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Application Note

# Quantitative Analysis of THC and its Metabolites in Whole Blood Using LC-MS/MS for Toxicology and Forensic Laboratories

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*For forensic toxicology use only.*

## Abstract

This application note details the extraction of THC and its metabolites from whole blood samples using a novel SPE sorbent, Oasis PRiME HLB, in a µElution format for forensic toxicology applications.

### Benefits

- Faster, simpler sample preparation workflow compared to traditional SPE sorbents
- Efficient and consistent recoveries and minimal matrix effects
- No evaporation or reconstitution necessary with µElution plate format
- Linear, accurate, and precise results for all analytes
- Cleaner eluates with removal of over 99% of phospholipids


## Introduction

Cannabis continues to be a highly abused recreational drug. The increasing number of states legalizing it for medical use combined with the trend towards legalization for recreational purposes, means that there is a growing need for analytical methods for the quantification of Δ-9-tetrahydrocannabinol (THC) and its metabolites. Furthermore, a growing number of laboratories are interested in the quantification of THC and its metabolites in whole blood for toxicology and forensic purposes. The complex nature of whole blood introduces many unique challenges that must be addressed to achieve consistent and reproducible results.

Sample preparation is an important consideration for any bioanalytical LC-MS/MS method designed for forensic toxicology. Waters has developed a novel sample preparation sorbent, Oasis PRiME HLB, which is designed to provide several key advantages over traditional SPE sorbents. These include the ability to eliminate sorbent preconditioning and equilibration, creating a faster workflow compared to traditional SPE products. It also has the ability to remove more matrix interferences, particularly phospholipids, resulting in a cleaner extracts and reducing the risk of short column lifetimes or MS source fouling.

This method details the extraction and analysis of THC and its major metabolites, 11-hydroxy Δ-9-THC (THC-OH) and 11-nor-9-Carboxy-Δ-9-THC (THC-COOH)[1] from whole blood using an Oasis PRiME HLB µElution Plate, followed by UPLC-MS/MS analysis. The SPE procedure is simple and very efficient, with elution in LC

compatible solvents, allowing for direct injection without evaporation and reconstitution of samples. Analysis is rapid and highly consistent, with all analytes eluting in less than 3 minutes. Recoveries were excellent and matrix effects were minimal for all compounds. Quantitative results were highly reproducible. Quality control results were within 10% of expected concentrations and average %RSDs within 2–4%.

While this application was performed with UPLC-MS/MS system, an HPLC separation method for THC and its metabolites was developed to provide HPLC-MS/MS users guidance as well.

## Experimental

### Materials

All standards and stable isotope labelled internal standards were purchased from Cerilliant (Round Rock, TX, USA). Stock standards at 100 µg/mL were prepared in 40% methanol (THC, THC-OH and THC-COOH). A working internal standard solution, consisting of 100 ng/mL THC-D3, THC-OH-D3 and THC-COOH-D3 was also prepared in 40% methanol. Individual calibrators and quality control standards were prepared daily in 40% methanol.

### Spiked whole blood solution

100 µL of each working calibrator or QC standard and 100µL internal standard (I.S.) were added to 1800 µL of rat whole blood to make calibration curves and QC samples. Calibrator concentrations ranged from 0.05–100 ng/mL for all analytes. Quality control samples were prepared at 0.375, 2, 7.5, 20 and 37.5 ng/mL, in whole blood.

### Sample Preparation

Sample pretreatment

Samples were extracted using Oasis PRiME HLB µElution Plates. 100 µL spiked whole blood was added to 25 µL of a solution of 0.1 M zinc sulfate/ammonium acetate, and the mixture was vortexed for 5 seconds to lyse the cells. All samples were then precipitated by adding 375 µL 0.1% formic acid in ACN. The entire sample was vortexed for 10 seconds and centrifuged for 5 min at 7000 rcf. The supernatant was then diluted with 800 µL water prior to loading.

SPE with Oasis PRiME HLB µElution Plate

The entire pretreated sample was directly loaded on to the Oasis PRiME HLB µElution Plate in 2 aliquots

without conditioning or equilibration. All wells were then washed with 2 x 250 µL aliquots of 25:75 methanol:water. All the wells were then eluted with 2 x 25 µL aliquots of 90:10 ACN:IPA and diluted with 50 µL of water. 5 µL was injected onto the UPLC-MS/MS system. The SPE extraction procedure is summarized in Figure 1.

