Cannabinoid Quantitation Using an Agilent 6430 LC/MS/MS

Application Note

Forensics

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Abstract

A method was developed for the quantitation of cannabinoids with LC/MS/MS using an Agilent 6430 Triple Quadrupole LC/MS system. The method displays excellent accuracy and precision using a linear calibration for the analysis of THC, Carboxy-THC, and OH-THC in whole blood. Sufficient resolution and peak shape for the targets can be achieved within an overall run time of 12.5 minutes. Additional validation studies confirmed that this method meets all criteria required for routine analysis of cannabinoids in whole blood.

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Introduction

Cannabinoids are analyzed in urine, oral fluid, and blood in many forensic toxicology laboratories. Quantitative analysis of cannabinoids in blood, specifically THC, is necessary for meaningful toxicological interpretation in the investigation of Driving Under the Influence of Drugs (DUID) cases. The quantitation and confirmation of cannabinoids for DUID cases constitutes a significant portion of the workload for many forensic toxicology laboratories worldwide. Cannabinoid analysis in blood has been driven by advances in GC/MS technology, notably 2D-GC/MS [1] and GC/MS/MS with column backflushing [2]. During the last decade, the role and progress of liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) in forensic and clinical toxicology has been assessed several times by leading experts in the field. This technique is becoming increasingly useful in routine toxicological analysis given its accuracy and sensitivity [3].

This application addresses the development of an LC/MS/MS method for the quantitation of THC and its metabolites. The primary pharmacologically active natural cannabinoid is Δ9-THC, and the primary (inactive) metabolite is 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC). Another (active) metabolite of forensic interest is 11-hydoxy- Δ9-tetrahydrocannbinol (OH-THC). A mathematical model using THC and carboxy-THC concentrations has been explored as a means of estimating time of last use of cannabis [4]. Recent research has also shown that a glucuronide conjugate of carboxy-THC is present in relatively high concentrations in the blood of cannabis users. Sample preparation methods that expose carboxy-THC glucuronide to basic pH result in its conversion to carboxy-THC and elevate the apparent carboxy-THC concentration by approximately a factor of two [5].

The LC/MS/MS method described within this application note has the advantage of simpler sample preparation, without derivatization, compared to standard GC/MS and GC/MS/MS methods. Liquid-liquid extraction at acidic pH does not result in artifactual elevation of carboxy-THC concentration due to hydrolysis of carboxy-THC glucuronide. Validation studies were conducted using the SWGTOX guidelines [6] in conjunction with the Virginia Department of Forensic Science validation guidelines. This method was determined to meet all criteria for the qualitative and quantitative analysis of cannabinoids [7].

Experimental

Equipment and Instrumentation

- Agilent 6430 LC/MS/MS system
- Agilent 1260 Infinity LC with an Agilent Poroshell 120 EC-18, 2.1 × 75 mm, 2.7 µm column
- Agilent 1260 Automatic Liquid Sampler
- Autosampler vials with inserts
- Agilent MassHunter Optimizer Software
- Zymark TurboVap Evaporator
- Screw capped extraction tubes with 12 mL or greater capacity
- Kimble/Chase tapered glass tubes for evaporation and reconstitution (p/n 73785-5)
- Glass Pasteur pipets
- Pipets for accurate dispensing of volumes 10 µL to 250 µL, and 1 mL to 10 mL
- Test tube rocker or rotator
- Centrifuge

Materials

- Water, Type 1 or HPLC grade
- Acetonitrile, Optima grade or higher
- Methanol, HPLC grade or higher
- Formic acid, eluent additive for LC/MS ~ 98%
- Hexane, Optima grade or higher
- Ethyl acetate, Optima grade or higher
- Glacial acetic acid, ACS plus grade or higher
- Blank blood

The calibrators, controls, and internal standards for this method were purchased from Cerilliant and Grace-Alltech and are listed in Table 1. The calibrators are prepared using a different source than controls.

Mobile phase solutions

- 0.1% formic acid in water (mobile phase A)
- 0.1% formic acid in acetonitrile (mobile phase B)

Table 1. Targets and Corresponding Internal Standards

Target	Internal standard
THC	THC-d3
Carboxy-THC	Carboxy-THC-d3
OH-THC	OH-THC-d3

Sample Preparation

Blood samples were prepared according to the procedure detailed in Figure 1. Whole blood samples were extracted using 9:1 hexane:ethyl acetate with 10% acetic acid. Extracts were reconstituted in 50:50 acetonitrile/water then separated with an Agilent 1260 Infinity LC with an Agilent Poroshell 120 EC-18, 2.1 × 75 mm, 2.7 µm column.

Internal Standard and Calibrator Preparation

Working internal standard solution (1 µg/mL): Pipette 100 µL of the 0.1 mg/mL (or 10 µL of 1.0 mg/mL) stock solution of deuterated standards into a 10-mL volumetric flask and qs to volume with methanol.

Working internal standard solution (0.1 µg/mL): Pipette 1 mL of the 1 µg/mL working internal standard solution of deuterated standards into a 10-mL volumetric flask and qs to volume with methanol.

Working standard solution (1/5 µg/mL): Pipette 25 µL/125 µL of the 1.0 mg/mL stock solution standards (THC, OH-THC/Carboxy-THC) into a 25-mL volumetric flask and qs to volume with methanol.

Working standard solution (0.1/0.5 µg/mL): Pipette 1 mL of the 1/5 µg/mL working standard solution into a 10-mL volumetric flask and qs to volume with methanol.

