SAMHSA-Compliant LC/MS/MS Analysis of 11-nor-9-carboxy-Δ^9-Tetrahydrocannabinol in Urine with Agilent Bond Elut Plexa PCX and Agilent Poroshell 120

Application Note

Forensic Toxicology

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Abstract

Guidelines from the US Substance Abuse and Mental Health Services Administration (SAMHSA) effective October 2010, allowed LC/MS/MS methods to be used for confirmation of initial drug tests [1]. LC/MS/MS methods are often less complicated than previously employed GC/MS methods because they do not typically require a derivatization step. This application note presents a method for analysis of 11-nor-9-carboxy-Δ^9– tetrahydrocannabinol that meets SAMHSA guidelines to demonstrate linearity, limit of detection (LOD), accuracy and precision, as well as measurement of matrix effects, extraction recovery and overall process efficiency. This is one of a suite of six simplified methods covering all classes of SAMHSA-regulated drugs and using premier Agilent products such as Agilent Bond Elut Plexa PCX mixed-mode polymeric SPE sorbent, Agilent Poroshell 120 EC-C18 2.7 µm superficially porous LC column, Agilent 1200 Infinity LC system, and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source.

Agilent Technologies

Introduction

11-Nor-9-carboxy-Δ^9– tetrahydrocannabinol (THCA, "THC-acid", THC-COOH) is a metabolite of tetrahydrocannabinol (Δ^9–THC), an active constituent of marijuana. In the form of its glucuronide conjugates, THCA is excreted in urine for several weeks [2]. The SAMHSA confirmation cutoff concentration for THCA is 15 ng/mL and a LOD at 10% of the cutoff would be 1.5 ng/mL.

Sample preparation for 11-nor-9-carboxy-Δ^9–THC analysis requires base hydrolysis of urine to convert glucuronides back to THCA. Although THCA is a carboxylic acid, for the sake of a single method setup for all SAMHSA-regulated drugs, the Agilent sorbent chosen for extraction is Agilent Bond Elut Plexa PCX, a mixed-mode cation-exchange polymer. It efficiently retains THCA by hydrophobic interaction.

The extraction method provides reproducible high recoveries of THCA due to the unique properties of the Plexa sorbent. Unlike other polymeric sorbents, Plexa possesses an amide-free hydroxylated particle surface that excludes protein binding. This results in minimized ion suppression and maximum sensitivity. Fast flow and reproducible performance are due to the narrow particle size distribution with no fines to cause blockages.

An Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm column was chosen due to its high capacity and excellent separation properties. With superficially porous 2.7 µm particles, the Poroshell 120 provides similar efficiency to sub-2 µm UHPLC columns, with approximately 40% less back pressure, thereby allowing the users of even 400 bar LC systems to increase resolution and shorten analysis and re-equilibration times by applying a higher flow rate.

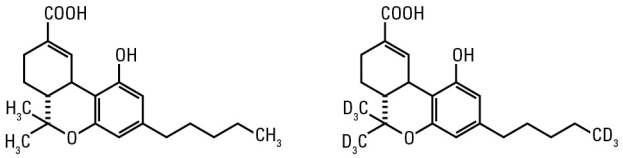
Being essentially nonpolar (log P>6), cannabinoids are not ideally suited for electrospray ionization and are often analyzed using APCI. However, due to its carboxylic moiety, THCA is much more efficiently ionized in negative ion mode than Δ^9–THC and 11-hydroxy- Δ^9–THC. A choice of electrospray source for THCA detection is warranted by the convenience of a single mass spectrometer configuration for all SAMHSA drugs.

With a low sample injection volume of 10 µL and no sample preconcentration, the method demonstrates excellent signal-to-noise ratios for cutoff and 10% of the cutoff concentrations (approximately 100:1 and 10:1, respectively) due to the enhanced sensitivity of the Agilent 6460 Triple Quadrupole LC/MS system with the Jet Stream electrospray source.

Previous methods from Agilent [3,4] used the Agilent 6410 Triple Quadrupole LC/MS system and other SPE/LC products and procedures.

Experimental

Analytes



11-nor-9-carboxy-Δ^9-THC
Log P (predicted) 6.06-6.36, pKa 4.5

11-nor-9-carboxy-Δ^9-THC-D_9

Figure 1. 11-nor- carboxy-Δ^9- tetrahydrocannabinol analytes and their structures.

Drug standards were purchased from Cerilliant Corporation as 1 mg/mL (11-nor-9-carboxy-Δ^9-THC) and 100 µg/mL (11-nor-9-carboxy-Δ^9-THC-D_9 and 11-nor-9-carboxy-Δ^9-THC-glucuronide) solutions in methanol.

