QUANTITATIVE ANALYSIS OF THC AND RELATED CANNABINOIDS IN MULTIPLE MATRICES USING SOLID PHASE EXTRACTION WITH UPLC/MS/MS FOR CLINICAL RESEARCH

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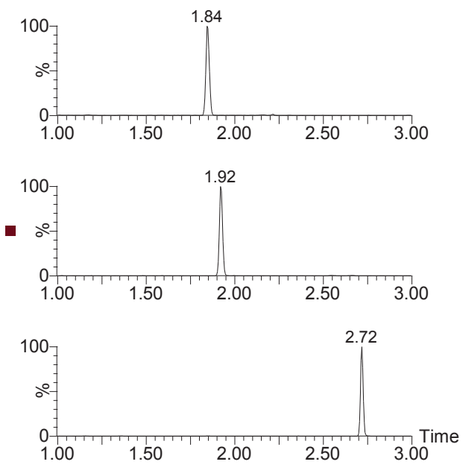
INTRODUCTION

Cannabis continues to be a highly abused recreational drug. In addition, the increasing number of states legalizing it for medical use, combined with the trend towards legalization for recreational purposes means than analytical methods for the quantification of Δ-9-tetrahydrocannabinol (THC), its metabolites and related cannabinoids continue to be necessary in clinical research. Among drugs of abuse, natural cannabinoids present some unique analytical challenges. Excreted THC and related compounds are highly glucuronidated, requiring efficient deconjugation before analysis. In addition, the highly hydrophobic nature of natural cannabinoids makes them exceptionally susceptible to loss via non-specific binding, meaning that care must be taken with sample handling and processing of prepared extracts. Finally, matrix effects can be a challenge to control for these compounds, and can vary significantly in different biological matrices.

This work uses a novel reversed-phase solid phase extraction (SPE) sorbent, Oasis PRiME HLB, which has been developed to enable simpler and faster SPE protocols, while at the same time generating cleaner extracts than other sample preparation methods. 3 step load-wash-elute SPE protocols, eliminating conditioning and equilibration, were successfully employed to extract THC, THC-OH and THC-COOH from multiple matrices, including plasma, oral fluid (OF), whole blood and urine.

INSTRUMENTAL CONDITIONS

UPLC System:	ACQUITY UPLC® I-Class-FL
MS:	XEVO TQ-S
Column:	ACQUITY UPLC® BEH C_{18}, 1.7 µm; 2.1 x 50 mm
Mobile Phase A (MPA)	0.1% Formic Acid in Water
Mobile Phase B (MPB)	0.1% Formic Acid in ACN
Column Temp:	40 °C7
Sample Temp:	10 °C
Strong Needle Wash	2% Formic Acid in 70:30 ACN:Water
Weak Needle Wash	10% ACN



Mass spectral parameters for all analytes and internal standards

Analyte	MRM transitions (m/z)	Con (V)	Col eV
THC-OH	331.3>313.1 331.3>193.1	40 40	18 30
THC-OH -d3	334.3>316.1	40	18
THC-COOH	345.3>327.3 345.3>299.3	50 50	20 25
THC-COOH-d3	348.3>330.3	50	20
THC	315.1>193.2 315.1>135.1	40 40	25 25
THC-d3	318.1>196.2	40	25

SAMPLE PREPARATION

Urinary sample: Glucuronide hydrolysis: 40 µL internal standards was added to 2 mL spiked human urine sample in a glass vial, then 2.4 mL 0.1M potassium phosphate buffer (pH 6.8) containing 10 µL β-Glucoronidase was added. Vials were capped, vortex mixed, and incubated at 37°C water bath for 16 hours. After allowing samples to cool down to room temperature, 150 µL of 10M NaOH was added, vortex mixed and hydrolyzed in a dry heating block for 30 min at 70 ° C. Once the samples had cooled, 850 µL glacial acetic acid was added to the samples and vortex mixed. Solid phase extraction with Oasis PRiME µElution plate: 500 µL pretreated sample (equivalent to 180 µL urine) was directly applied to the Oasis PRiME µElution plate. All wells of the SPE plate were then washed with 2 x 300 µL aliquots of 25% methanol. The samples were then eluted with 2 x 25 µL aliquots of 60:40 ACN:IPA and diluted with 50 µL of water.

