APPLICATION NOTE

MicroPorous Extraction Technology for the Analysis of Carboxy-THC in Urine

INTRODUCTION

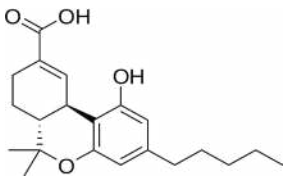
MicroPorous Extraction Technology (MPX) integrates a membrane or disk into an easy-to-use extraction device. MPX is a versatile technology that can be utilized in manual pipetting workflows for low throughput, or with fully automated liquid handling systems for high throughput. MPX devices can be integrated with vacuum systems, and allows for processing of samples with low volumes (50 µL) or high volumes (1 mL or more). Presented herein is an MPX device with a C18 disk chemistry for solid phase extraction of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (C-THC) from urine.

Recent literature has shown that the presence of acid during sample preparation can result in the conversion of cannabidiol (CBD) and its metabolites to THC and its respective metabolites [1,2]. Therefore, we performed hydrolysis using a neutral pH beta-glucuronidase, with no additional acid prior to sample extraction, to avoid potential conversion in case samples. This method greatly reduces sample matrix introduced into the LC-MS/MS system, thereby improving the quality of chromatographic data and minimizing instrument downtime. For these reasons, the MPX device is a great option for labs involved in pre-employment drug testing, DUID analysis, and other applications.

MATERIAL AND METHODS

The C18 disk was composed of glass fiber embedded with surface-modified silica with a 30 µm average particle diameter and 70Å mean pore size. The disk thickness was 0.6 mm.

Using a Hamilton NIMBUS96 automated liquid handler (ALH), a 20 µL aliquot of B-One™ buffer stabilized beta-glucuronidase enzyme solution (Kura Biotech) was added to 50 µL urine sample (fortified at various levels of C-THC), 30 µL of water, and 10 µL of d3-C-THC internal standard solution (0.5 ng/mL in methanol) in a well. The sample was allowed to stand for 10 minutes at room temperature for hydrolysis. The total sample volume was 110 µL.



Carboxy THC ($C_{21}H_{28}O_4$)

MW: 344.451 amu

LogP: 6.3 (ref. PubChem)

Figure 1. *An overview of C-THC, the analyte of interest*

While the sample was hydrolyzing, the MPX devices were loaded onto the vacuum block by the ALH.

The conditioning of the disk was performed by adding 500 µL of acetonitrile, and subsequently adding 100 µL of 30% methanol in water. Solutions were added to the MPX device in one direction (top down) during the entirety of the sample preparation method. Upon completion of hydrolysis, the sample solution was added to the device. Once the urine sample was passed through the disk, a 100 µL aliquot of 30% methanol in water was passed through the membrane to wash off matrix and interferents. The vacuum was stopped and vented. The 100 µL acetonitrile elution solvent was added to the device and directly dispensed into a well plate using the ALH. The eluate was diluted with 400 µL of water for a final volume of 500 µL. A low volume injection (5 µL) was used to readily achieve the necessary sensitivity for pre-employment drug testing and DUID analysis.

