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### Analysis of Cannabinoids in Vitreous Fluid

Haley Berkland

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## Abstract and Introduction

- Cannabis is the most commonly abused illicit drug in the world
- In the U.S., 33 states have legalized medicinal marijuana use and 11 states and the District of Columbia have legalized recreational marijuana use
- As medical and recreational uses of this substance become increasingly legal, there is a need for reliable analytical methods that can detect and analyze cannabinoids in death cases where there is a question relating to the cause and manner of death when typical matrices are not available
- Vitreous humor is a gelatinous fluid located in the eyeball that is used as a matrix to test for drugs and alcohol
- In this study, a method was developed and validated to quantitate THC, 11-OH-THC, THCCOOH, and CBD in vitreous humor and whole blood utilizing a liquid-liquid extraction and analysis by LC-MS/MS

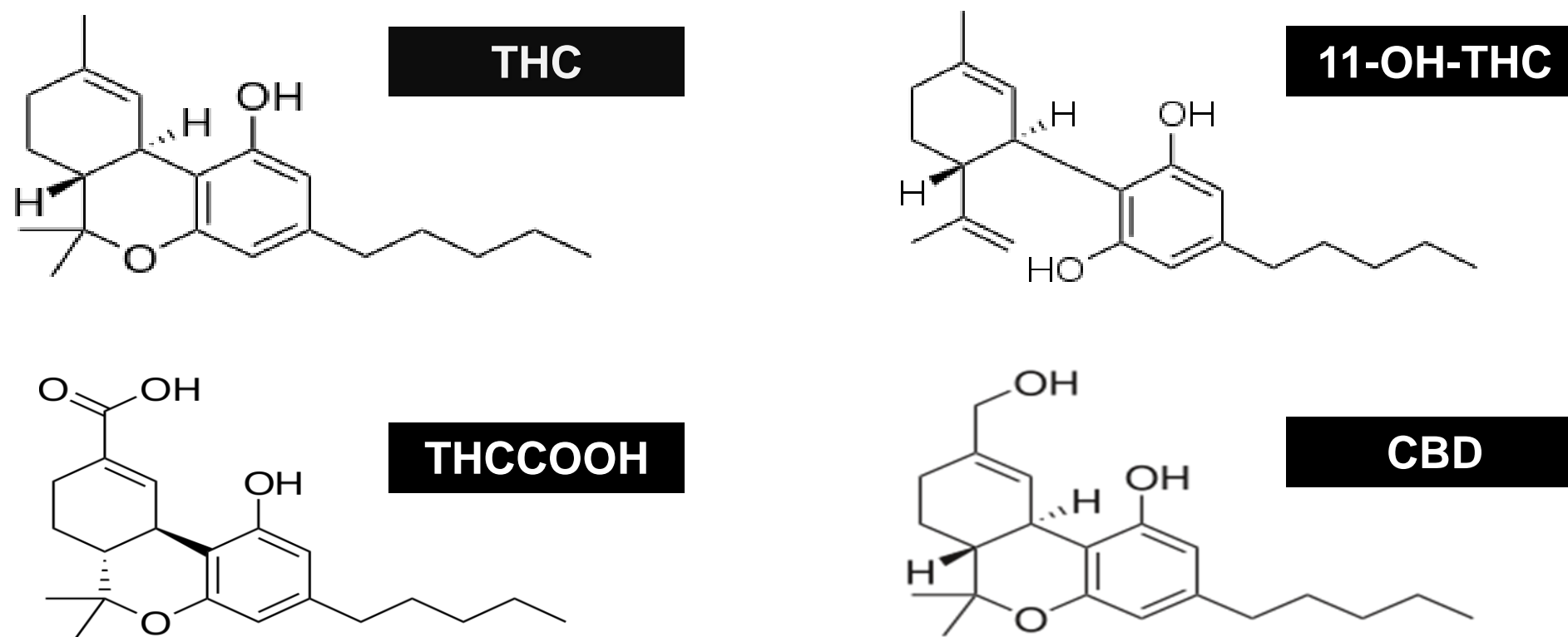
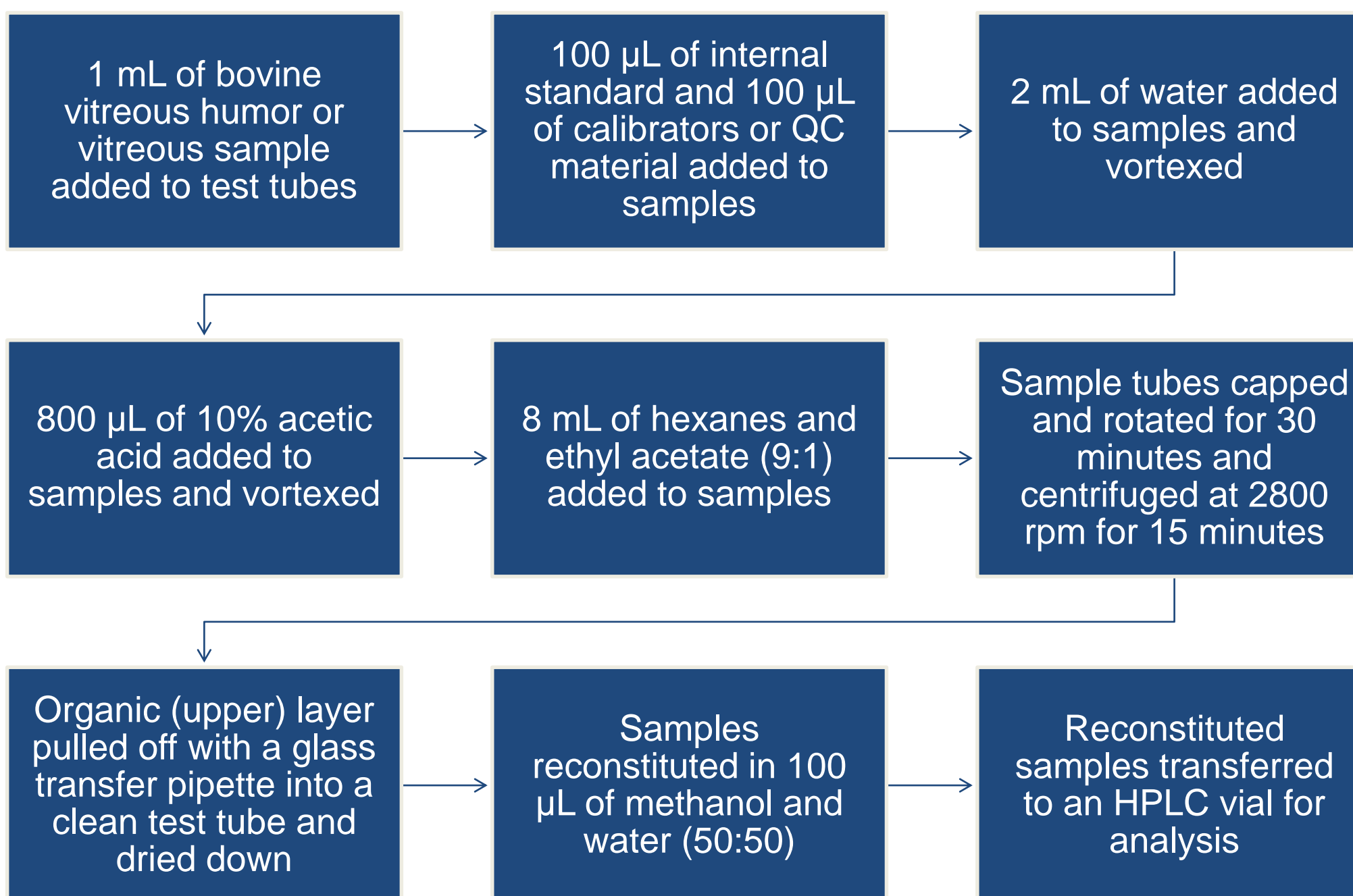


Figure 1. Cannabinoids included in developed method.