Materials and instrumentation

SPE

- Agilent Bond Elut Plexa PCX cartridges 30 mg, 3 mL (p/n 12108303)
- Agilent vacuum manifold VacElut 20 (p/n 12234100)
- Agilent stopcock valves (p/n 12234520)
- Agilent silanized 2 mL autosampler vials (p/n 5183-2072)
- Agilent screw caps for AS vials (p/n 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm (p/n 699975-302)
- Agilent 1260 Infinity LC system (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, G1330B thermostat)

MS

- Agilent 6460A Triple Quadrupole LC/MS system with AJST electrospray ionization source

Sample preparation

Hydrolysis and sample pretreatment

1. Spike 0.5 mL of urine with ISTD at 50 ng/mL; use of methanol-rinsed and 12 × 75 mm dried glass tubes is recommended.
2. Add 100 µL 7 N KOH, vortex.
3. Incubate in the heating block at 60 ±5 °C for 30 minutes.
4. Cool. Add 125 µL methanol, vortex.
5. Add 1.5 mL of 0.2 M sodium acetate buffer (pH 4).
6. Neutralize with 100 µL glacial acetic acid, vortex.
7. Centrifuge if cloudy.

Extraction

1. Condition Bond Elut Plexa PCX column with 0.5 mL methanol–soak, then let drip.
2. Load sample/supernatants.
3. Wash 1: 2 × 2 mL 10:90 ACN: 2% acetic acid.
4. Wash 2: 2 mL 30:70 ACN: 2% acetic acid.
5. Dry 5–10 minutes under high vacuum (10–15 in Hg).
6. Wash with 200 µL hexane, pull through with low vacuum (2–3 in Hg).
7. Dry under high vacuum, 3 to 4 minutes.
8. Elute with 0.5 mL 80:20 ethyl acetate:isopropanol. Soak, let eluate drip into collection vials, then apply low vacuum (2–3 in Hg).
9. Add 1 mL more of the same eluent, repeat soaking-elution procedure.
10. Evaporate to dryness at 40 °C.
11. Reconstitute in 0.5 mL initial mobile phase (30% methanol, 70% 5 mM ammonium formate).

LC/MS/MS

LC conditions

Mobile phase A	5 mM ammonium formate in water
Mobile phase B	methanol
Flow rate	0.8 mL/min

Gradient	Time (min)	% B
	0.0	30
	1	95
	5	95
	5.1	30

Stop time	5.2 minutes
Post time	2 minutes
Max pump pressure	400 bar
Injection volume	10 µL
Needle wash	Flush port 75:25 methanol:water for 10 seconds

Disable overlapped injection

No automatic delay volume reduction

MS conditions

ES Source Parameters

Ionization mode	negative
Capillary voltage	4,000 V
Drying gas flow	11 L/min
Drying gas temperature	320 °C
Nebulizer gas	18 psi
Sheath gas flow	12 L/min
Sheath gas temperature	320 °C
Nozzle voltage	0 V

MS parameters

Scan type	MRM

Pre-run script
SCP_MSDiverterValveToWaste(){MH_Acq_Scripts.exe}

Time segments	#1: 1.4 minutes - diverter valve to MS
Delta EMV (-)	800 V

Results and Discussion

The cannabinoids are notorious for their adsorption to glass and plastic. To minimize losses and to ensure method reproducibility, we strongly recommend the use of only freshly prepared stock solutions and calibrators, silanized or thoroughly washed, methanol-rinsed and dried glassware, and analyze final extracts immediately after reconstitution.

THCA is retained on a cation-exchange mixed mode Plexa PCX by hydrophobic interactions. The 100% methanol wash, commonly employed in ion-exchange SPE, is not practical for THCA extraction as high organic will elute the compound from the sorbent.

To minimize matrix interferences, 10 to 30% acetonitrile is added to wash one and two, respectively. The hexane wash serves the same purpose. When used alone and in a small amount (200 µL), hexane elutes most lipids but does not lead to analyte desorption, because THCA is very hydrophobic (log P>6) and is retained at the hydrophobic core of the Plexa particles very strongly. A soaking procedure is recommended at the elution step to enhance the solvent-analyte interaction and improve analyte recoveries.

The Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm column provides fast separation of THCA in urine extract and good peak shape (Figure 2). The LC separation intentionally begins with a relatively low fraction of organic solvent (30%) to allow salts and other polar components of urine to elute at the beginning of the sample run. Due to a steep gradient, the remaining hydrophobic interferences largely elute before the analyte, thus reducing matrix effect at the time of peak elution (1.96 minutes). A flow rate of 0.8 mL/min allows for a short retention and re-equilibration time. Each sample run begins with diverting a first portion of flow (0 to 1.4 minutes) to waste to minimize source contamination. Data collection begins at 1.4 minutes, immediately after the diverter valve switch.