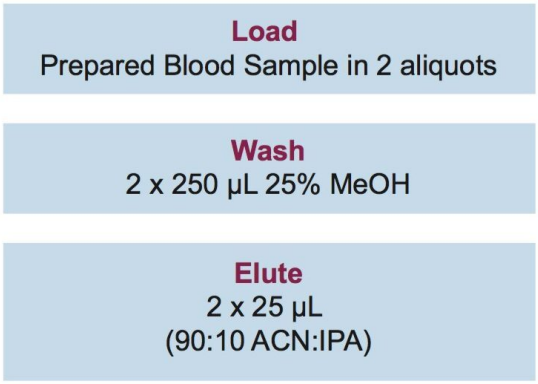



*Figure 1. Oasis PRiME HLB extraction methodology for THC, COOH-THC, and OH-THC from whole blood. With no conditioning and equilibration, sample extraction is simplified to just three steps.*

Analyte recovery was calculated according to the following equation:

$$\%\text{Recovery} = \left(\frac{\text{Area A}}{\text{Area B}}\right) \times 100\%$$

Where A equals the peak area of an extracted sample and B equals the peak area of an extracted blank matrix sample in which the compounds were added post-extraction.

Matrix effects were calculated according to the following equation:

$$\text{Matrix Effects} = \left(\left(\frac{\text{Peak area in the presence of matrix}}{\text{Peak area in the absence of matrix}}\right)\right) - 1 \times 100\%$$

The peak area in the presence of matrix refers to the peak area of an extracted matrix sample in which the compounds were added post-extraction. The peak area in the absence of matrix refers to analytes in a neat solvent solution.

## LC conditions

| | |
|---|---|
| UPLC system: | ACQUITY I-Class UPLC System |
| Column: | ACQUITY UPLC BEH $C_{18}$ Column, 1.7 µm, 2.1 x 50 mm |
| Column temp.: | 40 °C |
| Sample temp.: | 10 °C |
| Mobile phase A (MPA): | Water with 0.1% formic acid |
| Mobile phase B (MPB): | ACN with 0.1% formic acid |
| Strong wash solvent: | 70:30 ACN:Water with 2% formic acid |
| Weak wash solvent: | 10% ACN |
| Injection volume: | 5 µL |

The gradient ramp is shown in Table 1.

## Mass spectrometry

| | |
|---|---|
| MS system: | Xevo TQ-S Mass Spectrometer |
| Ionization mode: | ESI Positive |
| Capillary voltage: | 2.0 kV |

| | |
|---|---|
| Cone voltage: | Optimized for each analyte |
| Desolvation gas: | 1000 L/hr |
| Cone gas: | 150 L/hr |
| Desolvation temp.: | 500 °C |
| Source temp.: | 150 °C |

Data were acquired and analyzed using MassLynx. Software (V4.1). Quantification was performed using TargetLynx.

### Gradient

| Time (min) | Flow (mL/min) | %A | %B |
|---|---|---|---|
| 0 | 0.6 | 50 | 50 |
| 1.0 | 0.6 | 50 | 50 |
| 3.0 | 0.6 | 5 | 95 |
| 3.5 | 0.6 | 5 | 95 |
| 3.6 | 0.6 | 50 | 50 |
| 4.0 | 0.6 | 50 | 50 |