Plasma sample: 200 µL 0.1% FA in ACN was added to 100 µL spiked plasma to precipitate out the protein in a micro centrifuge tube. Then the mixture was vortexed for 5 seconds and centrifuged for 5 min at 7000 rcf. The supernatant was then diluted with 400 µL water prior to loading. The entire pre-treated sample was directly loaded on to the Oasis PRiME HLB µElution plate 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:MeOH and diluted with 50 µL of water prior to analysis.

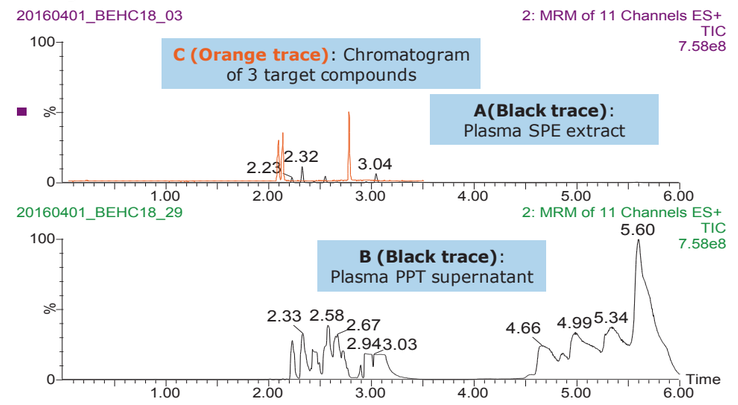
Blood sample:100 µL spiked whole blood was added to 25 µL of a solution of 0.1 M zinc sulfate/ammonium acetate, and the mixture was vortexed for 5 seconds to lyse the cells. All samples were then precipitated by adding 375 µL 0.1% formic acid in ACN. The entire sample was vortexed for 10 seconds and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

Oral fluid sample: Oral fluid samples were collected with Quantisal collection device from Immunalysis. The collection applicator was saturated with oral fluid (spiked), and then placed in a collection vial, which contained 3.0 mL of sample stabilization buffer. Per Quantisal instruction, this was claimed to be the equivalent of collecting 1.0±0.1 mL of sample. 1 mL acetonitrile was then added to the collection vial to help improve extraction. The collection kit was stored in a refrigerator overnight. 500 µL aliquots of buffer stabilized oral fluid samples (equivalent to 100 µL oral fluid) were pre-treated by adding 200 µL 4% H_3PO_4 and 10 µL of working IS mixture (100 ng/mL in 40% MeOH). The entire pre-treated sample (total of 710 µL) was directly loaded on to the Oasis PRiME HLB µElution plate without conditioning or equilibration, followed by washing with 2 x 250 µL 5% NH4OH in 25:75 methanol:water. All the wells were then eluted with 2 x 25 µL 90:10 ACN:MeOH and diluted with 50 µL of water. A CORTECS C18 column was used to minimize matrix effects.

Different matrices may require different sample pretreatment, such as urine requires deglucuronation hydrolysis, oral fluid requires complete extraction with extra ACN, blood and plasma require extra PPT to completely release drugs. Once the samples were done with pretreatment, the SPE procedure with Oasis PRiME HLB is as simple and straightforward as LOAD, WASH and ELUTE! With the unique µElution plate, non-specific binding were minimized with no evaporation or reconstitution.

