a simple input of the weighed powder into the vessel with the internal standard and introduction of the solvent followed by extraction in the microwave cavity per the protocol described above. Caplets (No Doz) were a degree less easy to extract than Goody’s. The binders did not pose any complications once the caplets were crushed as per the US Pharmacopoeial requirements. Coated tablets (Excedrin migraine) posed more complications than the caplets. The binders used for the tableting process as well as the additives added during the coating of the tablets seemed to interfere during the analysis process. These extracts had to be re-filtered using syringe filters. Finally, gel tabs were the most difficult to extract. This was especially true of the Midol menstrual geltabs. The gelatin contained in the geltabs possibly changes structure during the extraction and becomes a soft, pliable mass which entraps the extractant. The extractant had to be “pried” out of the mass by a syringe needed followed by filtration using syringe filters. Addition of internal standard made the extraction quantitative.

However, the coated tablets and geltabs released some of the additives into the extraction solvent, making the extraction less specific. This possibly explains why the GC/MS analysis ran into several problems, and we changed the analysis instrument to HPLC eventually. All the coated tablet extracts and geltab extracts seem to get the coloration from the pigments used to make the medications. Goody’s extracts were the only clear extracts. The results presented in Figure 22 are in mg/unit dose. Error is expressed as 95% CL, n=4.

4.3.8.3 Conclusions
A range of six different caffeine products were chosen to carry out the study of the influence of different matrix types upon the extraction recovery. Acetonitrile was used as
a solvent. A method blank was processed along with each batch of brand extractions. Sample equivalent to two unit doses was used per vessel, and the extractions were performed in replicates of four at each time point. 10-ml of solvent was added to obtain an optimal solute-solvent ratio (in most cases, the low dose amounts resulted in lower ratios). Internal standard was added. Temperature selected was 110°C for the extractions. Results obtained from literature indicate that matrix plays an important role. This study proves that matrix does play an important role; however, the ways the extractions are performed differ for different matrices. The efficiencies were not affected by different matrices as the results show close agreement with the amount of caffeine on the product labels.

4.4. Part 2: A Theoretical Model and Experimental Verification of Temperature Dependence of Recovery of MAE from Solid Materials

4.4.1. Introduction

Temperature is one of the most significant parameters influencing extraction, and hence is presented here in a separate section.

Normally, high temperature not only shortens the extraction time, but also increases the recovery of extraction. In most cases\textsuperscript{42,43} the extraction temperature is limited by boiling point of the solvent in traditional methods of extraction (e.g. Soxhlet). Closed-shell extraction methods, such as Microwave Assisted Extraction (MAE), enable extractions to be performed at higher temperatures than the boiling point of the extracting solvent.\textsuperscript{17,44,45} Microwave-assisted extraction consists of heating the extractant (mostly liquid organic solvents) in contact with the sample with microwave energy. The partitioning of the analytes of interest from the sample matrix to the extractant depends on the temperature and the nature of the extractant. For a proper understanding of the technique, the effects of microwaves on the sample-solvent mixture are presented below. It must be realized that, unlike classical heating, microwaves heat the entire sample simultaneously without heating the vessel. Therefore, the solution reaches its boiling point very rapidly, leading to very short extraction times.
4.4.2. Effects of microwaves

Microwave energy is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles. The theory of microwave effect has already been discussed (Chapter 3). The effect of microwave energy is strongly dependent on the nature of both the solvent and the matrix. Most of the time, the solvent chosen has a high dielectric constant, so that it strongly absorbs the microwave energy. However, in some cases, only the sample matrix may be heated, so that the solutes are released in a cold solvent (this is particularly useful for thermolabile components, to prevent their degradation). Evidence has been presented that during the extraction of essential oils from plant materials\cite{17,45}; MAE allows the migration of the compounds out of the matrix. In fact, microwaves interact selectively with the free water molecules present in the gland and vascular systems; this leads to localized heating, and the temperature increases rapidly near or above the boiling point of water. Thus, such systems undergo a dramatic expansion, with subsequent rupture of their walls, allowing the essential oil to flow towards the organic solvent. This process is quite different from classical solvent extraction, where the solvent diffuses into the matrix and extracts the components by solubilization. In addition, in MAE a wider range of solvents could be used, as the technique should be less dependent on a high solvent affinity. Similar mechanisms are suspected to occur in soils and sediments. Microwave heating of the clay, oxides, and water in the matrix should lead to the formation of gas bubbles, with subsequent local pressure build-ups. This should result in destruction of the macrostructure of the matrix, thereby increasing the surface available for the extraction solvent.

![Figure 23. Temperature profile for extraction of PAHs (115°C, 20 minutes)\(^1\)](image-url)
MAE has been successfully used in the digestion of inorganic and organic analytes from environmental and biological samples\textsuperscript{46-56}. Experiments show that MAE has many advantages over the traditional Soxhlet extraction\textsuperscript{57}. Vazquez et al performed a Factorial Experimental Design to study the effects of the experimental parameters over extraction recovery of methylmercury from freeze-dried marine sediments\textsuperscript{58}. Optimized experimental results showed that temperature and the amount of hydrochloric acid used were statistically significant to the recovery, but extraction time and solvent volume as well as the interactions between factors were not significant\textsuperscript{58}. Meanwhile research has been carried out to study the temperature-dependence of recoveries using MAE. Hoogerbrugge et al gave a model based on their experimental design results of extracting triazines from soil\textsuperscript{59}. They found that there was an optimal temperature in the range of experimental temperature, and the extraction efficiency has linear relationship with the squared difference between the actual and the optimal temperature. Researches by other groups have found that the recovery would be better at a higher temperature\textsuperscript{1, 12, 58, 60}. But in most cases, as extractions were done at only two or three temperature points, it is impossible get a model based on the sparse data. Owing to the complexity and variety of solid matrices, it is difficult to find a model for the relationship. Some research groups have attempted to build a theoretical model for temperature dependence of extraction recovery that will benefit extraction experiment work\textsuperscript{61, 62}. Most of them were empirical models.

Previously, work has been carried out by Lopez-Avila and co-workers that demonstrate the influence of temperature on extraction recoveries\textsuperscript{1}. Their results have been analyzed in Figure 23. In this work, we attempted to build a theoretical model of temperature dependence of extraction recoveries (efficiencies) from selected solid materials based on thermodynamic concept followed by the design and performance of an MAE experiment to test the temperature dependence model.

### 4.4.3. Theoretical Model

Experimentally, the extraction process could not be at thermodynamic equilibrium as the extraction was too slow and extraction time was not long enough. In this theoretical
model, all processes are treated as thermodynamic equilibria. To an equilibria extraction process, the partition coefficient (K) of the extraction process can be calculated by the free energy change (ΔG) in the extraction process.

\[ K = e^{\frac{\Delta G}{RT}} \]

Equation 15

\[ \Delta G = \Delta H_e - T\Delta S_e \]

Equation 16

\( \Delta G \) is the free energy change during the process of the extraction of solute from matrix into the solvent. \( \Delta H_e \) is the enthalpy change and \( \Delta S_e \) is entropy change in the process. The enthalpy change (\( \Delta H_e \)) mainly results from the internal energy changes of solute and solvent during the extraction process. The solid matrix is treated as unchanged for simplifying the energy calculation. Strong cohesive interactions exist between the molecular particles compared to vapor phase particles. The enthalpy change in mixture can been described by cohesion parameter of Hildebrand\(^{62}\)

\[ \Delta H_m = V_m \left( \delta_1 - \delta_2 \right) \phi_1 \phi_2 \]

Equation 17

where \( \Delta H_m \) is the change in enthalpy of mixing, and \( V_m \) is the total volume of mixing. \( \phi_1 \) is the volume fraction of solvent, and \( \phi_2 \) is the volume fraction of solute. \( \delta_1 \) is the Hildebrand solubility parameter of the solvent, and \( \delta_2 \) is the Hildebrand solubility parameter of the solute.

There are some empirical and semi-empirical equations suggested to describe the temperature dependence of Hildebrand parameter, one of them can be expressed by following equation\(^{62}\)
\[ \delta = \delta_0 \left[ 1 + 1.13\alpha (T_0 - T) \right] \]

Equation 18

where \( \alpha \) is the coefficient of expansion.

If the temperature coefficients of volume of mixing is small, the volume of mixing can be treat as constant in the normal range of extraction temperature. Hence, equation (5) can be used to describe the temperature dependence of enthalpy change:

\[ \Delta H_e = B(T_1 - T)^2 \]

Equation 19

where \( B \) is a coefficient and \( T_1 \) is temperature coefficient.

The entropy of solute increases during the process of entry of solute molecules into the solvent from the matrix. The whole procedure can be treated as the reverse process of solute being dissolved into matrix plus the process of solute being dissolved into solvent.

In the first step, the solid matrix is treated as unchanged and has no contribution to entropy change. The entropy change can be described with following equation:

\[ \Delta S_1 = R(n_A \ln x_A) \]

Equation 20

The entropy change in the process of solute is dissolved into solvent can be described as following equation:

\[ \Delta S_2 = -R(n_B \ln x_B + n_C \ln x_C) \]

Equation 21

Thus, the entropy change can be calculated by following equation:

\[ \Delta S_{\text{total}} = \Delta S_1 + \Delta S_2 = R(n_A \ln x_A - n_B \ln x_B - n_C \ln x_C) \]

Equation 22
From equation (1), (2), (7) and (8), the partition coefficient $K$ can be calculated by following equation:

$$K = e^{\frac{B(T_r-T)^2 + TB(n_B \ln x_B + n_c \ln x_C - n_A \ln x_A)}{RT}} = A_1 e^{\frac{B(T_r-T)^2}{RT}}$$

Equation 23

where $A_1 = x_B^n_B + x_C^n_C - x_A^n_A$

In the extraction, the recovery (R) of solute can be expressed as follows:

$$R = \frac{C_v V_l}{C_v V_l + C_s V_s} = \frac{1}{1 + \frac{C_s V_s}{C_v V_l}} = \frac{1}{1 + CK^{-1}}$$

Equation 24

Equation (9) is plugged into Equation (10). The temperature dependence of recovery for chemical extraction of solute from solid matrix can be obtained as follows:

$$R = \frac{1}{\frac{B(T_r-T)^2}{1 + A e^{\frac{-B(T_r-T)^2}{RT}}}}$$

Equation 25

where $A = \frac{C}{A_1} = \frac{V}{V_1 (x_B^n_B + x_C^n_C - x_A^n_A)}$

Equation 13 is the model we use for the prediction of temperature dependence of recoveries. It is predicted that as the temperature increases, recoveries will increase. The total increase will be dependent on the analyte and the temperatures the analyte can withstand. Experimental verification was then performed on the model, described as follows in Section 4.5 (Experimental)
4.5. Experimental Verification

4.5.1. Instrumentation

Experiments demonstrated that MAE process could be equilibrated within normal extraction times (10-15 minutes) for many systems, as extraction recoveries did not change when extraction time was increased\(^{48, 58}\).

MAE experiment was performed to verify the feasibility of the theoretical model. The microwave-assisted extraction system used for this work was the Ethos SEL (Milestone Inc., Monroe, CT) which is an integrated microwave solvent extraction system. This system consisted of an Ethos laboratory microwave unit with a built-in magnetic stirrer, a fiber optic temperature sensor, and a solvent sensor, which terminates the heating program in the event of a vessel leak or over-pressurization. The sample rotor used was the basic 12-position extraction rotor consisting of 100 ml, fluoropolymer lined, TFM vessels that have a maximum operating temperature and pressure of 220°C and 30 bar (500 psi) respectively. The software uses PID (Proportional Integrating Derivative) algorithms for precise temperature and process control that delivers the minimum power required to sustain the set temperature\(^{63}\).

The goal of the experiment was to determine the temperature dependence of recovery, if any, rather than the best recovery. The extraction was performed in a large range of temperatures which would be suitable for the solvent used in the extraction\(^{54, 57, 64, 65}\). The larger the temperature range and the more the temperature points, the better the experiment to test our model. Peanut-lipoidal material was selected for the experiment with crushed peanuts as the matrix, lipoidal material being the analyte. The composition of peanuts is substantially lipoidal material-based, making larger values possible for extraction recoveries. If an efficient solvent had been selected, the recovery would be very high even at lower temperatures. This would make the recovery range too narrow to be used to verify the model. Hence, the lesser efficient methanol was selected as the extraction solvent.
4.5.2. Samples and reagents
The peanuts sample, purchased from supermarket, was pulverized into particle size of approximately 0.3mm by sieving out the larger size particles. In this pulverization process, care was taken to avoid making the particle size extremely small as lipoidal material would be pressed out from the sample. All the reagents used in the experiment were analytical grade.

4.5.3. Procedure
2.00g of pulverized peanut was placed into an extraction vessel. Around 20g of methanol was then added into the vessel. Subsequently, a magnetic stirrer was placed into the extraction vessel. Four replicate samples were prepared and extracted for each temperature point. The vessel was inserted into a PTFE liner with the sleeve and subsequently sealed with the pressure spring in place. These closed vessels were then introduced into the rotor segments of microwave and extraction with stirring was commenced. The extraction procedure is as follows: 5-minute ramp to heat the system to the set/desired extraction temperature followed by a 15-minute hold time at that extraction temperature. The PID algorithm controls the microwave power. After the extraction was over, the vessels were allowed to cool to room temperature before opening. The extraction samples were filtered using pre-weighed Whatman quantitative filter paper. These filters were washed using 5ml of acetone and 5ml Hexane. After they were dried in microwave, the filter paper with the dried extracted samples was weighed accurately.

4.5.4. Results and discussion
Five temperature points were selected to perform the extraction. For every temperature point, four replicate samples were extracted. Thus, a set of four extraction results were obtained for each temperature point. A simple data process was performed on these raw results. The mean values of the extraction at each level with error expressed as 95% confidence intervals are listed in Table 1.
Table 25. Experimental results of extraction of lipoidal material from peanuts using methanol

<table>
<thead>
<tr>
<th>Temperature/K</th>
<th>333</th>
<th>353</th>
<th>373</th>
<th>393</th>
<th>413</th>
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<tr>
<td>Ground Matrix (g)</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Methanol (g)</td>
<td>20.68</td>
<td>20.76</td>
<td>20.65</td>
<td>20.60</td>
<td>20.60</td>
</tr>
<tr>
<td>Matrix after (g)</td>
<td>1.67</td>
<td>1.51</td>
<td>1.39</td>
<td>1.31</td>
<td>1.26</td>
</tr>
<tr>
<td>Lipoidal material (g)</td>
<td>0.33</td>
<td>0.49</td>
<td>0.61</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
<td>Recovery %</td>
<td>36</td>
<td>54</td>
<td>68</td>
<td>77</td>
<td>82</td>
</tr>
</tbody>
</table>

From the extraction results, we can see that the recovery of lipoidal material increases while extraction temperature increases. The highest temperature was limited in accordance with the safety protocol of the microwave unit; thus very high temperatures could not be attempted. Also, so as not to be detrimental to the analyte, the temperature range selected was 333-413K.

In the theoretical model (equation (11)), we know that \( A = \frac{V_s}{[V_l(\frac{x_B^n}{x_t^n} + \frac{x_c^n}{x_t^n} - \frac{x_A^n}{x_t^n})]} \).

In the experiment, 2g peanut and 20g solvent were used. The total lipoidal material constitutes 45% of peanuts. Thus, from the above equation, it is evident that \( A \approx 0.1-0.2 \). From Equation (5), we know that the temperature coefficient \( T_I \) has relation with critical temperatures \( T_0 \) of solute and solvent. An empirical value of \( T_I \) needs to be determined by experimental data. It is a system dependent coefficient.

Using the extraction data, values of \( A, T_I, \) and \( B \) of Equation (11) were determined by regression fitting analysis. The equation for the extraction system can be written as follows:

\[
R = \frac{1}{1 + 0.122e^{-\frac{0.0319(500-T)}{T}}} 
\]

Equation 26
The predicted recovery by the equation in the temperature range from 330 K to 415 K is shown by the curve in Figure 24 against the experimental recovery points. The curve illustrates the model prediction and the points correspond to experimental results. From the plot, we can see that our model fits the experimental values very well with correlation coefficient of 0.999 and the model shows the same trend as illustrated by the values obtained by experimental extraction of the lipoidal material.

The difference between predicted values and experimental values at experimental temperatures is also shown in Figure 24. From the plot, it is seen again that the theoretical model predictions are in agreement with the experimental results. This demonstrates that the model can be used to describe the temperature dependence of recovery of the experimented system and the assumptions and approximations are reasonable to some systems. The extraction process in the model is treated as an equilibrium process. If an extraction is very slow because solute is difficult to remove into solvent and extraction time is not long enough, the result can not be used in the model. As these assumptions and approximations were introduced into the model, the model may not be true to other systems in that these assumptions and approximations are not reasonable. The model needs to be tested further.

**4.5.5. Conclusion**

Temperature is of prime importance in ensuring efficient extraction, as elevated values usually enhance the extraction, as a result of an increased diffusivity of the solvent into the internal part of the matrix under high temperatures, as well as an enhanced desorption of the components from the active sites of the matrix. In closed systems, pressure is also an important variable; however, this is directly dependent on the temperature. So, the latter parameter is preferably controlled to avoid degradation of the extracted compounds.
Temperature was found to be a strongly influential parameter on the extraction efficiency of triazines using MAE; values of 80-100°C were found acceptable. The MAE of methylmercury from sediments was also strongly dependent on the temperature. However, at the same time, increased amounts of matrix materials were also extracted, leading to less selective extractions. So, a compromise must be found between high extraction efficiency and selectivity. In addition, in some cases, increasing the temperature may be prejudicial to the extraction, due to the degradation of the selected components.

From the observation that the experimental results are in agreement with the theoretical model, it can be said that the assumptions and approximation are reasonable and the simplified theoretical model can give a satisfactory prediction of the temperature dependence of recovery. Because an empirical Hildebrand parameter function and certain assumptions were introduced into the theoretical model, the validation of the model is contingent upon the validation of the empirical Hildebrand parameter function and of the assumption conditions in an experimental set-up. Based on the fact that the temperature dependence of interactions between the different materials is complex, further testing of the hypothesis is necessary.

4.6. List of Tables and Figures

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<thead>
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<th>Description</th>
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<tbody>
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<td>Physical properties of the solvents selected</td>
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<td>2</td>
<td>Extraction sample design</td>
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<td>Extraction protocol for analyte chemistry</td>
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<td>Physical properties of the solvents selected</td>
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<td>Extraction sample design for analyte chemistry</td>
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<td>Extraction protocol for sample size study</td>
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4.7. References

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4.8. Appendix
Table 26. Extraction using polar solvents

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methanol</th>
<th>95%CL</th>
<th>Acetone</th>
<th>95%CL</th>
<th>Acetonitrile</th>
<th>95%CL</th>
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<th>95%CL</th>
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<td>357</td>
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<td>264</td>
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Table 27. Extraction using non-polar solvents

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<th>Compound</th>
<th>Hexane</th>
<th>95%CL</th>
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Table 28. Comparison of all solvents for the extraction of PAHs

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<th>Acetonitrile</th>
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<td>1075</td>
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Table 29. Evaporation Recoveries for Polar Solvents

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<th>Acetonitrile</th>
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<td>Naphthalene</td>
<td>7.3 ± 0.7</td>
<td>7.9 ± 0.7</td>
<td>5.2 ± 0.3</td>
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<tr>
<td>Acenaphthene</td>
<td>16.9 ± 1.0</td>
<td>16.2 ± 1.3</td>
<td>13.1 ± 0.5</td>
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<tr>
<td>Fluorene</td>
<td>13.9 ± 1.5</td>
<td>14.4 ± 1.2</td>
<td>14.9 ± 1.2</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>17.6 ± 2.9</td>
<td>16.7 ± 2.9</td>
<td>11.9 ± 1.2</td>
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<tr>
<td>Anthracene</td>
<td>5.02 ± 0.8</td>
<td>5.2 ± 1.3</td>
<td>2.5 ± 0.41</td>
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<tr>
<td>Fluoranthene</td>
<td>19.2 ± 3.4</td>
<td>15.5 ± 3.5</td>
<td>16.5 ± 1.4</td>
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<tr>
<td>Pyrene</td>
<td>11.7 ± 2.2</td>
<td>12.2 ± 1.2</td>
<td>10.1 ± 0.2</td>
</tr>
</tbody>
</table>
Table 30. Evaporation Results for non-polar solvents

<table>
<thead>
<tr>
<th>Solvent--</th>
<th>Hexane</th>
<th>Toluene</th>
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</thead>
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<tr>
<td>Compound</td>
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<td>Expected</td>
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<td>8.8 ± 0.6</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>17.7 ± 1.6</td>
<td>18.5 ± 1.5</td>
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<td>Fluorene</td>
<td>15.8 ± 1.7</td>
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<td>12.6 ± 1.7</td>
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<td>Anthracene</td>
<td>14.7 ± 1.2</td>
<td>14.6 ± 2.6</td>
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<td>Fluoranthene</td>
<td>28.4 ± 1.9</td>
<td>27.3 ± 3.1</td>
</tr>
<tr>
<td>Pyrene</td>
<td>18.8 ± 0.9</td>
<td>18.8 ± 2.0</td>
</tr>
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- Plot that depicts influence of time of exposure on % Recovery.

Figure 15. Percent recovery vs. time for PAHs

Figure 15. Percent recovery vs. time for PAHs\(^1\)
Chapter 5 Overview
Performance and Prescription Based Extractions and GC/MS Analyses of Sediment Samples for Polycyclic Aromatic Hydrocarbons and Phenols: An Interlaboratory Study

5. PERFORMANCE AND PRESCRIPTION BASED EXTRACTIONS AND GC/MS ANALYSES OF SEDIMENT SAMPLES FOR POLYCYCLIC AROMATIC HYDROCARBONS AND PHENOLS: AN INTERLABORATORY STUDY

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Chapter 5

5. Performance and Prescription Based Extractions and GC/MS Analyses of Sediment Samples for Polycyclic Aromatic Hydrocarbons and Phenols: An Interlaboratory Study

5.1. Abstract

The current regulatory approach for collecting environmental monitoring data requires laboratories to follow analytical methods prescribed by the U.S. Environmental Protection Agency (EPA). The aim of the study was to check the feasibility of changing the environmental monitoring approach to focus on the quality of data (performance) rather than on the analytical method (the technology). Prescriptive methods have not exploited the opportunities to reduce the analysis cost, have been deterrent to the development as well as the use of innovative, faster and less costly analysis and sometimes resulted in data of less than desirable quality. This study was the result of a collaborative effort between the USEPA & ACS to begin to evaluate how the change to Performance Based Method System (PMBS) could affect data suitability cost of analysis and overcome impediments to innovation. As a participating laboratory, we evaluated the possibility of using a performance-oriented method (Integrated Microwave Extraction (IME)) as a replacement for a prescriptive method (Soxhlet-EPA Method 3540C; Resource Conservation and Recovery Act’s (RCRA) SW-846). Variables that were considered for the experimental design included: extraction efficiencies of both methods, effect of solvent changes in the performance method, influence of sample size on efficiencies, evaporation study for potential losses during the process and the effect of solvent changes on evaporation. Cost-effectiveness analysis is presented to examine the plausibility of replacing an existing method.

5.2. Introduction

5.2.1. Prescriptive Methods

Over the years, regulations have evolved to control the use of different materials, the methods of monitoring certain regulated chemicals, the remediation/ recovery methods,
etc. Generally, these regulations are prescriptive, stating what would be permitted and at what levels. These prescriptive methods are time-tested and have been approved by the regulating bodies.1

The current regulatory approach for collecting environmental monitoring data requires laboratories to follow analytical methods prescribed by the U.S. Environmental Protection Agency (EPA). These methods have been satisfactorily validated and well documented and are defined as prescriptive methods. Presently, most state and federal agencies require prescriptive methods in their monitoring or regulatory programs for several reasons, many of which are perhaps more pragmatic than scientifically based.

Prime reasons cited for using prescriptive methods are:

- They are generally well documented in terms of their performance characteristics (e.g., precision, bias, etc.), under certain known conditions or for certain matrices. Therefore, data with similar matrices can be evaluated using a prescriptive approach.2

- They have generally been used by many laboratories and organizations and so are familiar to the personnel collecting and interpreting the results of the method.2

- The agency requiring the data can have a relatively simple and clearly defined methodology structure and correspondingly, a less intensive and costly quality assurance program (i.e., fewer and simpler laboratory audits or data quality checks).2

All of the above reasons have been used by state and federal agencies to defend relatively cost-effective (though narrowly defined) laboratory certification programs and straightforward data quality control programs.

The U.S. Environmental Protection Agency planned to change its approach to compliance monitoring to emphasize the performance that must be achieved rather than the methods that must be used to collect the required data. This more flexible approach, it was hoped, will reduce the regulated community's compliance monitoring costs and will
encourage innovation in analytical technology while improving the quality of compliance monitoring.\textsuperscript{3} This study was the result of a cooperative effort between the American Chemical Society (ACS) and EPA to evaluate how the change to Performance-Based Measurement Systems (PBMS) would affect laboratory operations, costs, and the quality of compliance decisions.

\textbf{5.2.2. Performance Based Methods}

Several agencies (e.g., EPA, NOAA, USGS, USACE\textsuperscript{▲}) have independently recommended and emphasized the need of Data Quality Objectives (DQOs) or Measurement Quality Objectives (MQOs) for performing assessments\textsuperscript{2}. Both concepts are central to a performance-based system approach. MQOs are statements that contain specific units of measure such as: percent recovery, percent relative standard deviation, standard deviation of X micrograms per liter, or detection level of Y parts per billion. They should be thoroughly specified to allow specific comparisons of data to an MQO. DQOs are statements that define the confidence required in conclusions drawn from data produced by a project. The U.S. EPA’s DQO process is a seven-step strategic planning approach that is used to define what, how, when, and where data are collected and analyzed to ensure that the type, quantity, and quality of environmental data used in decision making will be appropriate for the intended application.

Several definitions of a PBMS have been proposed by different organizations. Various distinctions have been made between a performance-based methods system and a performance-based measurement system. The former generally implies the use of reference methods and their associated performance criteria as the standard of comparison to other methods while the latter requires only stated performance criteria as the comparison standard. Each of these definitions share the concept that PBMS is a framework that permits the use of any appropriate sampling and analytical technology that demonstrates the ability to meet established performance criteria and complies with

\textsuperscript{▲} NOAA: National Oceanic and Atmospheric Administration  
USGS: The United States Geological Survey  
USACE: The United States Army Corps of Engineers
specified DQOs and MQOs of the project in which the sampling and analytical technology is employed. To establish and preserve the credibility of performance-based systems, performance criteria, such as precision, bias, sensitivity, specificity, detection and quantitation levels, and rates of false positives and false negatives must be designated and a sample collection or sample analysis and method validation process must be documented. Whether we call PBMS a “methods” system or a “measurement” system, the basic goals are the same: to provide information of known quality that will satisfy user needs. The implementation of a PBMS, with corresponding required data qualifiers entered into a multi-user database, will allow divergent data from numerous environmental programs to be used for many purposes.

For the sake of consistency, this study will use the term “performance-based system” to highlight the fact that known data quality requires a systems approach whether it is based on method or measurement performance. There are differences between a performance method and a performance measurement system and that either form of performance-based system may be appropriate depending on the specific application. Therefore, unless specified differently in this study, the acronym PBMS is used in the more broad sense of a system approach.

The salient features of PBMS include:

- Use of a scientifically pertinent method (without EPA approval)

The method is application/project specific

- Responsibility for demonstrating compliance rests with the regulated entity

- Regulatory Authority retains the purview of the performance standards

5.2.3. Definition of Problem and Need for PBMS:

Prescriptive methods to environmental monitoring have failed to capitalize on opportunities to reduce the cost for laboratory analysis, have served as a barrier to the development and use of innovative, faster and less costly measurement technologies and have occasionally resulted in data of less than desired quality. EPA approached this problem through program-specific initiatives. In 1997, the agency announced its intent to
implement a PBMS approach for environmental monitoring in all of its media programs to a feasible extent.⁵ According to the report, in the PBMS system, a regulated entity may use any appropriate analytical technique to demonstrate compliance with regulatory requirements.

On the other hand, prescriptive methods have been in use for long periods of time. Some of the major reasons prescriptive methods have found such prolonged utility are based on the following assumptions:

- These methods are well documented for their performance characteristics, especially for known conditions and certain well-established matrices. Thus, there is a common platform for data evaluation and comparison in samples with similar matrices
- There is long-term familiarity with these methods for the personnel collecting and interpreting the data
- These methods, by virtue of being well-documented and with a clearly defined methodology lead to a less-intensive and less costly quality assurance program due to fewer and simpler laboratory audits or data quality checks

However, the validity of these assumptions is debatable. One of the main reasons is that the performance of any given method can vary when it is applied to the real world. Thus some of the drawbacks that these prescriptive methods face are:

- The capability to detect and quantify analytes with known accuracy can vary within any laboratory and more so among different laboratories
- Performance of a method for a certain known matrix may not be reproducible for other matrices. This drawback is more pronounced if the methodology has been documented for laboratory reagent water or other simpler matrices while the application is for groundwater, leachates, sediments, complex soil matrices or even drinking water containing high concentrations of dissolved solids.
- These methods can give a potentially false sense of known and acceptable data quality and may encourage less rigorous quality control programs than actually needed
The degree of comparability in data among programs decreases as different agencies or programs employ different prescriptive methods for the same analyte

- Laboratories and regulated entities have less incentive to design and evaluate potentially better analytical techniques that could be more sensitive, faster, more reliable or cheaper unless they can be readily adopted by the monitoring agency

Even if method improvements are well documented, they are difficult to implement because of regulatory and administrative constraints associated with using a prescriptive method

- Actual method performance and associated data quality is often unknown, especially in some of the older established methods

A performance-based measurement system could help solve many of the shortcomings of a prescriptive approach. Where it is feasible to implement PBMS appropriately, this approach should ensure that a) the method chosen is appropriate for the matrix being tested as well as the analyte being evaluated, b) new technologies are adopted much more readily than when using prescriptive methods, and c) laboratories can readily modify methods where such modifications are documented as still being effective and reliable.

