APPLICATIONS

Extraction and Analysis of THC and Metabolites from Whole Blood and Urine by GC/MS

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Zeshan loves to collect watches and the Back to the Future Trilogy. He has twin boys which drive him crazy! He is an Apple Fanboy for life and he likes being in the lab more than anywhere else.

Overview

- *High recovery of THC, 11-OH-THC and THC-COOH*
- *Extremely short run time*
- *Methods for both blood and urine sample preparation*

Introduction

In this technical note we demonstrate a high recovery method for Δ(9)-tetrahydrocannabinol (THC) and its metabolites 11-hydroxy-Δ(9)-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ(9)-tetrahydrocannabinol (THC-COOH). We offer sample prep methods for urine using Strata® Screen-C and for whole blood on Strata XL-A, followed by GC/MS using the Zebron™ ZB-5*PLUS*™ GC column. In both cases we achieved good separation of analytes and high recovery for all compounds.

When heated, tetrahydrocannabinolic acid (THCA) is converted to the primary psychoactive compound THC. The body metabolizes THC into the less psychoactive 11-OH-THC, which in turn is converted to inactive THC-COOH. Within seconds of inhalation THC is detectable in plasma, reaching peak concentration 8 minutes thereafter and rapidly decreasing after peak. The initial metabolite, 11-OH-THC, is seen after the first few inhalations and reaches peak concentration at 13.5 minutes. THC-COOH concentrations peak in plasma within the first hour but remain detectable for several weeks in the plasma of chronic users.[1, 2]

Materials

Analytical reference standards and biological matrices (human urine and whole blood) were purchased from Cerilliant Corporation (Round Rock, TX, USA) and BioreclamationIVT (Chestertown, MD, USA). Instrumentation used was purchased from Shimadzu (Kyoto, Japan). All other chemicals were obtained from the Sigma-Aldrich Company (St. Louis, MO). D.I. water via Sartorius Arium® Comfort II, courtesy of Sartorius Corporation (Bohemia, NY).

Urine Methods

Sample Pretreatment

Add internal standards and 100 µL of 10N NaOH to 2 mL of urine. Mix/vortex. Hydrolyze for 20 minutes at 60 °C. Cool. Adjust pH to 3.0 with ~1.0 mL of glacial acetic acid.

Sample Preparation

Step	
Product Name:	Strata® Screen-C (55 µm, 70 Å), 200 mg / 3 mL
Catalog Number:	8B-S016-FBJ
Load:	Load 3 mL at 1-2 mL/minute
Weak Wash:	2 mL D.I. water
Strong Wash	1 mL 100 mM HCl/acetonitrile (95:5)
Dry down:	5 minutes at >10" Hg
Elute:	1 x 3 mL hexane/ethyl acetate (50:50); collect eluate at 1-2 mL/minute
Evaporate:	Evaporate until dry under nitrogen
Reconstitute:	Add 100 µL of 50:50 Ethyl Acetate/BSTFA w/ 1 % TMCS. Mix/vortex. Heat at 70 °C for 30 minutes. Store at 40° C for 10 minutes. Centrifuge at 3500 RPM for 10 seconds.

GC/MS Conditions

Column: Zebron ZB-5*PLUS*
Dimensions: 10 meter x 0.18 mm x 0.18 µm
Part No.: 7CD-G032-08
Liner: Zebron Plus Liner for Agilent & Thermo 4 mm ID Single Taper Wool on Bottom
Liner Part No.: AG2-0A11-25
Injection: Split 10:1 @ 280°C, 1 mL
Carrier Gas: Helium @ 0.7 mL/min (constant flow)
Oven Program: 200 to 300 °C @ 20 °C/min
Detector: MSD @ 320 °C
Analytes
1. THC
2. THC-D3
3. 11-OH-THC
4. 11-OH-THC-D3
5. COOH-THC
6. COOH-THC-D3

[1] Marilyn A. Huestis, et al. Blood Cannabinoids. I. Absorption of THC and Formation of 11-OH-THC AND THCCOOH During and After Smoking Marijuana. Journal of Analytical Toxicology, 1992 September; (16) 276-282.

[2] Marilyn A Huestis. Human Cannabinoid Pharmacokinetics. Chem Biodivers. 2007 August ; 4(8): 1770–1804.

