[APPLICATION NOTE]

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Quantitative Analysis of THC and Metabolites in Urine With a Simple, Fast, and Clean Oasis PRiME HLB µElution Plate

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APPLICATION BENEFITS

- Faster, simplified sample preparation workflow
- Consistent recovery and minimal matrix effects
- No evaporation or reconstitution necessary
- Linear, accurate, and precise results for all analytes

WATERS SOLUTIONS

Oasis PRiME HLB 96-well µElution Plate (p/n 186008052)

96-well Sample Collection Plate, 700 µL, Round well (p/n 186005837)

ACQUITY UPLC® BEH C_{18} Column, 130Å, 1.7 µm, 2.1 x 50 mm (p/n 186002350)

ACQUITY UPLC I-Class System

Xevo® TQ-S Mass Spectrometer

KEY WORDS

Δ-9-tetrahydrocannabinol (THC) and its metabolites, THC-COOH, THC-OH, urine, Oasis PRiME HLB, µElution plate, sample preparation, LC-MS

INTRODUCTION

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, which is designed to have some key advantages over traditional SPE sorbents. These include the ability to eliminate sorbent preconditioning and equilibration, allowing a more rapid workflow compared to traditional SPE products, and the ability to remove more interferences, resulting in a cleaner extracts and reducing the risk of short column lifetimes or MS source fouling.

This application note details the extraction and UPLC-MS/MS analysis of Δ-9-tetrahydrocannabinol (THC) and its metabolites, 11-hydroxy- Δ-9-THC (THC-OH) and 11-nor-9-Carboxy-Δ-9-THC (THC-COOH) from urine using Oasis PRiME µElution Plates. Δ-9-tetrahydrocannabinol (THC) is the main psychoactive element present in the plant Cannabis sativa. Quantitative analysis of these compounds in urine is an indicator of cannabis consumption, with high levels indicating recent and/or chronic use.

The use of Oasis PRiME resulted in consistent and highly reproducible recoveries of all compounds with minimal matrix effects. The µElution format allowed for the concentration of the sample on the SPE column, eliminating the need to evaporate and reconstitute the sample, minimizing the risk of analyte loss due to nonspecific binding and streamlining the laboratory workflow. This resulted in a method that was linear, accurate and precise for all analytes, with limits of quantification of 0.1 ng/mL for THC and its metabolites.

EXPERIMENTAL

Methods

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 of 1 µg/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. 80 µL of each working calibrator or QC standard was added to 1920 µL of human urine to make calibration curves and QC samples.

β-Glucoronidase from *E. Coli* K 12 was purchased from Roche Life Science (Indianapolis, IN)

Sample preparation

Glucuronide hydrolysis: 40 µL internal standards was added to 2 mL spiked human urine sample in a glass vial, then 2.4 mL 0.1 M 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 10 M 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. 5 µL was injected onto the UPLC-MS/MS system. The SPE extraction procedure is summarized in Figure 1.

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:

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

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

LC system:	ACQUITY I-Class UPLC System
Column:	ACQUITY UPLC BEH C_{18} Column, 130Å, 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 vol.:	5 µL

The gradient ramp is shown in Table 1.

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.

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™

RESULTS AND DISCUSSION

Chromatography

Figure 2 shows chromatography of the three cannabinoids from an extracted calibrator at 2 ng/mL. All compounds eluted within 3 minutes with all peak widths were under 3 seconds at 5% of baseline. All peaks were symmetrical with symmetries between 0.95–1.15.

Table 2 lists the retention time 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).

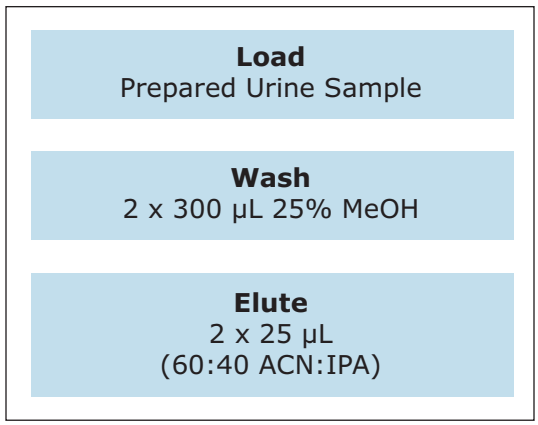

Figure 1. Oasis PRiME extraction methodology for urine THCs. With no conditioning and equilibration, sample extraction is simplified to just three steps.

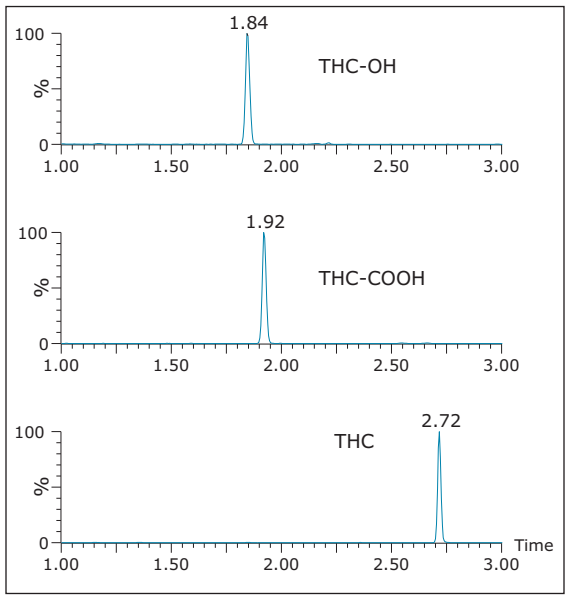

Figure 2. Chromatography of THC-OH, THC-COOH and THC from an extracted urine sample on the ACQUITY UPLC BEH C_{18} column, 1.8 µm; 2.1 x 50 mm. The concentrations are 4 ng/mL for all compounds.