The regulated entity is responsible for demonstrating and documenting that the chosen technique meets whatever performance criteria are established for the particular application. These criteria focus on the quality of data needed for the particular project rather than on the particular analytical method, thereby focusing on the performance (quality of data) as opposed to the technology (analytical methodology). PBMS will allow the regulated entity to choose the least costly, simplest or the most practical method that can meet the specified performance requirements. EPA would establish quantitative or qualitative performance criteria without prescribing specific procedures, techniques or instrumentation. These criteria would be published in regulations, permits or technical guidance documents. Performance criteria may be based on either Data Quality Objectives (DQO) or on Measurement Quality Objectives (MQO). DQO define the statistical confidence required in conclusions drawn from data while MQO establish measurement system performance requirements such as sensitivity, precision or bias.
Both objectives depend on the question or decisions to be addressed by the measurement, the level of uncertainty that is acceptable, the ease with which the performance can be verified as well other factors. Thus, a performance-based approach permits the use of any scientifically appropriate method that can demonstrate compliance whether or not the method has received prior EPA approval.

*Origin of the Study is included in Appendix*

5.2.4. **Original and Modified Goals of the Study**

*Original Goals of the Study*: The task force initially sought to compare three approaches to environmental monitoring:
- Current EPA-approved prescriptive methods
- Current EPA methods modified to the extent permitted under the "Streamlined Reference Methods" approach proposed the Office of Water, and
- Any method that would meet the performance requirements established for the study by the task force.

It is imperative to the acceptability of a non-prescriptive approach in compliance monitoring to demonstrate that the flexibility thus granted does not compromise the quality of the associated and subsequent decisions. In other words, PBMS should not allow flawed monitoring methods to prevent detection of poor environmental performance. Therefore, the study initially aimed to compare the three approaches and determine:
- The degree to which data generated with each approach satisfactorily answered the regulatory questions for which monitoring was being conducted
- The ability of PBMS to encourage innovation and the advantages and drawbacks of each approach
- The ease of implementation by the regulated community, the laboratory community, EPA, and state and local regulatory agencies
- The quality of data (i.e., precision, accuracy, etc.) generated with each approach, and
The minimum quality control data and verification procedures that must be specified in order to determine or verify method performance.

The project was designed to focus on compliance monitoring at normal permit levels for water samples and on a remediation-type scenario for soil samples.

Changes to the Original Goals of the Study: The original goals of the study were modified after the task force found that few commercial monitoring laboratories were interested in participating in the study. By selecting predominantly commercial laboratories to participate in the study, the task force intended to identify problems that are inherent in each of the approaches and might be commonly experienced by these facilities and to suggest some ways to overcome these challenges, and was also able to perform a limited evaluation of the relative advantages and disadvantages of the prescriptive and PBMS approaches. However, with this assumption, the study tended to be biased towards the functioning and regulatory compliance techniques of commercial labs only. Two changes were made to the study design:

- Since EPA decided to no longer pursue the streamlining option, only two approaches were evaluated, current prescriptive methods and PBMS
- Laboratories were not asked to assess compliance with real world permit requirements. The task force decided instead to use the analyses to determine whether any of the analytes in the samples exceed a hypothetical Project Decision Level (PDL)

Analytes were selected based on historical information on those likely to present in real world samples. Water (inorganic analyses) and soil (organic analyses) were selected as matrices. For the purpose of this study, only organic analyses will be discussed, and hence the matrix evaluated will be soil. Analyte concentrations were generally well above or below regulatory action levels.

5.2.5. Requirements of a PBMS

For a successful PBMS, the following criteria must be met:
• DQOs or MQOs must realistically define and measure the quality of data needed. These objectives must be compared to the attributes of the data to be used in the performance-based system.

• Validated methods must be made available that meet these objectives, or objectives should be dependent on results of multiple measurements on known samples using different methods.

• The performance of selected methods, used reasonably, must be adequate to meet the DQOs or MQOs and be well documented. Adequacy can be defined as meeting various performance goals including: analytical precision, accuracy, sensitivity; applicability to the measurement analyte(s) within the applicable matrix; number and type of parameters addressed; and sample collection, preservation, and storage requirements.

• Reference materials covering a variety of relevant matrices containing the analytes of interest, should be available either through preparation using known concentrations or through round-robin testing of unknowns. Concentrations of reference materials must be at or near expected quantitation levels or at levels expected in the environment. (Lack of availability of such reference materials is a limitation for both, prescriptive and performance-based methods).

• The chosen method must demonstrate ruggedness. The parameter of ruggedness has been defined as a measure of reproducibility of test results under normal, expected operational condition, from laboratory to laboratory and from analyst to analyst as well as normal, expected variations within one laboratory by one analyst. The higher the ruggedness of the method, the more suited it is for application across a wider variety of matrices.

The American Chemical Society Committee on Environmental Improvement established a Task Force to evaluate the impact that a possible shift to PBMS can have on the quality and costs of environmental monitoring. As per the Draft Report¹, this Task Force set out its plans in a four-phase approach. This approach will be discussed in more detail under Section 5.3.2. Phase 1 was the selection of evaluation of two approaches to be examined:
current prescriptive methods and PBMS. Phase 2 was the development of Project Decision Levels (PDLs) to represent plausible standards. These PDLs would permit the Task Force to avoid possible compromise of the legal acceptability of data developed by prescriptive methods and associated regulatory decisions. This phase also included the selection of analytes and matrices like natural waters and soil. These matrices were then analyzed for the analytes of interest. Phase 3 was the design of the sample sets. Once the matrices had been analyzed for the presence of absence of the analytes of interest, concentrations of analytes to be added to the matrices were selected. The basis for this selection was that the project was aimed at maximizing the amount of data that could be extracted from a relatively small number of samples. Three sets of samples were sent to participating laboratories. Two samples in each set were blind duplicates. Also included were Youden6 samples, which contained the same analytes as the primary samples, but at differing concentrations. Separate soil samples were also provided as analytical blanks. Phase 4 involved the selection of sample concentrations. The Task Force decided to spike the samples with relatively high concentrations of some analytes and low concentrations of others, on the assumption that this would closely simulate a real-world environmental sample, taking into consideration the background concentrations in sample matrices. PDLs were selected for each analyte and used to test how frequently the analytical results would correctly indicate that analytes were at or above their PDL concentrations. This comparison is vital in terms of consequential decisions of remedial techniques to be made on the basis of environmental monitoring data. EPA-determined Maximum Contaminant Levels (MCLs)7 (where available) or Preliminary Remediation Goals (PRGs) established by EPA Region IX8 (where MCLs were not available) were used as PDLs. When neither MCLs nor PRGs were available, professional judgment was used to provide reasonable target values for the laboratories. Following the completion of this four-phase process, the Task Force solicited and evaluated proposals to manufacture samples and perform water and soil analyses. This will be further discussed under the subsection of Methods.

Center for Microwave and Analytical Chemistry (CMAC) was one of the laboratories selected to participate in this study. (Our Research Group is referred to as Laboratory 1 in the Draft Report1 submitted to us). The study incorporated two sections to it, namely,
Organic Section consisting of Semi-volatile Organic Compounds (SVOCs) and Inorganic Section consisting of different elements and their isotopes. However, for the purpose of this chapter and dissertation, only the Organic Section will be discussed. Comparison between prescriptive and performance-based methods for organic analytes was the goal of this section, and for this purpose we selected the following: Soxhlet (EPA Method 3540C) as our prescriptive method, while Integrated Microwave Extraction (IME) was selected as our performance-based method.

Organic Extractions using liquid/liquid and Soxhlet methodology have been the most widely used techniques, the latter being in existence for over 150 years. Extractions performed using these methods often require the sample to be dried by the addition of sodium sulfate before extraction and filtered after extraction. The extracted samples must also be concentrated or reconstituted in an appropriate solvent for analysis. The whole process requires several pieces of glassware and/or other equipment while taking several hours to days for total processing of samples. Recently accelerated solvent and supercritical fluid extraction emerged as viable alternatives to the traditional methods. Accelerated solvent extraction requires smaller volumes and extractions are in the order of minutes to hours. Supercritical fluid extractions have an additional advantage in that there are no solvent disposal costs or related problems. One major disadvantage of these techniques is that they are only single sample extraction techniques.

CMAC selected Integrated Microwave Extraction (IME) as its performance-based method. A microwave extraction approach can reduce the extraction time from hours to minutes. The ability to control the extraction temperature to a ± 2°C will ensure a reproducible and accurate extraction procedure. In addition, IME uses the same piece of equipment for drying, extraction, filtering and evaporation. This design minimizes sample manipulation and reduces contamination. The ability to process 12-samples simultaneously leads to a semi-automated process. A unique feature of this technology is that both polar and non-polar solvents can be used for extraction. The utilization of a microwave absorbing inert material in the extraction vessels should enable the extraction to be performed without the problems associated with the use of additional microwave
absorbing co-solvent, resulting in an extract that can be more accurately and precisely analyzed by GC/MS. The use of microwave absorbing inert material allows the tailoring of the extraction process to specific compounds of interest. IME is an attractive EPA PBM because the microwave system used in this method is multi-functional. The same microwave can be used for, as mentioned above, all processes related to and dealing with organic extractions, post-extraction sample-processing including filtration and pre-concentration as well as methods used for inorganic sample preparation. In addition, IME allows for the recycling of solvents, which will prove the method to be environmentally friendly and a green process, along with economic advantages due to the reduction or removal of the post-extraction solvent disposal costs.

5.3. Methods and Experimental:

5.3.1. Study Design

As discussed in Section 5.2.5, the study was designed to begin to address some key ramifications of the implementation of performance-based approaches: data suitability cost of analyses and ability to overcome impediments to innovation. Because of its small size, the study was not intended to provide a definitive analysis of performance-based approaches or identify the best ways to implement them. Rather, it was an initial evaluation of the advantages and disadvantages of the PBMS approach, of the problems that may be expected as laboratories are given the capability to modify current methods used for environmental analysis and of possible solutions to these problems.

Evaluation of environmental analytical data typically uses a pass/fail system in which the results either meet or fail specified criteria. Thus, direct comparison of prescriptive methods modified under a PBMS approach was not an objective of the study design. In fact, the method that provides the best meta-data in terms of accuracy and precision is not always the method of choice. This initial evaluation was accomplished by a side-by-side comparison of current prescriptive methods and methods that the laboratories selected to meet a set of performance requirements specified by the task force. Laboratories could modify existing EPA methods or employ completely different techniques to carry out the analyses under the latter approach.
5.3.2. Sample Design
This sample design was accomplished in four phases.

5.3.2.1 Phase 1: Selection of Matrix
For the first phase of the design, selection of appropriate matrices, two aqueous sample types and two soil matrices were chosen. For soils, common sand/clay topsoil was selected. This soil is referred to in the report as "soil without oil". This matrix had been used by ERA (Environmental Resources Associates, Arvada, CO which prepared all of the samples) for over eight years to produce soil quality control and proficiency testing standards. Oil was added to a split of the topsoil to make a second, "more challenging" solid matrix. These samples are referred to as "oily soil" throughout the context of this study. At this point, it is imperative to clarify that Laboratory1 (as we are referred to), after studying the matrices (results presented in Section 5.4), none were found to be containing oil, and made a mention in its draft response to the Task Force. Also, when a second batch of samples was dispatched to our laboratory, many of these samples were found to be mislabeled.

5.3.2.2 Phase 2: Selection of Analytes
The second phase of the sample design was to select the analytes and concentrations of interest. The analytes were chosen to be representative of analytes of concern in a typical refinery effluent or a soil remediation project. Once the task force selected the analytes, the natural waters and soils were analyzed for the analytes of interest by contract laboratories (as discussed under Section 5.3.3). Concentrations of analytes added to the matrices were selected as described in Section 5.3.2.4.

5.3.2.3 Phase 3: Design of Sample Sets
The third phase was the design of the sample sets. Laboratories analyzed identical sample sets that were submitted as blind samples. To maximize the amount of data that could be extracted from a relatively small number of samples, two in each set were blind duplicates. The laboratories also received a single Youden sample for each set. This
sample contained the same analytes as the primary sample (the blind duplicates) but at slightly different concentrations than those in the primary sample\(^6\). For data analysis, the Youden sample was paired with each of the blind duplicate samples, effectively doubling the amount of information that could be obtained for the study. Separate soil samples were also provided as analytical blanks.

**5.3.2.4 Phase 4: Selection of Concentration**

The fourth phase of the sample design was the selection of the sample concentration. The task force decided to spike the samples with relatively high concentration of some analytes and low concentrations of others, just as would be expected to occur in many environmental samples, taking into account the background concentration in sample matrices. PDL were selected for each analyte and used to test how frequently the analytical results would correctly indicate that analytes were at or above their PDL concentrations. This is considered to be an important comparison because consequential decisions of many types are often made on the basis of environmental monitoring data. The task force used EPA-determined Maximum Contaminant Levels (MCL), where available, as the PDL\(^7\). Where the EPA has not determined an MCL, a hypothetical PDL value was estimated for other analytes based on Preliminary Remediation Goals (PRG) established by EPA Region IX\(^8\). Where MCL or PRG data were not available, the task force used its best professional judgment to provide reasonable target values for the laboratories.

**5.3.3. Methods**

*Sample Preparation by Commercial Standard Manufacturer is included in the Appendix*

**5.3.3.1 Sample Analysis Protocol**

5.3.3.1.1. Documentation

We were directed to analyze samples for specific inorganic, semi-volatile, or volatile constituents and asked to evaluate our analytical data against Method Quality Objective, and to conclude which analytes exceeded the PDL. We were directed to use standard
EPA methods (prescriptive) and any other approach we would like to use (PBMS). For the prescriptive approach, the laboratories were expected to follow the quality control (QC) requirements of the applicable EPA-approved methods as published in SW-846 or 40 CFR 136. We were free to select any approach for the PBMS analysis as long as we believed the selected technique could meet the required measurement quality objectives. The task force asked the laboratories to demonstrate that their PBMS methods were sensitive enough to quantify any analyte if that analyte were present at the PDL. We could perform the QC activities we believed appropriate for the PBMS approach used. Formal method validation of these approaches was not required or deemed necessary for the scope of this study.

The task force provided forms to the laboratories to report the following:

- **Analyte concentration**: Based on dry weights for soil samples
- **Analytical precision**: Based on matrix spikes
- **Blank concentration**: Based on sample processing and analysis of background samples for matrix blanks and untreated solvents (that were used for extraction) for method blanks
- **Matrix Spike Recoveries**: Based on formulae provided
- **Method Detection Limits**: Based on formulae provided

Apart from these, the task force requested sufficiently detailed descriptions of PBMS method so that another laboratory would be able to repeat the work. Laboratories also were asked to provide a narrative discussion of the costs (in time) of the PBMS approach relative to the prescriptive method as well as the advantages and disadvantages of the selected alternatives.

5.3.3.1.2. Quality Control for Soil

Laboratories were asked to perform an MS and an MSD (Matrix Spike and Matrix Spike Duplicate respectively) on each soil type for the prescriptive approach. Spike levels were requested to be between 25% and 50% of the PDL. A background soil sample was provided for this purpose. Laboratories were also requested to provide information about
their MDL and QL. We were asked to provide instrument calibration data, QC sample results, and copies of standard operating procedures. Data evaluation would be based on whether the UB (Upper Bound) of the analyte concentration exceeded the PDL. The UB could be estimated using the precision of the laboratory duplicate analysis based on historical laboratory performance.

For the PBMS approach, laboratories were requested to provide the information needed to access whether the upper bound of contaminant concentration in each of the samples was less than the PDL, considering the method variability (the precision from the MS/MSD analyses). Example calculations were provided. The calculations assumed an MS and MSD would be analyzed for each matrix type and analyte, and the reporting forms sent to the laboratories contained areas for providing MS and MSD recoveries.

### 5.3.3.2 List of participating laboratories

- Center for Microwave & Analytical Chemistry, Duquesne University (Laboratory 1)
- EAS Laboratories, Watertown, CT
- Environmental Health Laboratories, South Bend, IN
- Katahdin Analytical Services, Portsmouth, NH
- Mountain Sales Analytical, Inc., Salt Lake City, UT
- TriMatrix Laboratories, Inc., Grand Rapids, MI

### 5.3.4. Summary of Methods Used

The following table (Table A) presents a summary of the different methods employed by the labs. Only the labs participating in soil and semi-volatile compounds analysis are cited. Our research group is designated as Lab 1.
### Table A: Summary of methods used

<table>
<thead>
<tr>
<th>Prescriptive</th>
<th>LAB1 (CMAC)</th>
<th>LAB2</th>
<th>LAB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
<td>Semi Volatile Compounds and Herbicides</td>
<td>Semi Volatile Compounds and Herbicides</td>
<td>Semi Volatile Compounds and Herbicides</td>
</tr>
<tr>
<td>Matrix</td>
<td>Soils</td>
<td>Soils</td>
<td>Soils</td>
</tr>
<tr>
<td>Method</td>
<td>Method 3540C followed by analysis with 8270D</td>
<td>EPA 8270C</td>
<td>EPA 8270C</td>
</tr>
<tr>
<td>PBMS</td>
<td></td>
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</tr>
<tr>
<td>Analyte</td>
<td>Semi Volatile Compounds and Herbicides</td>
<td>Semi Volatile Compounds and Herbicides</td>
<td>Semi Volatile Compounds and Herbicides</td>
</tr>
<tr>
<td>Matrix</td>
<td>Soils</td>
<td>Soils</td>
<td>Soils</td>
</tr>
<tr>
<td>Method</td>
<td>Integrated Microwave Extraction followed by analysis using EPA 8270D</td>
<td>Microwave extraction following proposed EPA 3546. Analysis using ion-trap MS-MS with high volume injections</td>
<td>Sample aliquot (3-g extracted in 10-ml methylene chloride and analyzed by GC/MS in SIM mode)</td>
</tr>
</tbody>
</table>

### 5.3.5. Lab 1 Experimental Design

#### 5.3.5.1 Overall Experimental Design

The need for using designs ensues from the possibility of alternative relationships, consequences or causes. The purpose of the design is to rule out these alternative causes, leaving only the actual factor that is the real cause. There were different factors evaluated in each module. The following parameters were investigated:

1. Extraction Studies
   - Soxhlet vs. IME (Hexane/Acetone (H/A))
   - CRM vs. IME (H/A)
   - Soxhlet vs. IME (Hexane)
   - CRM vs. IME (Hexane)
   - H/A vs. Hexane (samples)
   - H/A vs. Hexane (CRM)

2. Evaporation studies
   - H/A recoveries (varying volumes)
   - Hexane recoveries (varying volumes)
• Comparison data (H/A vs. Hexane)

3. Sample Size study
   • Extraction using different matrix sizes (1, 2, 5, 10g)

4. Cost Analysis
   • Prescriptive Time/Cost study
   • PBMS Time/Cost Study

5.3.5.2 Experimental (for both methods)

*The solvents:*

The solvent selected for the study was 1:1 hexane/acetone, (per EPA Method 3540C)

• Polar solvents: A mixture of 1:1 Hexane: Acetone (for both methods)
• Non-polar solvents: hexane (only for the PBMS method)

All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

*The sediment sample:*

The sediment matrix used for this study was a sample randomly selected from the samples that were sent for the ACS/EPA study as described in Chapter 5. The sediment sample that was chosen was MC2427.

*Miscellaneous Supplies:*

• Whatman Extraction Thimbles, 09-656E, Fisher Scientific, Fairlawn, NJ and Supelco (6-4840-U), Bellefonte, PA
• GC/MS consumables: Agilent (HP), Palo Alto, CA and Supelco, Bellefonte, PA
• Microwave Consumables: Milestone Inc., Shelton, CT

*The Standards and Reagents:*

• Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
• EPA Method 620 Diphenylamine 70314 (nominal concentration of 1000 µg/ml in methanol) from Absolute Standards, Inc., Hamden, CT
• Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles GC/MS Tuning Standard GCM-150 (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
• Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

**Certified Reference Material:**
Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

**Analysis (GC/MS Determination)**
GC/MS analysis was carried out on Agilent (HP) 5972 equipped with an autosampler (courtesy: Dr. F. Fochtman, Mylan School of Pharmacy, Duquesne University). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 series II GC which was equipped with a DB-5ms capillary column ((30 m × 0.25 mm I.D. ×0.5 µm. (5%-Phenyl)-methyIsiloxane) : J & W Scientific, 122-5536, Folsom, CA). The GC oven program started at 40°C for 5 minutes, 40-290°C at 12°C/minutes, 290°C for 6 minutes, 290-325°C at 20°C/minutes, 325°C for 5 minutes. Injector: Splitless, 250°C. A Hewlett Packard 5972 MSD was with a source temperature at 325°C to monitor PAHs in the Selected Ion Monitoring (SIM) mode. The instrument was tuned daily with decafluorotriphenylphosphine (DFTPP) at a concentration of 50ng/µl introduced. The DFTPP mass intensity criteria as given in Table 3, EPA Method 8270 C, page 36 were used as tuning acceptance criteria. The calibration relationship established during the initial calibration was verified at periodic intervals. As a general rule, the initial calibration must be verified at the beginning of each 12-hour analytical shift during which samples are analyzed10, 11. If the response (or calculated concentration) for an analyte is within ±15% of the response obtained during the initial calibration, then the initial calibration is considered still valid. In any case, a one-point calibration (with a
standard at 5.00 ng/µl) was performed daily for quantitative analysis. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve.

**Preparation**

The preparation for this experiment is the same as that described in the section for Extractant (Chapter 4). Glass wool was used for the filtration process.

5.3.5.3 Prescriptive Approach (Soxhlet: EPA 3540C)

The objective of Soxhlet is to extract Semi Volatile Organic Compounds (SVOCs), pesticides and PCBs from solid such as soil, sediments, sludge and solid waste for GC/MS analysis. This technique is by far the most widely used method for solid-sample pretreatment	extsuperscript{12-16}. In this method, the solid sample is placed in a Soxhlet thimble, which is a disposable, porous container made of stiffened filter paper. The thimble is placed in a Soxhlet apparatus, in which refluxing extraction solvent condenses into the thimble and the soluble components leach out. The Soxhlet apparatus is designed to siphon the solvent with the extracted components after the inner chamber holding the thimble is filled to a specific volume with solution. The siphoned solution containing the dissolved analytes then is returned to the boiling flask, and the process is repeated until the analyte is successfully removed from the solid sample.

Soxhlet extractions usually require 24 hours or more. Samples can only be extracted one at a time for each apparatus. It uses hundreds of milliliters of very pure solvent, which is
expensive. Disposal of these solvents is as expensive, since they have to be disposed as hazardous waste. Because the dissolved analyte is allowed to accumulate in the flask, the sample must be stable at the boiling point of the solvent. The extraction methods require some method development. Solvent extractions are concentrated during most soil extractions, excess solvent unless other collection arrangements are made, is usually evaporated in a hood and vented to the atmosphere, potentially leading to environmental concerns. This method is usually applicable only to solid samples.

5.3.5.3.1. Definition of Matrix, Analytes, Extractants

The following samples were used as matrices for the Soxhlet experiments: MC 4910, MC 1049, MC 2968, MC 6829, MC 2427, MC 2724 (provided as samples prepared by ERA); MC 5770, MC 7057 (provided by ERA as background soils to carry out project specific QC requirements); CRM 104-100 (Resource Technology Corporation) for Method QC analysis.

The Project Decision Levels for the different analytes are included in the report\(^\text{17}\). The analytes chosen were a mixture of PAHs and phenols, given in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Analytes chosen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acenaphthene</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
</tr>
<tr>
<td>Benzy alcohol</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
</tr>
<tr>
<td>Naphthalene</td>
</tr>
</tbody>
</table>

Extractants selected for the prescriptive method were dictated by the method itself. In this case, the solvent used was a 1:1 v/v mixture of hexane/acetone. The other alternative given by the method is the use of 1:1 v/v mixture of methylene chloride/acetone. However, the method recommends that the toxicity of hexane/acetone combination is lower and so are the disposal costs, and as such was our choice of solvent system.

5.3.5.3.2. Extraction

Soxhlet apparatus was set-up as illustrated in Figure 1. Solvent in the round bottom flask is heated to boiling. The vapors of the solvent rise through the outer chamber and proceed into the condenser. Here, they condense and fall back to the bottom of the Soxhlet
chamber. As the distilled solvent rises in the chamber, it seeps through the permeable cellulose extraction thimble that holds the matrix. The solvent extracts the compounds of interest and leaves the solid mass behind. As the solvent level rises, the solution is forced through the small inner tube, and the chamber is flushed due to a siphoning effect. The solvent is redistilled from the solution in the flask and condenses in the chamber, repeating the extraction with fresh solvent. The process is repeated as many times as necessary, usually for 24-48 hours or as prescribed by the method.

5.3.5.3.3. Procedure

% Dry weight was calculated for all the samples as follows: (5 g sample was dried in an oven at 105°C overnight, cooled in a desiccator)

\[
\%\text{Dry Weight} = \frac{\text{grams of dry sample}}{\text{grams of sample}} \times 100
\]

Equation 27

10 g of the solid sample was blended with 10 g of anhydrous sodium sulfate and placed in an extraction thimble. The extraction thimble must drain freely for the duration of the extraction period. A glass wool plug was inserted above and below the sample in the Soxhlet extractor. Surrogate standard spiking solution was added onto the sample matrix spiking standard. Approximately 350 mL of the extraction solvent was added to a round bottom flask containing boiling chips. The flask was attached to the extractor and the sample extracted for 24 hours. The extract was allowed to cool after the extraction was complete. This extract was further dried using sodium sulfate.

5.3.5.3.4. Analysis (8270C)

Method 8270 can be used to quantitate most neutral, acidic, and basic organic compounds that are soluble in methylene chloride and capable of being eluted, without derivatization, as sharp peaks from a gas chromatographic fused-silica capillary column coated with a slightly polar silicone. Such compounds include, among other compounds, polynuclear aromatic hydrocarbons, and phenols, including nitrophenols. The entire text of the method can be found at [http://www.epa.gov/epaoswer/hazwaste/test/8_series.htm](http://www.epa.gov/epaoswer/hazwaste/test/8_series.htm)

For the prescriptive approach, the Upper Bound (UB) of analyte concentration is defined by the following equation (when EPA 8270C is used for organics)\(^\text{17}\):
\[ UB \leq X_{EPA} + 2(RPD_{lab}) \]

Equation 28

where \( X_{EPA} \) = analyte concentration from single analysis using prescriptive methods and \( RPD_{lab} \) = relative percent difference between lab duplicates based on historical lab performance

5.3.5.3.5. Variables Evaluated

For the prescriptive method, the variables evaluated were the extraction efficiency using 1:1 v/v mixture of hexane/acetone. Evaluation of extraction recoveries using pure hexanes was also carried out (though not a part of the study) and compared with PBMS method. Finally, QC data evaluation was done by CRM extractions using Soxhlet.

5.3.5.4 PBMS Approach (Integrated Microwave Extraction)

Integrated microwave extraction (IME) is a new approach to extraction of organic compounds from different matrices. This process has been extensively discussed in Chapter 3. IME incorporates the chemistry of the traditional methods with the benefits of microwave heating. IME uses a dual vessel design. The inner extraction vessel (Figure 2) is fitted with a glass fiber filter so that the sample can be filtered directly into the collection bottle or evaporation vessel (Figure). The inner extraction vessel, with cover (not shown) is placed inside the outer Teflon microwave vessel. The dual vessel allows extractions to be performed with as little as 5ml of extraction solvent in the inner vessel and 20-25ml of solvent in the outer vessel (used as a heat transfer agent). The solvent in the outer can be used multiple times. The dual vessel design also allows for stirring of the sample during extraction and the tailoring of the extraction conditions for the analytes of interest.

![Figure 36. Schematic of IME](image-url)
Often the extract must be concentrated or exchanged into a suitable solvent or derivatized before analysis. Using IME, this process can be completed in 10-25 minutes without significant sample loss or transferring the sample to another piece of equipment. The collection/evaporation vials are fitted with a Weflon™ cover, which allow the samples to be evaporated inside the microwave under vacuum in the presence of air or an inert atmosphere (Figure 2). The evaporated solvent is then collected and recycled.

IME as described above will be used for the extraction process. The extraction process will be optimized for sample size, extraction temperature, and extraction solvent. Six replicate extractions will be performed on each sample to ensure good statistics. Extracts will be analyzed using RCRA SW-846 EPA Method 8270D "Semi Volatile Organic Compounds by gas chromatography/mass spectrometry"  

5.3.5.4.1. Definition of Matrix, Analytes, Extractants
The definition of matrix and analytes is the same as discussed in the Prescriptive Section. Extractants: The same solvent mixture as that used for the Prescriptive method was used here for purposes of valid comparison. Apart from these comparison extractions, other factors were evaluated. Either pure hexane (no mixture) was used for this purpose or the same 1:1 v/v mixture of hexane/acetonel was used.

5.3.5.4.2. Extraction
Microwave Instrument and Apparatus: Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos labstation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

Procedure
A precisely and appropriately weighed amount of the sample was placed in a prepared extraction vessel as per the description given in Section 4.3.1.1.1. 1.00g of Na₂SO₄ was
introduced along with the sample. Surrogate/ Internal Standards were introduced into the extraction vessel as per the procedure given by EPA Method 8270C (“Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)”). 10 ml of 1:1 mixture of hexane/acetone was introduced in the extraction chamber. 15 ml of the same solvent was placed in the extraction liner. The chamber was capped and inserted into the liner and the assembly was sealed by placing it into the rotor segment. One method blank sample was run with each extraction. The extraction protocol was as follows:

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (1:1Hex: Act) 5 minutes (Hexane)</td>
<td>RT to 110°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>110°C to 110°C (Hold)</td>
</tr>
</tbody>
</table>

Table 2. Extraction protocol for IME

Once the samples cooled down to room temperature, they were opened filtered into evaporation vials. Post filtration, EvapEX™ lid was inserted and the samples evaporated using the pulsing evaporation protocol given in the previous section (Extractant).

Post-evaporation, the extracts were weighed to determine the final weight of the extracts. Final volume of the extractions was calculated based on the density of the solvent, which was determined on the same day as the extraction. Internal Standard (EPA Method 8270 C) was introduced and the sample placed in an appropriate vial for GC/MS analysis.