Urine Results

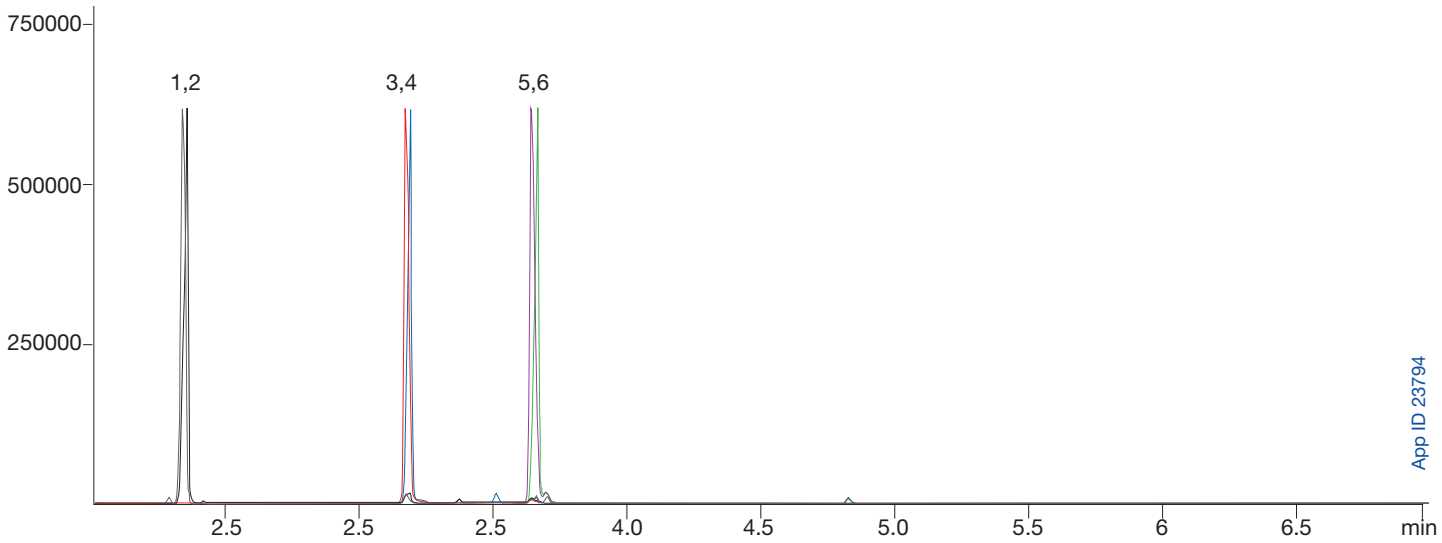

Figure 1. XIC of THC and metabolites extracted from urine

Whole Blood Methods

Sample Pretreatment

Add 100 µL of each internal standard to 1 mL of blood. Mix/vortex while adding (drop-wise) 2 mL of acetonitrile. Centrifuge at 4700 RPM for 10 minutes. Transfer supernatant to a new vial and add 2 mL of 2 % aqueous ammonia. Mix/vortex.

Sample Preparation

Step	
Product Name:	Strata®-XL-A 100 µm Polymeric Strong Anion, 100 mg / 6 mL
Catalog Number:	8B-S053-ECH
Condition:	2 mL of aqueous ammonia to Strata-XL-A
Load:	Load sample
Weak Wash:	1 mL methanol / D.I. water (50:50)
Strong Wash:	2 mL Hexane
Dry down:	5 minutes at >10" Hg
Elute:	2 x 1.5 mL EtOAc/Hexane/Acetic Acid (65:35:2); collect eluate at 1-2 mL/minute
Evaporate:	Evaporate until dry under Nitrogen
Reconstitute:	Add 100 µL of 50:50 Ethyl Acetate/BSTFA w/ 1 % TMCS. Mix/vortex. Heat at 70 °C for 30 minutes. Store at 4 °C for 10 minutes. Centrifuge at 4700 RPM for 10 seconds.

GC/MS Conditions

Column: Zebron™ ZB-5*PLUS*™
Dimensions: 10 meter x 0.18 mm x 0.18 µm
Part No.: 7CD-G032-08
Liner: Zebron Plus Liner for Agilent & Thermo 4 mm ID Single Taper Wool on Bottom
Liner Part No.: AG2-0A11-25
Injection: Split 10:1 @ 280 °C, 1 mL
Carrier Gas: Helium @ 0.7 mL/min (constant flow)
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Analytes: 1.THC
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5. COOH-THC
6. COOH-THC-D3