SAMHSA guidelines require the use of one quantifier and at least one qualifier ion for both target compound and ISTD. A third transition for target analyte is provided for additional confidence (Table 1). Agilent MassHunter Quantitative software automatically calculates qualifier ion ratios, highlighting those out of acceptable range.

Table 1. MRM Transitions

Compound	Parent	Product	Fragmentor	Collision energy
11-nor-9-carboxy-Δ^9-THC	343.2	299.2	135	18
	343.2	245.1	135	30
	343.2	191.1	135	33
11-nor-9-carboxy-Δ^9-THC-D_9	352.2	308.2	145	18
	352.2	254.2	145	30
11-nor-9-carboxy-Δ^9-THC glucuronide	519.2	343.2	160	22
	519.2	299.2	160	36

When processed according to the protocol, urine samples spiked with 11-nor-9-carboxy-Δ^9-THC-glucuronide at 1,000 ng/mL tested negative for this compound. Instead, they displayed a very large THCA peak, far beyond the upper calibration level of 600 ng/mL. This is proof of full conversion of glucuronides to THCA by the base hydrolysis step. MS parameters for the detection of 11-nor-9-carboxy-Δ^9-THC-glucuronide are included in Table 1 for analysts interested in testing the hydrolysis efficiency.

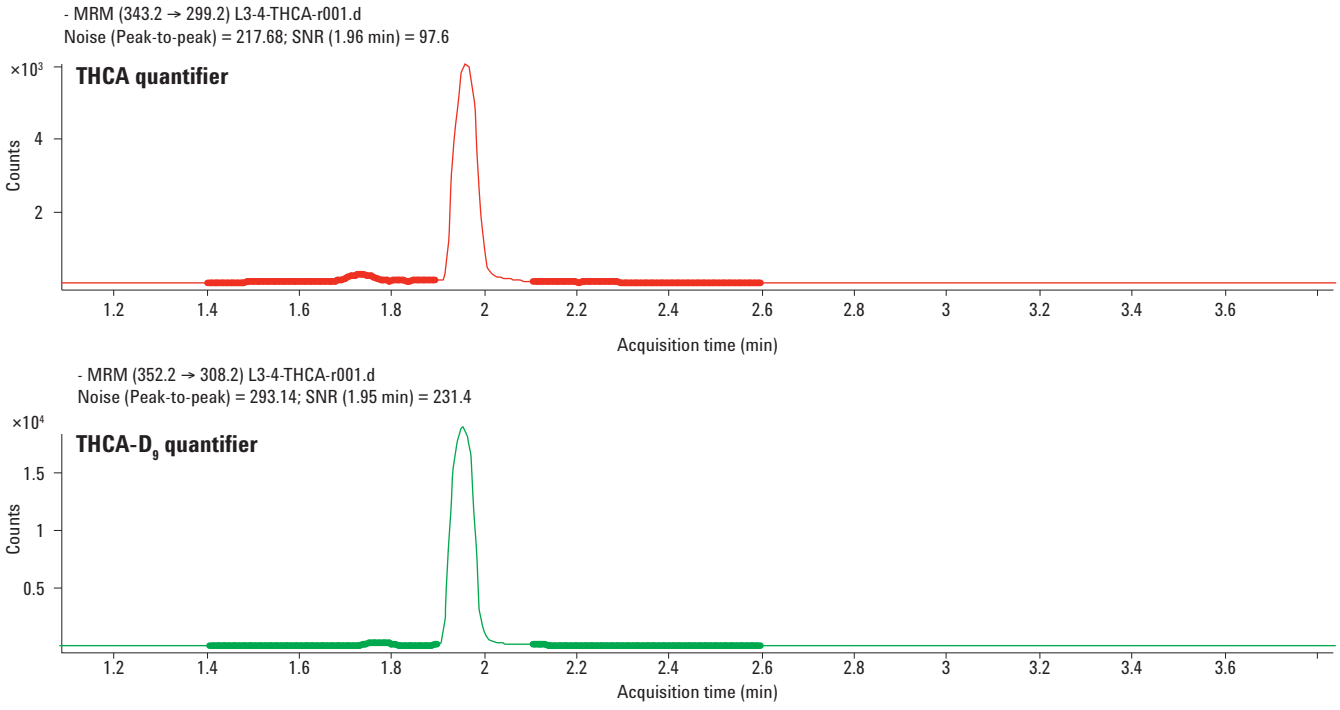


Figure 2. MRM extracted ion chromatograms for THCA (15 ng/mL) THCA-D_9 (50 ng/mL) in urine extract. Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm column. Noise regions are shown in bold.