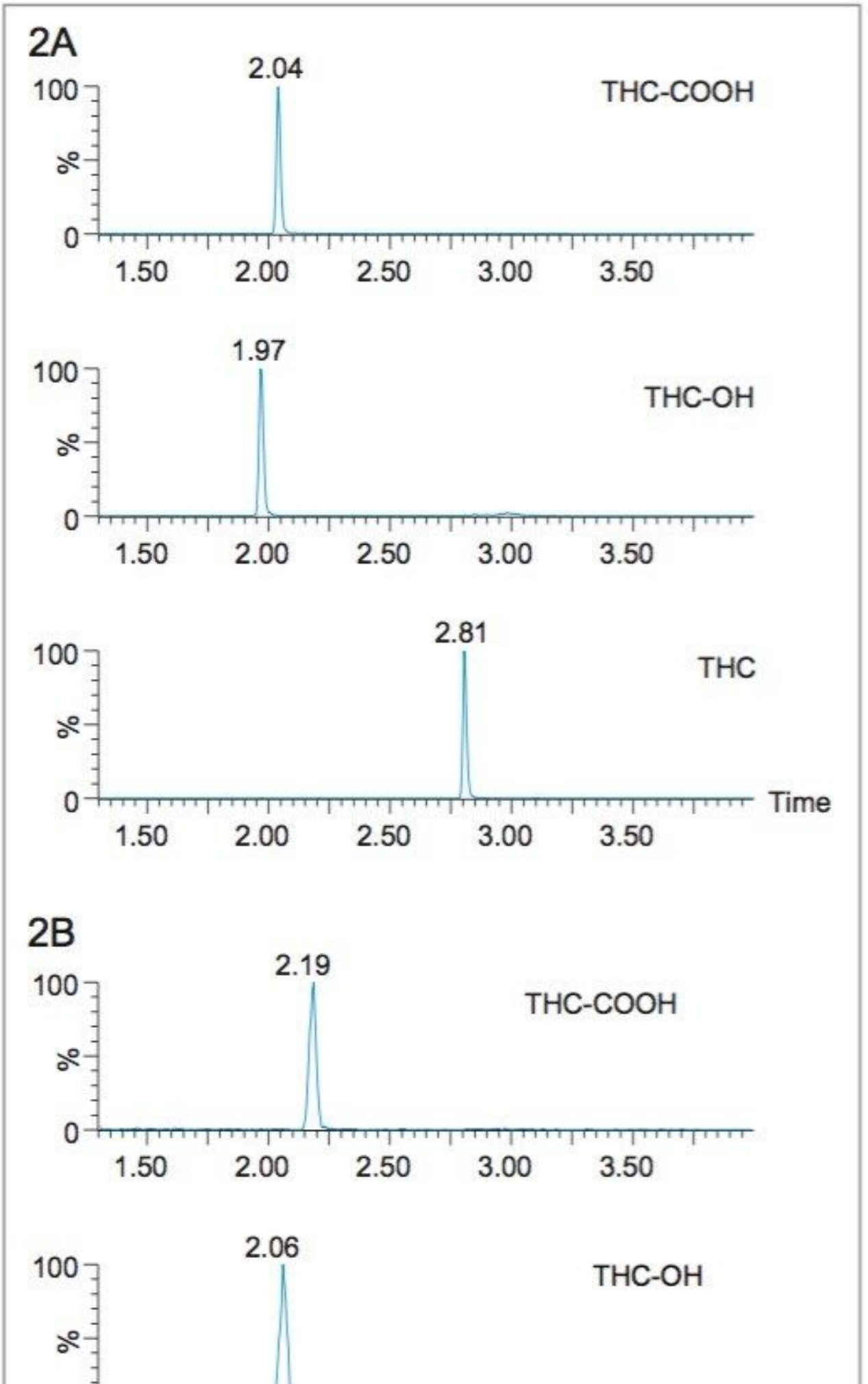
*Table 1. Mobile phase gradient. The compositions of MPA and MPB are listed in the Methods section.*

## Results and Discussion

### Chromatography

Figure 2A shows UPLC chromatograms of the three cannabinoids from an extracted calibrator at 1 ng/mL.

All compounds eluted within 3 minutes and all peak widths were between 2.2–2.6 seconds at 5% of baseline. All peaks were symmetrical with symmetries between 0.95–1.15. Figure 2B shows an HPLC chromatogram conducted with an XBridge BEH $C_{18}$, 2.5 µm; 2.1 x 50 mm Column with ACQUITY UPLC H-Class/Xevo TQD with a maximum system pressure of 4,000 psi. All peak widths were between 3.6–4.8 seconds at 5% baseline, an average of around 60% more than UPLC chromatogram. Similar symmetries were obtained. The slight difference in analyte retention time between UPLC and HPLC is due to the different system dwell volume (system delay volume, an ACQUITY UPLC I-Class dwell volume is around 100 µL and an H-Class is 300 µL).


