



Automated Solid Phase Extraction of THC and Metabolites from Whole Blood using the Gilson GX-274 ASPEC™ System

Application Note CL0311

We report the use of the Gilson GX-274 ASPEC to automate the solid phase extraction (SPE) of THC metabolites from whole blood. Four samples can be extracted in parallel, improving throughput of the procedure. The time-saving method includes only three simple SPE steps and does not require cartridge conditioning or multiple wash steps. After extraction, THC analysis is achieved with rapid LC/MS-MS analysis that takes less than 5 minutes.

Introduction

Tetrahydrocannabinol (THC) (see Figure 1) is the principal active component in marijuana. It is rapidly absorbed by inhalation and also through 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

Positive identification of tetrahydrocannabinol is commonly performed through screening tests using a urine sample, with confirmatory tests performed using mostly urine and blood samples.² Urine analysis does have a false positive problem with a known drug Protonix™ which is used to treat gastroesophageal reflux disease (GERD).³ Blood testing is commonly performed to detect the recent use of THC, and therefore is commonly used to investigate accidents and suspicion of driving under the influence (DUI) because blood testing can provide an indication of whether the subject was actually under the

influence. Studies have shown that high THC blood levels are correlated with impaired driving.⁴

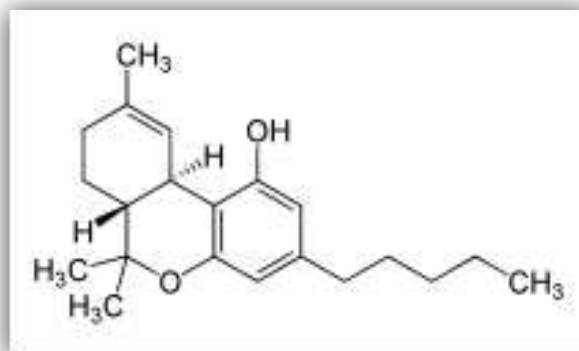


Figure 1. Chemical Structure of Tetrahydrocannabinol

This application note discusses a simple and effective automated solid phase extraction method using the GX-274 ASPEC™ (see 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.



Figure 2. Image of the Gilson GX-274 ASPEC System

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

Materials & 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 @ 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 - NO CONDITIONING REQUIRED

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



Figure 3. TRILUTION® LH THC Solid Phase Extraction Method Using the Gilson GX-274 ASPEC™ System

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 No.	Time (min)	Pct A	Pct 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: 25ms

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.

The application reported here involves three simple SPE steps, 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 data generated using this method.⁶ Recoveries are reported in Table 1.

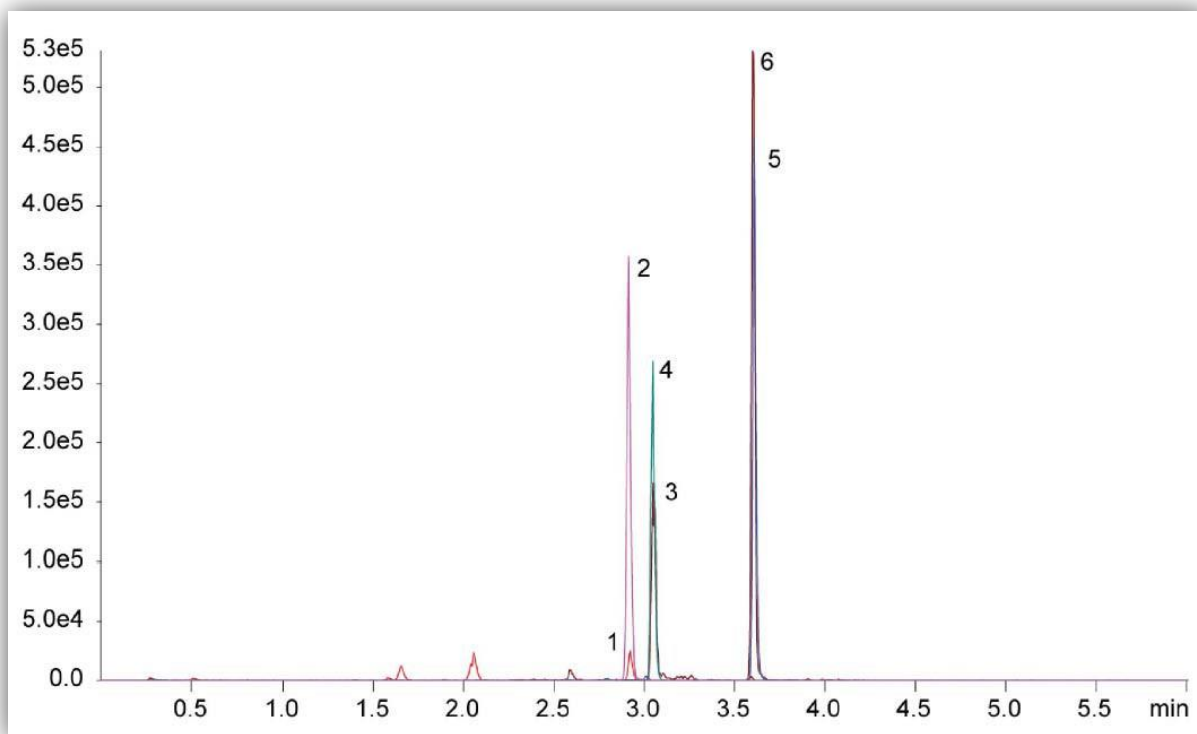


Figure 4. Detection of THC metabolites after SPE isolation. Peaks are numbered as follows: 1: THC-OH, 2: THC-OH-D3, 3: THC-COOH, 4: THC-COOH-D3, 5: THC, 6: THC-D3



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	NA	NA
3 / THC-COOH	345.2>327.0	103.9	5.4
4 / THC-COOH-D3	348.0>330.1	NA	NA
5 / THC	315.2>193.1	99.3	3.9
6 / THC-D3	318.2>196.1	NA	NA

Table 1. Example Chromatogram of Extracted THC Analytes/Metabolites from Whole Blood

References

1. Wall, ME. The *in vitro* and *in vivo* metabolism of tetrahydrocannabinol (THC). *Annals NY Acad. Sci.* **191**, 23-39 (1971)
2. United Nations Office on Drugs and Crime. Recommended methods for the identification and analysis of cannabis and cannabis products. www.unodc.org/documents/sciescient/ST-NAR-40-Ebook.pdf.
3. Protonix Label, Food & Drug Admin. www.accessdata.fda.gov/drugsatfda_docs/label/2012/020987s045lbl.pdf

Summary and Conclusions

- The Gilson GX-274 ASPEC 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.
- The GX-274 ASPEC includes four probes, allowing parallel processing of samples.
- Efficient THC analysis is achieved with fast LC/MS-MS analysis that takes less than 5 minutes.

4. Walsh JM, de Gier JJ, Christopherson AS, Verstraete AG. Drugs and driving. *Traffic Inj. Prev.* **5**,241-253 (2004)
5. Kreutz DS, Axelrod J. Delta-9-tetrahydrocannabinol: 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

The data in this application note was generated by Phenomenex (www.phenomenex.com) and referenced in application note # 19947.

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