## Materials and Methods

### Liquid-Liquid Extraction



### Sample Analysis

- All analysis performed with a Phenomenex Kinetex C18 (2.1x100 mm, 2.6 µm particle size) with Vanquish High Pressure Liquid Chromatography System
- Identification/quantitation performed with a TSQ Endura QqQ Mass Spectrometer operating in selective reaction monitoring mode
- Gradient elution performed with acetonitrile (Mobile Phase A) and 0.1% formic acid in water (Mobile Phase B)
- SRM transitions optimized by direct infusion

## Materials and Methods (cont.)

- Table 1. Chromatographic elution gradient.

Time	%A	%B
-1	40	60
0	40	60
7	100	0
9	100	0
9.1	40	60

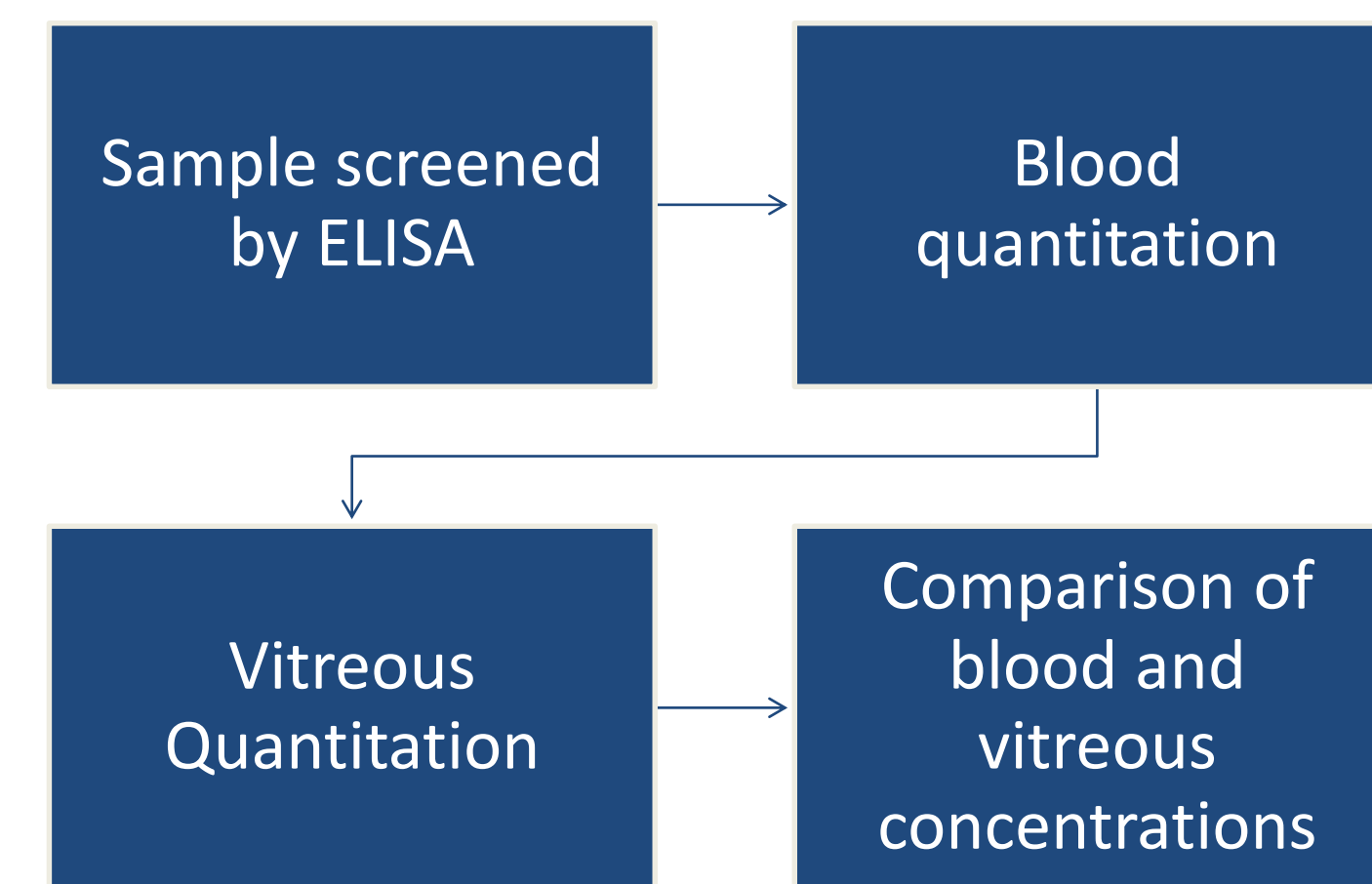
- Table 2: Optimized MS parameters.

