ASPEC® System: Fast, Accurate, and Reliable Quantification of THC and Metabolites from Blood in Forensics Testing with Fully-Automated SPE

APPLICATION NOTE AN1039

APPLICATION BENEFITS

- Able to manipulate whole blood
- Elevated throughput with 4 samples tested in parallel
- Quick SPE protocol
- High recoveries

ADDRESSED ISSUES

- Accurately quantify THC and metabolites for forensic purposes
- Traceability and confidence in the results

INTRODUCTION

Tetrahydrocannabinol (THC) is the principal active component in marijuana (Figure 1). It is rapidly absorbed by inhalation and the gastrointestinal tract. Being stored in the fat tissue of the body, it is released over a long period of time. THC is metabolized into two main analytes: 11-OH-THC (a.k.a. 11-hydroxy-THC) and 11-Nor-9-carboxy-THC (a.k.a. THC-COOH).[1]
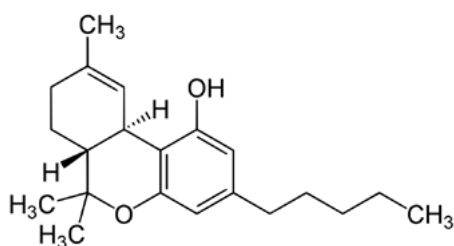

Figure 1
Chemical structure of Tetrahydrocannabinol

Positive identification of tetrahydrocannabinol is commonly performed through screening tests using a urine sample, with confirmatory tests typically performed using urine and blood samples.[2] Urine analysis can yield false positive results when patients are treated with Protonix®, a drug used to treat gastroesophageal reflux disease (GERD).[3] Blood testing is commonly performed to detect the recent use of THC. It is used to investigate accidents when driving under the influence (DUI) is suspected

because blood testing can provide an indication of whether the subject was actually under the influence at the time of the accident. Studies have shown that high THC blood levels are correlated with impaired driving.[4]

Figure 2
Image of Gilson's ASPEC® 274 System

GILSON®

This application note discusses a simple and effective automated solid phase extraction method using the ASPEC 274 (Figure 2) prior to sample analysis via LC/MS-MS for THC and its major metabolites in whole blood. The SPE method is diagramed in Figure 3.

Quantitative analytical testing for THC using urine and blood tests can be complementary to one another, as urine testing can detect THC levels because THC is continuously released from fat cells over time.[5]

MATERIALS AND METHODS

SPE Materials

SPE Cartridges
Phenomenex Strata™ X-Drug B 33u Polymeric Strong Cation 60 mg / 6 mL

SPE Solutions
Wash: Acetonitrile:Water (15:85)

Elute: Ethyl Acetate:Isopropyl Alcohol (85:15)

Pre-Sample Treatment
1. Samples were spiked at 100 ng/mL with 6 THC analytes/metabolites
2. Protein precipitation was performed by adding 1.0 mL cold ACN:MeOH (85:15 v:v) to 0.5 mL whole blood sample
3. Vortex for 1–2 min (using a maximix II, from Barnstead) with the maximum force possible
4. Centrifuge at 10,000 rpm, for 10 min
5. Discard pellet
6. Dilute sample obtained from protein precipitation step with 3 mL of 1% Formic acid in water
7. Load directly onto SPE cartridge with conditioning

SPE Method
1. Load 4.5 mL of pre-treated sample onto SPE cartridge at 3 mL/min
2. Wash with 2 mL solution at 6 mL/min
3. Dry for 5 minutes at 7–15 psi regulated gas (nitrogen, argon, or purified air)
4. Elute SPE cartridge with 2 mL solution at 3 mL/min

Final Sample Treatment Prior to Analysis
1. Evaporate eluent to dryness under 50°C nitrogen
2. Reconstitute in 500 µL of 50:50 Mobile Phase A:Mobile Phase B
3. Inject 5 µL on LC/MS-MS

Analytical LC/MS-MS Materials

HPLC System
Binary Gradient Mobile Phase Pumps
MS-MS Detection: API 3000

Mobile Phase
A: 1 mM Ammonium Formate with 0.1% Formic Acid

B: 0.1% Formic Acid/Methanol: Acetonitrile (1:1)

