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

Application Note CL0311

Keywords

Gilson GX-274 ASPEC™, TRILUTION® LH, Solid Phase Extraction (SPE), LC/MS-MS Analysis, Tetrahydrocannabinol (THC), Metabolites, Whole Blood

Introduction

This application note information was performed by Phenomenex (www.phenomenex.com) and referenced in application note # 19947.

Tetrahydrocannabinol (THC) (see figure 1) is the principle 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 (11-hydroxy-THC) and 11-Nor-9-carboxy-THC (THC-COOH)[3].

Positive identification of tetrahydrocannabinol is commonly performed through screening tests using a urine sample, with confirmatory tests performed using mostly urine and blood samples. Urine analysis does have a false positive problem with a known drug Protonix™ which is used to treat gastroesophageal reflux disease (GERD).[4] 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.[5]

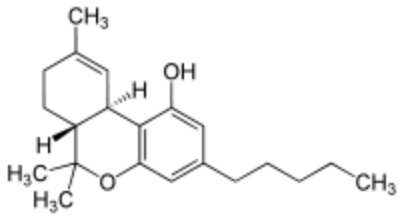

Figure 1. Chemical Structure of Tetrahydrocannabinol[3]

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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.

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

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

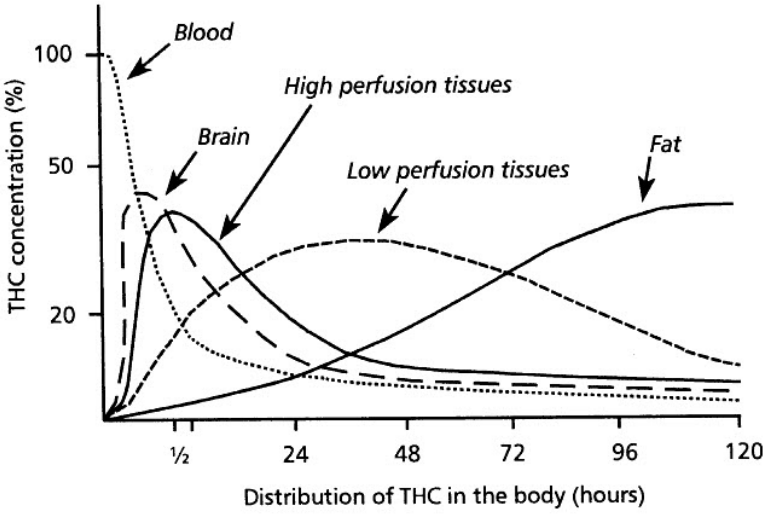


Figure 3. Distribution of THC in the body (Kreutz & Axelrod 1973) [6]

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 mins (using a maximix II, from Barnstead) with the maximum force possible
4. Centrifuge at 10,000 rpm, for 10 mins
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

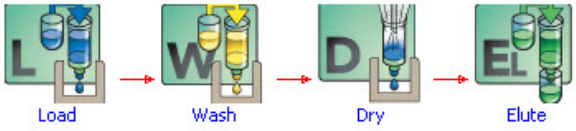


Figure 4. TRILUTION® LH THC Solid Phase Extraction Method Using the Gilson GX-274 ASPEC™ System

Final Sample Treatment Prior to Analysis:

1. Evaporate eluant to dryness under 50 C nitrogen
2. Reconstitute in 500 uL of 50:50 Mobile Phase A:Mobile Phase B
3. Inject 5 uL 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

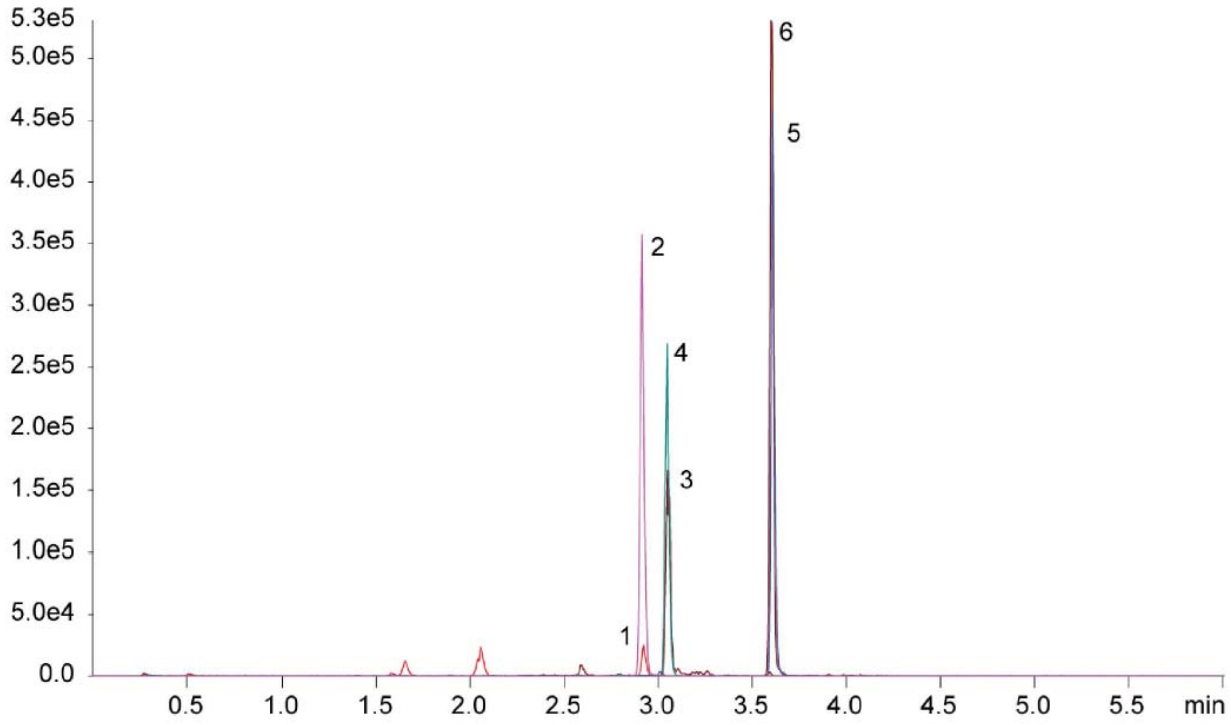
Flow rate: 0.4 mL/min

Column Temperature: ambient

Detection: CAD: 7 GS1: 60 GS2: 45 TEM: 600 CUR: 25 IS: 5500
Dwell: 25ms
Polarity: Positive

Results

Figure 5. Example Chromatogram of Extracted THC Analytes/Metabolites from Whole Blood



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

Summary

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 most notable for drivers under the influence. This application involves three simple SPE steps, saving time by not requiring the general SPE cartridge condition steps or mutliple wash steps. Fast LC/MS-MS analysis in under five minutes creates efficiency for THC analysis.

References

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

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

3 Wikepedia

4 http://www.canorml.org/healthfacts/testing.tips.html#wrong

5 http://www.canorml.org/healthfacts/drugtestguide/drugtestdetection.html

6 Kreutz DS & Axelrod J (1073) Delta-9-tetrahydrocannabinol: localization in body fat. Science 179 pp 391-392