2A
2.04
THC-COOH
100
%
0
1.50
2.00
2.50
3.00
3.50
1.97
THC-OH
2.81
THC
Time
2B
2.19
2.06

*, 2.5 µm; 2.1 x 50mm with system back pressure around 4000 psi. The LC solvent gradient and flows were the same as the UPLC separation.*

Table 2 lists the UPLC retention times and individualized MS parameters of the cannabinoids and their stable isotope labelled internal standards, including MRM transitions, cone voltage, and collision energy. Two MRM transitions were used for each compound, a primary (listed first) and a confirmatory transition (listed second). Compared to HPLC, UPLC offers improved resolution and sensitivity, higher efficiency, a faster run time, and reduced solvent use. In this application, all recoveries, matrix effects, phospholipid removal and method validation were performed on the ACQUITY UPLC I-Class/Xevo-TQ-S System to maximize the aforementioned benefits.

| Analyte | RT (min) | MRM transitions (*m/z*) | Cone voltage (V) | Collision energy (eV) |
|---|---|---|---|---|
| THC-OH | 1.84 | 331.3>313.1<br>331.3>193.1 | 40<br>40 | 18<br>30 |
| THC-OH-d3 | 1.84 | 334.3>316.1 | 40 | 18 |
| THC-COOH | 1.92 | 345.3>327.3<br>345.3>299.3 | 50<br>50 | 20<br>25 |
| THC-COOH-d3 | 1.92 | 348.3>330.3 | 50 | 20 |
| THC | 2.72 | 315.1>193.2<br>315.1>135.1 | 40<br>40 | 25<br>25 |
| THC-d3 | 2.72 | 318.1>196.2 | 40 | 25 |

*Table 2. Mass spectral parameters for all analytes and internal standards.*

## Recovery and matrix effects

Extraction recoveries were high and consistent. As Figure 3 shows, recovery for all analytes was greater than 85% with average RSDs within 5–7%, demonstrating the high reproducibility of Oasis PRiME HLB. Matrix effects were minimal, at less than 15% for all compounds. Once again, the low standard deviations (average at 5–7%) demonstrate the consistency of extraction and cleanup seen with Oasis PRiME HLB. All recovery and matrix effect data are summarized in Table 3. Oasis PRiME HLB provided comparable recovery, variability and matrix effects as mixed-mode SPE, with a more simplified procedure than previously published.[3]