Column
Phenomenex Kinetex™ 2.6u C18 100A, 50 x 2.1 mm ID

Analytical LC/MS-MS Method

Mobile Phase Gradient

Step Number	Time (min)	% A	%B
1	0	50	50
2	3	5	95
3	4.5	5	95
4	4.51	50	50
5	6	50	50

Flow Rate
0.4 mL/min

Column Temperature
Ambient

Detection
CAD: 7
GS1: 60
GS2: 45
TEM: 600
CUR: 25
IS: 5500

Dwell
25 ms

Polarity
Positive

RESULTS AND DISCUSSION

Automated solid phase extraction (SPE) prior to LC/MS-MS analysis of THC analytes/metabolites in whole blood provides a confirmatory test for the positive or negative presence of THC. This testing is often used to determine whether drivers were operating a vehicle under the influence.
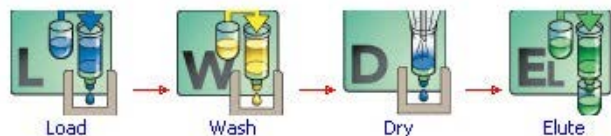

Figure 3
THC Solid Phase Extraction Method in TRILUTION® LH Software Using the Gilson ASPEC® 274 System

The application reported here involves three simple SPE steps (Figure 3), and saves time since it does not require the general SPE cartridge condition steps or multiple wash steps. Fast LC/MS-MS analysis in under five minutes creates efficiency for THC analysis. Figure 4 shows the detection of the metabolite peaks following SPE isolation.[6] Recoveries are reported in Table 1.
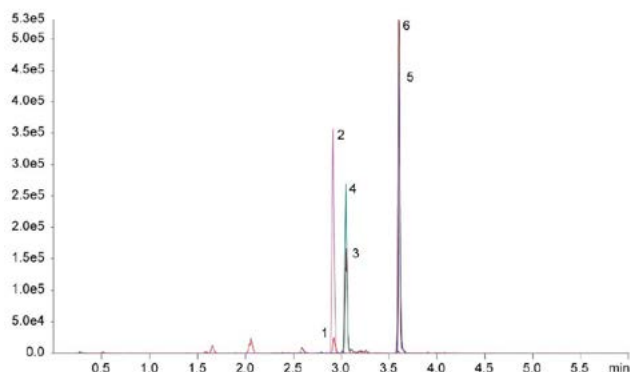

Figure 4
Detection of THC metabolites after SPE isolation. Peaks are numbered as follows: 1: THC-OH, 2: THC-OH-D3, 3: THC-COOH, 4: THC-COOH-D3, 5: THC, 6: THC-D3

Table 1
Recoveries of metabolites isolated using ASPEC® and detected by LC/MS-MS

Peak #/Analyte	Mass Range	% Recovery	% RSD (n=3)
1 / THC-OH	331.0 > 193.3	100.3	3.1
2 / THC-OH-D3	334.0 > 196.3	100	0.1
3 / THC-COOH	345.2 > 327.0	103.9	5.4
4 / THC-COOH-D3	348.0 > 330.1	100	0.1
5 / THC	315.2>193.1	99.3	3.9
6 / THC-D3	318.2>196.1	100	0.1

CONCLUSIONS AND BENEFITS

Gilson's ASPEC® 274 was used to automate the solid phase extraction (SPE) isolation of THC metabolites from whole blood. This simple SPE method saves time because cartridge conditioning and multiple wash steps are not required. Gilson's ASPEC 274 also includes four probes, allowing for samples to be processed in parallel. Additionally, efficient THC analysis is achieved with fast LC/MS-MS analysis that takes less than five minutes.

REFERENCES

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4. J. M. Walsh, J. J. de Gier, A. S. Christopherson, A.G. Verstraete. Drugs and driving. *Traffic Inj. Prev.* **5**, 241–253 (2004)

5. D. S. Kreutz, J. Axelrod. Delta-9-tetra-hydrocannabinol: localization in body fat. *Science* **179**, 391–392 (1973)

6. Phenomenex Application Note 19947: Analysis of THC & Metabolites from Whole Blood on Strata-X-Drug B and Kinetex C18 by LC-MS-MS.

ACKNOWLEDGMENTS

ORDERING INFORMATION

Description	Part Number	QTY
ASPEC 274, 2-DUAL 4260, WITH Z DRVE	2614010	1

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