Whole Blood Results

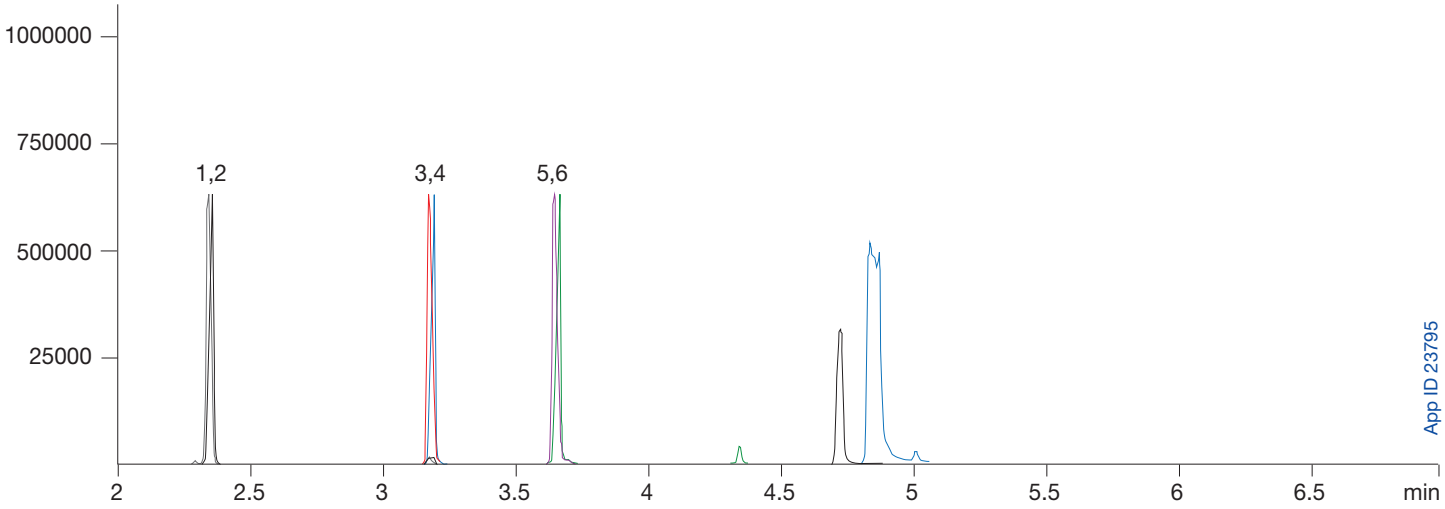

Figure 2. XIC of THC and metabolites extracted from blood

Table 1. Peak Areas and Recoveries for Urine and Whole Blood

	Neat Peak	Urine PA	Urine % Recovery	Blood PA	Blood % Recovery
Δ-THC	202758	185300	91.390	187337	92.394
11-OH-THC	25633	23240	90.664	25634	100.004
COOH-THC	87357	87302	99.937	85727	98.134
Δ-THC-d3	190587	180587	94.753	178450	93.632
11-OH-THC-d3	29202	26154	89.562	28031	95.990
COOH-THC-d3	96307	101361	105.248	95779	99.452

Conclusion

In this technical note we demonstrate a complete sample prep and GC/MS solution for the analysis of THC and its primary metabolites from both urine and whole blood. In both matrices, we achieved a > 90 % recovery for all analytes. GC analysis time, using the Zebron™ ZB-5*PLUS*™, was less than 4 minutes. When compared to other published methods, this method offers higher recovery and a faster run time.

APPLICATIONS

Ordering Information

Zebron™ ZB-5PLUS™ GC Columns

Length (m)	ID (mm)	Film (µm)	Temp. Limits °C	Part No.
10	0.10	0.10	-60 to 360/370	7CB-G032-02
10	0.18	0.18	-60 to 360/370	7CD-G032-08
15	0.25	0.25	-60 to 360/370	7EG-G032-11
20	0.18	0.18	-60 to 360/370	7FD-G032-08
30	0.25	0.25	-60 to 360/370	7HG-G032-11
30	0.25	0.50	-60 to 360/370	7HG-G032-17
30	0.25	1.00	-60 to 360/370	7HG-G032-22
30	0.32	0.25	-60 to 360/370	7HM-G032-11
30	0.32	0.50	-60 to 360/370	7HM-G032-17
60	0.25	0.25	-60 to 360/370	7KG-G032-11

Strata®-XL-A

Format	Sorbent Mass	Part Number	Unit
Tube			
	30 mg	8B-S053-TAK	1 mL (100/box)
	60 mg	8B-S053-UBJ	3 mL (50/box)
	100 mg	8B-S053-EBJ	3 mL (50/box)
	100 mg	8B-S053-ECH	6 mL (30/box)
	200 mg	8B-S053-FBJ	3 mL (50/box)
	200 mg	8B-S053-FCH	6 mL (30/box)
	500 mg	8B-S053-HCH	6 mL (30/box)
Giga™ Tube			
	2 g	8B-S053-KEG	20 mL (20/box)
	5 g	8B-S053-LFF	60 mL (16/box)
	10 g	8B-S053-MFF	60 mL (16/box)
96-Well Plate			
	30 mg	8E-S053-TGB	2 Plates/Box

Strata Screen-C

Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S016-EAK**	1 mL (100/box)
	100 mg	8B-S016-EBJ	3 mL (50/box)
	150 mg	8B-S016-SBJ	3 mL (50/box)
	150 mg	8B-S016-SCH	6 mL (30/box)
	200 mg	8B-S016-FBJ	3 mL (50/box)
	200 mg	8B-S016-FCH	6 mL (30/box)
	300 mg	8B-S016-RBJ	3 mL (50/box)
	300 mg	8B-S016-RCH	6 mL (30/box)
	500 mg	8B-S016-HCH	6 mL (30/box)
96-Well Plate			
	50 mg	8E-S016-DGB	2 Plates/Box

**Tab-less tubes available. Contact Phenomenex for details.

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