5.3.5.4.3. Analysis (8270C)

The analytical method is as described under the Analysis Section for Prescriptive Method.

For the PBMS analytical approach, the Upper Bound (UB) of analyte concentration is defined by the following equation (when EPA 8270C is used for organics)\textsuperscript{17}:

\[
UB \leq \bar{X}_{PBMS} + 2(RPD_{PBMS})
\]

Equation 29

where \(X_{PBMS}\) = average analyte concentration from sample duplicate analysis using PBMS methods

\(RPD_{PBMS}\) = relative percent difference between sample duplicates using PBMS methods
5.3.5.4.4. **Variables Evaluated**

The variables evaluated for the purpose of the ACS/EPA study included the extraction recoveries using 1:1 v/v hexane/acetone for comparison with Prescriptive method using the same variables. Evaluation of extraction recoveries using pure hexanes was also carried out (though not a part of the study) and compared with Prescriptive method. Finally, QC data evaluation was done by CRM extractions using PBMS. Time/ Cost analysis was also done for PBMS.

5.3.5.4.5. **Additional Variables Evaluated:**

In addition to the variable mentioned in the previous subsection, some other variables were evaluated:

- Evaporation studies
  - H/A recoveries (varying volumes)
  - Hexane recoveries (varying volumes)
  - Comparison data (H/A vs. Hexane)
- Sample Size study
  - Extraction using different matrix sizes (1, 2, 5, 10g)
- Moisture Study
  - Effect of added moisture on recoveries
  - Effect of added moisture on recoveries using different solvents
  - Effect of moisture present naturally in sediments/soils/sludges

5.4. **Results and Discussion**

5.4.1. **Results: Prescriptive Approach**

5.4.1.1.1. **Extraction Recoveries**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MC 2968 Avg</th>
<th>95%CL</th>
<th>MC 6829 Avg</th>
<th>95%CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>2.25</td>
<td>1.2</td>
<td>Phenol</td>
<td>2.13</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>1.07</td>
<td>0.94</td>
<td>2-chlorophenol</td>
<td>0.87</td>
</tr>
<tr>
<td>Isophorone</td>
<td>2.56</td>
<td>2.12</td>
<td>Isophorone</td>
<td>2.63</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>1.62</td>
<td>0.39</td>
<td>2-chloronaphthalene</td>
<td>1.61</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.61</td>
<td>0.24</td>
<td>Anthracene</td>
<td>0.39</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>1.18</td>
<td>0.88</td>
<td>Benzo(b)fluoranthene</td>
<td>1.2</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>1.08</td>
<td>0.72</td>
<td>Benzo(k)fluoranthene</td>
<td>1.07</td>
</tr>
</tbody>
</table>
Benzo(a)pyrene 0.76 0.35 Benzo(a)pyrene 0.59 0.12
MC 2427 Avg. 95%CL MC 2724 Avg. 95%CL
Phenol 2.49 0.43 Phenol 2.4 0.38
2-chlorophenol 1.69 0.37 2-chlorophenol 1.54 0.41
Isophorone 2.45 1.93 Isophorone 2.29 1.8
2-chloronaphthalene 1.43 0.3 2-chloronaphthalene 1.4 0.4
Anthracene 0.83 0.19 Anthracene 0.53 0.33
Benzo(b)fluoranthene 0.98 0.78 Benzo(b)fluoranthene 0.91 0.69
Benzo(k)fluoranthene 0.88 0.65 Benzo(k)fluoranthene 0.83 0.53
Benzo(a)pyrene 0.96 0.37 Benzo(a)pyrene 0.76 0.56

All concentrations in µg/g. Error expressed as 95%CL, n=4

5.4.1.1.2. Prescriptive CRM Results (Method QC data)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Soxhlet</th>
<th>Certified</th>
<th>Soxhlet(SD)</th>
<th>Certified(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dib(a,h)anthracene</td>
<td>4.25</td>
<td>1.55</td>
<td>2.56</td>
<td>1.69</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.19</td>
<td>1.44</td>
<td>0.94</td>
<td>0.87</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.78</td>
<td>0.77</td>
<td>0.12</td>
<td>0.21</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>1.64</td>
<td>1.21</td>
<td>0.08</td>
<td>0.77</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.64</td>
<td>0.77</td>
<td>0.69</td>
<td>0.35</td>
</tr>
</tbody>
</table>

All concentrations in µg/g. Error expressed as Std. Dev, n=3

5.4.2. Results: PBMS Approach

5.4.2.1.1. Extraction Recoveries

<table>
<thead>
<tr>
<th>Compound</th>
<th>IME</th>
<th>95%CL</th>
<th>IME</th>
<th>95%CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>2.84</td>
<td>0.61</td>
<td>Phenol</td>
<td>2.3</td>
</tr>
<tr>
<td>2-Cl phenol</td>
<td>1.49</td>
<td>0.49</td>
<td>2-Cl phenol</td>
<td>0.99</td>
</tr>
<tr>
<td>Isophorone</td>
<td>3.9</td>
<td>3.84</td>
<td>Isophorone</td>
<td>3.25</td>
</tr>
<tr>
<td>2-Cl naphthalene</td>
<td>1.98</td>
<td>0.3</td>
<td>2-Cl naphthalene</td>
<td>2</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.74</td>
<td>1.41</td>
<td>Anthracene</td>
<td>0.78</td>
</tr>
<tr>
<td>B(b)fluoranthene</td>
<td>1.85</td>
<td>1.77</td>
<td>B(b)fluoranthene</td>
<td>1.57</td>
</tr>
<tr>
<td>B(k)fluoranthene</td>
<td>1.6</td>
<td>1.45</td>
<td>B(k)fluoranthene</td>
<td>1.35</td>
</tr>
<tr>
<td>B(a)pyrene</td>
<td>2.47</td>
<td>2.37</td>
<td>B(a)pyrene</td>
<td>1.51</td>
</tr>
</tbody>
</table>

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### 5.4.2.1.2. PBMS CRM Results (Method QC Data)

**Table 6. Method QC Data (PBMS)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IME</th>
<th>Certified</th>
<th>IME(SD)</th>
<th>Certified(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>1.68</td>
<td>1.55</td>
<td>1.06</td>
<td>1.69</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.53</td>
<td>1.44</td>
<td>0.41</td>
<td>0.87</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.51</td>
<td>0.77</td>
<td>0.17</td>
<td>0.21</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>1.15</td>
<td>1.21</td>
<td>0.27</td>
<td>0.77</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.67</td>
<td>0.77</td>
<td>0.28</td>
<td>0.35</td>
</tr>
</tbody>
</table>

All concentrations in µg/g. Error expressed as 95%CL, n=4
5.4.3. Results: Moisture Study: Laboratory Samples

All concentrations in \( \mu g/g \). Error expressed as Std. Dev, \( n=3 \)

Figure 3. Moisture study (H/A)  
Figure 4. Moisture study (hexane)

5.4.4. Discussion (and Comparison)

5.4.4.1 Prescriptive

The plots depicted in Figure 5 and 6 are representative sample plots randomly selected. From the plots, it is evident that precision for some compounds is good, but for others is very low. However, no trend is detectable. A case in point is 2-chlorophenol for MC 1049.
For example, for MC 2968, 2-chlorophenol error is ~88% of the total concentration, and for 1049 it exceeds 110%. On the other hand, it was as low as 22% for MC 2427. The samples have obviously been spiked with differing concentrations, but it is also necessary to note here that the matrix that was received by CMAC was not very homogeneous and the particle size was high. There were also pieces of wood chips present in the samples. Thus, sampling plays a very important role. The method performance seems to be satisfactory, as is evident from Figure 7 (Method QC Data), and hence the method variable can be excluded. All compounds meet the CRM values within confidence intervals, and the precision values mimic those of the CRM (with the exception of naphthalene).

5.4.4.2 Performance Based Method Study

The plots depicted in Figure 8 are representative sample plots randomly selected. As discussed in Prescriptive methods, from the plots it is evident that precision for some compounds is good, but for others is very low. However, no trend is detectable. A case in point is anthracene. For example, for MC 1049, anthracene error is ~73% of the total...
concentration. On the other hand, it was as low as 18% for MC 4910. Again, keeping in view that the samples have obviously been spiked with differing concentrations, but again the same factors need to be noted: sample was not very homogeneous and the particle size was high with the presence of organic (wood) material. Thus, the role of sampling cannot be emphasized enough. We tried to keep the sampling as random as possible and the samples were thoroughly mixed prior to withdrawal for extraction. Again, the method performance seems to be satisfactory, as is evident from Figure 9 (Method QC Data), and hence the method variable can be excluded. All compounds meet the CRM values within confidence intervals, and the precision values mimic those of the CRM (with the exception of naphthalene).

**5.4.4.3 Prescriptive vs. PBMS**

Figure 10 depicts the results in a comparative mode (as was required by the study). Again, these followed the randomly selected samples above. (MC 1049 and MC 4910). The red bars represent Integrated Microwave Extraction efficiencies. The blue bars represent Prescriptive Method. In both cases, it can be seen that for the absolute values of the extraction efficiencies, IME performs better than the prescriptive method. With confidence limits, IME gives comparable results. Precision-wise, both IME and prescriptive gave comparable results.
5.4.4 Extractant comparison

Data evaluation was done for both approaches using the guidelines given at the start of the study. The matrix spike was required to be between 25-50% of the PDL. To assure that PDL decision is reliable, the matrix spike recovery data was used to adjust the measured analyte levels for recovery efficiency, according to the following equation:

\[
\frac{\text{UB}}{\%\text{Recovery}} \leq \text{PDL}
\]

Equation 30

The RPD between the MS and MSD samples were calculated using absolute value of output of the following equation:

\[
\text{RPD} = \left(\frac{\text{MS} - \text{MSD}}{\text{MS} + \text{MSD}}\right) \times 100
\]

\[
\%\text{Recovery} = \left(\frac{\text{ MSSample}_{\text{conc}} - \text{Sample}_{\text{conc}}}{\text{Spike}_{\text{conc}}}\right) \times 100
\]

Equation 31 & Equation 32

The plots in the Figure are results obtained for MC 1049 and MC 4910 (representative, selected to keep “constant “with the selection in the above discussion). The red bars in the above Figure 11 and 12 represent...
recoveries obtained from 1:1 v/v hexane/acetone, while the blue bars represent hexane recoveries. The trend reconfirms results discussed in Chapter 4, wherein the solvents exhibited a preference for analytes that were chemically similar. Thus, hexane showed a preference for the non-polar PAHs, while phenols showed higher extraction values with the chemically similar environment of hexane/aceticone. All extractions were performed using Microwave Extraction.

5.4.4.5 Moisture study

The analytes chosen were: Naphthalene, acenaphthene, anthracene and fluoranthene ranging from molecular weight of 128 to 202. (Figure 14 in Section on Time Study; Chapter 4). Detailed version of this section can be found in Chapter 4. The solvent selected for evaluation had physical properties as given in Table 7 (Section on Sample Size Study, Chapter 4). The samples were each run in three replicates, thus the experimental design could be represented as:

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Moisture Content (%w/w)</th>
<th>Replicates</th>
<th>Blanks Solvent</th>
<th>Blanks Sediment</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>20</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>40</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hex/Act</td>
<td>Spiked Sediment</td>
<td>2</td>
<td>50</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Moisture content of the matrix is bound to have some effect on the final recoveries. Depending on the method of extraction chosen, the effect can be either detrimental or
advantageous. For microwave extraction, we obtained results which indicate a direct proportionality between the recoveries and the amount of moisture present. The extracts gave higher percent recoveries as summarized in the plot in Figure 3. (The results are given in \( \mu g/g \); error expressed as one Standard Deviation for \( n=3 \)). The study was done using closed vessel extraction using a 1:5 sample-solvent ratio with 1:1 hexane/acetone as the extractant. As is apparent from Figure 4, the trend is discernible. The compounds show a general trend of lower efficiencies for 0% moisture while the efficiency keeps increasing and is maximum for 50% moisture. For 0 to 20%, within confidence intervals, the recoveries are comparable. However, 30-50% show marked improvement in efficiency. Additionally, while hexane recoveries do not show this trend as markedly (possibly due to the mixed nature of the analytes as well as the fact that hexane and water are immiscible), it is evident even from the hexane results that the presence of moisture is beneficial. This could possibly be due to the fact that water absorbs microwave energy and can set up its own heating independent of the solvent by conduction and convection. The water present in the matrix can allow local heating which could favor the expansion of the pores and “liberate” the molecules in the solvent, possibly accelerating the extraction. It has however been reported in literature\(^{18}\) that if the amount of water in the matrix gets too significant, there could be problems of miscibility with the organic solvent used for extraction. The water acts as a barrier and hinders the transfer of analytes from the matrix to the solvent. This is especially evident from related moisture study done by other group members (David Lineman\(^{19}\)). Precision values on the other hand are generally best for the 20% extractions, but do not show a trend for the other extraction moisture points.

### 5.4.4.6 Sample Size Study

The analytes chosen were: Acenaphthylene, acenaphthene, anthracene, benzo(a)anthracene and benzo(a)pyrene ranging from molecular weight of 152 to 228. (Figure 11 in Chapter 4; this topic is more extensively discussed in Chapter 4). The solvents selected for evaluation had physical properties as given in Table 7 (Chapter 4).

Hexane and acetone are miscible with each other (Chapter 2), and at a 1:1 proportion form an azeotropic mixture that boils at 49°C (determined experimentally). Based on the
calculations described in the section on analyte chemistry, the density of the solvent mixture was found to be close to the theoretical density of 0.72 g/ml, and the polarity will also be the same as calculated above, 3.45 on the Snyder scale. The samples were each run in four replicates (with the exception of the 5.00 g point), thus the experimental design could be represented as:

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Matrix</th>
<th>Sample Size (g)</th>
<th>Replicates</th>
<th>Method Blank</th>
<th>Total MW Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hexane: Acetone</td>
<td>CRM 104-100</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

From the results obtained by running these extracts on the GC/MS, it was evident that the sample size does not play a predictable role in the extraction. It is however, essential to note that the linearity range falls in the 1-2g sample size; and as such, for all application in this and related studies, 2g sample size was used. The solvent platform was kept constant, i.e., all samples used 1:1 hexane/acetone as the extracting medium. Soxhlet (CRM reported Soxhlet values) used a 1:35 matrix: solvent ratio while for IME the different ratios evaluated were 1:1 (10 g) to 1:10 (1 g). As can be seen from the above table and a representative plot given in Figure 12, it is clear that the sample size does not play any significant role in the extraction. However, this holds true for homogeneous solids. Extensive sampling study will need to be done for non-homogeneous matrices.
The problem encountered for the 10-g sample of incomplete matrix-wetting could possibly have led to channel formation, and can explain the reason for the large values on the 95%CL error bars for 10 g as evident from the representative plot (Figure 13). This was especially true of the late eluting molecule, Benzo(a)pyrene. (This particular PAH however, had peak tailing problems on the chromatograph, and precision for this molecule was affected across the board).

Precision values for the other compounds were typically better than those of CRM. In most cases, there was an appreciable decrease in the error of the extraction efficiencies, (and hence an increase in the precision values. Figure 13 indicates the improvement in precision in percent terms over the numbers reported on the Certificate of Analysis supplied with the CRM. For example, for the 2-g sample the improvement in precision was 86% for anthracene as well as acenaphthylene (Figure 14). The 10-g samples suffer from precision as well as accuracy, but it can be assumed that these were not typical results. The numbers for 1-g tend to be lower than 2-g for the precision values. Keeping in mind that the linearity range was observed to be between 1 and 2 grams and also that the 2-g sample may be the most optimal solvent-solute ratio in interest of both precision and accuracy (1-g sample could show lower precision values), 2-g sample range was selected for all related applications.

5.4.5. Cost Analysis

a. Labor Costs
Method 3540C (Attended Labor Time)

Weigh samples, add reagents, and setup: 60 min.
Post-extraction processing: 30 min.
Evaporation Setup: 20 min.
Post-evaporation processing: 30 min.

Total Labor time for 4 samples: 2.33 hours
Total Labor time for 12 samples: 7 hours
Total Labor Cost for 12 samples $140.00
(7 hours at $20.00 per hour)

Method 3540C (Unattended Labor Time)

Extraction time for 4 samples: 23 hours
Evaporation time for 4 samples: 1 hour
Total time to process 4 samples: 26.33 hours

Total time to process 12 samples 316 hours

Integrated Microwave Extraction (Attended Labor Time)

Weigh samples, add reagents, and setup: 60 min.
Post-extraction processing: 20 min.
Evaporation Setup: 15 min.
Post-evaporation processing: 30 min.

Total Labor time for 12 samples 2.08 hours
Total Labor Cost for 12 samples $40.60

Integrated Microwave Extraction (Unattended Labor Time)

Extraction time for 12 samples 40 min
Evaporation time for 12 samples 20 min

Total time to process 12 samples 3.08 hours

b. Reagent Costs

High purity Hexane/Acetone 1:1 = $0.02 per ml
Method 3540C

450ml per sample = $9.00

**Total Cost for 12 Samples** = $108.00

**Integrated Microwave Extraction**

20ml per sample in outer vessel\(^1\) = $0.40
15ml per sample for extraction and rinse = $0.30
Total cost per sample = $0.70

**Total Cost for 12 samples** = $8.40

\(^1\) This outer vessel solution can be used for multiple extractions.

### 5.4.5.1.1. Equipment Costs

**Method 3540C**

- Hot Plate for 4 samples = $300.00
- Extraction apparatus for 4 samples = $1,022.00
- Evaporation apparatus for 4 samples = $660.00
- Water Bath for Evaporation = $700.00

**Total Cost for 4 Samples** = **$2,682.00**

**Integrated Microwave Extraction**

- Microwave system = $20,000
- Microwave extraction apparatus = ~$5,000.00

**Total cost for 12 samples** = **$25,000**

### Table 9 Cost Analysis Summary

<table>
<thead>
<tr>
<th></th>
<th>Method 3540C 12 Samples</th>
<th>Method 3540C 1000 Samples</th>
<th>Integrated Microwave Extraction 12 Samples</th>
<th>Integrated Microwave Extraction 1000 Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attended Labor Cost</td>
<td>$140</td>
<td>$11,666</td>
<td>$40.60</td>
<td>$3,383</td>
</tr>
<tr>
<td>Solvent Cost</td>
<td>$108</td>
<td>$9,000</td>
<td>$8.40</td>
<td>$400(^*)</td>
</tr>
<tr>
<td>Equipment Cost</td>
<td>$2,682</td>
<td>$2682</td>
<td>$25,000</td>
<td>$25,000</td>
</tr>
<tr>
<td>Total Cost</td>
<td>$2,930</td>
<td>$29,666</td>
<td>$25,049</td>
<td>$28,783</td>
</tr>
</tbody>
</table>
Table 10. Cost if Microwave is available

<table>
<thead>
<tr>
<th>Method 3540C</th>
<th>Integrated Microwave Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 Samples</td>
</tr>
<tr>
<td>Attended Labor Cost</td>
<td>$140</td>
</tr>
<tr>
<td>Solvent Cost</td>
<td>$108</td>
</tr>
<tr>
<td>Total Cost</td>
<td>$248</td>
</tr>
<tr>
<td>Total Time Required</td>
<td>316 hours</td>
</tr>
<tr>
<td>Total Solvent Consumed</td>
<td>5.4 L</td>
</tr>
</tbody>
</table>

†This number was calculated using the outer vessel solvent for 4 extractions then discarding.

Comments

An additional cost that has not been factored into the above projections is electricity. A hot plate will use ~400W of power for the full 23 hours required to evaporate 4 samples using Method 3540C. While the microwave will use ~400W of power for the 40 minutes required to extract and evaporate the samples.

Finally, cost involved with organic extractions is solvent disposal. This will increase the cost for doing 1000 samples as given below:

Solvent Disposal Costs²⁰:

Table 11. Solvent Disposal Costs

<table>
<thead>
<tr>
<th>Total Cost: $520.00</th>
<th>185.63 L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost/450L</td>
<td>$1260.61</td>
</tr>
<tr>
<td>Cost/16L</td>
<td>$44.82</td>
</tr>
<tr>
<td>Savings</td>
<td>96.44%</td>
</tr>
</tbody>
</table>

- Calculation based on consolidated solvent waste in 55-gallon drums
- Includes: Supplies (drum), Mobilization fee (trucks & supplies), Field supervisor, Field technician, Transportation & Disposal
- DU Hazardous Waste company: middle price range

*Task Force Interpretations are included in the Appendix.*
5.5. Data Evaluation

The statistical approach to determine whether laboratory results were above or below the PDL at 95% CL, considering the bias from the method is defined by the equation:

\[ X_{UB} = C_s + [12.7 \times C_s \times RSD] \]

Equation 33

where,

\( C_s \) = mean concentration in the replicate samples

\( RSD \) = relative standard deviation

12.7 = the Student-t value for a 95% confidence interval for two measurements.

This upper boundary of the mean was then adjusted for the bias in the method to generate the upper boundary of the corrected result, CUB, as follows:

\[ C_{UB} = \left( \frac{X_{UB}}{X_{MS}} \right) \times 100 \]

Equation 34

\( X_{MS} \) = mean recovery from the MS and MSD analyses;

If \( C_{UB} < PDL \), then the sample value was less than the established performance criteria at 95% CL. If this result were obtained in an actual monitoring program, it would have demonstrated that a facility was in compliance.

The approach takes into account neither the uncertainty of the standard deviation estimate nor the uncertainty in the mean recovery from the MS and MSD analyses. Further, because the MS and MSD analyses were performed on a background soil sample, and not the actual sample replicates, additional error was introduced. The propagated error of these factors could lead to upper confidence limits much greater than those calculated.

According to the Task Force, the analysis of laboratory data indicated that in cases where the PDL value was much greater than the reported laboratory result, the method performance had little effect on whether a correct decision was made. By contrast, in cases where the PDL was close to the measured concentration, both the PBMS and
prescriptive approaches gave results where the 95% CL was above the PDL. If sample concentrations are close to the action level then a more accurate method or additional sample analyses would be required to demonstrate that the concentration was, in fact, below the action level. In such cases, a higher number of replicates would be needed to complete the analysis. If a method with 50% RSD is used, only 9 replicate analyses would be required. If one could develop a method with 10% RSD, less than 4 sample replicate analyses would be required.

Taken together, these analyses show the interaction between data quality requirements and proximity to the action level. If the true concentrations in a sample are near the action level, either the laboratory must analyze a large number of samples using a method with poor precision or it must seek a method with sufficient precision to minimize the number of samples. If the samples have true concentrations at low levels compared to the action level, the precision is much less important and one might anticipate cost savings through the use of less sophisticated methods. A review of the data indicates that PBMS and prescriptive data generally gave comparable results. The one exception was the PBMS approach for semi-volatile organic compounds where analytes were not detected. The laboratory established that its quantification levels (QL) were equal to the PDL.

Most of the changes proposed by the laboratories streamlined sample preparation (and thereby increased laboratory productivity) rather than altered the instrumental technique itself. The reports from the laboratories suggested that novel analytical techniques could eventually find their place in the laboratories. However, laboratories would have to invest more resources in the form of capital equipment and time to validate a method. It is the judgment of the task force that, at least initially, PBMS approaches will be minor improvements or modifications of existing methods.

5.5.1. Advantages & Disadvantages of Approaches

The task force attempted a direct comparison of relative costs in terms of time to analyze samples by both approaches. However, this created obvious bias in the data against the prescriptive method. One reason was that the laboratories already had developed and
validated their prescriptive methods, so the time required for these tasks was not always reflected in their reports. In addition, since the laboratories have worked with the prescriptive methods for a number of years, it was relatively easy for them to identify potential areas where time and other resources might be saved using PBMS.

In general, reports from the laboratories confirmed the logical presumption that a laboratory is given the freedom to modify a method; it will do so in ways that are likely to help it gain economic advantage. The laboratories described advantages of their PBMS approaches as saving time, labor, and sometimes supplies such as solvents and other materials. The reduced usage of consumable materials had benefits in addition to cost savings. Reducing the amount of chlorinated solvents translated into an additional safety factor by lowering potential exposure of employees to these substances. Reduction of chlorinated solvents also reduced waste disposal costs and lessened the contamination of ambient air through losses via evaporation of large amounts of solvents.

Thus, in cases where no major equipment purchases are needed to employ PBMS, performance-based approaches may offer clear financial advantages to a laboratory and faster sample turnaround for their customers. The reports from the laboratories with respect to the advantages and disadvantages of the PBMS methods made it clear that the laboratories would prefer to continue to use the PBMS approaches they developed because they saved time and other resources. When a disadvantage to a PBMS approach was mentioned it was always related to a specific technical factor involved in the method. In general, the laboratories viewed PBMS approaches as having overall positive attributes.

5.5.2.  Steps to be taken to improve PBMS implementation
According to the task force, laboratories and their clients need to work together to determine analytical performance requirements to avoid costly reanalysis of samples. In the current study, the need for clarity was reflected in correspondence between the task force and the laboratories concerning PDL and how they were to be verified.
The third requirement is better communication from the laboratories. Many of the laboratory reports failed to provide required data. Some laboratories made modifications or chose to delete spikes or analytes without prior approval of the client. Few laboratories demonstrated the efficacy or performance of their PBMS approaches in meeting the project requirements. Also, PDL concentrations ranged over four orders of magnitude. Background concentrations in the samples exceeded the PDL for some analytes. It also appears that routine laboratories may not be equipped to use PBMS methods as they are geared to production and following methods and not to refining the analytical procedures to optimize accuracy. This seems to be a criticism of the system and not of the PBMS methods. In interacting and talking with the laboratory personnel they are not treated as analytical professionals who are depended on to make critical judgments as they perform their professional skills.

5.6. Conclusions

Although limited in size and scope, this study begins to answer some of the questions related to the technical feasibility and implementation of PBMS. Data quality is dependent on the types of analyte and matrix, as well as the analytical method. Although PBMS approaches could improve the quality of environmental monitoring data, better data may not always be needed. In cases where the concentrations of the analytes are substantially below the regulatory action levels, laboratories and their clients (regulated facilities) might elect to employ methods that yield less accurate and less precise data than would be obtained using the conventional EPA methods.

Such projects need more time than was given by the Task Force. Instructions given were lacking in direction. Samples received were not labeled accurately. There also was an apparent bias in the data evaluation made by the Task Force. Some of the labs did not report statistical data, and as such any comparison made could not be statistically valid not only inter-laboratory but also between the two approaches from the same laboratory. This defeats the very purpose of the study. (Some of this data is included in the Appendix). Since the samples were not homogeneous in nature, statistical validation gains even more consequence.
Data from this study demonstrate the utility of a PBMS approach where action levels are much greater than method capability, both in terms of the number of samples analyzed as well as the accuracy requirements of the method. Conversely, where a method’s accuracy and sensitivity characteristics are close to those needed to demonstrate compliance, or where the sample concentration is close to an action level, then the data show the need for additional sample analyses and/or better performing methods. This latter finding applies equally well to prescriptive methods or those developed under PBMS. PBMS offers some clear advantages to environmental monitoring laboratories. PBMS provides new opportunities to develop and use new technologies, reference materials, and methods. PBMS would also allow a laboratory to modify and improve a current prescriptive method when it is clear that the prescriptive method does not produce data of desired quality or when more modern techniques will save time and costs due to more efficient protocols.

Unfortunately, the task force indicates that the complexity of evaluating data against performance-based criteria makes it unlikely state and federal regulators will accept data collected using performance-based approaches until training is provided. Thus, for the PBMS approach to be successfully implemented EPA will have to support training efforts aimed at laboratory personnel, as well as data users such as state and federal regulators who must accept the data produced using these methods. These users currently work from simple checklists of prescriptive tasks and will need to be trained to make technical evaluations of whether the methods used under PBMS are logical and produce data that meet specified objectives.

However, the main point is that PBMS approaches hold promise due to the following factors:

- Time-saving
- Labor-saving
- Saving on supplies such as solvents
- Cost savings
- Reduction in the amount of chlorinated solvents used
- Increase in the safety factor by lowering potential exposure to hazardous substances. Reduction in waste disposal costs
- Lessening environmental contamination

The results of this investigation should suggest that with adequate training the PBMS approach, on a case-by-case basis, should produce analytical data more quickly and less expensively that is comparable in quality to that produced by current prescriptive methods.

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5.8. References:


(2) National Water Quality Monitoring Council; Methods and Data Comparability Board (MDCB), August 2001.

(3) Environmental Protection Agency (EPA); Fact Sheet on Performance Based Method System, 1997.


(5) Environmental Protection Agency (EPA); Performance Based Method System Notice of Intent 1997, pp 52098-52100.


(7) Environmental Protection Agency (EPA); Current Drinking Water Standards, 2000.

(8) Environmental Protection Agency (EPA); Preliminary Remediation Goals, 1999.

(9) Center for Microwave and Analytical Chemistry (CMAC); Response to Task Force, Duquesne University: Pittsburgh, PA, 1998.


American Chemical Society, Committee on Environmental Improvement;
Evaluation of Approaches to Improve the Quality and Cost-Effectiveness of
Environmental Monitoring: Draft Report; ACS Task Force on Performance-Based

Budzinski, H.; Letellier, M.; Garrigues, P.; Le Menach, K. J. Chromatogr. A

Lineman, D. N.; Shah Iyer, S.; Kingston, H. M. In The Pittsburgh Conference on


5.9. Appendix

Method Limits of Quantitation and Detection

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>Quantitation Limits (ng/µL)</th>
<th>MDL (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5¹</td>
<td>2.5</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
</tbody>
</table>
Dibenzo(a,h)acridine | 0.25 | 0.125 |
Dibenzo(a,h)anthracene | 0.25 | 0.125 |

1 EPA Method 8500B states “the lowest concentration calibration standard that is analyzed during an initial calibration establishes the method quantitation limit based on the final volume of extract”. Samples for both methods were concentrated to 5ml.