Parameter	
Positive spray voltage (V)	4000
Negative spray voltage (V)	2500
Sheath gas	30
Aux gas	12
Sweep gas	2
Ion transfer tube temperature (°C)	375
Vaporizer temperature (°C)	425

### Method Validation

- Method validated according to SWG TOX validation guidelines
- Precision, accuracy, ion suppression, and interferences evaluated by various experiments
- Method was fully validated in vitreous fluid and cross validated into whole blood

### Comparison of Blood and Vitreous Concentrations



## Results

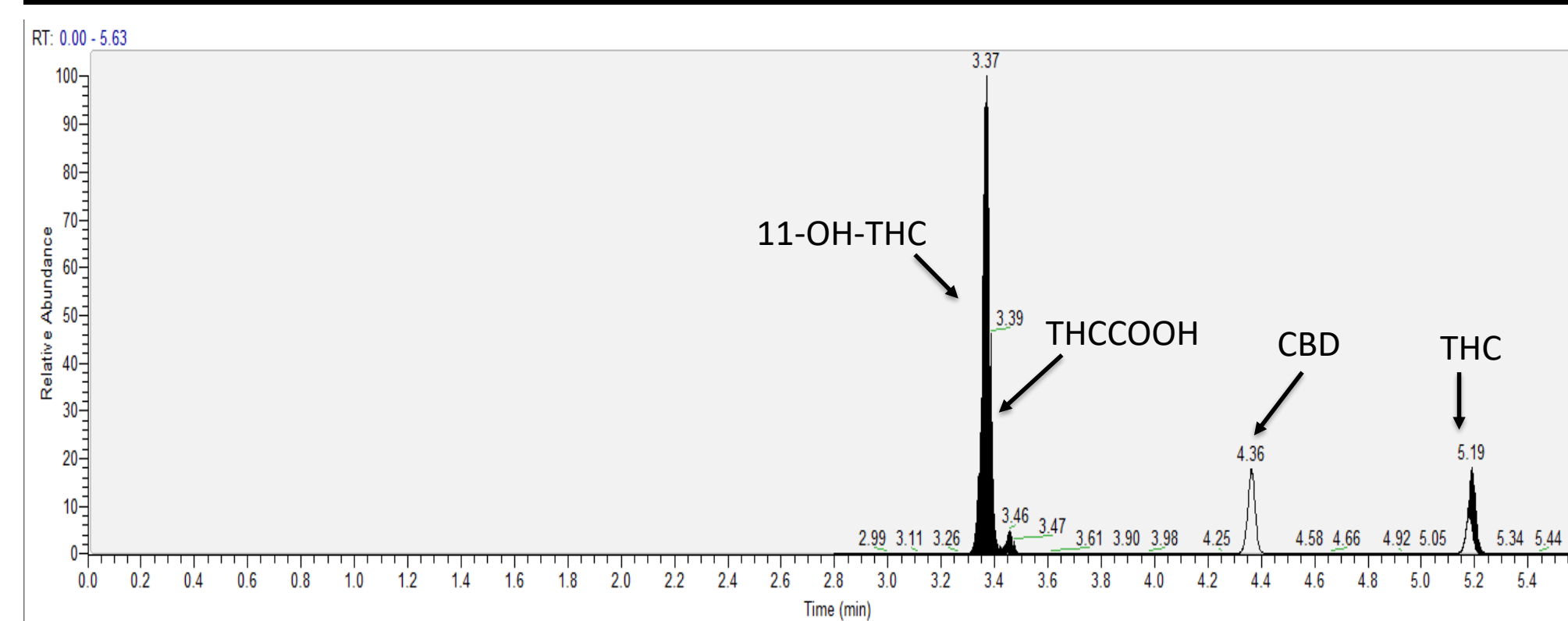


Figure 2. Total ion chromatogram of all four analytes using developed method.