Normal, rather than dynamic, MRM acquisition mode can be used with this method, since dynamic MRM has no advantages for detection of a single peak.

Due to its extreme hydrophobicity, THCA can adhere not only to glassware but also to injector parts and tubing. To avoid carryover, we recommend running a mobile phase blank after samples with high concentration, and to use the Injector Flush Pump option of the autosampler. If needed, the needle wash can be increased from 10 to 20 seconds.

A signal-to-noise ratio approximately 100:1 for the cutoff concentration of 15 ng/mL for THCA (Figure 2, upper panel) illustrates excellent performance of the 6460 Triple Quadrupole LC/MS system, capable of reliably detecting THCA at a small fraction (10%) of the SAMHSA cutoff concentration.

Figure 3 shows a calibration curve for extracted urine standards at five concentration levels. Calibration standards were prepared by spiking negative urine at 1.5, 15, 75, 300, and 600 ng/mL with THCA. Deuterated internal standard THCA-D_9 was added at 50 ng/mL. Excellent linear fit ($R^2 > 0.999$) demonstrates linearity of the method across a broad dynamic range of concentrations, as required by SAMHSA guidelines.

Method evaluation

Method performance metrics in Table 2 were calculated according to the principles laid out in Matuszewski *et al*. [5] and widely accepted as an industry standard approach for LC/MS/MS methods. Extraction procedure and LC/MS/MS measurement were performed for five replicates of negative urine spiked pre-extraction at the cutoff level, and five replicates of negative urine extract reconstituted in initial mobile phase and then fortified at 15 ng/mL with THCA (spiked post-SPE). The third measurement was of initial mobile phase (the reconstitution solvent) fortified to correspond to the cutoff concentration of 15 ng/mL in urine (spiked mobile phase).

Process efficiency (absolute recovery) is a ratio of a peak area of target analyte in urine sample spiked pre-SPE to its peak area in matrix-free spiked mobile phase. Extraction recovery is a ratio of a peak area of target analyte in urine extract spiked pre-SPE to its peak area in an extracted negative urine sample spiked post-SPE. Matrix effect is a ratio of a peak area of target analyte in urine extract spiked post-SPE to its peak area in spiked mobile phase.

Accuracy is a ratio of a measured concentration calculated using the calibration curve to the expected concentration in a sample spiked with a known amount of target analyte. Precision or coefficient of variation (CV) is a measure of reproducibility and is calculated as a percent standard deviation over the mean of the five measurements.

The method is characterized by good recoveries together with very high accuracy (98%) and precision (2.2%) of the data (Table 2). Matrix effect in excess of 100% indicates ionization enhancement as opposed to ionization suppression. Signal enhancement of only 13% confirms cleanliness of Plexa PCX extracts. Overall process efficiency of 73% is rather high due to analytical challenge associated with the cannabinoid family.

Table 2. Method Performance for 11-nor- carboxy-Δ^9- tetrahydrocannabinol at the Cutoff Level, n = 5

	%
Process efficiency	73
Extraction recovery	65
Matrix effect	113
Accuracy	98.2
Precision (CV)	2.2

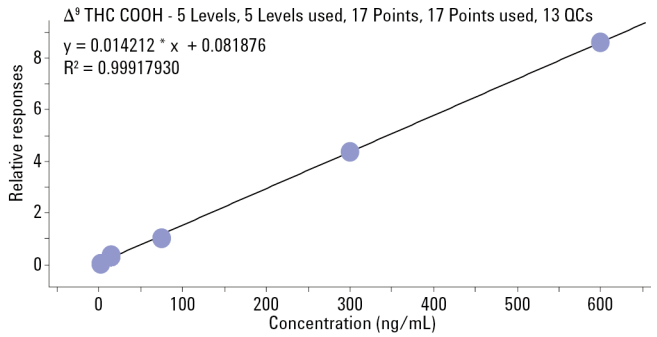


Figure 3. Example calibration curve for THCA in urine extract. Calibration range 1.5 to 600 ng/mL. Linear fit, R^2>0.999.

Conclusions

The solid phase extraction procedure coupled with the LC/MS/MS detection method described is SAMHSA-compliant and provides reproducible results for forensic toxicology or other analytical environments with similar requirements for legally defensible data. The THCA method uses the same hardware setup as the other Agilent SAMHSA methods. These methods are usable with all models of Agilent 1100 and Agilent 1200 LC series, since the back pressure in the LC system does not exceed 400 bar. Source parameters can be easily modified to use this method with other models of Agilent Triple Quadrupole LC/MS systems. Electronic copies of the LC/MS/MS acquisition and quantitation methods are available from Agilent Technologies.

References

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