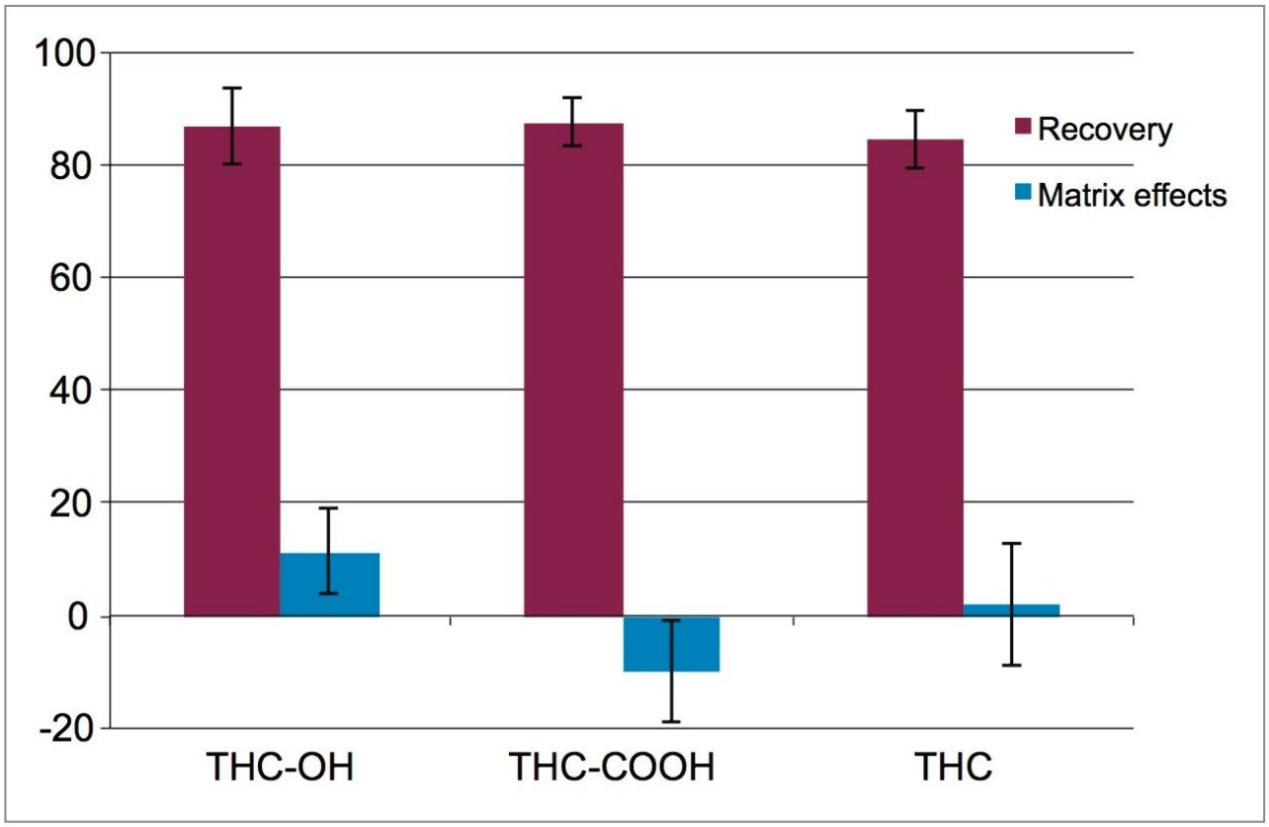



*Figure 3. Recovery and matrix effects for THC-OH, THC-COOH, and THC after extraction using the Oasis PRiME HLB µElution Plate. Low average RSDs between 5–7% for all compounds. Matrix effects were all less than 15% with RSDs under 12%.*

| | $R^2$ | Mean % dev. | Range (ng/mL) | Curve type |
|---|---|---|---|---|
| THC-OH | 0.998 | 7.8 | 0.1–100 | Linear |
| THC-COOH | 0.999 | 4.0 | 0.05–100 | Linear |
| THC | 0.998 | 2.4 | 0.05–100 | Linear |

*Table 3. Recovery and Matrix effects for THC and its metabolites (N=4 for all tests).*

## Phospholipid removal

One of the key attributes of Oasis PRiME HLB is its ability to deliver cleaner extracts than other sample preparation methods.  One way that this is achieved is by removing endogenous phospholipids. Figure 4 shows chromatograms of combined phospholipid traces from an Oasis PRiME HLB extract (B) and an

identical sample subject to protein precipitation (C). Compared with protein precipitation (PPT), Oasis PRiME HLB removes over 99% phospholipids, resulting in a much cleaner extraction. This can translate to reduced matrix effects, longer column lifetimes, and less mass spectrometer source maintenance. The chromatography of the three target compounds is also shown (A), demonstrating the potential interference with phospholipids if they were not removed during the extraction.