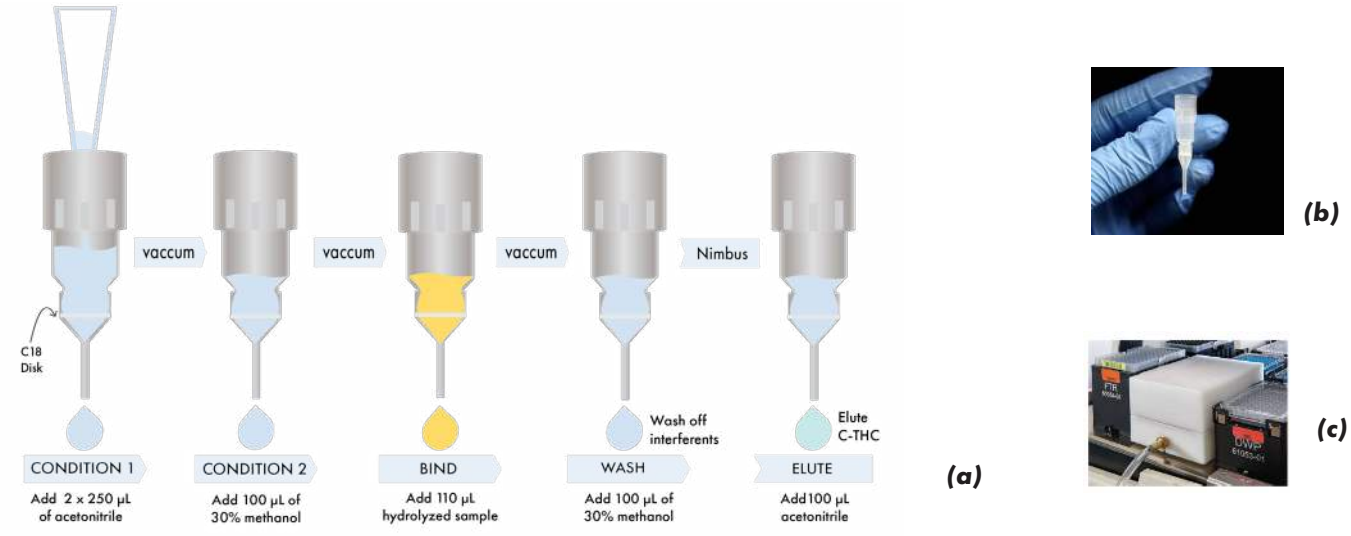


Figure 2. *A schematic of the extraction protocol (a), the MPX device (b), and the vacuum block used for the protocol (c).*

ANALYSIS

The final eluate was injected (5 µL) into the LC-MS/MS system (SCIEX 6500+) equipped with an Agilent 1260 LC system and an analytical column from Phenomenex (polar C18, 2.1 µm, 3 mm x 50 mm). The MS parameters and LC methods are shown in **Tables 1** and **2,** respectively.

Table 1. *The mass spectrometry (MS) parameters used for The analysis of C-THC extracted from urine*

Compound	Parent Ion (m/z)	Product Ion (m/z	Dwell (ms)	DP (v)	EP (v)	CE (v)	CXP (V)
C-THC (Quant)	343.15	299.20	300	-60	-10	-30	-15
C-THC (Qual)	343.15	245.10	300	-60	-10	-38	-21
C-THC D_3 (ISTD)	46.18	302.20	300	-60	-10	-30	-21

Table 2. *The liquid chromatography (LC) method used for the analysis of C-THC extracted from urine.*

Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0.00	500	80.0	20.0
0.40	500	80.0	200
1.00	500	15.0	85.0
4.40	500	2.0	98.0
7.40	500	2.0	98.0
7.50	500	80.0	20.0
10.00	500	80.0	20.0

Table 3. *The resulting recoveries and matrix effects (ion enhancement or suppression) from 3-days of triplicate calibration curves. A positive (+) number for matrix effects indicates ion enhancement while a negative (-) number for matrix effects indicates ion suppression.*

Concentration	Recovery	Matrix Effects
5 ng/mL	65%	-2%
10 ng/mL	65%	-3%
25 ng/mL	65%	-4%
50 ng/mL	65%	4%
100 ng/mL	71%	0%
250 ng/mL	68%	10%
500 ng/mL	82%	9%
1000 ng/mL	81%	9%

RESULTS AND DISCUSSION

The recovery of C-THC in urine was 81% for the highest concentration tested of 1000 ng/mL, even though the solution pH was not adjusted from the neutral pH hydrolysis conditions. In this method, the calibration plot was linear from 5 ng/mL to 1,000 ng/mL with a linear regression of 0.999. Representative chromatographic data are shown in **Figure 3**.

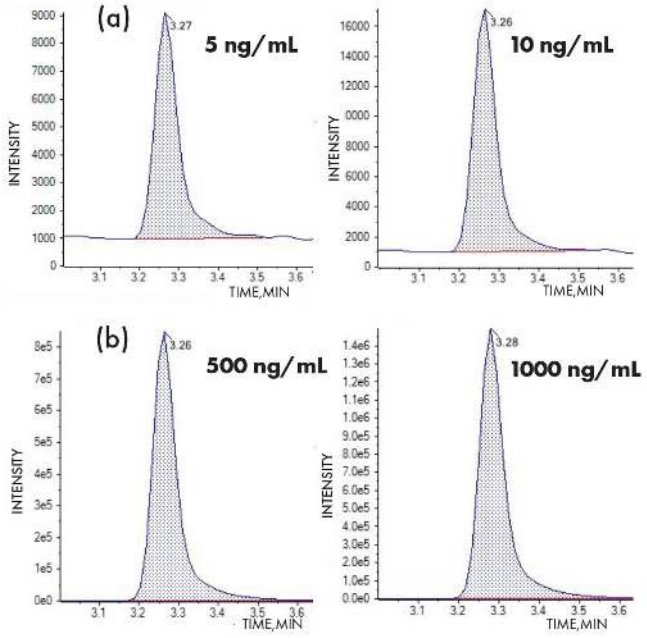


Figure 3. *The first two calibration levels (a) and the last two calibration levels (b) are shown.*