2 This number is based on 10 grams of extracted soil.

3 This was the lowest calibration standard that could be quantified.

### METHOD BLANKS (Concentrations in ng/µL)

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>Prescriptive EPA Method 3540C</th>
<th>PBMS IME</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Phenol</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Isophorone</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Anthracene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>1.4 ± 1.3</td>
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</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>&lt; 0.25</td>
<td>&lt; 0.25</td>
</tr>
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<td>quantitation limits mg/kg, dry weight</td>
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<tr>
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<tr>
<td>p-benzoquinone</td>
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<td>0.125</td>
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<tr>
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</tr>
<tr>
<td>2-chlorophenol</td>
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<td>0.125</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>isophorone</td>
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</tr>
<tr>
<td>hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
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</tr>
<tr>
<td>2,6-dinitrotoluene</td>
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<td>0.125</td>
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<tr>
<td>acenaphthalene</td>
<td>0.25</td>
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<tr>
<td>acenaphthene</td>
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<td>0.125</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
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<td>0.125</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
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<tr>
<td>diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>benz(a)anthracene</td>
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</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
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<td>MDL mg/kg, dry weight</td>
<td>Quantitation Limits mg/kg, dry weight</td>
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<tr>
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<tr>
<td>p-benzoquinone</td>
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<td>0.125</td>
</tr>
<tr>
<td>Phenol</td>
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<td>0.125</td>
</tr>
<tr>
<td>2-chlorophenol</td>
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<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
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</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Hydroquinone</td>
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<td>0.125</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
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<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
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</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
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<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
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Error expressed as 95% Confidence Interval (n=4)

**SAMPLE ID:** MC 2968  
**METHOD:** Prescriptive  
**EXTRACTION:** EPA Method 3540C  
**ANALYSIS:** EPA Method 8270D
### ANALYTIC DATA

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
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<tbody>
<tr>
<td>phthalate</td>
<td></td>
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</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.18 ± 0.88</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.08 ± 0.72</td>
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<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.76 ± 0.35</td>
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<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Error expressed as 95% Confidence Interval (n=4)</td>
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**SAMPLE ID:** MC 6829  
**METHOD:** Prescriptive  
**EXTRACTION:** EPA Method 3540C  
**ANALYSIS:** EPA Method 8270D

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<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>2.13 ± 0.59</td>
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<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>0.87 ± 0.54</td>
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<td>Benzylalcohol</td>
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<td>0.25 ± 0.24</td>
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<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>2.63 ± 2.14</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
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<td>0.66 ± 0.80</td>
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<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
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<td>1.61 ± 0.35</td>
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<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
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<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.67 ± 1.76</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.39 ± 0.12</td>
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<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
<td>0.25</td>
<td>0.125</td>
<td>1.27 ± 2.02</td>
</tr>
<tr>
<td>ANALYTE</td>
<td>MDL mg/kg, dry weight</td>
<td>Quantitation Limits mg/kg, dry weight</td>
<td>Sample Concentration mg/kg, dry weight</td>
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<tr>
<td>-----------------------------</td>
<td>-----------------------</td>
<td>--------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>2.49 ± 0.43</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>1.69 ± 0.37</td>
</tr>
<tr>
<td>Benzylic alcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>0.24 ± 0.26</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>2.45 ± 1.93</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
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<td>0.125</td>
<td>0.33 ± 0.31</td>
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<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
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<td>0.125</td>
<td>&lt;0.125 ±</td>
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<td>2-chloronaphthalene</td>
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<td>0.125</td>
<td>1.43 ± 0.30</td>
</tr>
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<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.83 ± 0.19</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
<td>0.25</td>
<td>0.125</td>
<td>4.99 ± 7.93</td>
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</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>phthalate</td>
<td></td>
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</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.98 ± 0.78</td>
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<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.88 ± 0.65</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.96 ± 0.37</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
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Error expressed as 95% Confidence Interval (n=4)

**SAMPLE ID:** MC 2724

**METHOD:** Prescriptive

**EXTRACTION:** EPA Method 3540C

**ANALYSIS:** EPA Method 8270D
<table>
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<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
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<tbody>
<tr>
<td>phthalate</td>
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<tr>
<td>Benzo(b)fluoranthene</td>
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<td>0.91 ± 0.69</td>
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<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.83 ± 0.53</td>
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<tr>
<td>Benzo(a)pyrene</td>
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<td>0.125</td>
<td>0.76 ± 0.56</td>
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<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
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<tr>
<td>Dibenzo(a,h)anthracene</td>
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<td>0.125</td>
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Error expressed as 95% Confidence Interval (n=4)

**SAMPLE ID:** MC 4910  
**METHOD:** Prescriptive  
**EXTRACTION:** EPA Method 3540C  
**ANALYSIS:** EPA Method 8270D

<table>
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<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
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</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>2.34 ± 0.29</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>1.63 ± 0.40</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>2.25 ± 1.44</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
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<tr>
<td>2-chloronaphthalene</td>
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<td>1.41 ± 0.24</td>
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<td>1,4-dinitrobenzene</td>
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<tr>
<td>2,6-dinitrotoluene</td>
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<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalamide</td>
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<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
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<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.18 ± 0.48</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.69 ± 0.76</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
<td>0.25</td>
<td>0.125</td>
<td>18.04 ± 40.02</td>
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### Table

<table>
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<tr>
<th>Analyte</th>
<th>MDL</th>
<th>Quantitation Limits</th>
<th>Sample Concentration</th>
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<tbody>
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<td>mg/kg, dry weight</td>
<td>mg/kg, dry weight</td>
<td>mg/kg, dry weight</td>
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<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>0.48 ± 0.40</td>
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<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
<td>0.25</td>
<td>0.125</td>
<td>0.61 ± 0.16</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)
<table>
<thead>
<tr>
<th>Analyte</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

**SAMPLE ID:** MC 5770  
**METHOD:** Prescriptive  
**EXTRACTION:** EPA Method 3540C  
**ANALYSIS:** EPA Method 8270D
<table>
<thead>
<tr>
<th>Phthalate</th>
<th>Percent Recovery</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25 0.125 &lt;0.125</td>
<td>±</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25 0.125 &lt;0.125</td>
<td>±</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25 0.125 &lt;0.125</td>
<td>±</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25 0.125 &lt;0.125</td>
<td>±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25 0.125 &lt;0.125</td>
<td>±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

**METHOD:** Prescriptive

**EXTRACTION:** EPA Method 3540C

**ANALYSIS:** EPA Method 8270D

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>Percent Recovery</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>116 ±</td>
<td>61</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>53 ±</td>
<td>133</td>
</tr>
<tr>
<td>Benzyalcohol</td>
<td>40 ±</td>
<td></td>
</tr>
<tr>
<td>Isophorone</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>2-chloroanaphthalene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>ND ±</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>108.67 ±</td>
<td>60</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>192.08 ±</td>
<td>53</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>ND ±</td>
<td>120</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>ND ±</td>
<td>30</td>
</tr>
<tr>
<td>ANALYTE</td>
<td>MDL mg/kg, dry weight</td>
<td>Quantitation Limits mg/kg, dry weight</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Error expressed as standard deviation (n=2)

ND = Sample was below the limit of quantitation

SAMPLE ID: MC 1049
METHOD: PBMS
EXTRACTION: Integrated Microwave Extraction
ANALYSIS: EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL</th>
<th>Quantitation Limits</th>
<th>Sample Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/kg, dry weight</td>
<td>Mg/kg, dry weight</td>
<td>mg/kg, dry weight</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>18.52 ± 24.55</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.08 ± 0.68</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.97 ± 0.44</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.33 ± 0.78</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

SAMPLE ID: MC 2968

METHOD: PBMS

EXTRACTION: Integrated Microwave Extraction

ANALYSIS: EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL (mg/kg, dry weight)</th>
<th>Quantitation Limits (mg/kg, dry weight)</th>
<th>Sample Concentration (mg/kg, dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>1.96 ± 1.54</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>20.65 ± 34.53</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.85 ± 1.77</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.60 ± 1.45</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>2.47 ± 2.37</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Error expressed as 95% Confidence Interval (n=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SAMPLE ID:** MC 6829  
**METHOD:** PBMS  
**EXTRACTION:** Integrated Microwave Extraction  
**ANALYSIS:** EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits Mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>15.58 ± 2.77</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.57 ± 0.27</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.35 ± 0.20</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.51 ± 0.21</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>2.63 ± 0.41</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>1.72 ± 0.64</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>2.67 ± 0.58</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>2.29 ± 0.08</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>0.18 ± 0.15</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.74 ± 0.14</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.20 ± 0.16</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.82 ± 0.20</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>2.26 ± 0.29</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

SAMPLE ID: MC 2427  
METHOD: PBMS  
EXTRACTION: Integrated Microwave Extraction  
ANALYSIS: EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits Mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>18.05 ± 2.89</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.38 ± 0.31</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.21 ± 0.23</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>2.18 ± 0.25</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

SAMPLE ID: MC 2724
METHOD: PBMS
EXTRACTION: Integrated Microwave Extraction
ANALYSIS: EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>16.5 ± 1.71</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.07 ± 0.24</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>0.97 ± 0.16</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.24 ± 0.32</td>
</tr>
<tr>
<td>Dibenz[a,h]acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Error expressed as 95% Confidence Interval (n=4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SAMPLE ID:** MC 4910  
**METHOD:** PBMS  
**EXTRACTION:** Integrated Microwave Extraction  
**ANALYSIS:** EPA Method 8270D
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits Mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>22.31 ± 4.44</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.32 ± 0.39</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>1.21 ± 0.34</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>2.22 ± 0.54</td>
</tr>
<tr>
<td>Dibenzo(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

SAMPLE ID: MC 7057
METHOD: PBMS
EXTRACTION: Integrated Microwave Extraction
ANALYSIS: EPA Method 8270D

p-benzoquinone 0.25 0.125 <0.125 ±
Phenol 0.25 0.125 <0.125 ±
2-chlorophenol 0.25 0.125 <0.125 ±
Benzylic alcohol 0.25 0.125 <0.125 ±
Isophorone 0.25 0.125 <0.125 ±
Naphthalene 0.25 0.125 <0.125 ±
Hydroquinone 0.25 0.125 <0.125 ±
1,2,4,5-tetrachlorobenzene 0.25 0.125 <0.125 ±
2-chloronaphthalene 0.25 0.125 <0.125 ±
1,4-dinitrobenzene 0.25 0.125 <0.125 ±
2,6-dinitrotoluene 0.25 0.125 <0.125 ±
Acenaphthalene 0.25 0.125 <0.125 ±
Acenaphthene 0.25 0.125 <0.125 ±
2,4-dinitrotoluene 0.25 0.125 <0.125 ±
2,3,4,6-tetrachlorophenol 5 2.5 <2.5 ±
Diphenylamine 0.25 0.125 <0.125 ±
Anthracene 0.25 0.125 <0.125 ±
Benz(a)anthracene 0.25 0.125 <0.125 ±
<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>MDL mg/kg, dry weight</th>
<th>Quantitation Limits Mg/kg, dry weight</th>
<th>Sample Concentration mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenz(a,h)acridine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Dibenz(a,h)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>p-benzoquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Isophorone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
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<td>2-chloronaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>5</td>
<td>2.5</td>
<td>&lt;2.5 ±</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.25</td>
<td>0.125</td>
<td>&lt;0.125 ±</td>
</tr>
</tbody>
</table>

Error expressed as 95% Confidence Interval (n=4)

SAMPLE ID:  MC 5770

METHOD:  PBMS

EXTRACTION:  Integrated Microwave Extraction

ANALYSIS:  EPA Method 8270D
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Percent Recovery</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>0 ±</td>
<td>0</td>
</tr>
<tr>
<td>Phenol</td>
<td>78 ±</td>
<td>3</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>93 ±</td>
<td>7</td>
</tr>
<tr>
<td>Benzylalcohol</td>
<td>82 ±</td>
<td>32</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>97 ±</td>
<td>15</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>78 ±</td>
<td>35</td>
</tr>
<tr>
<td>1,2,4,5-tetrachlorobenzene</td>
<td>106 ±</td>
<td>27</td>
</tr>
<tr>
<td>2-chloronaphthalene</td>
<td>104 ±</td>
<td>22</td>
</tr>
<tr>
<td>1,4-dinitrobenzene</td>
<td>111 ±</td>
<td>30</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>70 ±</td>
<td>4</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>85 ±</td>
<td>4</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>85 ±</td>
<td>4</td>
</tr>
<tr>
<td>2,4-dinitrotoluene</td>
<td>115 ±</td>
<td>6</td>
</tr>
<tr>
<td>2,3,4,6-tetrachlorophenol</td>
<td>ND ±</td>
<td>ND</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td>74 ±</td>
<td>20</td>
</tr>
<tr>
<td>Anthracene</td>
<td>94 ±</td>
<td>25</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>97 ±</td>
<td>30</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>47 ±</td>
<td>54</td>
</tr>
<tr>
<td>Benzo(b)fluorantheine</td>
<td>86 ±</td>
<td>30</td>
</tr>
<tr>
<td>ANALYTE</td>
<td>Concentration sample Mg/kg, dry weight</td>
<td>Certified Values Mg/kg</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>0.67 ± 0.28</td>
<td>0.77 ± 0.35</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>1.15 ± 0.27</td>
<td>1.21 ± 0.77</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.51 ± 0.17</td>
<td>0.77 ± 0.21</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.53 ± 0.41</td>
<td>1.44 ± 0.87</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>5.61 ± 1.37</td>
<td>7.98 ± 2.56</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl) phthalate</td>
<td>1.51 ± 1.64</td>
<td>(1.64) ±</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>5.66 ± 1.05</td>
<td>(9.69) ±</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>7.36 ± 1.90</td>
<td>(5.1) ±</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>3.64 ± 1.06</td>
<td>5.09 ± 1.69</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>1.68 ± 0.72</td>
<td>(1.55) ±</td>
</tr>
</tbody>
</table>

Error is expressed as 95% Confidence Interval

5.9.1. Appendix B: QC Data

Method QC Data

SAMPLE ID: Certified Reference Material
METHOD: PBMS
EXTRACTION: Integrated Microwave Extraction
ANALYSIS: EPA Method 8270D

Error expressed as standard deviation (n=2)

ND = Sample was below the limit of quantitation
Method QC Data

SAMPLE ID: Certified Reference Material
METHOD: Prescriptive
EXTRACTION: EPA Method 3540C
ANALYSIS: EPA Method 8270D

<table>
<thead>
<tr>
<th>ANALYTE</th>
<th>Concentration sample Mg/kg, dry weight</th>
<th>Certified Value Mg/kg, dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.64 ± 0.69</td>
<td>0.77 ± 0.35</td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>1.64 ± 0.08</td>
<td>1.21 ± 0.77</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.78 ± 0.12</td>
<td>0.77 ± 0.21</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.19 ± 0.94</td>
<td>1.44 ± 0.87</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>8.98 ± 4.85</td>
<td>7.98 ± 2.56</td>
</tr>
<tr>
<td>Bis(2-ethylhexyl)phthalate</td>
<td>2.04 ± 3.36</td>
<td>(1.64)* ±</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>2.93 ± 9.23</td>
<td>(9.69)* ±</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>10.15 ± 8.46</td>
<td>(5.1)* ±</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>6.36 ± 2.56</td>
<td>5.09 ± 1.69</td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>4.25 ± 3.18</td>
<td>(1.55)* ±</td>
</tr>
</tbody>
</table>

Error is expressed as 95% Confidence Interval

* = Information Value Only
5.9.2. Comparison Results (All concentrations in µg/g; Error expressed as 95%CL, n=4; Lab 1=CMAC)

The context of these results can be found under Conclusions
### Hexane Results (IME)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MC 1049</th>
<th>+/-</th>
<th>Compound</th>
<th>2968</th>
<th>+/-</th>
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</thead>
<tbody>
<tr>
<td>p-benzoquinone</td>
<td>1.90</td>
<td></td>
<td>p-benzoquinone</td>
<td>1.85</td>
<td>0.09</td>
</tr>
<tr>
<td>Phenol</td>
<td>1.01</td>
<td>0.11</td>
<td>Phenol</td>
<td>1.44</td>
<td>0.52</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>1.62</td>
<td>0.67</td>
<td>2-chlorophenol</td>
<td>1.92</td>
<td>0.39</td>
</tr>
<tr>
<td>Isophorone</td>
<td>2.22</td>
<td>0.56</td>
<td>Isophorone</td>
<td>2.33</td>
<td>0.78</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2.89</td>
<td>0.79</td>
<td>Naphthalene</td>
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</tr>
<tr>
<td>2-chloronaphthalene</td>
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<td>0.75</td>
<td>2-chloronaphthalene</td>
<td>3.23</td>
<td>0.38</td>
</tr>
<tr>
<td>2,6-dinitrotoluene</td>
<td>5.93</td>
<td>1.87</td>
<td>2,6-dinitrotoluene</td>
<td>9.26</td>
<td>5.66</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.55</td>
<td>0.40</td>
<td>Anthracene</td>
<td>2.20</td>
<td>0.35</td>
</tr>
<tr>
<td>Bis(2-ethylhexylphthalate)</td>
<td>19.14</td>
<td>0.28</td>
<td>Bis(2-ethylhexylphthalate)</td>
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</tr>
<tr>
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<td>Benzo(b)fluoranthene</td>
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<tr>
<td>Benzo(a)pyrene</td>
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<td>0.58</td>
<td>Benzo(a)pyrene</td>
<td>3.23</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>MC 6829</th>
<th>+/-</th>
<th>Compound</th>
<th>MC 2427</th>
<th>+/-</th>
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<tbody>
<tr>
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<td>0.15</td>
<td>p-benzoquinone</td>
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</tr>
<tr>
<td>Phenol</td>
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<td>Phenol</td>
<td>0.88</td>
<td>0.79</td>
</tr>
<tr>
<td>2-chlorophenol</td>
<td>1.69</td>
<td>0.27</td>
<td>2-chlorophenol</td>
<td>1.56</td>
<td>0.63</td>
</tr>
<tr>
<td>Isophorone</td>
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<td>0.53</td>
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<td>1.08</td>
</tr>
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<td>Naphthalene</td>
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<td>Naphthalene</td>
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<td>0.79</td>
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<td>0.69</td>
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<td>0.89</td>
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<td>Anthracene</td>
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<td>0.27</td>
<td>Anthracene</td>
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<td>0.39</td>
</tr>
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<td>1.53</td>
<td>Bis(2-ethylhexylphthalate)</td>
<td>10.89</td>
<td>6.62</td>
</tr>
<tr>
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<td>Benzo(k)fluoranthene</td>
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<td>0.39</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>2.85</td>
<td>0.23</td>
<td>Benzo(a)pyrene</td>
<td>3.25</td>
<td>0.57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
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<th>MC 2724</th>
<th>+/-</th>
<th>Compound</th>
<th>MC 4910</th>
<th>+/-</th>
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</tr>
<tr>
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<td>Naphthalene</td>
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<tr>
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<tr>
<td>Anthracene</td>
<td>1.56</td>
<td>0.49</td>
<td>Anthracene</td>
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<td>0.39</td>
</tr>
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<td>Bis(2-ethylhexylphthalate)</td>
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<td>0.42</td>
<td>Benzo(b)fluoranthene</td>
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<td>Benzo(k)fluoranthene</td>
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<td>Benzo(k)fluoranthene</td>
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<td>0.18</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>2.35</td>
<td>0.73</td>
<td>Benzo(a)pyrene</td>
<td>3.04</td>
<td>0.64</td>
</tr>
</tbody>
</table>
5.9.3. **Origin of Study**

The Committee on Environmental Improvement established a task force to evaluate how the shift to performance based approaches would affect the quality and costs of environmental monitoring. This task force was established following the awareness of EPA's decision to move to PBMS in 1996. The task force comprised of members from regulated industry, environmental reference material manufacturers, environmental consultants and a liaison from EPA. After initial evaluation the task force concluded that a real world evaluation of performance based approaches was needed to determine whether problems might be encountered during widespread implementation of PBMS and to identify possible solution. In 1997, EPA and ACS agreed to enter into a cooperative agreement to start exploring the ramifications of moving to a performance-based approach for environmental monitoring\(^1\).

5.9.4. **Sample Preparation by Commercial Standard Manufacturer**

ERA, a manufacturer of environmental reference materials, prepared aqueous and soil samples for analysis by participating laboratories. The starting materials used in the manufacturing procedures were verified and certified for homogeneity, accuracy and stability, and were analytically traced to NIST Standard Reference Materials (SRMs) where available. If NIST SRMs were not available, ERA analytically verified the starting materials against an independent reference material. After manufacturing, the standards were individually packaged following ERA's documented procedures.

Background levels of analytes in the sample matrices were assessed using EPA methods and other methods like neutron-activation analysis. Prescribed methods were employed if they had documented Method Detection Limit (MDL) the Project Decision Level (PDL) established for each contaminant. Background concentrations in sample matrices were taken into account when choosing the concentration of contaminants to add to the samples. Quanterra (Denver, CO) analyzed the organic compounds in the soil.
The ERA-certified values in the samples sent to the laboratories represent the 100% "made to" concentrations for each analyte, as determined by gravimetric and volumetric measurements made during the manufacturing process plus background concentrations of each analyte. Certified values were corrected for the purity of the starting materials. The certified value was established as the central value within the manufacturing uncertainty (4% for each analyte, estimated from historic performance) Manufacturing uncertainties were calculated per the procedures described in NIST (1994).

5.9.5. Task Force Interpretations
One of the primary objectives of this study was to assess the degree to which the laboratories’ data could be used to determine satisfactorily if each of the samples was above or below the applicable PDL described in the section. This objective was consistent with EPA’s intent for PBMS to “specify performance criteria that are not linked to methods, techniques, or instruments.” Laboratories were asked to determine whether sample concentrations were above or below the PDL. The task force instructed laboratories that a sample concentration was below the PDL if the upper boundary analyte concentration was less than or equal to the PDL.

A key component of the assessment is analytical precision, determined from the mean and relative standard deviation (RSD) of duplicate sample analysis. Since samples were submitted as blind samples, the laboratories did not know which samples were duplicates and consequently could not perform the requested calculation unless replicate analyses of a sample were performed. The instructions for performing the MS and MSD analyses were difficult to implement given the range of analyte concentrations in the samples. The PDL values ranged by orders of magnitude, making it difficult for the laboratories to determine appropriate spike concentrations.

The task force clearly articulated its intent for the laboratories to estimate the imprecision and then correct for the bias, but the example calculations provided were incorrect. Although calculations that incorporate method bias and take upper boundary
concentrations into account may be important elements of testing whether a PBMS approach meets measurement or data quality objectives established for a permit, clean-up project, or regulation, most laboratories have not had to perform them. In cases where the PDL was much greater than the laboratory reported value, poor method performance was tolerable. When the PDL was close to the laboratory-reported value, imprecision in the analyses almost always led to a conclusion that the UB concentration was above the PDL. A more accurate and precise method would be needed to demonstrate compliance in these cases.
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Chapter 6

6. Clinical Application of Microwave Extraction

6.1. Abstract
Timely analysis of drugs of abuse is of vital importance today. Forensic analysis typically requires very fast turn-around times. Minimizing extraction times is invaluable to analysts and, consequently, to law enforcement. Opiates like morphine are common targets of analysis for forensic analysts. Some of the techniques used to extract morphine from matrices in practice today take anywhere from one to two days for the completion of analysis. This study was undertaken to evaluate the feasibility of using microwave sample preparation techniques to facilitate and/or enhance the extraction of morphine from biological fluid matrices. The liquid/liquid extraction (LLE) scheme from the Pittsburgh Criminalistics Labs was used as a platform for comparison of Microwave Assisted Extraction results in order to validate the microwave results. Following LLE, Solid Phase Extraction (SPE) is also presented as an additional extraction platform for comparison, as SPE is a widely used technique, and as such, it is a natural comparison platform for validation. Analytical techniques employed are GC/MS, HPLC and LC/MS.

6.2. Introduction

6.2.1. Part 1: Sample preparation for Biomedical Analysis
The analytical process is the means by which chemical information is obtained from a sample. Sample preparation is required before analysis to improve the specificity of the assay by removing the majority of the matrix whilst concentrating the analyte. This removal of extraneous matrix and subsequent specificity of a sample preparation technique is very significant for biomedical analysis because of the degree of complexity of matrices of biological origin. Over the past decades, technological advances have allowed analytical techniques to accurately measure lower quantities of analyte; computer control of instruments has enabled the data produced to be managed efficiently. Until recently, these advances were not matched by improved sample preparation procedures; this means that sample clean-up remains the rate-limiting step for a laboratory.
The isolation and measurement of organic compounds present in a biological matrix, especially at low concentrations, presents a significant analytical challenge. Therefore, a sample preparation scheme should have several objectives, including:

1. Removal of unwanted protein or other material that would interfere with analyte determination.
2. Removal of material if the resolving power of the chromatographic column is insufficient to separate all of the components in the sample with appropriate resolution or in a reasonably practical time.
3. Removal of material that would affect chromatographic resolution or reproducibility.
4. Solubilization of compounds to enable injection under the initial chromatographic conditions.
5. Concentration of the analyte to surpass the detection limits of the analytical instrument.
6. Dilution to reduce solvent strength or to avoid solvent incompatibility.
7. Removal of material that could block the chromatograph tubing, valve, column or frits.
8. Stabilization of the analyte to avoid hydrolytic or enzymatic degradation.

Some of the factors to consider during sample preparation are the concentration of the analyte, the matrix involved, and the assay specificity required. A balance should be struck between the specificity that is obtained by the sample preparation scheme with that requires for the instrumental assay: insufficient sample clean-up may result in interference with the analysis or, on the other hand, too great a sample preparation effort may result in the chromatograph being under-utilized, or loss, conversion, or degradation of the analyte.

6.2.2. Unit operations of sample preparation

Operations that can be utilized for sample preparation can be classified into four groups:

1. Stabilization and release of analyte from the matrix.
2. Removal of endogenous compounds
3. Addition, mixing, separation or removal of liquids.
4. Enhancement of assay selectivity or sensitivity.

Table 31. Unit operations of sample preparation for biomedical analyses

<table>
<thead>
<tr>
<th>Group 1 Release</th>
<th>Group 2 Removal</th>
<th>Group 3 Procedures</th>
<th>Group 4 Enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis, sonication</td>
<td>LLE, SPE, HPLC, precipitation</td>
<td>Aspiration, centrifugation, dilution, evaporation</td>
<td>Pre and post column derivatization (esp. for HPLC and GC)</td>
</tr>
</tbody>
</table>

6.2.2.1 Release of analyte from the matrix

The unit operations in this group are either to cleave a molecule into a more convenient form, to release an analyte by breakdown of the biological matrix, or to stabilize the analyte to avoid artifact formation by undesirable reactions or enzymatic degradation.

Molecular cleavage is used to cleave the conjugate and release the original compound for assay. This can be achieved by enzymatic hydrolysis or chemical (acid/base) hydrolysis.

Breakdown of biological matrix is performed where the analyte is bound to a component of the matrix. Enzymes like proteases can be used to break down the components of the matrix and release bound compounds.

6.2.2.2 Removal of endogenous material

A biological matrix may be solid or particulate, e.g. muscle, tissue, milk, feces, or blood, or a mixed composition of organic compounds in an aqueous solution, e.g. urine or plasma. Unit operations in this group are considered to be sample preparation techniques and are responsible for removing the majority of the biological material from the sample matrix prior to analysis. Techniques include a variety of physico-chemical procedures such as adsorption or partition mechanisms that aim to selectively isolate the analyte in preference to components of the sample matrix, e.g. LLE, SPE, or HPLC. Other techniques use ultrafiltration or precipitation to remove proteins and other macromolecules.
6.2.2.3 Liquid handling procedures
Methods in this group are mainly involved with the addition, mixing, removal or transfer of liquids and provide the links between the techniques in other groups. Liquid handling procedures can often be the rate-limiting steps in a sample preparation scheme as too many of them will result in a tedious and labor-intensive assay.

6.2.2.4 Enhancement of selectivity and sensitivity
Operations in this group are mainly concerned with derivatization of an analyte to enhance the assay sensitivity and specificity such as pre-column derivatization reactions and post-column derivatization and reaction detectors.

6.2.3. Techniques for Sample Preparation in Biomedical Analysis
The main methods for the removal of endogenous material include dilution, precipitation, ultrafiltration, LLE, SPE, and HPLC. These techniques require that the sample be a liquid. Homogenization methods are used for converting solids or semi-solids into liquids.

6.2.3.1 Dilution
When an analyte is present in a sufficiently high concentration, dilution is a very simple and effective means of sample preparation. A diluting fluid, such as water or a buffer, is added to the sample, which is mixed or centrifuged and then assayed. The diluting fluid can also disrupt weak bonding between the analyte and plasma proteins.

6.2.3.2 Precipitation and Deproteinization methods
a. Protein precipitation: done using acids, organic solvents, or combinations thereof. These techniques are effective particularly on plasma or blood samples prior to analysis to prevent technical problems like precipitation during the analytical procedure.

b. Precipitation of urine pigments and bile salts: prevents high backgrounds thus helping quantification of analytes
c. **Ultrafiltration**: uses filters and centrifugation to exclude molecules with mass exceeding a particular value, e.g. 25,000 or 50,000 mass units.

d. **Dialysis**: separates an analyte from the matrix by diffusion through a semi-permeable membrane.

### 6.2.3.3 Liquid-liquid extraction

This method entails the extraction of the biological material with a water-immiscible solvent. The isolation of the analyte is achieved by partitioning it between the organic phase and an aqueous medium. The distributing ratio, which follows the Nernst Distribution Law, will be influenced by the choice of the extracting solvent, pH value of the aqueous phase, and the ratio of the volumes of the organic to aqueous phases.

### 6.2.3.4 Solid Phase Extraction

SPE consists of mixing the biological fluid with an absorbent, separating the solid phase, and eluting the analyte with an appropriate solvent. The success of this approach depends on the relative affinities of the analyte for the biological matrix and for the adsorbent, and on the relative ease of eluting the compound for subsequent analysis. SPE can have a higher throughput than a comparable LLE because of the ease of handling the solid phase (as pre-packed cartridges).

### 6.2.3.5 High-performance Liquid Chromatography

Liquid-chromatography can be used to perform separation and clean-up, so its use can either enhance any preparation scheme already undertaken or perform both the extraction and quantification stages.