### Validation Results

- Precision experiment showed that all analytes have a coefficient of variance within 20% of target concentration
- Accuracy experiment showed that analytes typically have an overall bias within 20% of target concentration
- Limit of quantitation (LOQ) for each analyte established based on lowest concentration at which analyte passes both precision and accuracy criteria

## Results (cont.)

- Table 3. LOQs for all analytes.

Analyte	LOQ (ng/mL)
THC	0.5
11-OH-THC	0.5
THCCOOH	5
CBD	0.5

- Ion suppression was required to be within ±25%
- All analytes fell above the upper limit of 25%
- Parallel studies performed to show that ion suppression not affecting quantitation

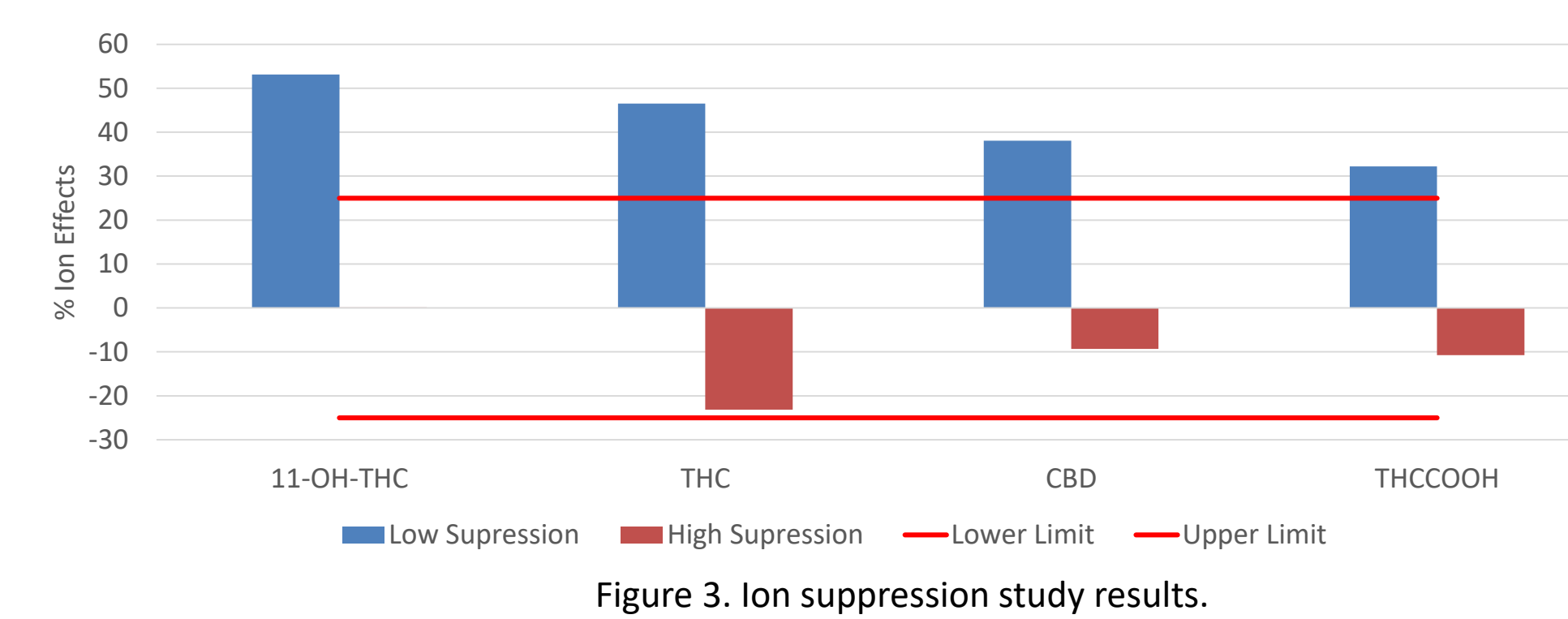


Figure 3. Ion suppression study results.