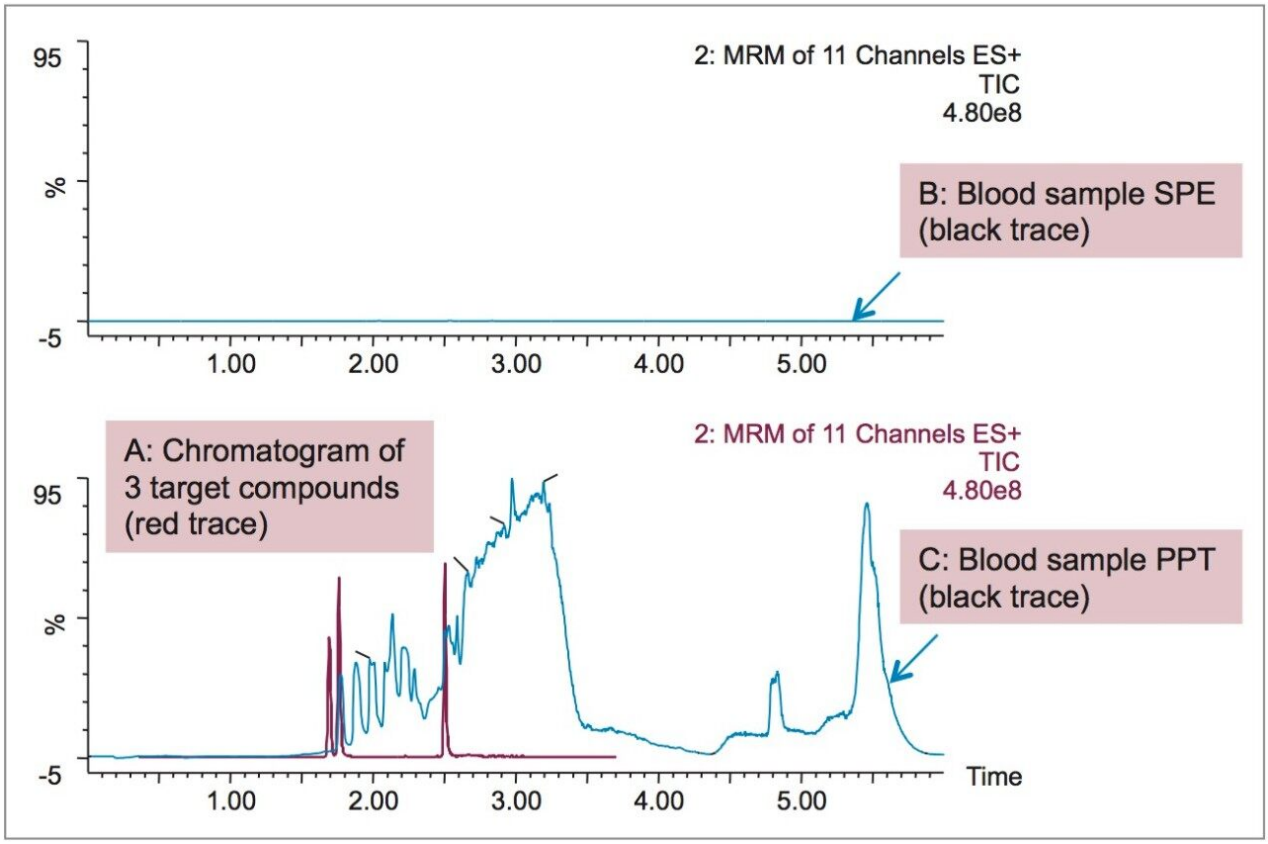



*Figure 4. Chromatography of phospholipids remaining in Oasis PRiME HLB extraction vs. whole blood protein precipitation. Scales are linked. An overlaid chromatogram shows the retention times of THC-OH, THC-COOH, and THC in relation to the phospholipid traces. A: Chromatogram of 3 target compounds; B: blood sample SPE extract; C: blood sample PPT supernatant.*

## Quantitative results

The SPE method developed has been shown to deliver high and consistent extraction recoveries from whole blood. Research data shows that 2–3 ng/mL THCs are an indicator of recent marijuana exposure (cut off concentration).[1] This method detects THC and its metabolites down to 0.1 ng/mL, well below threshold for recent marijuana exposure.

Calibration samples were prepared as previously described in the materials section. Calibration ranges were from 0.1–100 ng/mL for THC-OH and 0.05–100 ng/mL for THC and THC-COOH. All compounds had linear responses over the entire calibration range with $R^2$ values of 0.99 or greater with 1/x weighting. Figure 5 shows the calibration curves and Table 4 summarizes the data from these curves for all the compounds. Lower limits of quantification (LLOQ) were 0.1 ng/mL for THC-OH and 0.05 ng/mL for THC and THC-COOH, which are much lower than cut off concentration. In each case, all FDA recommendations for accuracy, precision, linearity and analytical sensitivity were met for validated methods.[2]