### 6.2.3.6 Miscellaneous techniques

a. **Lyophilization or freeze-drying** is the removal of water and other volatile compounds by vacuum sublimation. Once the water is removed, the residue is easier to manipulate. The technique can also be applied to semi-liquid matrices (e.g. plasma) and tissue homogenates.
b. **Saponification** is the hydrolysis of an ester with either sodium hydroxide or potassium hydroxide. Fats (e.g. lipids in biological samples) form water-soluble soaps that can be easily removed.

### 6.2.4. Part II: Opiates, Opioids and Other Synthetic Narcotic Analgesics

Opiates are alkaloids derived from opium, which is the partly dried latex from incised unripe capsules of *Papaver somniferum*. Some of the naturally occurring opiate alkaloids are morphine, codeine, thebaine and papaverine. Morphine is the most important constituent of opium and the therapeutic efficiency of opium products is dependent on their morphine content[^5]. According to the Drug Abuse Warning Network (DAWN), opiate abuse-related deaths are steadily on the rise. Heroin toxicity now accounts for nearly half of all drug-related deaths[^4]. Their main activity is analgesia, whereby they abolish pain without loss of consciousness. Their site of action is within the central nervous system (CNS), which distinguishes them for other painkillers like aspirin, which have a peripheral site of action. Formerly the terms opiates and opioids were used interchangeably; however, a distinction is now made as applied to central analgesics. The term *opiates* refers to agents derived from opium or one of its constituents, while opioid is a more general term for any directly acting agent the effects of which are stereospecifically antagonized by naloxone. Agents that block the actions of opioid analgesics are called opioid agonists, in spite of the fact that they inhibit rather than promote a pharmacological response[^5].

Forensic Chemistry classifies opiates on the basis of their source as either naturally occurring (morphine and codeine); semi-synthetic, morphine based (heroin or hydromorphone); semi-synthetic thebane based (oxymorphone or oxycodone) or purely synthetic (meperidine). Toxicology classification is based on them being either opiates or opioids. Opiates are peptides derived from the morphine molecule that bind to the opioid receptors, while opioids describe non-peptide agents binding at the same sites[^4].
6.2.5. Mechanism of Action\textsuperscript{4}

The body produces endogenous pain-relieving substances that have molecular structures similar to that of morphine. These substances, called endorphins or enkephalins, along with opiates such as morphine bind to the opioid receptors located in the brain and the rest of the body. Depending on which receptor is activated, the result may vary. There are five basic classes of opioid receptors: mu, kappa, delta, sigma and epsilon. Of concern for the scope of this study is the mu receptor, named so because morphine binds to it. The effects associated with mu receptor activation are analgesia, euphoria, moderate sedation, and respiratory depression. Morphine does bind to the other receptors. Activation of kappa receptor causes the same effects as mu receptors, but less marked depression. Delta receptors produce spinal analgesia. Sigma receptors do not relieve pain; instead they produce undesirable effects like dysphoria and hallucinations. However, the direct toxicity of morphine is related to mu receptor activation.

6.2.6. Pharmacological Action\textsuperscript{3, 6}:

Effects on CNS are biphasic because of a complex combination of depression and stimulation with the former predominating. In the human subject, psychological studies indicate personality changes in the direction of introversion as manifested by increased fantasy living. Small therapeutic doses adversely affect mental performance with regard to speed. With repeated administration, tolerance develops to the depressant but not to the stimulant effects. Depression of the cerebral cortex, brain stem, and hypothalamus produce sedation, drowsiness and diminution of pain perception. The respiratory center is depressed, with the raising of its threshold to CO\textsubscript{2}, producing at first, slow, deep respiration and later, slow, shallow and quite inadequate respiration. There is also simultaneous stimulation of the vomiting center and nucleus of the third cranial nerve, which produces the characteristic constriction of pupils. Its effect of smooth muscle on gastro-intestinal tract (GIT) decreases peristalsis and produces severe constipation. Postural hypotension and subnormal body temperature due to peripheral vasodilation are observed\textsuperscript{3, 6}.
6.2.7. **Clinical Manifestations**\(^4,6\)

Profound coma with marked respiratory depression is common. Also noticeable is intense constriction of pupils. Dry mouth and diminished urinary output results in delayed excretion of the opium alkaloids with prolongation of their effect. Cheyne-Stokes breathing\(^\Delta\) is observed which may even lead to cyanosis and death due to asphyxia. Subnormal body temperature due to decreased metabolism and peripheral vasodilation is a common feature\(^5\).

6.2.8. **Individual Narcotic Agents**

6.2.8.1 **Morphine**\(^4,6\)

Morphine was isolated from opium by Setürner in 1805. It was first characterized in 1927 and the total synthesis was only accomplished in 1952. The principle site of metabolism is the liver. Morphine’s elimination is a biphasic process. During an initial phase, lasting only a few minutes, morphine is rapidly distributed throughout the tissues with the highest blood flow. During a second phase, morphine is quickly converted to its principal metabolite, morphine-3-glucuronide (M3G), and somewhat more slowly to smaller amounts of morphine-6-glucuronide (M6G) (from one to eight hours). Conversion of morphine to the M3G form is rapid. Within six minutes after intravenous (IV) administration, there is more metabolite than morphine circulating in the blood stream. M6G is pharmacologically active, and possibly more active than morphine itself. Opiate receptor studies have shown that the 3-position in the morphine moiety must remain accessible for a molecule to have opiate activity. Since the Carbon-3 position is open in the M6G molecule, its analgesic effects are only to be expected.

6.2.8.2 **Heroin**\(^3,4,7\)

Heroin is a synthetic morphine derivative. It was first marketed by Bayer in 1898. It is produced by acetylating morphine’s two hydroxyl groups. Once in the body, heroin is

\(\Delta\) *Cheyne-Stokes respiration is an abnormality of the pattern of breathing. It qualifies as a form of sleep apnea. The condition was named after John Cheyne & William Stokes, the physicians who first classified it.*
very rapidly converted by deacetylation to 6-acetylmorphine and then to morphine. Conversion to 6-acetylmorphine is completed within 10-15 minutes. The complete conversion of heroin to morphine typically requires a few hours. The metabolism is depicted in Figure 3.

6.2.8.3 Codeine\textsuperscript{3, 4, 7} Codeine is one of the naturally occurring alkaloids found in opium. Codeine has pain-relieving properties that are about one-fifth of morphine’s. These pain-relieving properties arise from the fact that codeine is converted to morphine. Most of the codeine that is consumed in antitussive and analgesic mixtures is of semi-synthetic origin, obtained by the methylation of morphine. Codeine metabolism is given in Figure 4.

6.2.9. Absorption, Distribution, Metabolism and Excretion\textsuperscript{6, 8, 9}

6.2.9.1 Absorption
Almost all of the opiates are well-absorbed, no matter the route of administration. Absorption takes place from nearly all mucous membranes. Absorbed actively from the GIT, morphine is therapeutically active for 6-8 hours. Some of the routes of absorption include intravenous, subcutaneous, oral, and rectal. Following IV administration, peak levels are achieved in minutes; however, levels also decline rapidly, and reach the lowest in 30 minutes\textsuperscript{4}. It is readily absorbed following oral administration, whereas absorption from stomach depends on the pH of the contents. Bioavailability is significantly reduced because of the hepatic first-pass metabolism.

6.2.9.2 Distribution
Distribution appears to be uniform in most tissues. Differential distribution occurs in kidney and liver. Morphine readily traverses the placental barrier. It was interesting to note that in spite of the powerful action that this drug exerts on the CNS it does not concentrate here to any large extent. Even the cerebrospinal fluid and fat contained very little of the drug in either form. Peak levels in the CNS however, correlated with the pharmacological activity as measured by the pain-reaction time method. The extremely small amount of morphine in CNS indicates that this tissue must be exceptionally
sensitive to this drug. Both free and bound morphine can be detected in plasma.
(Conjugation is discussed in ‘Chemistry of Morphine’). Following IV administration of
30 mg/kg body weight of free base, plasma level of free morphine reached about 10 µg/
ml. Bound morphine in the same animal was thrice the free morphine.

**6.2.9.3 Metabolism**

![Morphine metabolism diagram]

*Morphine is readily depleted from blood, and conjugation is rapid, as is evident from the
fact that within fifteen minutes of oral administration, free as well as bound morphine is
excreted in urine. Morphine gets metabolized to normorphine to a very small extent;
however, morphine gets converted (almost all) to 3 and 6 glucuronides (Figure 1). M3G=
Morphine-3-glucuronide and M6G= Morphine-6-glucuronide

**6.2.9.4 Excretion**

Kidney is the principal route of excretion of morphine and elimination commences
promptly after administration. Most rapid excretion occurs during the first two hours.
90% of morphine is eliminated in excreta (6-7% is fecal excretion, rest is urinary). A small amount of morphine is converted to normorphine. Urinary excretion products: Morphine-6-glucuronide: <1%, Morphine-3-glucuronide: 54-74%, Free morphine: 7.5-12.5%, Free normorphine: 0.5-1.5%. A large portion of the dose undergoes conjugation in the liver. Whole blood or serum with hemoglobin or H₂O₂ precipitated pseudomorphine from a solution of morphine, whereas plain serum did not. Morphine does not seem to be excreted in human milk.

Route of administration seems to have an influence not only on the speed with which the peak levels are attained, but also on the levels attained in the plasma. Following IV administration of 30 mg/kg body weight of free base, plasma level of free morphine reached about 10 µg/ml. Bound morphine in the same animal was thrice the free morphine. Concentration declined slowly and free morphine was not detectable 5 hours after injection. With subcutaneous injection, lower plasma levels were obtained and following oral administration, morphine was barely detectable. % Excretion was also higher during subcutaneous excretion than oral administration.

6.2.10. Chemistry of Morphine and Related Compounds

Opium alkaloids are classified as natural compounds, semi-synthetic compounds and synthetic compounds. Chemical structure for selected compounds is given in Figure 2.

1. Natural Compounds include Morphine, Codeine, Thebaine, Papaverine and Narcotine, etc.
2. Semi-synthetic Compounds include Heroin, Dionine, Dilaudid, Apomorphine, Nalorphine, etc.
3. Synthetic Compounds include Meperidine, Methadone, Levorphan, etc.
4. Two types of basic structures are recognized among the opium alkaloids, i.e., the *phenanthrene* (morphine) type and the *benzyl-isoquinoline* (papaverine) type. Phenanthrene types exert a biphasic pharmacological effect (depressant and stimulant), whereas benzyl-isoquinoline types exert an anti-spasmodic effect. As evident from the structures, the reactive groups are arranged in a partially hydrogenated phenanthrene skeleton, which carries an ethenamine chain, -CH₂CH₂N(CH₃).

Conjugation of morphine in vivo takes place only in the liver. Both the alcoholic and phenolic hydroxyl groups of morphine appear to be involved in the conjugation. Morphine is excreted as a glucuronide (since the amount of glucuronic acid increases proportionately with increasing dosage of morphine). The union of morphine with glucuronic acid is probably a glucosidic linkage through the aldehyde group to the alkaloid on either the phenolic or the secondary alcoholic hydroxyl group. Two forms are excreted:

1. Phenolic monoglucuronide
2. Alcoholic hydroxy conjugated as glucuronide with phenolic hydroxyl as ethereal sulfate.

### 6.2.11. Blood Levels of Morphine\textsuperscript{7,10}

#### Table 32. Blood levels of morphine

<table>
<thead>
<tr>
<th></th>
<th>Therapeutic</th>
<th>Toxic</th>
<th>Lethal</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Tolerance</td>
<td>0.1 µg/ml</td>
<td>-</td>
<td>0.05-4.00 µg/ml</td>
<td>Pittsburgh Criminalistics Labs</td>
</tr>
<tr>
<td>Speed Shooting</td>
<td>60 ng/ml</td>
<td>0.8-2.6 µg/ml</td>
<td>10 µg/ml</td>
<td>Ellenborn</td>
</tr>
</tbody>
</table>

**Therapeutic Blood Level** is the concentration of drug or chemical present in the blood (serum/plasma) following therapeutically effective doses in humans.

**Toxic Blood Level** is the concentration of drug or chemical present in the blood (serum/plasma) that is associated with serious toxic symptoms in humans.

**Lethal Blood Level** is the concentration of drug or chemical present in the blood (serum/plasma) that has been reported to cause death, or is so far above the reported therapeutic or toxic concentrations that one can judge that it might cause death in humans.\textsuperscript{8}

All blood levels vary for each subject. However, among these, toxic blood levels vary from individual to individual. There is a rapid onset of tolerance for opium alkaloids in
humans. Thus, the toxicity will vary with the degree of abuse. Chronic opium users are
tolerant to higher doses of opiates, while non-users cannot tolerate even a small dose.

6.2.12. Metabolism of Heroin

A strong motivation for development of extraction methods for morphine and codeine is
the metabolism pathway of commonly abused drugs like heroin. As shown in the figure
above, heroin is rapidly deacylated to 6-monoacetylmorphine and then to morphine.
Body fluid analyses of a subject suspected of heroin abuse shows the presence of
morphine. 6-acetylmorphine is a unique metabolite of heroin in that it has a very short
half-life and is usually not quantitated (unless the sample is withdrawn in a timely
manner for analysis). Toxicological investigations of opiate-related deaths continue to
rely on measurements of free morphine concentrations in blood, liver, urine, and bile.
(Figure 4).

6.2.13. Metabolism of Codeine

The major metabolic pathways for codeine are glucuronidation and
demethylation, but most of the given dose
is converted to codeine-6-glucuronide, an
inactive metabolite. Much smaller
amounts are converted to norcodeine,
which is believed to be psychoactive.
Significant amounts of codeine may also
be shunted to pathways yielding
pharmacologically active products like
normorphine and morphine. All
compounds are eventually excreted via
urine. Thus, the metabolism is of
significance in that it produces three different compounds with known psycho-activity.
Figure 4 represents the pathways of codeine metabolism. M3G and M6G stand for
morphine-3 and morphine-6-glucuronides respectively, while N3G and N6G represent normorphine-3 and normorphine-6-glucuronides, respectively.

6.3. Experimental

This project investigated the extraction of drugs, medicaments and their metabolites from human blood through the use of microwaves. The analyte selected was morphine.

Traditional methods of extraction for these substances are liquid/liquid extraction and solid-phase extraction. In previous studies, microwave extractions have shown appreciable advantages over conventional extraction methods regarding recoveries, time and solvent consumption. A proposed sterilization effect of microwaves may also facilitate the performance of this innovative microwave extraction method.

All the microwave extraction experiments for obtaining calibration curves; recovery studies, etc., were performed with bovine serum. However, to test the feasibility of the newly developed microwave extraction method, “real world” human blood samples were extracted. These samples were provided by Pittsburgh Criminalistics (PC) Laboratories and consisted of proficiency samples and samples from hospitals and police stations. The maximum amount of human blood needed for extractions was approximately 30 ml.

6.3.1. Samples, Reagents and Standards

**Solvents:**

The following solvents were used for this study: Isopropyl alcohol, methanol, diethyl ether, and methylene chloride. All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

**Reagents:**

The following reagents were prepared for use in this study: Sulfuric acid (10N) (stock), potassium hydroxide (10N) (stock), propyl iodide, potassium phosphate, sodium acetate, ammonium hydroxide, sodium tetraborate. All chemicals were ACS reagent grade obtained from Fisher Scientific, Fairlawn, NJ.
**Matrices:**
The matrices used in the study were human and bovine serum. Water was used as a matrix in the preliminary studies to verify the initial stages of each extraction. Human serum was obtained from Pittsburgh Criminalistics Labs. Bovine serum was used for the Solid Phase Extraction evaluation, and was obtained from Fisher Scientific, Fairlawn, NJ (BW14492E). †

**Standards and Reagents:**
Morphine sulfate USP was gifted from PCL. This was used for the preliminary studies. Morphine salt (1 mg/ml in methanol), Morphine d3 Hydrochloride (100 µg/ml), Codeine Solution (1 mg/ml), Codeine d3 Solution (100 µg/ml) were obtained from Sigma-Aldrich, (Life Sciences), St. Louis, MO.

**GC Supplies:**
The column used for the SPE analysis was RTX-5Sil-MS (Restek 12723) 30m x 0.25mm x 0.25µm. The column and other GC accessories (septa, liners, etc.) were obtained from Restek Corporation, Bellefonte, PA.

**HPLC/LCMS Supplies: Filters, Columns and Accessories:**
- 0.2 µm, 47mm Polycarbonate Membrane filters for the HPLC were procured from Osmonics (Poretics09-732-35) from Sigma-Aldrich, St. Louis, MO.
- Millipore Glass Fiber Filters, 25mm, 1.0 µm (PFB02500) were obtained from Sigma-Aldrich, St. Louis, MO.
- Acrodisc® GHP Syringe Filters, PP, 13 mm, 0.45 µm, mini spiking fitting, (Z26,036-30) were obtained from Sigma-Aldrich, St. Louis, MO.
- PP/PE Syringe, 1.0 ml, All PP/PE, Sterilized (Z23,072-3) were obtained from Sigma-Aldrich, St. Louis, MO.

† Plasma refers to the liquid that remains after red blood cells have been removed from whole blood, while serum refers to plasma from which clotting factors like fibrinogen have been removed.
- Waters Symmetry C$_{18}$ column (5µm, 3.9 x 150mm) (Waters WAT046980), Waters Milford, MA.

**Microwave Instrument and Apparatus**

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos LabStation is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

**GC/MS Determination**

**LLE analysis.** (This part of the project was done in collaboration with Dr. Charles Winek, Mylan School of Pharmacy, Duquesne University, Pittsburgh, PA). GC/MS analysis was carried out on Agilent (HP) 5973 (courtesy of Dr. Charles Winek, Pittsburgh Criminalistics Labs, Pittsburgh, PA). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 series II GC which was equipped with a DB-5 capillary column (30 m × 0.25 mm I.D. ×0.5 mm). The GC oven program started at 60 °C for 3 minutes, ramped from 60 °C to 325 °C at 5 °C/minute, and held 325 °C for 3 minutes. The injector was splitless, held at 250 °C. A Hewlett Packard 5973 MSD was used with a source temperature at 325 °C to monitor morphine in Selected Ion Monitoring (SIM) mode (parent ion 327). Each morphine injection was followed by holding the column at high temperature (300°C) for 20 minutes. This was then followed by analyzing a methanol blank in Total Ion Monitoring mode. The last two steps were necessary to clean the column of cholesterol, because extraction clean-up is not able to entirely remove the cholesterol and it gets injected by default with the matrix. This was done in order to ensure that the column was clean for the next injection and that sensitivity was not compromised. The linear dynamic range was established using a 5-point calibration curve. Data were collected using HP ChemStation Software.
**SPE GC/MS Analysis:** (This part of the project was done in collaboration with David Lineman, Hermitage, PA). GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy of: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. A Hewlett Packard 5970B MSD with a source temperature at 325°C was used to monitor the analytes. Data were collected using HP ChemStation Software. A 5-point calibration curve was used for quantitation purposes.

**HPLC-UV and LC/MS Analysis:** To determine the λ_max of morphine, a 0.1 µg/ml sample in methanol was scanned on a Cary 3 double beam absorption spectrophotometers, using a range from 200 to 900 nm, with computer control. A Waters HPLC (Waters, Milford, MA) was used for this purpose equipped with a Waters 600 quaternary gradient system with manual injector, helium sparge degassing, and a Waters 2487 dual wavelength detector. For LC/MS, a Waters LCMS equipped with a Waters Alliance 2695 pump with an auto-injector with a Micromass ZMD MS equipped with Waters 2487 dual wavelength detector was used.

### 6.3.2. Preparation
For this study, modification had to be made to the extraction vessels as the matrix size was too small to use the glass extraction vessels described previously (Section 4.3.1.1.1). Since the processing involved eventual transfer to a centrifuge tube, to avoid potential loss we used centrifuge tubes as extraction vessels. Thus, a small cylindrical holder was fashioned out of Teflon; three apertures were made in the holder (two for the monitor vessel), each aperture serving as a holding place for a centrifuge tube containing the matrix, analyte, and a stir bar which was fashioned out of paper clip sections encased in a Teflon tubing fused at both ends. The centrifuge tubes were sealed with Teflon tape, with two holes made in the tape so as not to cause overpressure. Figure 5 shows the holder with the centrifuge tubes.
6.3.3. Extraction Procedure

6.3.3.1 Conventional method of morphine extraction for GC/MS

The conventional procedure involved eight steps as follows:

1. Introduction of hydromorphone as an internal standard into the bovine serum along with isopropyl alcohol as the solvent. The mixture was vortex-mixed and centrifuged.

2. The organic layer was transferred to a screw top vial (15 ml), followed by introduction of sodium tetraborate for derivatization. This solution was again vortex-mixed and centrifuged.

3. The top layer was transferred to another screw top vial (15 ml) to which was added 50 µl of propyl iodide. This mixture was then heated in a heating block for 30 minutes.

4. After cooling, ether was added to the same vial and the contents were vortex-mixed and centrifuged.

5. To the organic layer was then added 0.5 N sulfuric acid. Again, the mixture was vortex-mixed and centrifuged.

6. To the aqueous layer was added potassium hydroxide and ether. The mixture was vortex mixed and centrifuged.

7. The top layer was dried in Barb tubes, washed with ether, vortex mixed, and re-dried.

8. The residue was then reconstituted with methanol and subsequently injected into the GC/MS.

6.3.3.2 Extraction using Microwave:

1. The internal standard was introduced into serum along with IPA in an extraction vessel. This assembly was inserted into the microwave cavity. The stirring option was utilized. It was cooled and filtered, then washed with small amounts of isopropyl alcohol. This was followed by the addition of sodium tetraborate for derivatization. This solution was vortex-mixed and centrifuged. Propyl iodide was then added and the system inserted into the microwave cavity to perform a derivatization reaction. The reaction was conducted by heating the mixture to X °C and holding for X
minutes, followed by cooling to room temperature. This mixture was centrifuged and the top layer separated.

2. Ether was added and the mixture was vortex-mixed and centrifuged, and the aqueous layer was removed. To the organic layer was added 0.5 N sulfuric acid and the mixture was vortex-mixed and centrifuged.

3. To the aqueous layer was added potassium hydroxide and ether and the mixture vortex-mixed and centrifuged. The top layer was dried and washed with ether, vortex-mixed and re-dried.

4. The residue was then reconstituted with methanol and subsequently injected into the GC/MS.

Table 33. Extraction protocol for analyte chemistry

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (IPA)</td>
<td>RT to 85°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>10 minutes</td>
<td>85°C to 85°C (Hold)</td>
</tr>
<tr>
<td>3 (Post pH change &amp; reagent addition)</td>
<td>3 minutes</td>
<td>RT to 70°C</td>
</tr>
<tr>
<td>4</td>
<td>30 minutes (derivatization)</td>
<td>70°C to 70°C</td>
</tr>
</tbody>
</table>

Once the samples were cooled, they were opened and centrifuged. An aliquot was further filtered with a syringe filter and injected into the GC/MS.

6.4. Results and Discussion

Following extensive sample preparation with both LLE and IME at a concentration range of 0.5-4 µg/ml, it was evident that at low concentrations IME gave better performance in terms of accuracy and precision. This data is shown in Figure 6. The optimal performance was exhibited at 2 µg/ml, where IME efficiency was nearly equal to 100%. At higher concentrations, the same trend was evident, where IME efficiency was equal to or higher than the LLE efficiency. The precision values were lower across the board than those for LLE. However, at high concentrations (3-4 µg/ml)
both extraction protocols show a decrease in extraction efficiency. It is hypothesized that the derivatization\textsuperscript{13} reaction that is needed during the sample preparation process leads to increased variation in recoveries, as explained below.

There is a possibility that this reaction (Figure 7) does not go to completion at high concentrations. Derivatization was necessary to permit the detection of compounds (e.g. morphine) that are not directly amenable to analysis due to inadequate volatility or stability problems. The derivatization reaction was needed for GCMS analysis of morphine since free morphine tends to degrade on the GCMS column and produce a single “blob” which is not possible to quantitate\textsuperscript{14}.

The other problem encountered during the procedure was the precipitation of protein at high temperatures. This precipitation made post-extraction processing cumbersome. Thus extraction temperature optimization was needed. Eventually, it was also found that the morphine standards supplied to us had degraded. At this point, a new standard solution of morphine needed to be ordered and a new analysis procedure was developed to overcome the derivatization issue.

To overcome these problems, HPLC was chosen as the method of analysis\textsuperscript{15-19}. Figure 8 gives the preliminary results for analysis of free morphine using HPLC. Since $n=2$, \[ \text{Figure 44. Recovery of morphine using HPLC} \]
statistical validation is not presented in these plots. However, the average of the two runs proves that by using HPLC, system is capable of better recoveries. A Symmetry C_{18} column (Waters, Milford, MA) was used. The mobile phase was acetonitrile and 0.001 M ammonium formate (1% v/v formic acid) buffer under isocratic conditions of 6 (Acetonitrile): 94 (Formate) at a flow rate of 0.3 mL/min. The concentration range was 2-4 µg/ml. In this instance, the recoveries ranged from 80-92 % across the concentration range. This confirmed our earlier hypothesis of where the problem lay. The sensitivity problems can be overcome by using LCMS.\textsuperscript{18,20}

6.5. \hspace{1cm} \textbf{Part 2: Solid phase extraction}

A survey of extraction methods for drugs of abuse by the “Steering Committee for the United Kingdom National External Quality Assessment scheme for Drug Assay” found that about 70 % of respondents used solid-phase extraction (SPE) as their method of choice for extraction of drugs of abuse from urine\textsuperscript{17}. (Figure 9) The advantages of SPE have become even more pronounced in recent years with the advent of semi-automated and automated SPE instruments. One study found that in general, SPE was twelve-fold

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig45.png}
\caption{Extraction methods conventionally employed}
\end{figure}
less time-consuming and five-fold less expensive than LLE. Automated methods have been shown to offer higher drug recoveries and greater precision. Overall, SPE has been shown to offer many advantages over traditional LLE, including amenability to automation, higher selectivity, improved reproducibility, and cleaner extracts.

6.5.1. Experimental (Solid Phase Extraction)
SPE cartridges were gifted by Agilent. The salient features for the EVIDEX II SPE cartridges are as follows:

- Proprietary bonding chemistry
  - Mixed RP and cation-exchange bonded phase
- Designed for NIDA-5 Drug Classes
  - Morphine and Codeine
- Two cartridge configurations
  - 200 mg/3ml & 400 mg/6 ml (for varying sample sizes)
- GC-MS analysis using column specific for DOA
  - DB-5ms equivalent purchased from Restek

These cartridges were specifically designed for NIDA-5 (National Institute of Drug Abuse) list drugs that include codeine and morphine. These cartridges are supposed to ensure lot-to-lot reproducibility with high recoveries and clean extractions.

6.5.1.1 Procedure
- Cartridge: 400 mg/ 6ml
- Cartridge Preconditioning:
  - 6 ml methanol
  - 6 ml 0.1 M potassium phosphate (pH 6.0)
- Loading
  - Add 3 ml 0.1 M potassium phosphate (pH 6.0) to the cartridge
  - Attach an 8 ml reservoir
  - Add the urine sample
- Rinse
- Remove reservoir
- ml water
- ml 0.1 M sodium acetate (pH 4.5)
- ml methanol

**Elution**
- Place a collection tube beneath cartridge
- ml methylene chloride/isopropyl alcohol/ NH₄OH (78/20/2)
- Collect the eluate

The GCMS column used was obtained from Restek Corp. (12723 RTX-5Sil-MS 30m x 0.25mm x 0.25µm). The results obtained from this procedure are summarized in Figure 10. The first two bars in both figures represent the extraction efficiency obtained from spiking water at two different concentrations. At 2 µg/ml, the concentration recovered is slightly less within error than that recovered at 4 µg/ml. This can also be observed from the second figure. Figure 10 represents the recoveries and percent. There cannot be a direct comparison of the precision values of microwave with SPE as the SPE error is reported as standard deviation (n=3). The last bar in both figures represents the recoveries obtained from the extraction of serum. Thus, recoveries for microwave (IME?) are in agreement with the recoveries obtained with Solid Phase Extraction.

6.6. **Conclusions and Summary**

From the discussion above, it is evident that the results obtained for microwave extraction of morphine from biological fluids are comparable to the results obtained by Liquid-
Liquid Extraction, and in some cases are improved. The precision values are improved as compared to LLE. Both methods suffered from a decrease in efficiency at high concentrations. This was possibly a drawback of the method of analysis rather than the extraction methods. HPLC was therefore used for the analysis of the extracts. While the concentration range was higher (because of the decreased sensitivity), the percent recovery values were much better as compared to those obtained by the use of GC/MS.

There is also appreciable time-savings with MAE as compared to LLE, and a reduction in the number of transfer steps which decreases the chances for loss of analyte for MAE. The decreased number of steps could also explain the improvement in precision. The overall method was also less tedious to perform as compared to LLE. When the analysis was moved to HPLC from GC/MS, the procedure was even less tedious than the original.
The flowcharts in Figure 11 denote the different procedures used for the extraction of morphine, and to some extent are directly related to the time savings achieved by the respective procedures.

For wider acceptance of the microwave method, SPE was sought as a comparison platform due to the extensive usage SPE finds among clinical chemistry. The results obtained with SPE are comparable to the results obtained with microwave extraction. The SPE method also had high precision values. Thus, microwave extraction method was validated by comparison with two conventionally-used methods. Accuracy and precision for MAE proved to be comparable to SPE and better than LLE.

This study is being revised into a shorter manuscript for publication purposes. We anticipate further study of both the extraction and analysis methods to produce a new method for morphine analysis in the future. We also anticipate morphine extraction to be extended into green chemistry application; i.e., use of ionic liquids as extraction media for morphine and codeine.

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Chapter 7

7. Applications of Integrated Microwave Extraction

7.1. Introduction
This dissertation has examined the development of IME, optimized the factors affecting the extraction, and examined the process integration. We have studied in depth the science of sample preparation; we have studied the theory of traditional microwave extraction, related it to integration microwave extraction, as well as the theory of solvent extraction. We have examined how various factors affect the efficiency of extraction. After the evaluation of these factors, we optimized the parameters for the most advantageous extraction recoveries using different analytes. We have applied IME towards checking the feasibility of using performance based method system for compliance monitoring as opposed to prescriptive methods. The results of this study have corroborated our optimization protocols and have validated IME as a feasible option for the extraction of a variety of analytes. The results of the study have also provided invaluable information which helped us to further optimize the IME technique. This final version resulted from a confluence of our understanding of the theoretical basis of microwave extraction and real-world application of this concept. This honed tool to improve of optimized IME was then applied to different analytes, environments and products.