Analyte	RT (min)	MRM transitions (*m/z*)	Cone voltage (V)	Collision energy (eV)
THC-OH	1.84	331.3>313.1 331.3>193.1	40 40	18 30
THC-OH-d3	1.84	334.3>316.1	40	18
THC-COOH	1.92	345.3>327.3 345.3>299.3	50 50	20 25
THC_COOH-d3	1.92	348.3>330.3	50	20
THC	2.72	315.1>193.2 315.1>135.1	40 40	25 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 very consistent. As Figure 3 shows, recovery for THC-OH and THC-COOH was around 90% and THC was 60% with all %RSDs under 7.5%, demonstrating the reproducibility of Oasis PRiME. Matrix effects were minimal, at less than 15% for all compounds. Once again, the low standard deviations (7.5% or less) and high recoveries for THC-OH and THC-COOH 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 also provided better recovery, variability and matrix effects than LLE, with a more simplified procedure.[1]

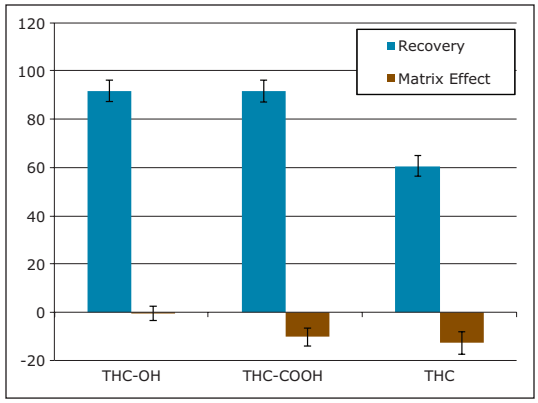

Figure 3. Recovery and matrix effects of THC-OH, THC-COOH, and THC after extraction using the Oasis PRiME µElution plate. %RSDs for extraction recovery were less than 5% for all compounds. Matrix effects were all within 20%.

Quantitative results

Calibration and quality control samples were prepared as previously described in the materials and method section. Calibration ranges were from 0.1–100.0 ng/mL for THC-COOH and THC-OH and 0.2–100.0 ng/mL for THC. Quality control samples were prepared at low, medium, and high concentrations as appropriate for the calibration ranges.

All compounds had linear responses over the entire calibration range with R^2 values of 0.99 or greater with 1/x weighting. Table 4 summarizes the data from the calibration curves. Lower limits of quantification (LLOQ) were 0.1 ng/mL for THC-COOH and THC-OH and 0.2 ng/mL for THC. In each case, all FDA recommendations for accuracy, precision, linearity and analytical sensitivity were met for validated methods.[2]

Quality control samples were accurate and precise. All results were within 15% of expected values and %RSDs were under 2% (N=6). This data can be seen in Table 5. The excellent accuracy and precision demonstrate the consistency and robustness of this sorbent.

	Recovery			Matrix effects	
	Mean	S.D.	%RSD	Mean	S.D.
THC-OH	91.9	4.5	4.9	-0.4	3.0
THC-COOH	91.5	4.5	4.9	-10.4	3.7
THC	60.6	4.5	7.4	-12.9	4.5

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

	R^2	Mean % dev.
THC-OH	0.997	2.8
THC-COOH	0.998	2.0
THC	0.998	1.2

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

	Accuracy and precision								
N=6	THC-OH			THC-COOH			THC		
QC level (ng/mL)	Mean (ng/mL)	%Acc.	%RSD	Mean (ng/mL)	%Acc.	%RSD	Mean (ng/mL)	%Acc.	%RSD
0.75	0.66	88.6	1.7	0.76	100.8	1.4	0.72	96.3	0.4
7.5	6.70	89.3	1.3	7.37	98.3	1.3	7.15	95.3	1.2
75	73.4	97.9	1.8	73.6	98.2	0.7	75.5	100.7	0.8
Mean		91.7			99.7			97.4	

Table 5. Quality control results from extracted urine samples. (N=6 for each compound at all three levels).

CONCLUSIONS

Calibration and quality control samples were prepared as previously described in the materials and method section. Calibration ranges were from 0.1–100.0 ng/mL for THC-COOH and THC-OH and 0.2–100.0 ng/mL for THC. Quality control samples were prepared at low, medium, and high concentrations as appropriate for the calibration ranges.

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References

1. Lee, R., Traynor, A., LeCount, J., Wood, M., Quantitative analysis of 11-nor-carboxy Δ9-THC in urine using UPLC-MS/MS. Waters Application Note 720004808EN (2012).
2. Bansal, S., DeStefano, A., Key elements of bioanalytical method validation for small molecules. *The AAPS Journal* 9(1), E109-E114 (2007).

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