- Area count for each analyte required to be <25% of the LOQ for that analyte
- Interference seen from 11-OH-THC-d3 likely due to isotope of this analyte
- Parallel studies performed to show that ion suppression not affecting quantitation

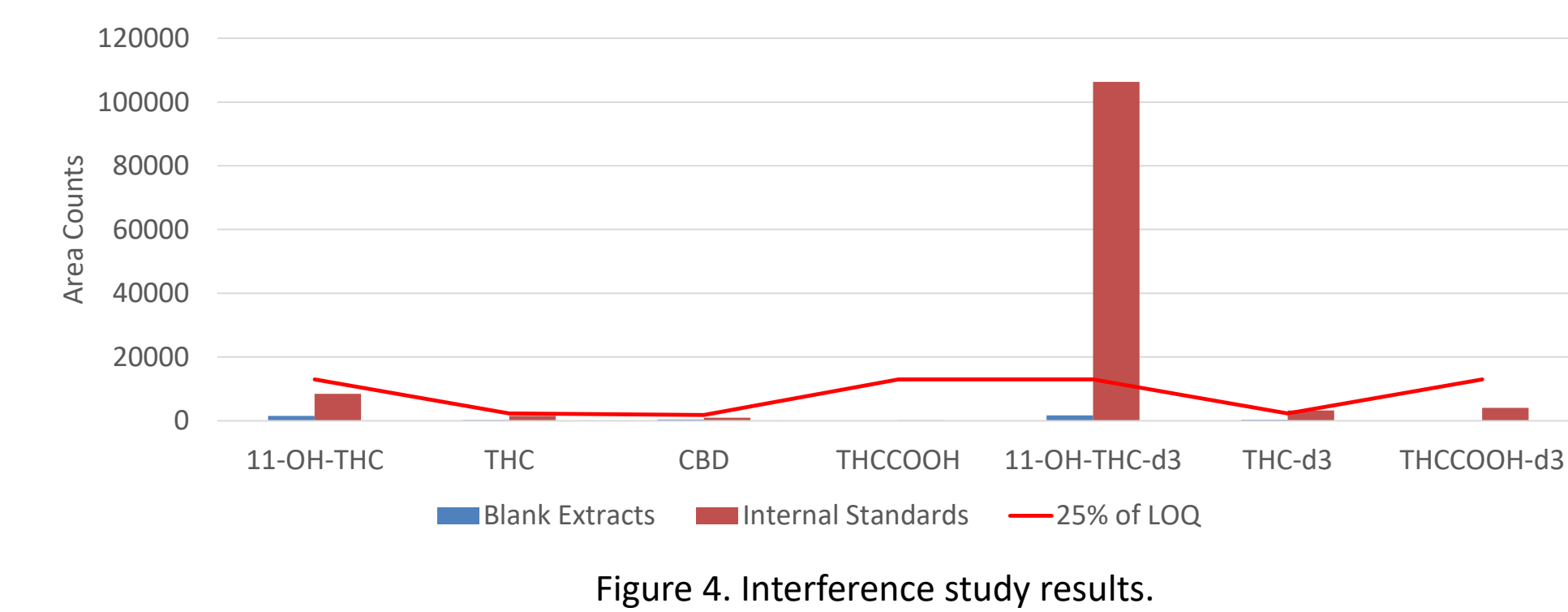


Figure 4. Interference study results.