In its final form, we wanted to use IME for solving some analytical/extraction problems or improving the efficacy of existing procedures. The following were the applications of IME that were successfully attempted:

1. Extraction of additives from polymers
2. Extraction of pesticides and integration of equipment
3. Fat from food products
4. ACS meat and cheese application
7.2. Application 1: Use of microwave-assisted extraction for batch quality control in the production of styrene-butadiene oil extended rubber

7.2.1. Introduction

Synthetic polymer materials are becoming the materials of choice for many industrial and commercial applications. The current world consumption of synthetic polymers is greater than 70 million metric tons per year\(^1\). As the demand for polymer materials increases, manufacturers are looking for methods to improve production and processing. One area of interest is batch quality control. Batch quality control is important because polymer production involves a precise blend of monomers, initiators, cross-linking agents, and other additives\(^2\). Changes in the reaction stoichiometry alter the properties of the polymer and result in a compromised or undesired product. The ability to find a production problem early and correct it translates into significant cost savings for the manufacturer\(^3\).

The most common method of batch quality control is monitoring the percentage of additive(s) or production materials incorporated into the polymer matrix\(^1\). This method requires the additive(s) or production materials to be extracted from the polymer matrix and then analyzed. The analysis techniques vary from simple gravimetric, which requires minutes to complete, to HPLC or GC/MS analysis, which can be completed in 15 to 30 minutes. The major disadvantage of the additive monitoring technique is the extraction process. The typical extraction process involves refluxing the polymer in an appropriate solvent for 1 to 48 hours\(^4\text{-}^7\). Upon cooling, the extraction solution and remaining polymer matrix are separated by filtration. At this time the polymer matrix is either dried to determine total extractable or re-extracted if the first
extraction was incomplete\textsuperscript{8-12}. In addition to being time consuming, the extraction methods usually require large amounts of expensive and hazardous solvents. In contrast, microwave-assisted extractions can be performed in 10 to 20 minutes and use as little as 10 ml of solvent\textsuperscript{13-15}. The major advantage of microwave-assisted extraction is that solvents can be heated to 2 to 3 times their atmospheric boiling point. These results in an increase in extraction efficiency which allows the extraction solvent to be chosen based on its chemical properties not its boiling point. In this report we describe the optimization of a microwave-assisted extraction procedure for styrene-butadiene oil extended rubber.

7.2.2. Instrumentation

7.2.2.1 Microwave Extraction System

The microwave-assisted extraction system used for this work was the Ethos SEL system (Milestone INC., Monroe, CT). This system consists of an Ethos laboratory microwave unit with a built-in magnetic stirrer for homogenous mixing of the sample, a fiber optic temperature sensor, as well as a solvent sensor, which terminates the heating program in the event of a vessel leak or over-pressurization. Two different sample rotors were used for this work. The first rotor was the basic 12-position extraction rotor consisting of 100 ml TFM vessels that have a maximum operating temperature and
pressure of 220°C and 30 bar (500 psi) respectively. The second rotor was the large volume 6-position extraction rotor consisting of 270 ml TFM vessels that have a maximum operating temperature and pressure of 200°C and 10 bar (150 psi) respectively. The optional EvapEX™ evaporation rotor was used in conjunction with the Ethos SEL system for drying. This system is shown in Figure 1. EasyWAVE™ control software (Figure 2) was used to monitor and control the microwave system. The EasyWAVE program PID algorithm automatically adjusts the microwave power to follow the desired heating profile. The pink line is the target profile. The red line is the actual heating profile. The user can change the microwave parameters during the run, which allows for real time optimization during method development. Additionally, this software has sophisticated PID algorithms for precise process control that delivers only the minimum power required to sustain the set temperature or conditions. This is important when performing extractions with organic solvents.

7.2.2.2 Experimental

7.2.2.2.1. Solvent test procedure

0.1 grams of styrene-butadiene oil extended rubber was placed in 5 ml of either pure solvent or solvent mixture. The 1 to 50 sample to solvent ratio is the same as the manufacturer’s current procedure (see below). The samples were extracted at room temperature, with occasional shaking, for 1 hour. The solvents and solvent mixtures used in this study are listed in the captions for Figure 3 and 4.

7.2.2.2.2. Manufacturer’s extraction procedure
The milled styrene-butadiene oil extended rubber sample was cut into approximately 0.5 cm x 4 cm strips. Six grams of the cut rubber were placed in an extraction vessel with 100 ml of 40:60 Ethanol/Toluene extraction solvent. The rubber was extracted three times at 77°C for 30, 30, and 15 minutes. A fresh 100mL portion of solvent was used for each extraction. The extracted rubber was dried under vacuum at 100°C for 45 minutes. Upon cooling, the sample was weighed to determine the percentage of extractable material.

**7.2.2.3 Microwave Assisted Extraction Method**

*Extraction Process:* The milled styrene-butadiene oil extended rubber sample was first cut into approximately 2 cm x 2 cm squares. Between 0.95 and 1.00 grams (usually two squares) was accurately weighed and subsequently placed into the microwave vessel. The appropriate volume extraction solvent was added:

- Initial test – 50 ml
- Volume test – 100 ml
- Optimization: 45 ml for IPA/Hex/ACE and 50 ml for IPA/ACE

If the extraction was performed using stirring, a Teflon™ coated stir bar was also added to the microwave vessel. The styrene-butadiene oil extended rubber was extracted using a 5-minute ramp and a 15-minute hold at the target temperature. When the microwave-heating program was finished, the microwave vessel was removed and allowed to cool to 25°C in a 4°C ice-water bath before opening. 

*Figure 60. styrene-butadiene oil extended rubber sample after 1 hour at room temperature with mixed solvents. 1 = Toluene/Ethanol (60:40), 2 = Hexane/Acetone (50:50), 3 = Isopropanol/Acetone (50:50), 4 = Isopropanol/Hexane/Acetone (38:57:5). Toluene/ Ethanol*
Post extraction processing and drying: The extraction solvent was decanted into a 250-ml beaker. The extracted rubber sample was rinsed and then poured into a piece of pre-weighed Whatman-41 filter paper. The sample was rinsed 3 times with the extraction solvent. The filter paper containing the extracted sample was then placed in the EvapEX™ extraction vessel. The sample was then microwave dried under vacuum, with an argon purge, for 15 minutes at 750 W. Upon cooling, the filter paper containing the extracted sample was weighed to determine the percentage of extractable material.

7.2.3. Results and Discussions
The manufacturer’s current batch quality control requires the monitoring of both total extractable material and the individual process components (aromatic oil, soap, and organic acid) that are incorporated into the styrene-butadiene oil extended rubber matrix. For this work, we were interested only in the total extractable material since propriety formulation information would have been revealed with the determination of the individual components. The manufacturer’s requirements for the new microwave-assisted extraction batch quality procedure were to duplicate the results of their current batch quality control procedure while accomplishing the following goals:

1) Reduced extraction time.
2) An extraction procedure that uses a less hazardous solvent.
3) Reduction in the quantity of solvent used.
4) Simplification of the overall extraction process.

7.2.3.1 Solvent Selection
To develop an efficient microwave-assisted extraction procedure, it was necessary to carry out a preliminary investigation for a suitable solvent system. Since the microwave-assisted extraction process allows the extraction solvent to be chosen based on chemical properties and not its boiling point, we chose four appropriate solvents (hexane, acetone, ethanol, and isopropanol) and performed a simple room-temperature extraction (Figure 3 & 4). Both toluene and hexane exhibited a high solvating power for the production materials (indicated by the deep yellow-orange color), but degraded the styrene-butadiene
oil extended rubber sample (the sample in toluene was totally dissolved in 3 hours). Acetone and isopropanol exhibited some ability to extract the production materials, while ethanol exhibited little to no solvating power. The manufacturer’s batch quality control procedure uses a mixture of toluene (an extracting and degrading solvent) and ethanol (a non-extracting solvent). Complete extraction of the production materials may be dependent upon slight degradation (or swelling) of the rubber matrix. Solvent mixtures of a slightly degrading and a non-extracting were prepared and tested using the room temperature extraction procedure described above (Figure 3). All of the solvent mixtures exhibited some ability to extract the process materials from the styrene-butadiene oil extended rubber and appeared to be good candidates for microwave-assisted extraction.

7.2.3.2 Optimization of the microwave-assisted extraction procedure

The three-candidate solvent mixtures underwent initial testing to determine their suitability for microwave-assisted extraction. The styrene-butadiene oil extended rubber samples were extracted at 85°C without stirring. The isopropanol/acetone (IPA/ACE) mixture and the isopropanol/hexane/acetone (IPA/HEX/ACE) mixture were able to extract the production materials from the rubber sample without degrading the rubber sample too severely. The hexane/acetone (HEX/ACE) mixture, on the other hand, severely degraded the rubber sample turning it into a ‘liquid gel-like’ substance, which was extremely difficult to remove from the microwave-extraction vessel. Hence, the HEX/ACE mixture was eliminated from further consideration.

Table 34. Effect of extraction temperature on the total extractable material from Styrene-butadiene oil extended rubber

<table>
<thead>
<tr>
<th>Extraction Temperature</th>
<th>Extraction Solvent System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IPA/HEX/ACE</td>
</tr>
<tr>
<td></td>
<td>IPA/ACE</td>
</tr>
<tr>
<td>85°C</td>
<td>27.8 %</td>
</tr>
<tr>
<td></td>
<td>26.6 %</td>
</tr>
<tr>
<td>100°C</td>
<td>31.3 %</td>
</tr>
<tr>
<td></td>
<td>27.5 %</td>
</tr>
<tr>
<td>115°C</td>
<td>31.9 %</td>
</tr>
<tr>
<td></td>
<td>28.3 %</td>
</tr>
<tr>
<td>125°C</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>30.6 %</td>
</tr>
<tr>
<td>140°C</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>30.8 %</td>
</tr>
</tbody>
</table>

Manufacturer’s current procedure yields a value of 31.8%
The total extractable material obtained from the initial microwave extractions with IPA/ACE and IPA/HEX/ACE extractions was 10.5% and 12.3% respectively. These numbers are significantly lower than the manufacturer’s value of 31.8%. Previous work with microwave-assisted extraction has shown that stirring the sample during extraction improves both the extraction efficiency as well as reproducibility. The initial extraction was repeated, however, this time the stirring was applied during microwave heating. Stirring increased the total extractable material with the IPA/ACE mixture to 26.6% and to 27.8% with the IPA/HEX/ACE mixture. Although there was significant improvement in the extraction efficiency with the addition of stirring, the numbers still did not agree with the manufacturer’s value.

Since increasing the amount of extraction solvent and/or increasing the extraction temperature could also potentially improve the extraction efficiency, these parameters were also explored. The effect of increasing the volume of extraction solvent was tested first. The amount of extraction was increased from 50 ml to 100 ml for both solvent mixtures. The increase in solvent volume had no effect on the amount of process material extracted with the IPA/ACE mixture. In contrast, increasing the amount of IPA/HEX/ACE caused degradation of the rubber sample. Although the degradation was mild when compared to that with HEX/ACE (34.3%), it still caused the results to be biased high.

The effect of extraction temperature on the extraction efficiency was then investigated. Styrene-butadiene oil extended rubber samples were extracted at different temperatures. The amount of solvent used for the IPA/HEX/ACE extraction was decreased to 45 ml to limit the amount of sample degradation that may occur at higher temperatures. The results of this experiment are shown in Table 1. The extraction using IPA/HEX/ACE reached the target value with only a 30°C temperature increase, while the extraction with IPA/ACE fell short of the target value even at 140°C. In an attempt to achieve the target value with IPA/ACE, the extraction was repeated at 140°C, while increasing hold from 15 minutes to 25 minutes. Increasing the hold time did not improve the extraction
efficiency for the IPA/ACE mixture (30.7%) making the IPA/HEX/ACE mixture the best solvent for this application.

Finally, the proposed microwave-assisted extraction with IPA/HEX/ACE at 115°C was tested for repeatability. Four replicate samples were extracted and processed simultaneously. The average for the total extractable material was 31.7% with a standard deviation of 0.3%.

7.2.4. Conclusion

Microwave-assisted extraction was found to be a viable alternative for batch quality control of styrene-butadiene oil extended rubber. The microwave-assisted extraction procedure is a significant time saver when compared to the manufacturer’s current procedure (Table 2). These benefits can be explained by the fact that the microwave procedure improvises the current method by saving time in all aspects of the method (Figure 5) as well as decreasing the solvent consumption. The biggest time-saving is the extraction process itself (75 minutes to 20 minutes) as well as the drying process (45 minutes to 15 minutes). The microwave-assisted extraction procedure can extract all the process material in a single extraction, which simplifies the overall extraction process.
while improving the overall method precision. Switching the extraction solvent from a mixture of toluene/ethanol to a mixture of isopropanol/hexane/acetone and reducing the solvent volume from 300 ml to ~50 ml reduces the overall cost and eliminates the use of a hazardous solvent.

Table 35. Comparison of the time required to process four samples using the newly developed microwave assisted extraction procedure and the manufacturer’s extraction method

<table>
<thead>
<tr>
<th>Method</th>
<th>Microwave Method</th>
<th>Manufacturer’s Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Sample preparation</td>
<td>10 minutes</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Total extraction time</td>
<td>20 minutes</td>
<td>75 minutes</td>
</tr>
<tr>
<td>Post extraction cooling time</td>
<td>10 minutes</td>
<td>45 minutes</td>
</tr>
<tr>
<td>Additional sample processing</td>
<td>15 minutes</td>
<td>45 minutes</td>
</tr>
<tr>
<td>time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample drying</td>
<td>15 minutes</td>
<td>45 minutes</td>
</tr>
<tr>
<td>Total</td>
<td><strong>60 minutes (1 hour)</strong></td>
<td><strong>255 minutes (4.24 hours)</strong></td>
</tr>
</tbody>
</table>

This technique is not only limited to styrene-butadiene oil extended rubber. Microwave-assisted methods have been developed for the extraction of Irganox from polyethylene and polystyrene, softener’s from PVC, and other common additives. The use of microwave-assisted extraction for batch quality control in polymer production will continue its growth as methods are developed.
7.3. Application 2: Microwave Assisted Extraction and Evaporation: An Integrated Approach; Extraction and Preconcentration Studies of Environmental Contaminants

7.3.1. Introduction
The widespread use of organochlorine compounds around the world has led to their ubiquitous distribution in the environment. Perhaps one of the most pertinent issues is risk assessment of the effects of long-term exposure to trace levels of these chemical pollutants. Nowadays, it is unclear what relationship there may be between environmental exposure to these types of compounds and either the initiation or progression of certain diseases. However, it is suggested that environmental exposure to these pollutants during prenatal development and after birth may have adverse effects on children. Also, it is known that many of these compounds are able to disturb the development of the endocrine system and so they are more accurately named endocrine-disrupting compounds which occur at concentrations of $10^{-5}$ to $10^{-6}$ M and also a long half-life and lipophilic properties, which facilitate their accumulation in adipose tissues. These compounds have entered into the human body via the food chain or respiration and they have been detected in human tissues such as blood, milk or fat\textsuperscript{17-19}.

\textit{A short description of DDT and its history can be found in the appendix}

7.3.2. The Impact of Organochlorine Pesticides on Health and Environment
DDT (dichlorodiphenyltrichloroethane) is an organochlorine compound that persists in the environment and bioaccumulates in human and animal tissue. Aldrin and dieldrin are synthetic organochlorine insecticides with similar chemical structures. Aldrin quickly breaks down to dieldrin in the environment or in the body. Dieldrin persists in the environment and bio-accumulates in body fat and are highly toxic. Aldrin has been used as a soil insecticide to control root worms, beetles, and termites. Dieldrin has been used for soil and seed treatment in agriculture, for control of disease vectors such as mosquitoes and tsetse flies, and for the treatment of wood and the mothproofing of woolen products. Animal studies have linked these chemicals to liver damage, central nervous system effects, and suppression of the immune system. Aldrin and dieldrin also disrupt the endocrine system, with evidence that exposure of pregnant women may harm
the developing fetus. USEPA designates these chemicals as possible carcinogens. Endrin is a persistent, acutely toxic organochlorine insecticide used mainly on field crops. It is estimated that endrin can remain in soil for more than 14 years. Exposure to endrin can cause endocrine effects, liver damage, and disorders of the nervous system. Hexachlorobenzene (HCB) is a synthetic crystalline compound first produced in the 1940s for use as a fungicide. HCB is toxic by all routes of exposure and can damage the liver, thyroid, kidneys, as well as the endocrine, immune, reproductive, and nervous systems. There is evidence of increased susceptibility to infections, immune effects, and decreased survival rates in infants exposed to HCB. Heptachlor is characterized by its toxicity, environmental persistence, and ability to bioaccumulate in the fat of living organisms. It has been found in remote environments and has a half life of up to two years in soils. Studies on laboratory animals have shown that heptachlor can have adverse effects on reproduction and the endocrine system. Heptachlor is considered to cause cancer in animals, and may be linked to bladder cancer. Traditionally, a variety of extraction methods have been used for pesticides ranging from ASE to SFE. This application describes the use of IME for the extraction of pesticides.

The use of microwave-enhanced chemistry, the theory of which has been extensively discussed, offers many advantages over traditional heating methods. Rapid heating, heating to higher than boiling point temperatures, and less solvent consumption are some of the salient features of this technique. Stirring is possible which makes the extraction conditions more homogenous, promotes interaction with the solvent, and assists in releasing the analyte from the matrix. Utilization of a microwave absorbing component makes possible the use of non-polar solvents for microwave extraction.

7.3.3. Experimental

7.3.3.1 Samples, Reagents and Standards

The solvents:
The solvents selected for this application are hexanes and a solvent mixture of 1:1 Hexane: Acetone. All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

**The Standards and Reagents:**
- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

**Certified Reference Material:**
Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

**Microwave Instrument and Apparatus**

![Diagram](https://via.placeholder.com/150)

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Lab Station is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a
dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

**GC/MS Determination**

GC/MS analysis for PAHs was carried out on Agilent (HP) 5972 equipped with an autosampler (courtesy: Dr. F. Fochtman, Mylan School of Pharmacy, Duquesne University). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 series II GC which was equipped with a DB-5ms capillary column (30 m × 0.25 mm I.D. ×0.5 mm. ((5%-Phenyl)-methylpolysiloxane). A Hewlett Packard 5972 MSD was with a source temperature at 325°C to monitor PAHs in the Selected Ion Monitoring (SIM) mode. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve. Pesticide analysis was done on Saturn GC/MS (Varian Inc., Walnut Creek, CA). Saturn GCMS/ Varian 3410 high-temperature gas chromatograph coupled to a Varian Saturn II ion trap mass spectrometer and an autosampler was used for this analysis. Data collection and processing was done using Saturn and SaturnView software. A 1-µl aliquot was introduced into the Varian 3410 Gas Chromatograph (using autosampler).

7.3.4.  Procedure

7.3.4.1  Microwave Extraction

![Figure 63. Depiction of equipment configuration. A) Filtration system B) Cross section through a microwave cavity illustrating the evaporation system](image-url)
The extraction vessel is prepared by fitting the vessel into the secondary absorber base followed by the insertion of a suitable filtering medium (filter papers, frits, membranes, glass wool). Extraction chamber capacity ranges from 100 ml to 270 ml. For the 100 ml extraction chamber, the sample is prepared in the following manner: the soil sample (range: 1-5 g)/CRM (RTC, Laramie, WY) was introduced into the extraction chamber with the solvent (range: 10-15 ml). The extraction chamber contains the same solvent as the extractant, enough in volume to immerse the secondary absorber base and part of the vessel (~20ml). This solvent can be recycled for subsequent runs. The vessel is capped with a Teflon lid for separation of inner and outer solvents. Glass coated magnetic stir bars were added. Stirring was set to 40% of maximum. The closed extraction chambers were sealed into the individual rotor segments. The soil samples were extracted using the following temperature program: a 5-minute ramp to 100°C and a 15-minute hold at 100°C. After cooling to 25°C, the extraction chambers are opened and vessels are removed. The secondary absorber base is snapped off, and the vessel is then directly fitted into the slot in the filtration system lid. Samples were vacuum filtered (vacuum is applied in the central position, Fig. 2A) into vials in which evaporation was subsequently carried out. The Teflon cap can be removed for additional washings if necessary. After the completion of filtration, only the closure from the filtration system was replaced with the evaporation closure. (Profile depicted in Figure 8).
Table 36. Extraction protocol for microwave extraction

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 minutes (1:1Hex: Act)</td>
<td>RT to 100°C (Ramp)</td>
</tr>
<tr>
<td>2</td>
<td>20 minutes</td>
<td>100°C to 100°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>100°C to RT</td>
</tr>
</tbody>
</table>

### 7.3.4.2 Microwave Assisted Evaporation

Evaporation was carried out under argon (connected at the central position, Fig. 2B) using alternate heating and cooling steps of 700 W for 2 minutes and 0 W for 30 seconds. A cooling step was incorporated to avoid possible overheating of analytes, which could potentially cause thermal degradation. This cycle was repeated 4 to 5 times depending on the solvent used. The see-through microwave door provides easy real-time visual monitoring. Processing of 12 samples simultaneously can be accommodated in one rotor assembly for 25ml (approximate) extraction vial size using this current instrument configuration. The instrument also enables an integrated solvent recovery system to permit recycling of the solvents permitting a minimization of fresh solvent usage.

### 7.3.5 Results and Discussion

The effect of solvent on extraction of PAHs was tested. Data obtained (Figures 9, 10, 11) indicate analogous results between the two classes of solvents. For example, for pyrene, acetone extracted 2715±357, while hexane extracted 3025±106 (µg/g, 95%CL, n=6). Acetone recovery of fluoranthene was 3359±353, while hexane recovery was 3831±107 (µg/g, 95%CL, n=6)\(^3\). Thus, although the data indicates statistically equivalent recoveries for both classes of solvents, hexane extracts demonstrated an increase in average recoveries. It was also observed that hexane extracts gave cleaner chromatograms and spectra than the polar solvents used. Non-polar solvents can thus replace solvent combinations. The secondary microwave absorber base converts the microwave power to thermal energy and transfers this energy to the surrounding solvent which in turn heats the extracting solvent inside the glass extraction chamber. This secondary heating mechanism is illustrated in Figure 6. The ability to use either type of solvent allows one to tailor the extraction conditions to the analyte of interest or to mimic
the extraction conditions used in the traditional methods. It was also observed that recoveries using IME are comparable to those reported for the Certified Reference Materials (CRM's) which were obtained using Soxhlet extraction. As there was no transfer step throughout the extraction process, the error associated with each transfer step may have been eliminated, which is evident from the higher precision values associated with IME. To be accepted as a replacement for the traditional techniques, the results obtained from the method are expected to be comparable to those obtained from the traditional methods. Therefore, extraction of pesticides (Figure 12) was done using 1:1 hexane/acetone mixture to simulate the Soxhlet procedure closely. Results illustrate good agreement between IME and certified values while using only about 1/50th of the...
amount of time needed by Soxhlet. Twelve samples were extracted simultaneously in 15 minutes.

The use of the microwave for evaporation allowed good control over the evaporation conditions. The microwave power output is varied to produce slow heating, even at small solvent volumes (<2ml). Results from evaporation recovery of PAHs in hexane (Figure 6) verify complete recovery of the analytes where the analyte concentration ranged from 10-30 µg/ml each. 1:1 v/v Hexane/Acetone was then used for extraction. High concentration range (10-30 µg/ml) was used in the first design. In separate experiments, 15 ml was evaporated to 5, 2 and 1 ml. No analyte loss was observed (Figure 10). However, in the second design, low concentration range of 1:1 v/v H/A (1 µg/ml) was evaporated from 15 ml to 5, 2 and 1 ml. In this set of experiments, there seemed to be an appreciable loss of analyte. This is probably due to either uneven heating which resulted in the solvent depositing over the inside surface of the glass extraction vessel. At low concentrations, this solvent loss translated into evident analyte loss. (Figure 11). The recovered solvent when subjected to GC/MS analysis showed no analyte loss, thereby making it possible for the solvent to be recycled. (Miscellaneous and supporting tables in Appendix)
7.3.6. Conclusion

The Integrated Microwave Solvent Extraction system is demonstrated to be an attractive alternative to traditional solvent extraction techniques. Rapid processing of samples results in significant timesaving over traditional methods as demonstrated. Feedback real-time computer control of the extraction parameters increases precision and safety of the procedure. The integration of processes ensures enhanced extraction efficiency. The automation of sample processing minimizes sample manipulation thereby reducing potential for operator error. Use of a secondary microwave absorber allows the use of non-polar solvents, making it possible to design the extraction protocol so as to optimize the chemistry of the solvent and process. While the initial investment for a MASE system is higher as compared to Soxhlet apparatus, which is always a trade-off with modern extraction equipment, it is important to factor in the operating and solvent quantity used and disposal costs. The analyst's exposure to hazardous solvents is minimized in MASE. Consumption of these solvents and their subsequent disposal is substantially reduced, making it an economical and greener process.
7.4. Application 3: Evaluation of meat products for PAHs introduced during the grilling process and phthalates from cheese leached by the wrapping.

7.4.1. Introduction

PAHs are ubiquitous and consistently present in the environment and are typically formed during the incomplete burning of organic material including wood, coal, oil, gasoline and garbage. These compounds are also found in oil, coal tar and asphalt. Historically, PAHs have been associated with human activities such as cooking, heating and fuel for operating automobiles. While PAHs spread in environment are of anthropogenic origin, some are also present due to natural sources like forest fires. All emissions from incomplete combustion contain polycyclic aromatic hydrocarbons.

A brief account of PAH carcinogenicity is included in the Appendix

7.4.2. Impact of PAHs and Phthalates on Human Health

A few PAHs (e.g. benzo(a)pyrene) are confirmed carcinogens, while most others are on the suspected carcinogens list\textsuperscript{31, 32}. Percentage contribution of the PAH-fraction and benzo(a)pyrene to the carcinogenic potency of various emission condensates was evaluated by the topical application onto the skin of mice by analysis and is presented in Table 4.

\textbf{Table 37. Percentage contribution of the PAH-fraction and the benzo(a)pyrene to the carcinogenic potency of emission condensates}

<table>
<thead>
<tr>
<th>Source</th>
<th>PAH Fraction (%)</th>
<th>Benzo(a)pyrene (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automobile exhaust (3.5%)\textsuperscript{*}</td>
<td>85</td>
<td>6</td>
</tr>
<tr>
<td>Flue gas of coal-fired residential furnaces (15.2%)\textsuperscript{*}</td>
<td>&gt;90</td>
<td>11</td>
</tr>
<tr>
<td>Used lubricating oil (1.14%)\textsuperscript{*}</td>
<td>70</td>
<td>18</td>
</tr>
</tbody>
</table>

\textsuperscript{*} Weight % of the PAH-fraction related to the total emission extract

Phthalates are a class of chemicals added to a number of common consumer products. In 1994, close to 87\% of all phthalates in the United States were used as plasticizers, or
softening agents, in vinyl products. Humans are widely exposed to phthalates because vinyl is a ubiquitous plastic used to make anything from home furnishings (for example, flooring, wallpaper), medical devices (for example, catheters, IV- and blood bags), children's items (for example, infant feeding bottles, squeeze toys, changing mats, teethers) to packaging (for example, disposable bottles, food wrap). Beyond vinyl, humans are further exposed to phthalates in cosmetics and scented products such as perfumes, soaps, lotions and shampoos. Phthalates are also added to insecticides, adhesives, sealants and car-care products. According to the U.S. Environmental Protection Agency (EPA), eating is probably the main route by which humans are contaminated with diethylhexyl phthalate (DEHP), the most widely used phthalate plasticizer. DEHP also migrates into food from certain food wraps during storage. DEHP has been classified as a "probable human carcinogen" by the EPA. Rats and mice fed DEHP and DINP also showed an increase in liver cancers over animals that had not been fed the chemicals. The offspring of rats separately fed three different phthalates, (DEHP, DINP and BBP), do not follow normal patterns of sexual development. High doses of diethyl phthalate (DEP) given to female rats have been shown to cause the growth of an extra rib in their offspring. 

This application evaluated the possibility of the presence of PAHs in grilled meat introduced during the grilling process and leaching of phthalates into food products from wrappings.

7.4.3. Experimental

7.4.3.1 Part 1: Microwave Assisted Extractions

In this application, microwave-assisted extraction was used to extract Polynuclear Aromatic Hydrocarbons (PAHs), adipates, phthalates and chlorophyll from a variety of different matrices like grilled meat and cheese.

7.4.3.1.1. Samples, Reagents and Standards

The solvents:
The solvents selected for this application are hexanes, dichloromethane and a solvent mixture of 1:1 Hexane: Acetone

All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

**The Standards and Reagents:**

- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- EPA Method 620 Diphenylamine 70314 (nominal concentration of 1000 µg/ml in methanol) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Semi-Volatiles GC/MS Tuning Standard GCM-150 (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI

**Certified Reference Material:**

Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

**Real world sediment sample**

The preliminary phase of extraction was carried out on David Lineman’s sediments from Lowellville River, OH. The sediments were sampled from the river as well as the riverbank. The sediments (because of their origin) were rich in water, and sodium sulfate was added to counteract any additional barrier effect from water. (Moisture study was carried out separately). (Samples courtesy David Lineman).

**Microwave Instrument and Apparatus**
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Lab Station is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

**GC/MS Determination**

GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-µl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. A Hewlett Packard 5970B MSD used to monitor PAHs. Data were collected by a HP ChemStation Software. The linear dynamic range was established by 5-point calibration curve ranging from 2 µg/ml to 10µg/ml. The preliminary work was carried out using HPLC. Waters HPLC (Waters, Milford, MA) was used for this purpose equipped with a Waters 600 quaternary gradient system with manual injector, helium sparge degassing, and a Waters 2487 dual wavelength detector.