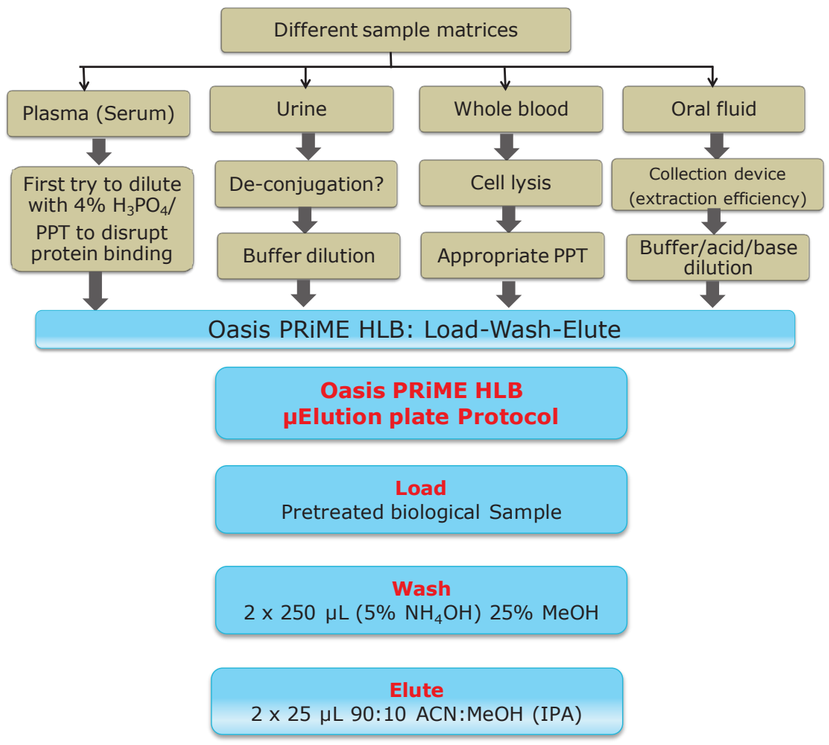
PHOSPHOLIPIDS REMOVAL

One of the key attributes of Oasis PRiME HLB is its ability to deliver cleaner extracts than other sample preparation methods. Figure below shows Oasis PRiME HLB extract removes over 99% of phospholipids, resulting in a much cleaner extraction.