- Method was tested for interferences with a fentanyl drug panel (FEN2), a hallucinogenic drug panel (HAL), a benzodiazepine panel (BZE), an antihistamine panel (AHS), and an antipsychotic medication panel (APS)
- All analytes within a 20% difference of target concentration in the presence of these other analytes

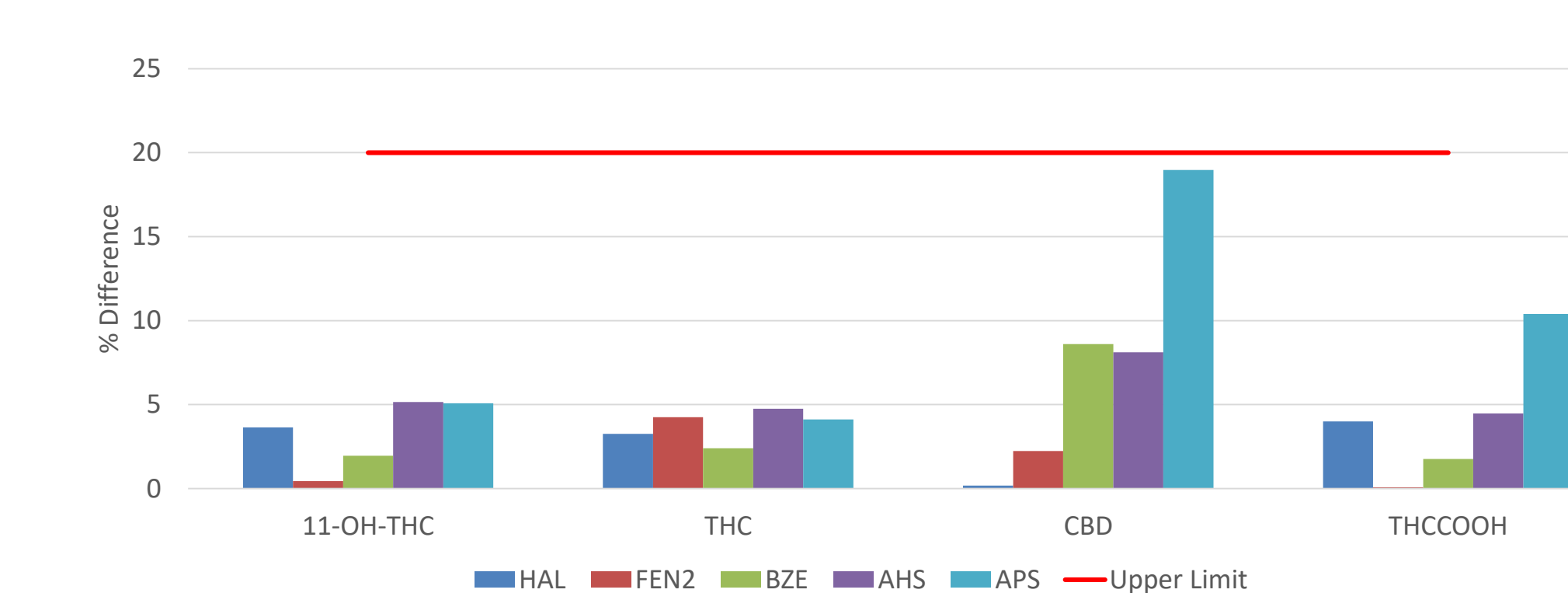


Figure 5. Cross panel interference study results.

### Comparison of Blood-Vitreous Concentrations

- Seven forensic casework samples extracted for comparison
- Blood samples showed amounts of THC (ranging from .71-8.3 ng/mL), 11-OH-THC (ranging from 0.51-1.7 ng/mL), and THCCOOH (ranging from 9.6-32.2 ng/mL)