7.4.4. Procedure

The glass wool is attached to the Weflon base by the Teflon outlet. Then, this Teflon outlet is first blocked with a filter/ glass wool. This filter is held in place with a stopper disc. Matrix is placed in the chamber along with a stir bar and appropriate amount of solvent. This lidded extraction assembly is then placed into the liner which contains the same solvent as inside the extraction chamber. This liner is then inserted into the sleeve, which is further capped. Pressure plate and spring are secured in place with the Teflon sleeve. This assembly is then inserted into its segment. Individual procedures are outlined as follows.

| Table 38. Extraction protocol for the meat and cheese products |
|-------------------|-----------------|-----------------|
| Sequence | Time | Temperature |
| 1 | 3 minutes (1:1Hex: Act) 5 minutes (Hexane) 3 minutes (Dichloromethane) | RT to 100°C (Ramp) |
| 2 | 20 minutes | 100°C to 100°C (Hold) |
| 3 | 20-25 minutes | 100°C to RT |
7.4.4.1.1. *Extraction of PAHs from grilled meat.*

Procedure

- The meat was grilled (ref: David Lineman) and homogenized in a blender.
- Approximately 2 grams of the blended sample was weighed into extraction vessel.
- 10 µl of surrogate standard was added.
- DCM was used as an extracting solvent (dichloromethane, 10 ml)
- Extracted using the extraction protocol depicted in extraction protocol
- Samples were cooled and subsequently filtered.
- Preconcentration was done using EvapEX™ as described in Chapter 4
- Extracts were inserted in vials and capped for GC-MS analysis

7.4.4.1.2. *Extraction of phthalates and adipates from cheese samples*

Procedure

- Different cheese samples were obtained from the supermarket. Outer 2mm of cheese samples was cut. This portion was used for extraction.
- Approximately 2 grams of the blended sample was weighed into extraction vessel.
- 10 µl of surrogate standard was added.
- DCM was used as an extracting solvent (dichloromethane, 10 ml)
- Extracted using the extraction protocol depicted in extraction protocol
- Samples were cooled and subsequently filtered.
- Preconcentration was done using EvapEX™ as described in Chapter 4
- Extracts were inserted in vials and capped for GC-MS analysis

7.4.4.2 Part 2: Evaporation/Drying

7.4.4.2.1. Procedure (outline)
Detailed procedure is described in Chapter 4.

1. The extracts were quantitatively transferred (using the filtration system and vacuum filtration) into evaporation vials
2. EvapEX™ was used for preconcentration
3. Pulsed microwave heating was employed. (700 W for 1 minute, 0 W for 1 minute; 400 W for 30 seconds, 0 W for 30 seconds...so on until desired volume was attained. Final evaporation time depends on the solvent of extraction and the original volume of the extract).

### 7.4.5. Results and Discussion

The profile presented in Figure 13 was produced during the extraction of PAHs on the EasyWAVE™ software. On analysis, the results obtained are given in Table 6. It can be said with reasonable certainty that PAHs were introduced during the grilling process. Some of the PAHs that were found included naphthalene, acenaphthylene, phenanthrene and benzo(a) pyrene among other compounds. Benzo(a)pyrene is a confirmed carcinogen, and most others are suspected carcinogens. In Figure 14, it was found that certain types of wrapping leached phthalates and adipates into the cheese products that had these wrappings covering them. Since these compounds are of medical concern in

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**Notes:**

- The extraction procedure is applicable to PCB’s and pesticides as well. This method can accommodate sample sizes up to 10 grams and solvent volumes up to 60 ml.

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**Figure 70. Microwave Extraction profile for the extraction of PAH contaminated soil**
that they can potentially cause cancer in humans, this application proved to be illuminating of how these harmful chemicals can be accidentally consumed by humans.

Table 39. Extraction Recoveries of PAHs from meat samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>2700</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>1700</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2000</td>
</tr>
<tr>
<td>Anthracene</td>
<td>77000</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>46000</td>
</tr>
<tr>
<td>Pyrene</td>
<td>2000</td>
</tr>
<tr>
<td>Chrysene</td>
<td>4700</td>
</tr>
<tr>
<td>Benzopyrene</td>
<td>1300</td>
</tr>
</tbody>
</table>

7.4.6. Conclusions:

The Integrated Microwave Solvent Extraction system is demonstrated to be an attractive alternative to traditional solvent extraction techniques. Rapid processing of samples
results in significant timesaving over traditional methods as demonstrated. From Table 6 and Figure 13, it is evident that carcinogenic compounds are introduced externally into these food products. Since PAHs are a by-product of incomplete combustion, it can be reasoned that the grease from the meat on the grill that releases smoke undergoes incomplete combustion, and the smoke plume carries these PAHs onto the meat. In case of the phthalates, only a certain kind of plastic leached them out onto the cheese. It was also evident that the compounds were leached into the cheese from the wrapping, as the phthalates were found on the surface of the cheese and not to a great degree towards the core of the cheese. The wrapper that was found to leach the compounds was the thin, flimsy polymer plastic (the type that a deli would use), and not the thicker version of the pre-packaged cheese.
7.5. Application 4: Application of Microwave Extraction for the isolation of lipoidal material from food products

The interest in dietary fat is a growing trend, and the determination of fatty compounds is a basic requirement in testing food material as a result. Consumers demand reduction of the total fat contents in food in order to improve human health, thus forcing government agencies to the use of more precise methods for fat determination which assure accuracy in labeling products. For nutrition labeling purposes, fat has been defined as triglycerides, substances extracted with ether or total lipids. To unify criteria, the US Food and Drug Determination (FDA) through the Nutritional Labeling and Education Act (NLEA) of 1990, defined “total fat” as the sum of all fatty acids obtained in the lipid extract, expressed as triglycerides. Hence, a complete extraction of lipids from the sample is a mandatory step. Lipid extraction is carried out in different ways depending on the sample characteristics. Thus, some extraction methods (namely, Weibull-Berntrop, Röse-Gottlieb, Mojonnier, Folch, Werner-Schmid, Bligh-Dyer methods, etc.,) are based on hydrolysis (either acid, alkaline or enzymatic) before solvent extraction but some others involve only the solvent extraction step (Soxhlet, Lickens-Nickerson, etc.). Despite several modifications in solvent mixtures and laboratory practice, the previous, conventional procedures have not been greatly improved, and long preparation times with a second re-extraction step to ensure complete removal have been required most times. The critical choice of the use of organic solvents and the by-side phenomena namely, co-extraction of non-lipid material such as sugar or sugar by-products, vitamins, color compounds, etc., and the chemical transformations of triglycerides associated to the long time and high temperature needed for classical digestion or extraction are the principal shortcomings. These methods provide a lipid extract that is usually quantified by gravimetry but there also are titration methods as Babcock or Gerber methods. At present, a tendency towards the use of supercritical fluid extraction (SFE), and accelerated solvent extraction (ASE) can be observed. Recently, a dynamic ultrasound-assisted extraction method has been proposed prior to the gravimetric determination of the total fat content in bakery products. Recoveries from 99.7 to 100.7% and shortening of the extraction time between five and eight times, depending of the type of sample, were obtained as compared with conventional Soxhlet. Microwave Extraction might
possibly accelerate the process, minimizing environmental pollution due to the small amount of solvent consumed, lower waste disposal, minimized solvent exposure and low degradation of thermolabile analytes.

7.5.1. Experimental

7.5.1.1 Samples, Reagents and Standards

The solvents:
The solvent selected for the extraction of lipoidal material from different matrices was n-hexan. The solvent used was Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

7.5.1.2 Microwave Instrument and Apparatus

Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Lab Station is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel. Post-extraction filtration and evaporation was done using Milestone FiltEX™ and EvapEX™ systems respectively without transferring the extracts. The evaporated solvent was collected and recycled using the EvapEX™ in conjunction with the Solvent Recovery System. EasyWAVE™ control software was used to monitor and control the microwave system which uses a PID algorithm for precise temperature and process control that delivers the minimum power required to sustain the set temperature.

7.5.1.3 Extraction Procedure

Three different products were purchased from local supermarkets. The different products tested as matrices were: Sandies® Cookies (Brand: Simply Shortbread; Keebler™ Cookies, Kellogs, Inc., Battle Creek, MI); Peanuts from Planters (10oz., Kraft Foods North America, Inc., East Hanover, NJ), and Chocolate bars from Hershey’s (Hershey’s
Milk Chocolate Bars, Hershey Foods, Hershey, PA). The product under study was homogenized in a blender; 50 g of sample was crushed in a blender and then was homogenized again and stored in a cold room in the dark until use. 2-g of the homogenized sample was weighed into the vessel followed by the introduction of 10 ml of hexane. Extraction was done using the protocol given in Table 7. Analysis was done gravimetrically.

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<th>Temperature</th>
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<tr>
<td>2</td>
<td>20 minutes</td>
<td>90°C to 90°C (Hold)</td>
</tr>
<tr>
<td>3</td>
<td>20-25 minutes</td>
<td>90°C to RT</td>
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7.5.2. Results and Discussion:

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<th>Sample</th>
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<th>Lipophilic Content (Extracted)</th>
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<tr>
<td>Cookies</td>
<td>36.9</td>
<td>26.2 ±0.3</td>
</tr>
<tr>
<td>Peanuts</td>
<td>48.5</td>
<td>47.9 ± 0.5</td>
</tr>
<tr>
<td>Chocolate</td>
<td>30.2</td>
<td>34.1 ± 3.4</td>
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</table>

All results given in Table 8 are in grams, error expressed as 95%CL, n=8. From the results Kraft food peanuts had a labeled amount of 48.5 g while the extraction values are close to 47.9g. This is a close agreement. In case of the chocolates, the higher percent could be due to the inability to properly reduce the sample size (lumping occurs). The sampling is not ideal in this case. However, for the cookies, it was evident that during the crushing phase, some of the fat is lost. This is reflected in the extraction efficiency that falls short of the labeled amount. For the most part however, the extracted values are in agreement with the labeled amounts.

7.5.3. Conclusions

This research focuses on the establishment of a method for the removal of fat from bakery products which was faster, cleaner and requiring less consumption of reagents than those presently used. Therefore, the optimization of the overall method here
proposed was concentrated on the leaching step. Microwave Extraction provides the following advantages:

1. Substantial shortening of the extraction time
2. Saving of extractant is such a way that only 25–30 ml is consumed per extraction.
3. Use of samples as received, without the moisture adjustment usually required in conventional Soxhlet methods.

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| Figure 76. Graphic representation of the processing time required by the two different methods |
7.7. References


(7) Nerin, C.; Asensio, E.; Fernandez, C.; Battle, R. *Quimica Analitica (Barcelona, Spain)* 2000, 19, 205-212.


(16) Richter, R.; Shah, S. *Am Lab (Shelton, Conn)* 2000, 32, 14-16.


Extraction of organochlorine pesticides

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<th>DDE</th>
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**Extraction of pesticides (setting up of equations)**

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<td>Dieldrin</td>
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<tr>
<td>Methoxychlor</td>
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<tr>
<td>Lindane</td>
<td>y = 150301x + 41082</td>
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</tr>
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<td>Aldrin</td>
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<td>p,p'-DDD</td>
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**Extraction of PAHs using polar solvents**

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|              | 95% CL   | 95% CL  | 95% CL       | 95% CL    |
Extraction of PAHs using non-polar solvents

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95%CL 95%CL 95%CL

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<td>Pyrene</td>
<td>106</td>
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History of DDT and EPA’s association with it

EPA's creation coincided with the culmination of the public debate over DDT (dichloro-diphenyl-trichloro-ethane). A chlorinated hydrocarbon, DDT proved to be a highly effective, but extremely persistent organic pesticide. Since the 1940s, DDT has been spread across the environment to control pests such as Mexican boll weevils, gypsy moths, and pesky suburban mosquitoes. Widespread public opposition to DDT began with the publication of Rachel Carson's influential *Silent Spring*. Reporting the effects of DDT on wildlife, Carson demonstrated that DDT not only infiltrated all areas of the ecological system, but was exponentially concentrated as it moved to higher levels in the food web. Through Carson, many citizens learned that humans faced DDT-induced risks. By 1968 several states had banned DDT use. The Environmental Defense Fund, which
began as a group of concerned scientists, spearheaded a campaign to force federal suspension of DDT registration--banning its use in the United States. DDT’s remaining legal use is for malaria control.

PAH and carcinogenicity

A scientist working on cancer research, E. L. Kennaway initiated the search for the carcinogenic constituents of coal-tar pitch. He produced tumors in mice with synthetic compound dibenzanthracene which thus proved to be the first polycyclic aromatic hydrocarbon of a long series of carcinogens of this type. However, it has not been possible to obtain experimental evidence for the intracellular formation of PAHs. Among the 450 compounds which were found to be carcinogenic, more than 100 were PAHs. These compounds have several features that distinguish them from some of the listed carcinogens. They act at the site of application, the effective dose is minute, (of the order of micrograms) and they have been found to induce tumors in almost every tissue and animal species in which they have been tested. Carcinogenic activity has been found mainly in certain appropriately substituted tri-and tetra cyclic aromatic hydrocarbons as well as some higher cyclic aromatic hydrocarbons31.
Chapter 8 Overview

Development of Green Analytical Extraction Method Using Ionic Liquids as Extraction Media

8. DEVELOPMENT OF GREEN ANALYTICAL EXTRACTION METHOD USING IONIC LIQUIDS FOR EXTRACTION

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8.2.1.2. Green Analytical Chemistry

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8.4. MICROWAVE ASSISTED EXTRACTION (MAE)

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Chapter 8

8. Development of Green Analytical Extraction Method using Ionic Liquids for Extraction

8.1. Abstract
Development of cleaner technologies is assuming increasing significance in today's research scenario. This proposal describes a novel alliance of ionic liquids and microwave technology, both of which are green approaches to separations. Green Chemistry efficiently utilizes renewable raw materials, eliminates waste and avoids the use of toxic and/or hazardous reagents and solvents in the manufacture and application of chemical products. Thus, the aim of Green Chemistry is to eliminate waste at source and utilize environmentally benign reagents in syntheses and analytical processes. The search for alternatives to volatile organic solvents has become a high priority since they are used in high quantities and are usually difficult to contain. Some of these solvents are toxic to human health and are even carcinogenic, while their release into the atmosphere causes ozone depletion. Ionic liquids (ILs) offer a solution to these problems. Significant forays have been made in the field of organic syntheses using ILs. However, little progress has been made on the development of extraction protocol avoiding the use of organic solvents. This reduces the overall "greenness" of the methodology. Microwave Assisted Extraction (MAE) processes are not only more efficient, but also consume significantly lower quantities of toxic solvents as compared to traditional extraction processes. This proposal explores the use of MAE using ILs as extraction solvents. The aim is to evaluate the possibility of equivalent or increased extraction efficiencies with a concurrent increase in the "greenness" of the process. The effectiveness of the methodology outlined in this proposal will be verified on two diverse applications. We expect to reap the synergistic benefits of coupling two environmentally friendly processes.

8.2. Introduction
Chemistry generates a staggering amount of solvent waste everyday. Organic solvent waste has huge negative impact on the environment. Green chemistry is an attempt by
practicing chemists to be environmentally friendly. Green chemistry aims to reduce, if not eliminate, waste generated by chemical procedures. There is a three-pronged approach to this problem; prevention of waste generation, reduction of quantity of waste generated and researching alternate sources to accomplish the same chemistry.

Figure 1 is a pie-chart showing the kinds of waste generated in chemical labs today. As can be seen, most of the waste is toxic and corrosive and can adversely affect the environment.

8.2.1. Green Chemistry

8.2.1.1 Background
Green Chemistry is an approach to the design, development and implementation of chemical products and processes with the aim to reduce or eliminate substances hazardous to human health and the environment. The U.S. chemical industry is the world’s largest producer of chemical products (1), and depends heavily on chemicals, which eventually contribute to pollution as toxic waste (such as Volatile Organic Compounds (VOCs)). For example, 42% of the waste generated by the Environmental Protection Agency (EPA) labs is toxic in nature (2). The toxic effects on human health range from skin irritations to cancer, while the effect on the environment encompasses air, water and soil pollution. Each year billions of dollars are spent on the treatment of these waste products. Thus, the consequences of chemistry do not stop with the properties of the target molecule or the efficacy of a particular reagent. This knowledge is now manifested in the different approaches that scientists are taking to ensure that the processes are less harmful to the global environment. Many innovative chemistry
techniques have been designed over the last several years that are effective, efficient and more environmentally benign, including new syntheses and analytical processes. In recent years, concentrated efforts have been made to control pollution; however, equal focus has not been placed on its prevention. Green Chemistry eliminates waste at the source and avoids the use of toxic and/or hazardous reagents and solvents in the manufacture and application of chemical products. The realization that pollution prevention is frequently more cost effective than remediation has catalyzed tremendous effort in the development of environmentally benign solvents and processes. The benefits to the industry, as well as the environment, are all part of the positive impact of Green Chemistry.

8.2.1.2 Green Analytical Chemistry

Analytical chemistry has a long history of dealing with environmental hazards. Analytical chemistry methodologies are often the basis of regulation for environmental protection and monitoring agencies. The process of monitoring environmental contaminants using the different analytical methods, more often than not, ironically contributes to further environmental problems. Right from the stage of sample preparation to the culmination in analytical measurement, use of hazardous substances is fast becoming a matter of concern. Also gaining significance is the analyst’s exposure to these hazardous substances as well as their mounting disposal costs, making many analytical methods economically unfeasible.

Risk has been summarized as the product of the hazard related to a particular substance and the exposure to that substance. Green Analytical Chemistry aims at reducing the hazards associated with the substance, thereby minimizing the exposure part of the equation. Some of the methods where green analytical chemistry approaches are being implemented include field analysis, screening, extraction, dilution, digestion and alternative mobile phase techniques (2). One such approach of the green method is the use of room temperature Ionic Liquids (ILs) for chemical syntheses, reactions, biotransformations and separations.
8.3. Ionic Liquids

Chemistry involves solvents in a variety of processes, from synthesis to analysis. Solvents are high on the list of damaging chemicals simply because they are used in large quantities and are usually volatile liquids that are difficult to contain. Although previously used polychlorinated solvents are done away with, solvents such as VOCs are still extensively used. The U.S. chemical industry uses more than 3.8 million tons of solvents per year, most of them designated as toxic (3). ILs are rapidly proving to be the answer to this challenge and are described as salts that are liquids at room temperature. As opposed to molecular solvents, these liquids are made entirely of loosely coordinated ionic species. Their high boiling points are accounted for by their relatively bulky organic cations. Their simple, inorganic anions determine their chemical properties to a large extent. These two components can be altered and designed for a specific end use.

8.3.1. Theoretical aspects

A typical ionic liquid is shown in figure 2. The cation consists of the imidazole ring with alkyl groups appended on the nitrogen. Anions can be varied from chloride to BF₄ to PF₆, each of which confers different properties to the parent cationic molecule.

For liquid/liquid extraction purposes, some of the properties of a solvent which need to be considered are its boiling/melting points, viscosity and density. To begin with, the melting point of a salt is directly related to its lattice energy. Since ionic liquids are salts, where one or both of its ions are large and have a low degree of symmetry, the lattice energy of the crystalline form of the salt is reduced, which in part explains its lower melting point. (Deviations from this rule are usually due to other forms of bonding within the structure). It is evident that by using larger anionic and cationic components in the salt it is possible to lower this energy and thus decrease the melting point by considering the Kapustinskii Equation (4),
Increasing the cation size is made possible by the use of organic cationic moieties. This in turn would decrease the lattice energy, thereby decreasing the melting point of the salt. Thus, the melting point of ILs is directly related to their lattice energy as shown in Figure 3. Larger the size of the ions, lower is the melting point.

Viscosity is another important physical characteristic that determines the handling of the solvent. It is desirable for a fluid to have only small changes in viscosity through the normal operating temperature range to help design the process especially when designing large-scale (industrial) extractions. The temperature dependence of the viscosity (4) can fit to the Arrhenius type equation:

\[ \eta = \eta_0 \exp \left( \frac{E_a}{RT} \right) \]  

(2)

Where,

- \( E_a \) energy of activation for viscous flow
- \( R \) gas constant
- \( T \) absolute temperature
Studies indicate linear dependence for N-alkylpyridinium salts; non-linear for chloro & bromoaluminate ILs (4). Many other parameters influence the viscosity of ILs, however an exhaustive study is needed to establish a correlation model.

The parameter of density is important to consider during the design of liquid/liquid extraction schemes. Density of ILs is relatively high compared to normal industrial solvents due to their bulky ions. Density is fitted (4) to the following equation:

\[ \rho = a + b \times T \]  

where,

- \( a \) and \( b \) are constants.
- \( T \) is the absolute temperature.

One of the most advantageous properties of ILs in the context of Green Chemistry is their negligible vapor pressure and they are therefore not lost to the atmosphere and cause air pollution. ILs are considered to be polar phases with the solvent properties being largely determined by the ability of the salt to act as a hydrogen bond donor or acceptor and the degree of localization of the charge on the anions. Furthermore, it was found that increasing the chain length of the alkyl substituent on both the cations and the anions leads to greater lipophilicity of the ILs (5). A recent study indicates that these liquids are more polar than acetonitrile yet less polar than methanol (6). These properties are important in for the proposed work in view of the fact that these are some of the aspects that need to be considered for the design of Microwave Assisted Extraction.

### 8.3.2. Advantages of Ionic Liquids

Some of the physical properties described in the previous section make ILs potentially interesting solvents with the following advantages that they are:

- Good solvents for a wide range of organic and inorganic materials allowing unusual combinations of reagents to be brought into the same phase
- Composed of poorly coordinating ions and hence they can be highly polar compounds
- Miscible with a number of organic solvents providing a non-aqueous polar alternative for two-phase systems
- Polar in nature (6); thus, coupling microwave energy to the solvent is possible. This is especially advantageous for the proposed work as discussed in later section.
- Non-volatile (due to negligible vapor pressure) thus can be used in high-vacuum processes and eliminate the containment problems faced by the current industrial solvents and thermally stable up to 200°C
- Recycled and reused, making them not only environmentally benign but also economically feasible

8.3.3. Current uses of Ionic Liquids

Ionic liquids are currently being used for a variety of processes. Interest in ILs has increased exponentially in the last couple of years as evidenced by an increased number of publications on this subject. ILs are used for organic reactions (Diels-Alder, alkylation, Friedel-Craft’s acylation (5), Stille coupling, etc.), catalysis (hydrogenation, hydroformylation, dimerization, Heck reactions (5)), syntheses (5) and separations (7, 8), along with the production of pharmaceutical compounds (9) and in a number of other processes and applications.

8.4. Microwave Assisted Extraction (MAE)

Microwaves are electromagnetic radiations, commonly used for heating and cooking food. Recent industrial applications of microwaves include materials processing, waste remediation and organic synthesis. Some of the criteria that an extraction technique needs to meet are: the ability to quantitatively extract analytes from any matrix, reproducibility, usage of minimum amount of hazardous solvents and being cost effective. MAE is able to meet most of the above criteria. Microwaves are now also used for the extraction of organic compounds from a variety of matrices. Interest in this technology is growing rapidly as evidenced by the increase in the number of publications.
8.4.1. Theoretical aspects (10, 11)

Microwaves are high-frequency electromagnetic waves located between radio frequency and the infrared regions of the electromagnetic spectrum. The microwave region of the electromagnetic spectrum corresponds to wavelengths between 0.1 cm and 1 m or frequencies between 300 MHz to 300 GHz respectively. Normally, the application of microwave ovens for domestic and scientific use is restricted to 2450 MHz. The heating effect in MAE is due to dielectric polarization (i.e. the displacement of opposite charges). While this polarization is due to a number of factors, only two are of any importance in MAE, namely, dipolar and interfacial polarization.

The microwave energy affects molecules by ionic conduction and dipole rotation. In ionic conduction, the ions in solution migrate when an electromagnetic field is applied. The solution's resistance to this flow of ions results in friction and, thus, heating of the solution. Dipole rotation is the realignment of the dipoles with the applied field. At 2450 MHz, the dipoles align and randomize $4.9 \times 10^9$ times per second; this forced molecular movement results in molecular “friction” and, thus, heating of the solution.

The polarizability of a molecule is represented in terms of the dielectric constant, $\varepsilon'$. This term, $\varepsilon'$ can be related to the dielectric loss, $\varepsilon''$ which is a measure of the efficiency with which the energy of the electromagnetic radiation can be converted to heat by considering $\tan \delta$, the dissipation factor, given by the equation,

$$\tan \delta = \frac{\varepsilon'}{\varepsilon''}$$

(4)

It is possible to estimate the ability of the microwave to couple to an organic solvent by considering $\varepsilon'$ values. In contrast to conventional heating where the heat penetrates slowly from the outside to the inside of an object, microwave energy produces in situ as heating takes place by dielectric loss. Therefore, the primary heating appears in the core of the molecules that are being irradiated, and the secondary heating results as this heat spreads from the inside to the outside of the body. In addition, because the MAE vessels are sealed, it is possible to achieve higher solvent boiling temperatures than are possible
under normal atmospheric conditions. The increase in solvent boiling temperature of as much as 100°C can result while rate of extraction doubles every 10°C, which should lead to increased extraction efficiency in a shorter interval of time (Refer to equation 5).

The extraction process can be treated as a thermodynamic equilibrium system. Hence it is possible to calculate the partition coefficient (K) of the extraction process. The partition coefficient is decided by the free energy (ΔG) of the process of solute molecules being extracted from the matrix into the solution (12).

\[
K = \exp\left(\frac{-\Delta G}{RT}\right) \tag{5}
\]

where,
- \(\Delta G\): Free energy of the system
- \(T\): Temperature

Determination of the partition coefficient for the analyte under study will make it possible to predict the kinetics of extraction and design the process more efficiently.

### 8.4.2. Advantages of MAE

Some of the most obvious advantages of MAE include:
- Rapid sample preparation,
- Simultaneous multiple-sample processing
- Increased accuracy and precision resulting from minimized sample manipulation as well as increased operating temperatures
- Reduced overhead costs due to appreciably lower consumption of solvents and multi-sample processing.

These benefits make MAE an environmentally friendly and economically feasible process. Use of ILs in conjunction is projected to be advantageous. Their polar nature makes coupling of microwave energy possible. This enables the analyst to use a solvent based on the solute-solvent interaction, rather than use a co-solvent to absorb the microwave energy in case of microwave-transparent solvents. Secondary heating
technology is also not needed. An advantage of MAE is that the solvent consumption of MAE is considerably less compared to traditional methods of extraction. For example, in different studies, MAE consumes between 4-10% of the total solvent consumed using Soxhlet extraction. This is the most relevant advantage of MAE to this proposed work, as this makes it a green sample preparation process.

8.4.3. Current uses of MAE
Microwave assisted extraction is being used for applications that include the extraction of additives from polymers (13), antinutritive compounds from plants, crude fat from food products, polycyclic aromatic hydrocarbons and pesticides from soil as well as for organic synthesis(14-16).

8.5. Proposal

8.5.1. Synergistic coupling of concepts
From the previous discussion, it can be seen that the synergistic linking of these two concepts (ILs and MAE) provides a distinct advantage over traditional methods. Both concepts are environmentally friendly processes and employing them in a complementary fashion is expected to increase the “greenness” of the entire procedure. The proposal uniquely integrates the use of ILs for MAE. The excellent precedents for each of these concepts individually lead one to have a healthy level of confidence in the feasibility of this project. The goal of this proposal is to perform microwave assisted extraction using ionic liquids as the extractants and thereby highlight the significance of the profitable value of combining these two essentially green techniques. To the author's knowledge, currently there are no publications on the use of microwave extraction using ionic liquids.

8.6. Experimental (Materials and Methods)

Reagents:
Methyl imidazole (ICN 151655901) Iodomethane (AA3187636) and 1-chlorobutane (MCX09153) were obtained from Fisher Scientific, Fairlawn, NJ.
Acetonitrile (LCMS) and Methylene chloride (GCMS) were used for analysis. All solvents were Optima Grade obtained from Fisher Scientific, Fairlawn, NJ.

Matrix:
The preliminary phase of extraction was carried out on David Lineman’s sediments from Lowellville River, OH. The sediments were sampled from the river as well as the riverbank. The second phase of the study uses medications available over the counter for the extraction of ingredients, mainly acetaminophen and caffeine.

Standards:
- Semi-Volatile Mix 92408 (nominal concentration of 1000 µg/ml in methylene chloride) from Absolute Standards, Inc., Hamden, CT
- EPA Method 620 Diphenylamine 70314 (nominal concentration of 1000 µg/ml in methanol) from Absolute Standards, Inc., Hamden, CT
- Base/Neutrals Surrogate Standard Mixture, ISM-280N (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Semi-Volatiles GC/MS Tuning Standard GCM-150 (nominal concentration of 1000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Semi-Volatiles Internal Standard Mixture US-108N (nominal concentration of 4000 µg/ml in methylene chloride) from Ultra Scientific, North Kingstown, RI
- Acetaminophen and caffeine standards were obtained from Fisher Scientific, Fairlawn, NJ.

Certified Reference Material:
Natural Matrix Certified Reference Material, PAH Contaminated Soil/Sediment CRM104-100 (individual concentrations on file from Certificate of Analysis for Lot No. CR912) from Resource Technology Corporation (RTC), Laramie, WY

Microwave Instrument and Apparatus
Apparatus and filters were obtained from Milestone, Inc., Shelton, CT. Ethos 900 was the microwave used for this study. Ethos Lab Station is a microwave mode stirrer to ensure a homogeneous field within the microwave cavity for even heating of all samples. Continuous stirring of solvent/sample and immiscible phases eliminates sample clumping and achieves uniform temperature inside vessels. The system is equipped with a dual magnetron with an ATC-400 FO Fiber-Optic Temperature Control, QPS-3000 Solvent Sensor and an ASM-400 Magnetic Stirring for homogenous mixing in every vessel.

**Solid Phase Extraction:**
The following SPE cartridges were used for this project: Discovery®DSC-8 SPE Tube (2g, volume 12ml, 52717U); Discovery®DSC-18 SPE Tube (2g, volume 12ml, 52607U); Supelclean™ LC-4 SPE Tubes (500mg, volume 3ml, 57089). All SPE tubes were obtained from Supelco, Bellefonte, PA.