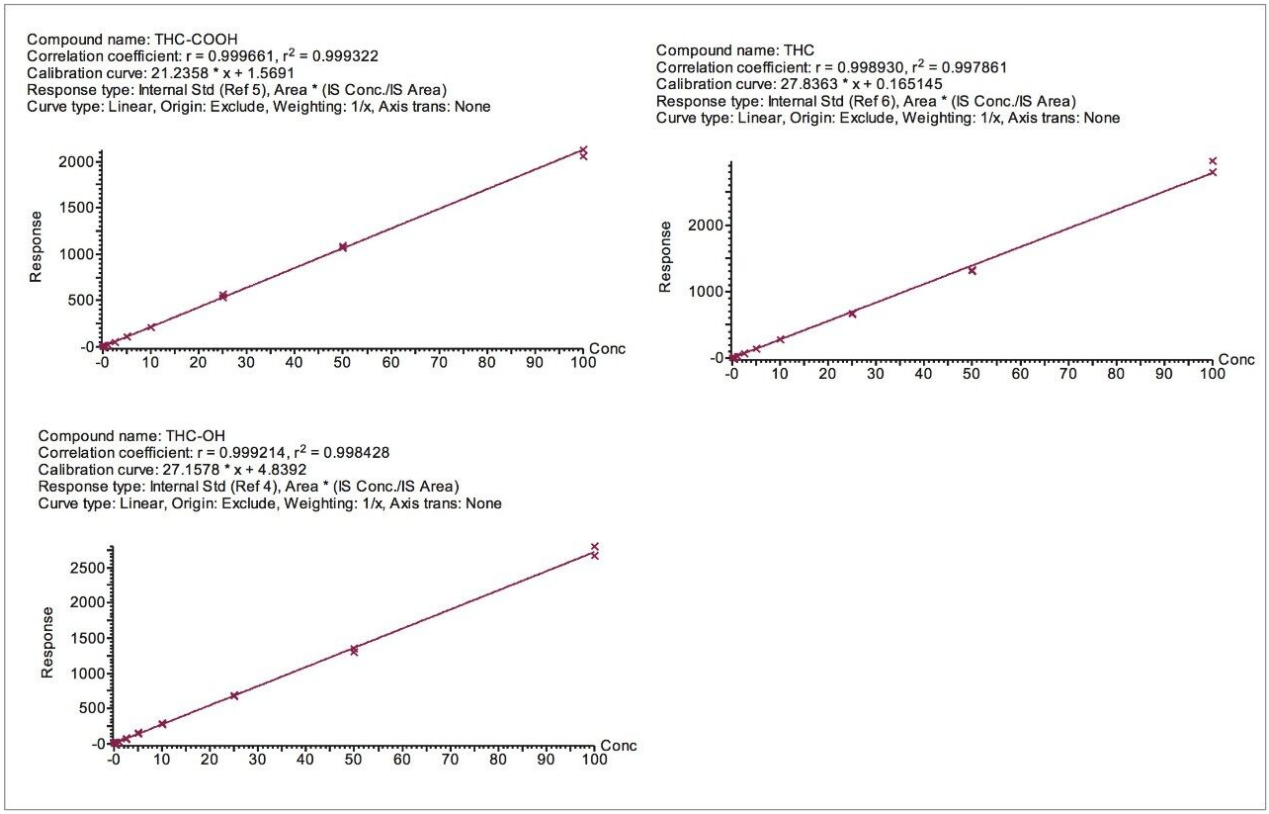



*Figure 5. Calibration curves for THC and its metabolites, $R^2$> 0.99, fit – linear 1/x weighting.*

| | $R^2$ | Mean % dev. | Range (ng/mL) | Curve type |
|---|---|---|---|---|
| THC-OH | 0.998 | 7.8 | 0.1–100 | Linear |
| THC-COOH | 0.999 | 4.0 | 0.05–100 | Linear |
| THC | 0.998 | 2.4 | 0.05–100 | Linear |

*Table 4. Calibration Curve Summary for THC and its metabolites with 1/x fit weighting.*

Quality control samples were prepared at low, medium, and high concentrations as appropriate for the calibration ranges. Quality control samples were accurate and precise. All results were within 10% of expected values with average RSDs between 2–4% (N=6). This data can be seen in Table 3. The excellent accuracy and precision demonstrate the consistency and robustness of this sorbent.

## Conclusion

This application note details the extraction of THC-OH, THC-COOH and THC from whole blood samples using a novel SPE sorbent, Oasis PRiME HLB, in a µElution format for forensic toxicology applications. The unique nature of this sorbent enabled the elimination of conditioning and equilibration steps, simplifying the extraction procedure and speeding up the sample preparation workflow. In addition, the µElution format enabled the direct injection of extracts without evaporation or reconstitution, minimizing the risk of nonspecific binding. One key attribute of this sorbent is its ability to retain phospholipids. As mentioned previously and shown in Figure 4, >99% of residual phospholipids were eliminated from extracted samples, some of which would have co-eluted with the target analytes in this assay.

Recoveries were very consistent, with recoveries >85% and average RSDs at 5–7%. Matrix effects were less than 15% for all compounds. Linearity, accuracy, precision and analytical sensitivities were excellent for all compounds. All accuracies were within 10% of target concentrations with an average RSDs between 2–4% for QC samples, demonstrating the high reproducibility of the combination of this sorbent and the UPLC-MS/MS method. In conclusion, Oasis PRiME HLB has been successfully used to achieve consistent recoveries with minimal matrix effects as well as accurate quantification over 4 orders of magnitude from whole blood samples.

While this application was conducted using UPLC conditions, the chromatography illustrated in Figure 2B shows that this assay can also be run on an HPLC scale at reduced backpressures.

## References

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3. Lee, R., Saussereau, E., Lacroix C., and Wood M.: Quantitative Analysis of Cannabinoids in Whole Blood using UPLC-MS/MS for Forensic Laboratories. Waters Application Note 720004700EN, (2013).

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