## Results (cont.)

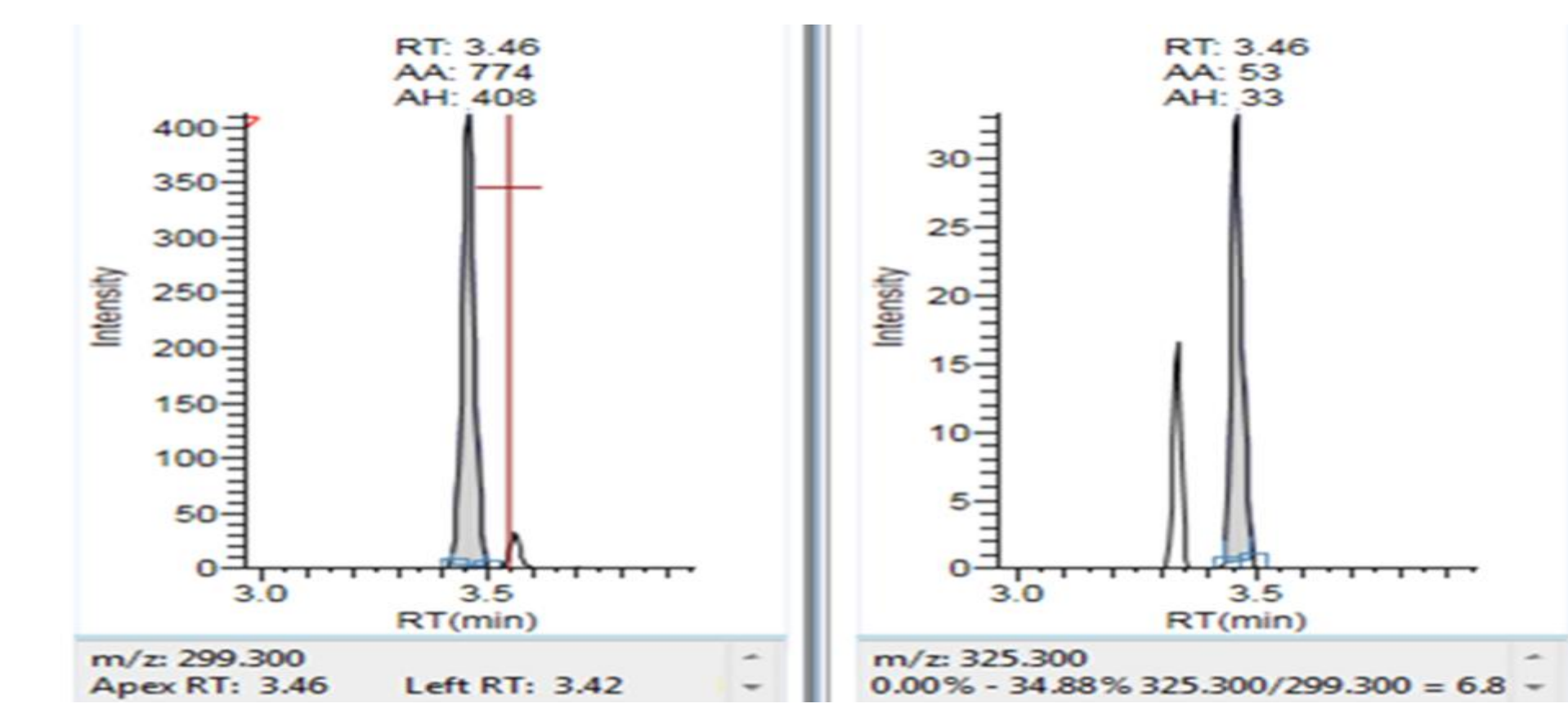


Figure 6. Quantitative ion peak (left) and confirmatory ion peak (right) for THCCOOH in vitreous case sample.

- In 5 of the 7 cases analyzed, trace levels of THCCOOH were detected in the vitreous sample below the LOQ (5 ng/mL)
- Possible to confirm that THCCOOH is present due to passing ion ratios and consistent retention times
- Blood and vitreous cannabinoid concentrations considerably different

## Discussion and Conclusions

- A method was developed and validated to quantitate THC, 11-OH-THC, THCCOOH, and CBD in vitreous fluid and whole blood by LC-MS/MS
- LOQs were typically 0.5 ng/mL with a measurement range reaching 250 ng/mL
- Agency issued proficiency test samples were tested using this method to confirm that the experienced ion suppression and interference were not affecting quantitation (parallel studies)
- Casework samples showed that blood samples testing positive for cannabinoids have a positive correlation for trace levels of THCCOOH in vitreous humor
- Low levels of cannabinoids in vitreous humor could be due to the compounds being mostly water insoluble or some other postmortem mechanism

## References

- United Nations Office on Drugs and Crime, World Drug Report 2016 (United Nations publication, Sales No. E.16.XI.7).

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