**SPE GC/MS Analysis:** (This part of the project was done in collaboration with David Lineman, Hermitage, PA). GC/MS analysis was carried out on Agilent (HP) 5970B (courtesy: Mr. David Lineman, Hickory High School, Hermitage, PA). A 1-μl volume of the aliquot was directly injected into a Hewlett Packard 5890 GC. A Hewlett Packard 5970B MSD was with a source temperature at 325°C to monitor the analytes. Data were collected by a HP ChemStation Software. 5-point calibration curve was used for quantitation purposes.

**HPLC-UV and LC/MS Analysis:** To determine the $\lambda_{max}$ of acetaminophen and caffeine, 0.1µg/ml sample in acetonitrile was scanned on Cary 3 double beam absorption spectrophotometers, 200-900 nm range, with computer control. Waters HPLC (Waters, Milford, MA) was used for this study equipped with a Waters 600 quaternary gradient system with manual injector, helium sparge degassing, and a Waters 2487 dual wavelength detector. For LC/MS: Waters LCMS - Waters Alliance 2695 pump with an auto-injector with a Micromass ZMD MS equipped with Waters 2487 dual wavelength detector was used.
8.6.1. Preparation of Ionic Liquid

The ionic liquid employed for the proposed work and applications is 1-butyl-3-methylimidazolium chloride, denoted as [bmim][Cl]. This IL is chosen mainly because of its stability on exposure to air and water (moisture). It is water miscible in nature, which is a very useful characteristic to have for the analysis described within the context of this study. Since the viscosity of the liquid is high, it was decided that we would instead use a 1:1 v/v mixture of the ionic liquid with water. The viscosity of this mixture was much less, making the “solvent” easier to use. The following procedure was modified from methods found in literature (17, 18). Equimolar 1-chlorobutane and 1-methylimidazole are placed in a round bottom flask and inserted into the microwave cavity. The reaction mixture will be exposed to microwave radiation at 120 to 240 W of power for 60 seconds followed by cooling for 30 seconds. Stirring is used to avoid localized heating. This cycle will be repeated for about 15 minutes. Upon microwave radiation, the ionic liquid begins to form, increasing the polarity of the reaction medium. This, in turn, increases the rate of the microwave absorption by the IL. The formation of IL can be measured visually as the mixture turns from clear to opaque to clear once again.

The resulting viscous liquid will be allowed to cool to room temperature and then washed three times with ethyl acetate to remove traces of starting material. After the last washing, the remaining ethyl acetate will be removed by heating to 70 °C under vacuum.

To prepare the ionic liquid, hexafluorophosphoric acid (1.3 mol) is added slowly to a mixture of 1-butyl-3-methylimidazolium chloride (1 mol) in 500 ml of water. After stirring for 12 h, the upper acidic aqueous layer is decanted and the lower ionic liquid portion washed with water (10 × 500 ml) until the washings are no longer acidic. The ionic liquid is then heated under vacuum at 70 °C to remove any excess water.

8.6.1.1 Modifications

A significant advantage of using ionic liquids as solvents is that they can be designed for the solvation of the desired compound. The R group in the figure below can be changed for the desired physical and chemical characteristics. For example, imidazolium cations,
such as those commonly used in preparing ionic liquids can easily be derivatized to include task-specific functionality. Metal ligating groups when used as part of the solvent or doped into less expensive ionic liquids, dramatically enhance the partitioning of targeted metal ions into the ionic liquid phase from water; the strategy of preparing task-specific ionic liquids is applicable to a wide range of designer solvent needs. In addition, the miscibility of organic compounds can be varied easily and extensively by altering the chain lengths of the alkyl substituents on the cations. Thus, ionic liquids are rightly termed as "designer solvents".

8.7. Microwave Extraction

8.7.1. Experimental protocol
Extraction protocol for microwave assisted extraction is different for each application and will depend on the solvent, its boiling point, the analytes of interest etc. In general, the system is ramped to the desired temperature in 5-7 minutes and held at that temperature for 15-25 minutes. Individual protocol will need to be optimized for maximum extraction efficiencies. The solvent employed in this project is an ionic liquid, which is polar by nature (dielectric constant of the solvents are not investigated, however, polarity and solvent strength has been confirmed to lie between acetonitrile and methanol) (6, 20) and hence [bmim][PF₆] will absorb microwave radiation. This eliminates the need of using either a polar co-solvent or moisture to absorb the microwaves or the use of secondary-heating techniques to circumvent the heating problems of non-polar solvents.

8.7.2. Equipment configuration
All experiments proposed can be carried out without any modification to the microwave apparatus. However, sample handling can be made easier with the configuration described herein.

The microwave apparatus used in this proposal (Ethos 900) is housed in the Dept. of Chemistry & Biochemistry, Duquesne University. It was obtained from Milestone, Inc., Monroe, CT. Teflon carousel tray equipped with Teflon shaft is used as provided by the manufacturer. The following will be fashioned from Teflon: a ring (of diameter so as to
fit the Teflon shaft) with radial arms. These radial arms end in Teflon rings. These end rings will serve as holders for separatory funnels made of glass. The radial arms and end rings will be detachable to enable use of separatory funnels of varying capacities. Separatory funnels are used since they are commonly available and are economical. This design will therefore retain the multiple-sample processing capacity of the microwave. Post-extraction processing will be tailored to the specific application and end-use.

8.7.2.1 Preparation of the Ionic Liquid

By choosing the right mix of anion and cation, we can design an array of solvents with different physico-chemical properties that can be used in a wide spectrum of applications, consequently making green chemistry easily adopted by the practicing chemist.

![Figure 6 Synthesized IL (conventional procedure)](image)

1-butyl-3-methylimidazolium hexafluorophosphate\(^{1,2}\) is a particularly popular ionic liquid because of its stability on exposure to air and water (moisture). We initially embarked on synthesizing this ionic liquid for use in extraction. But due to its water immiscible nature, it was not compatible with extraction protocols that used water. However, the chloride precursor proved to be soluble in water and an excellent medium for extraction. (Figure 5).
8.7.2.2 Synthesis

The synthesis followed an established protocol. 3 1-butyl-3-methylimidazolium chloride was prepared by the reaction of equal molar amounts of 1-methylimidazole and chlorobutane in a round-bottomed flask fitted with a reflux condensor by heating and stirring at 70°C for 48-72 hours. The resulting viscous liquid was allowed to cool to room temperature and then was washed three times with 200 ml portions of ethyl acetate. After the last washing, the remaining ethyl acetate was removed by heating to 70°C under vacuum. To prepare the ionic liquid, hexafluorophosphoric acid (1.3 mol) was added (slowly to prevent the temperature from rising significantly) to a mixture of 1-butyl-3-methylimidazolium chloride (1 mol) in 500 ml of water. After stirring for 12 hours, the upper acidic aqueous layer was decanted and the lower ionic liquid portion was washed with water (10 x 500 ml) until the washings were no longer acidic. The ionic liquid was then heated under vacuum at 70°C to remove any excess water. We modified this procedure and adapted it to microwave synthesis on a small scale. We were able to reduce reaction time to only 30 min to form the 1-butyl-3-methylimidazolium chloride in quantitative yields.

8.7.2.3 Reaction Details

<table>
<thead>
<tr>
<th></th>
<th>Mass (g)</th>
<th>Mol. Wt.</th>
<th>Moles</th>
<th>Equivalent</th>
<th>Volume (ml)</th>
<th>Density (g/ml)</th>
</tr>
</thead>
<tbody>
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<td>82.11</td>
<td>0.0122</td>
<td>1</td>
<td>0.97</td>
<td>1.03</td>
</tr>
<tr>
<td>1-chlorobutane</td>
<td>1.13</td>
<td>92.57</td>
<td>0.0122</td>
<td>1</td>
<td>1.27</td>
<td>0.886</td>
</tr>
<tr>
<td>Hexafluorophosphoric Acid</td>
<td>2.32</td>
<td>145.97</td>
<td>0.016</td>
<td>1.3</td>
<td>1.40</td>
<td>1.65</td>
</tr>
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</table>

The ionic liquids synthesized were checked by proton NMR. The spectral data was a good match to that found in literature. 2,4
Following the successful syntheses listed above, the next synthesis attempted was a change in the alkyl group chain length from butyl to methyl, i.e., 1,3-dimethylimidazolium iodide was carried out (iodomethane replaces the 1-chlorobutane in the previous reaction—Figure 8). This IL was found to be solid at room temperature and therefore not as useful as the butyl-analog (Figure 9). It was also found to be miscible with water and methanol.

We have thus synthesized different ionic liquids. For our subsequent extractions, we chose 1-butyl-3-methylimidazolium chloride for its favorable properties.

Figure 9. Synthesized DMIM Iodide

8.8. Results and Discussion

8.8.1. Preliminary Studies on compounds of environmental interest

<table>
<thead>
<tr>
<th>Compound</th>
<th>C-18 / DMIL</th>
<th>% Rec</th>
<th>C-8 / DMIL</th>
<th>% Rec</th>
<th>C-4 / DMIL</th>
<th>% Rec</th>
<th>C-4 / BMIL</th>
<th>% Rec</th>
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</thead>
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<td>Napthalene</td>
<td>104</td>
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<tr>
<td>Benz(b,k)fluoranthene</td>
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<td>Benz(b,k)fluoranthene</td>
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<td>-</td>
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<td>91</td>
<td>2,4,5-trichlorophenol</td>
<td>70</td>
<td></td>
</tr>
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</table>
The analytes selected were PAHs which have been discussed extensively in Chapters 4, 5 and 7. Two different ILs were evaluated: 1-butyl-3-methyl imidazolium chloride (BMIL) and dimethylimidazolium iodide (DMIL). All extractions were carried out using a 1:1 v/v ratio of IL/water to help with reducing the viscosity to an optimum level for operation. Table 2 comprises of data that were obtained when different cartridges were tried during the Solid Phase Extraction process to carry out a solvent exchange making it more suitable for GC/MS analysis. GC/MS analysis was preferred because it was hitherto not done, as well as because the analytes attempted have already been analyzed from the same matrix, using the same extraction instrument (Ethos 900), and so it was decided to keep the analytical instrument same so as keep all variables constant.
From the trial results obtained, it was found that C18 cartridges gave good recoveries for PAHs; however, suffered from a low phenol recovery. It was also found that extracts from C4 tubes showed higher recoveries for phenolic compounds. This can be explained since for C18, the longer alkyl chain length was favorable for the relatively non-polar PAHs. However, for polar phenolic compounds, C4 alkyl chain is short enough that secondary effects come into play in that phenols have intermolecular interactions with the silica moiety of the packing bed. Other results indicate that phenolic compounds show a lower precision value (which is predictable, as phenols typically give low precisions with a variety of extraction techniques.

Since C4 tubes give optimum results, the plots represented in Figures 10 and 11 show the recoveries obtained using C4 cartridges for both, BMIL and DMIL. The graphs were plotted based on analytes. Figure 8 depicts recovery of PAHs using the two ILs, while Figure 9 represents recovery of phenols using the same ILs. For both classes of analytes, BMIL shows the most optimal performance in terms of both, accuracy and precision. PAHs with lower molecular weights (e.g. naphthalene) show the best precision values (Error here is represented as 90%CL, n=3). DMIL shows better trend for compounds like phenanthrene, chrysene, etc., but since the recoveries exceed the expected values, no concrete conclusions can be made for these late-eluting higher molecular weight compounds. For phenols, however, the results were unequivocal: BMIL gave better recoveries than DMIL. Whether this phenomenon is due to the comparatively longer alkyl chain of BMIL as compared to DMIL, or whether there is a viscosity effect (DMIL viscosity is higher the BMIL), needs to be further evaluated. Also, the analytes were not a complete “match”, i.e., “like dissolves like” was not applied as the theoretical dielectric constant of the solvents is infinity, while the analytes chosen were relatively non-polar, especially in case of PAHs. However, these were preliminary results, and the study was undertaken to test the feasibility of the concept with the resources that were already available in the laboratory, both in terms of materials (solvents, SPE, etc.) as well as technical expertise. Once the concept was proven feasible, it was then extended to other compounds.
I would like to thank Mr. David Lineman for his help with this project; his technical input as well as discussions.

8.8.2. Evaluation of the concept coupling using compounds of pharmaceutical interest

Two pharmaceutical compounds that are found commonly were used for this study, namely acetaminophen and caffeine (Figure 10). Acetaminophen belongs to the class of analgesic and antipyretic drugs, commonly used for pain-relief and to bring down fever. Unlike other pain-killers however, this drug is not used for relief from arthritic pain, stiffness or any kind of inflammation. When administered with caffeine, it proves to be a synergistic mixture, and hence usually available in the market in combination. Caffeine is an alkaloid and can be obtained from plant sources (It is also synthesized). It is classified as an analgesic adjunct. It is present in a variety of products that are consumed daily, like carbonated beverages, coffee, tea, chocolates, etc. Some of the effects include Central Nervous System (CNS), cardiac muscle as well as respiratory system stimulant, diuretic and reduction of fatigue.

This time, the two different ILs used were: 1-butyl-3-methyl imidazolium chloride (BMIL) and 1-butyl-3-methylimidazolium BF₄. Waters LC/MS was used for the analysis. The solvents were used in a 1:1 v/v IL/Water combination to bring the viscosity down to a more convenient level.

Calibration curves for acetaminophen and caffeine were carried for a mixture of acetaminophen and caffeine at concentrations of 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml. The calibration curves were obtained by plotting the peak area against the concentration for runs in triplicates to check for reproducibility and a linear
equation was obtained. The calibration curves were obtained for two different mobile phases.

- Mobile phase I
  A-0.01M Ammonium acetate, pH 2.8
  B-Methanol

- Mobile Phase II
  A-0.5% bmimCl in water, pH 3.9
  B-Methanol

Extraction of acetaminophen and caffeine was done at different concentrations using 50:50 water: [bmim]Cl as the extracting solvent. The results for the two different phases and two different analytes are as follows:

<table>
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<th>Table 44. Acetaminophen results for two mobile phases</th>
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<tbody>
<tr>
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<td>3</td>
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<td>4</td>
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</table>

Tables 3 and 4 as well as Tables 6 through 9 in the appendix are results for acetaminophen and caffeine respectively. There does not seem to be an appreciable difference in the results between the two different mobile phases for acetaminophen, except for the extractions at high concentrations (200 mg/ml). Precision values are very high (low error) for all the results obtained. Also recovery at high concentration was not 100% and extraction and therefore, chances are, the solvent system is getting saturated at that concentration (solvent contains 50% water, and neither of the analytes are miscible to any appreciable degree in water). Caffeine on the other hand shows better consistency with accuracy. However, there does not seem to be consistency with precision values. Again, at high concentrations, there appears to be a saturation effect.
This project is in process and forms the focus of Ms. Pallavi Deshpande’s dissertation. 

*I wish to thank Pallavi Deshpande for the collaborative effort on this project.*

8.9. Conclusions

We attempt to contribute positively to the environment by developing analytical methods that will require lower levels of solvent usage. The fast-growing field of green chemistry has taken a keen interest in ionic liquids, its various forms and applications, as an important tool in developing “greener” ways of doing everyday chemistry. Ionic liquids are excellent candidates for the replacement of organic solvents in various facets of chemistry as well as other branches of science. The interest in ionic liquids is growing exponentially. The contribution of ionic liquids to Green Chemistry in general and Green Analytical Chemistry in particular, is significant. Not only will it be possible to execute waste prevention concept, but also waste generation can be minimized since these benign solvents can be recycled easily and reused. The variety of applications for these solvents is incredible. The field is in comparative infancy and various fundamental principles of solute-solvent interactions, solvent extraction mechanisms, basic physical parameters and structures need to be explored. MAE has already proven to be a greener technique as compared to traditional methods of extraction. Chemistry, as well as the society at large, can thus reap the potential benefits of bringing together these two green techniques. The linking of these two concepts has not been investigated so far. This work will therefore attempt to fill this void by the combining these two concepts. We have conclusively shown MAE to be superior to conventional techniques in terms of cost-effectiveness, time and environmental-friendliness. We aimed to couple MAE with ionic liquids to create an even better tool for green chemistry by harnessing the synergy of two environmentally-friendly techniques. The preliminary results presented herein show promise, and this project needs to be explored further.
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8.11. References


(20) Roberts, Nicola. Personal Communication


(23) Dr. Gary Lye, Personal Communication.


8.12. APPENDIX

Mechanism of Reaction (Proposed)

Table 5. Results for DMIL using C4

<table>
<thead>
<tr>
<th>C-4 / DMIL</th>
<th>Replicate 1</th>
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Table 46. Results for BMIL using C4

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Table 47. Acetaminophen Results for Mobile Phase 1

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<th>Concentration recovered (x)</th>
<th>SD</th>
<th>95%CL (n=4)</th>
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<td>0.693</td>
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<td>0.052</td>
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<tr>
<td>100 mg/ml</td>
<td>0.962</td>
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<td>0.012</td>
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<tr>
<td>150 mg/ml</td>
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Table 48. Acetaminophen Results for Mobile Phase 2

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<th>95%CL (n=4)</th>
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Table 49. Caffeine Results for Mobile Phase 1

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<th>95%CL n=4</th>
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<td>1.273</td>
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<td>0.151</td>
</tr>
<tr>
<td>100 mg/ml</td>
<td>2.182</td>
<td>0.062</td>
<td>0.058</td>
</tr>
<tr>
<td>150 mg/ml</td>
<td>2.540</td>
<td>0.447</td>
<td>0.438</td>
</tr>
<tr>
<td>200 mg/ml</td>
<td>3.004</td>
<td>0.040</td>
<td>0.107</td>
</tr>
</tbody>
</table>
Chapter 9

9. Summary and Conclusions

9.1. Synopsis

This dissertation has examined three aspects of analytical chemistry, namely environmental chemistry, clinical chemistry, and green chemistry, in the context of using microwave enhanced extractions in an integrated theme.

Chapter 1 included an introduction of sample preparation with a brief account of its history. It then discussed the analytical process and sampling process sequence. This was followed by a description of the stages of sampling, sample transport, storage and secondary sampling. Subsequently, a mention was made of the goals and objectives of sample preparation; analyte quantitation and sample preconcentration.

Chapter 2 began by introducing some background information about traditional and modern methods. Comparison of the merits and drawbacks of the aforementioned techniques was also given. It delved into the principles of extraction and the theory governing the process, and further explored some of the factors affecting extraction such as polarity of solvents and solutes. Many properties significant to extractions, such as the various intermolecular interactions and peripheral properties of solvents have been examined, and factors affecting solvent selectivity have been discussed. A separate section has been devoted to discussing the extraction theory as it relates to microwave heating. The hypothesis that was presented, as related to me by Dr. Kingston, is under evaluation. Based on experimental data from works published over the last few years, chemists have found that reaction rates can be faster than those of conventional heating methods by as much as 1000-fold. The temperature enhancements needed to increase the energy levels can be provided by microwave energy instantly. These instantaneous temperatures are very consistent with the temperatures that would be expected in a microwave system and are directly responsible for the reaction rate and yield enhancements. Thus, microwave heating greatly expands the options for extraction in a
variety of fields including environmental, clinical, pharmaceutical, and food industries. Some of these applications are discussed in this dissertation in the following chapters.

**Chapter 3** was an extension of Chapter 2 in the context of specific form of extraction, viz., microwave extraction. This chapter focused on microwave assisted extraction and examined in detail all the factors affecting the extraction. Further, there was given a description of the theory behind microwave energy including dielectric loss, effects on dipole rotation like relaxation time and sample viscosity. The effects of sample size on heating were examined, as well as effects of polarity and dielectric compatibility. The technique that was used for the entire length of this dissertation, Integrated Microwave Enhanced Extraction (IME) was introduced. The concept of IME shows promise to be a time saving method with the added advantages of being economical, safe and environmentally friendly process. The data that will be presented subsequently indicate equivalent recoveries for both classes of solvents (polar as well as non-polar) within a 95% confidence interval. Comparable accuracy with increased precision and enabling of a greener environmental extraction process will promote acceptance for IME. The need for the use of co-solvents was rendered not necessary. Also, the number of sample manipulation steps needed to be streamlined in an effort to decrease error due to potential sample loss. IME addresses these drawbacks. Occasionally, the recoveries are higher than the values reported on the CRM, with better precision. CRM values reported were on the basis of Soxhlet extraction.

**Chapter 4** initiated the experimental verification of some of the theory discussed in Chapter 3. Factors affecting microwave extraction like nature of solvent, analyte chemistry, time, sample size, nature of matrix and the effect of moisture on the efficiency of extraction were studied in detail and were presented in Chapter 4. Microwave extraction and various evaporation systems were examined, and the optimization of parameters influencing microwave extraction were elucidated. A theoretical model for the temperature dependence of extraction was postulated. From the observation that the experimental results are in agreement with the theoretical model, it can be said that the
assumptions and approximation are reasonable and the simplified theoretical model can give a satisfactory prediction of the temperature dependence of recovery.

**Chapter 5** utilized some of the optimization discussed in Chapter 4 towards the implementation of this optimized technique and verification of the possibility of replacing prescriptive methods with performance based methods for environmental monitoring compliance. A description of the project was followed by a discussion on the design of the study as well as the design of the experiments. Both methods were studied individually, with results presented in that order. Some of the variables that were discussed included the comparison of two different methods (prescriptive vs. PBMS), comparison of extraction efficiency with a change in the polar nature of the solvent, the presence of moisture in different types of matrices (natural or spiked), as well as the method quality control data. Although limited in size and scope, this study begins to answer some of the questions related to the technical feasibility and implementation of PBMS. Data quality is dependent on the types of analyte and matrix, as well as the analytical method. Although PBMS approaches could improve the quality of environmental monitoring data, better data may not always be needed. PBMS approaches hold promise due to the following factors: They are time-saving, labor-saving, saving on supplies such as solvents, cost savings, reduction in the amount of chlorinated solvents used, and increase in the safety factor by lowering potential exposure to hazardous substances, reduction in waste disposal costs, lessening environmental contamination. PBMS also encourages innovation. The chapter outlined the conclusions of the ACS/EPA study and closed with a comparison of the cost analysis of the two methods of compliance monitoring.

**Chapter 6** presented the clinical aspect of the dissertation. In this chapter, we examined in detail the sample preparation needed for analyzing biomedical samples like morphine containing matrices. Results using various extraction techniques like LLE, SPE, HPLC and others were compared. We examined the unique physico-chemical and pharmacological properties of opiates, opioids and other synthetic narcotic analgesics. This chapter also described the ADME (Absorption, Distribution, Metabolism, Excretion)
profiles of morphine and some related narcotics like heroin and codeine (within the framework of morphine) as well as the chemistry of the above mentioned compounds. The various parameters involved in microwave extraction of these analytes from their biological matrices were examined and optimized.

Chapter 7 presented some of the applications of microwave enhanced chemistry in the form that has been studied so far. We have examined the development of IME, optimized the factors affecting the extraction, and examined the process integration. We have studied in depth the science of sample preparation; we have studied the theory of traditional microwave extraction, related it to integration microwave extraction, as well as the theory of solvent extraction. We have examined how various factors affect the efficiency of extraction. After the evaluation of these factors, we optimized the parameters for the most advantageous extraction recoveries using different analytes. We have applied IME towards checking the feasibility of using performance based method system for compliance monitoring as opposed to prescriptive methods. The results of this study have corroborated our optimization protocols and have validated IME as a feasible option for the extraction of a variety of analytes. The results of the study have also provided invaluable information which helped us to further optimize the IME technique. This final version resulted from a confluence of our understanding of the theoretical basis of microwave extraction and real-world application of this concept. This honed tool to improve of optimized IME was then applied to different analytes, environments and products.

In its final form, we wanted to use IME for solving some analytical/extraction problems or for improving the efficacy of existing procedures. The following were the applications of IME that were successfully attempted:

- Extraction of additives from polymers
- Extraction of pesticides and integration of equipment
- Fat from food products
- ACS meat and cheese application
Chapter 8 represented the green chemistry aspect of the above technique. We have examined the role of IME in green chemistry by using ionic liquids for extraction. We studied the background of green chemistry, ionic liquids and IME. The contribution of ionic liquids to green chemistry in general and green analytical chemistry in particular, is significant. Not only will it be possible to execute waste prevention concept, but also waste generation can be minimized since these benign solvents can be recycled easily and reused. MAE has already proven to be a greener technique as compared to traditional methods of extraction. Chemistry, as well as the society at large, can thus reap the potential benefits of bringing together these two green techniques. The linking of these two concepts has not been investigated so far. This work will therefore attempt to fill this void by the combining these two concepts. We have attempted to harness the synergy between IME and ionic liquids to develop an environmentally friendly analytical technique that was tested on compounds of pharmaceutical interest.

9.2. Publications and presentations

Some of the publications and presentations that we have authored and/or presented from the work described herein are as follows (four manuscripts are in various stages of preparation):


“Microwave Enhanced Sample Preparation” General, Analytical and Environmental Applications” Lab Manual, ACS Short Course Presented by H. M. Kingston August 2004, Pittsburgh, PA.

9.3. Final Remarks

The field of environmental analysis has reached a stage of development that is challenging and promising. More refined environmental analysis methods are required to tackle increasingly complex problems, necessitating the development of innovative approaches and state of the art tools. Changes in the sensitivity and types of environmental monitoring have increased our knowledge about the nature of the chemicals around us and our understanding of its complexity. The unintentional (as well
as the occasionally intentional!) contamination of the environment requires the continuous monitoring of our surroundings. Increasing regulation in the interest of consumer protection will continue to require monitoring for a broad range of pesticides, insecticides, fungicides and herbicides as part of the new approach to the evaluation of active substances in plant protection products. We are still concerned with the traditional environmental contaminants such as organochlorine and other semi-volatile and volatile organics. The developments are particularly valuable for environmental analysts who must incorporate novel approaches and technologies to enhance the scope and efficiency of their analyses. The approaches followed now are more elegant, the data gathering has become easier, the detection limits lower, and the instrumentation is more advanced and powerful. In the past, there were many attempts to speed up sample preparation and analysis. What had been a wish has become a reality. With a surer control of selectivity, specificity, levels of detection and modes of analysis, more and different determinations are possible.

The use of microwave energy in sample treatment has attracted growing interest in the past few years. Initially, it was applied to the mineralization of samples. In recent years, numerous applications have reported the use of microwaves for assisting the extraction of organic compounds from various matrices. The emergence of commercial microwave systems which are specifically designed for extraction is rather recent, and has encouraged renewed interest in the technique. Thus, in the past few years, numerous compounds have been extracted by microwave-assisted extraction (MAE) from several matrices, with special emphasis on environmental applications.

Numerous applications have been reported, with special emphasis on environmental matrices in the recent years. Hence, several classes of compounds (such as PAHs, PCBs, pesticides, phenol compounds) have been extracted efficiently from a variety of matrices (mainly soils, sediments, animal tissues, and food products), either spiked or containing native compounds. All the attempted applications have shown that microwave-assisted extraction is a viable alternative to conventional techniques for such matrices. Comparable efficiencies have been reported along with acceptable reproducibility. In
addition, MAE offers a great reduction in time and solvent consumption, as well as the opportunity to perform multiple extractions. The emergence of commercial systems, affords a high level of safety. Additionally, evidence has also been presented in literature that MAE may compete favorably with recent techniques, namely supercritical fluid extraction and accelerated solvent extraction. In particular, optimization of MAE conditions is rather easy, owing to the low number of parameters (i.e., matrix moisture, nature of solvent, time, temperature, etc.) as compared to some of the other more recent techniques like SFE. Using traditional MAE, less selectivity may be achieved using MAE, so a cleanup procedure was required before chromatographic analysis.

For clinical applications, the methodology of separating and isolating drugs from biological matrices is frequently of crucial importance. In general terms, analysis of body fluids for drugs of abuse takes place in two different environments: the clinical setting (therapeutic care) and the consequence setting (e.g., forensic medicine). In clinical practice, the analytical result is an important step in a series of factors that affect the decision-making process and must be assessed as a complement to the patient–physician relationship. Quick turnaround times for isolation and analysis are always appreciated in a clinical/hospital milieu, both for the patients as well as for the medical staff to aid in making decisions. The pharmaceutical industry, on the other hand, requires quick turnaround times during the Quality Control/Quality Assurance steps in the processing of medications. Rapid sample preparation and analysis in a pharmaceutical setting ensures faster movement of the finished products in the assembly line, and therefore lesser waste of time, resources and personnel. In the light of this, utilization of IME to clinical and pharmaceutical applications was successfully attempted.

IME was designed to solve selectivity problem. This also addressed the analytical issues related to non-selectivity of extractions. Since sample handling is decreased, sample manipulation and loss of analyte are minimized. It appears that the use of MAE in analytical laboratories should increase in the next few years, especially owing to the reasonable cost of the equipment. It is important to consider some significant factors: capital cost, operating costs, requirements for method development, environmental
impact and level of automation. It is likely that a cost-benefit analysis of the instrumental techniques might well enable the user to improve sample throughput and reduce solvent consumption (and subsequently, disposal costs). Since the entire operation is performed in closed cavity, exposure of the analyst to hazardous chemicals is considerably minimized. The low level of solvent consumption also makes this technique “easier” on the environment.

Green or Sustainable Chemistry is an umbrella concept that has grown substantially since it fully emerged a decade or so ago. Green Chemistry is the design, development and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment. The continued use of large quantities of organic solvents as liquid media for chemical reaction, extraction and formulation is a major concern in today’s chemical processing industry. The perceived harmful effects of these chemicals on human health, safety and environment combined with their volatility and flammability has led to increasing pressure for minimizing their use. One of the principles of green chemistry is to design safer chemicals (fallout of the tenet, prevention of waste is better than generation and treatment of waste). Thus, we see the emergence of ionic liquids as a replacement for conventionally used solvents. We have combined the concept of the use of ionic liquid as extraction media with microwave extraction, expecting that the effect is synergistic and positive. Preliminary results are encouraging, and this promises to be the one of the future trends of this interesting and stimulating field.

Thus, new mechanisms of MW are embodied in the microwave effect that is emerging. The microwave effect has been hypothesized and that the mechanisms are still being refined and are different and unique from conventional heating. This practical, automated and integrated concept for extraction is still a growing dynamic and a recent innovation.