Recovery and matrix effects were evaluated according to the post-extraction addition technique. The results are shown in **Table 3**, which indicate an average of 70% (ranging 65%-82%) recovery across the 8 concentration levels. Some loss in recovery is likely due to performing the extraction at a neutral pH; adding acid to the samples would risk conversion of C-CBD to C-THC (as stated previously)[1,2]. Matrix effects studies (using the equation: %ME = [((average sample)/(average neat)) – 1] x100) show an average of 3% enhancement across all concentrations, indicating effective sample clean up. Inter-day and intra-day precision and accuracy are shown in **Table 4.**

This method may be varied at multiple points to increase sensitivity for applicability across a variety of detectors and analysis requirements. Vacuum drying prior to elution allows for GC friendly solvents and concentration factors desired for GC-MS analysis (which generally requires larger sample volumes for analysis). As a proof-of-concept to show the applicability for larger sample volume, an extraction of 1 mL of urine for C-THC analysis was conducted using this MPX method (with LC-MS/MS analysis). Preliminary results (**Table 5**) indicate 95% recovery and adequate sample cleanup.

CONCLUSION

MPX with C18 can be used to provide accurate quantitation of C-THC rapidly from urine with LC-MS/MS analysis. This method presented eliminates addition of acid, extracts samples in a rapid and automated fashion (up to 96 samples in less than 15 minutes), and provides purified extracts to maximize LC-MS/MS cleanliness. Future studies will include analysis of case samples for validation.

Table 4. *The results of a 3-day interday and intra-day precision and accuracy study.*

	Intra-day Precision (%)	Inter-day Precision (%)	Accuracy (%)
QC 1 (5 ng/mL)	4.6	0.1	89.4
QC 2 (10 ng/mL)	4.0	1.1	101.5
QC 3 (1000 ng/mL)	3.1	0.4	98.9

Table 5. *The results of preliminary studies involving large volume urine analysis of C-THC with an MPX device.*

1 mL of Urine Extracted with C18 MXT 10 ng/mL (n=5)	
Recovery	95%
% RSD	5%
Matrix Effects	10%

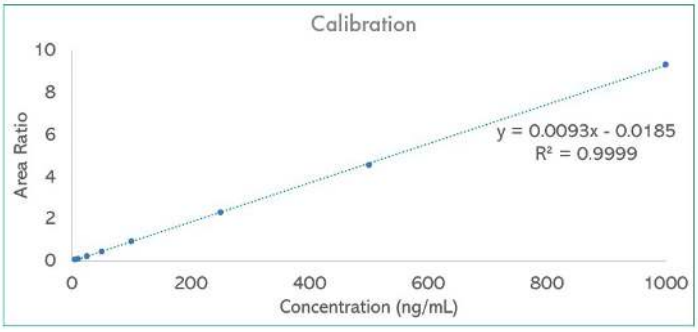


Figure 4. *The figure shows the average calibration of 1of the 3 days during the 3-day precision and accuracy study.* R^2 *averaged at 0.999 for triplicate calibration curves on 3 days.*

REFERENCES

1. Hart ED, Vikingsson S, Mitchell JM, Winecker RE, Flegel R, Hayes ED. Conversion of 7-Carboxy-Cannabidiol (7-COOH-CBD) to 11-Nor-9-Carboxy-Tetrahydrocannabinol (THC-COOH) during Sample Preparation for GC-MS Analysis. J Anal Toxicol. 2022 May 20;46(5):573-576. doi: 10.1093/jat/bkab046. PMID: 33987675.

2. Coulter C, Wagner JR. Cannabinoids in Oral Fluid: Limiting Potential Sources of Cannabidiol Conversion to Δ9- and Δ8-Tetrahydrocannabinol. J Anal Toxicol. 2021 Sep 17;45(8):807-812. doi: 10.1093/jat/bkab074. PMID: 34137890.