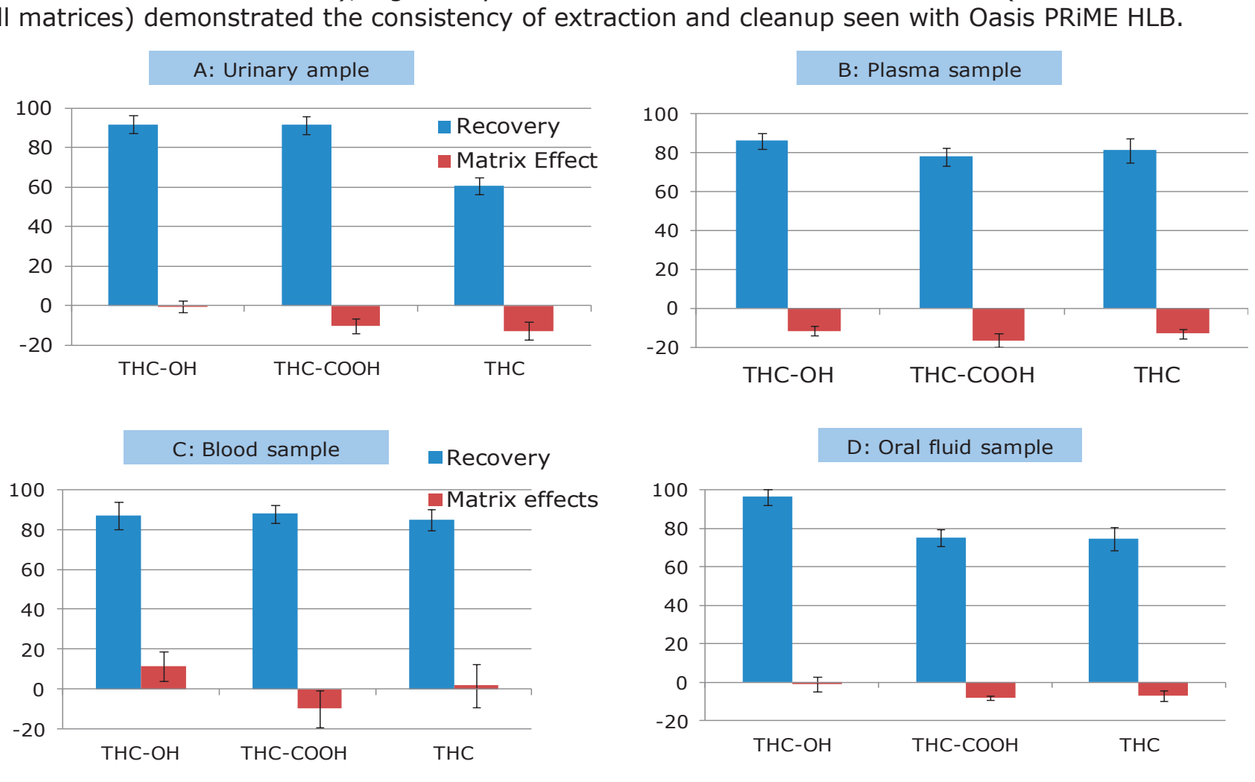
CONCLUSION

- **One SPE device worked for many matrices in clinical research.**
- **Employed a novel SPE sorbent extraction that is easier to perform, faster in analysis and cleaner in elution.**
- **Demonstrated accurate and precise validation data in the analysis of THC and its metabolites in multiple matrices.**
- **Proved accuracy over a wide of calibration range compared to a fully validated method from an external laboratory.**
- **Provided complete guidelines for sample pretreatment of different matrices including plasma, urine, whole blood and oral fluid.**



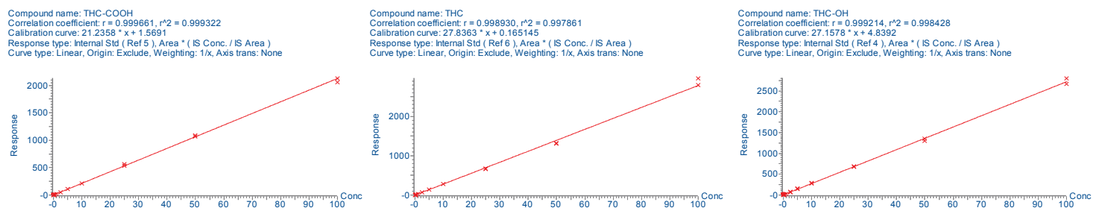
RESULTS—ANALYTE RECOVERY AND MATRIX EFFECT

Figure 2 below listed the recovery and matrix effects for THC and its metabolites in different matrix (A: urinary sample, B: plasma sample, C: blood sample, D: oral fluid sample). The low standard deviations (8% or less with all matrices), high analyte recoveries and excellent matrix effect (all lower than 20% with all matrices) demonstrated the consistency of extraction and cleanup seen with Oasis PRiME HLB.



RESULTS - METHOD VALIDATION

The SPE method has been shown to deliver high and consistent extraction recoveries. All compounds had linear response over the entire calibration range with R^2 values of 0.99 or more for all four matrices. Calibration and quality control (QC) results indicate that the method is linear, accurate and precise within 4 orders of magnitude. Figure 3 below is the validation data for whole blood sample with excellent linearity range, correlation and accurate and precise QC data. Similar accurate validation results for other matrices can be found in Waters application notes



	Accuracy and Precision								
N=6	THC-OH (0.1-100ng/mL)			THC-COOH (0.1-100ng/mL)			THC (0.05-100ng/mL)		
QC Level (ng/mL)	Mean (ng/mL)	%Acc.	%RSD	Mean (ng/mL)	% Acc.	%RSD	Mean (ng/mL)	% Acc.	%RSD
0.375	0.33	97.9	0.6%	0.40	105.8	8.1%	0.41	108.2	3.0%
2	1.92	96.0	3.7%	1.89	94.7	2.3%	2.01	100.5	3.7%
7.5	7.50	100.0	2.7%	7.34	98.9	2.8%	7.42	98.9	1.4%
20	19.87	99.3	3.2%	20.04	100.2	2.1%	19.56	97.8	1.2%
37.5	36.19	96.5	2.2%	37.95	101.2	3.0%	35.32	94.2	0.7%
Mean		**98**	**2%**		**100**	**4%**		**99.9**	**2%**

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