To prepare the calibration curve, pipette the following volumes of the 1/5 or the 0.1/0.5 µg/mL working cannabinoid standard solution into appropriately labeled 16 × 125 mm screw cap test tubes. To eliminate a solvent effect, calibrators and controls may be dried under nitrogen prior to the addition of blank blood. Add 1 mL blank blood to obtain the final concentrations listed in Table 2.

The samples eluted in a gradient of 0.1% formic acid in acetonitrile and 0.1% formic acid in water. Once separated, the liquid phase was analyzed using an Agilent 6430 Triple Quadrupole LC/MS in positive ion mode. Chromatography and transition ions were identified and optimized by Agilent MassHunter Optimizer Software, and used to determine the quantifier and qualifier transitions.

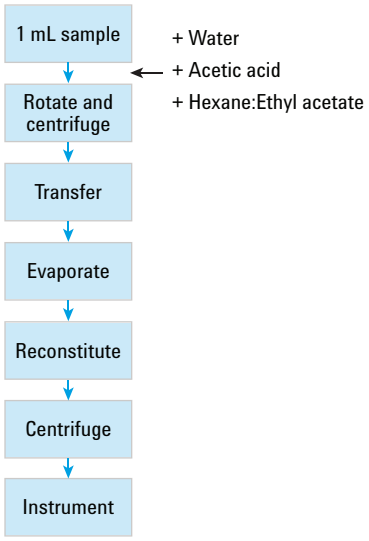

Figure 1. Sample preparation procedure.

Table 2. Preparation of Calibrators

Amount of 1/5 µg/mL stock solution (µL)	Amount of 0.1/0.5 µg/mL stock solution (µL)	Final concentration of cannabinoids (mg/L)
100		0.100/0.500
50		0.050/0.250
25		0.025/0.125
	100	0.010/0.050
	50	0.005/0.025
	25	0.0025/0.0125
	10	0.001/0.005

Procedure

1. Label clean 16 × 125 mm screw cap tubes appropriately with calibrators, controls, and case sample IDs.
2. Prepare calibrators and controls.
3. Add 1.0 mL case specimens/blank blood to the appropriately labeled tubes.
4. Add 100 µL of the 0.1 µg/mL internal standard working solution to each tube.
5. Add 2 mL of water and vortex briefly.
6. Add 800 µL of 10% acetic acid and vortex.
7. Add 8.0 mL of 9:1 hexane:ethyl acetate solution; cap and rotate tubes for 30 minutes.
8. Centrifuge at approximately 2,800 rpm for 15 minutes to achieve separation. Transfer organic (upper) layer to appropriately labeled tubes.
9. Evaporate samples to dryness at approximately 40–50 °C under nitrogen.
10. Reconstitute samples in 100 µL of 50:50 acetonitrile:water. Centrifuge at approximately 2,800 rpm for 15 minutes. Transfer to autosampler vials with inserts for LC/MS/MS analysis.
11. Run the calibrators, controls, and samples in a Worklist, according to the LC/MS/MS method described in Table 3.

Table 3. Instrument Conditions

MSD parameters

Parameter	Value (+)
Ionization	ESI
Polarity	Positive
Gas Temperature	350°C
Gas Flow	10 L/min
Nebulizer Pressure	40 psi
Capillary	4,000 V

LC parameters

Injection volume	10.0 µL	
Column	Agilent Poroshell 120 EC-18, 2.1 × 75 mm, 2.7 µm	
Column thermostat	40.0 °C	
Needle wash	5.0 seconds	
Mobile phase A	0.1 % formic acid in water	
Mobile phase B	0.1 % formic acid in acetonitrile	
Flow rate	0.5 mL/min	
Gradient	Initial	40% B
	1.0 minutes	40% B
	7.0 minutes	95% B
	10.0 minutes	95% B
	10.5 minutes	40% B
Stop time	10.5 minutes	
Post time	2.0 minutes	

Table 3. Instrument Conditions (continued)

Acquisition time segments

Index	Start time	Scan type	Ion mode	Div valve	Delta EMV	Store
1	0	MRM	ESI	To waste	0	No
2	4	MRM	ESI	To MS	400	Yes
3	6.15	MRM	ESI	To MS	400	Yes
4	8	MRM	ESI	To waste	0	No

Time segment 2

Compound	ISTD?	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell time (ms)	Fragmentor (V)	CE (V)
THC-COOH-d3	Yes	348.2	Unit	330.2	Unit	50	125	12
THC-COOH-d3	Yes	348.2	Unit	302.2	Unit	50	125	16
THC-COOH	No	345.2	Unit	299.2	Unit	50	125	16
THC-COOH	No	345.2	Unit	193.1	Unit	50	125	24
OH-THC-d3	Yes	334.2	Unit	316.2	Unit	50	120	8
OH-THC-d3	Yes	334.2	Unit	196.1	Unit	50	120	20
OH-THC	No	331.2	Unit	313.2	Unit	50	105	8
OH-THC	No	331.2	Unit	193.1	Unit	50	105	20

Time segment 3

Compound	ISTD?	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell time (ms)	Fragmentor (V)	CE (V)
THC-d3	Yes	318.2	Unit	196.1	Unit	100	120	20
THC-d3	Yes	318.2	Unit	123	Unit	100	120	32
THC	No	315.2	Unit	193.1	Unit	100	120	20
THC	No	315.2	Unit	123	Unit	100	120	32

Results and Discussion

The method achieved acceptable resolution of target compounds in an overall run time of 12.5 minutes. Figures 2–5 illustrate the resolution and MRM transitions achieved with this method. Peak shape is excellent with no significant tailing or other chromatographic abnormalities.

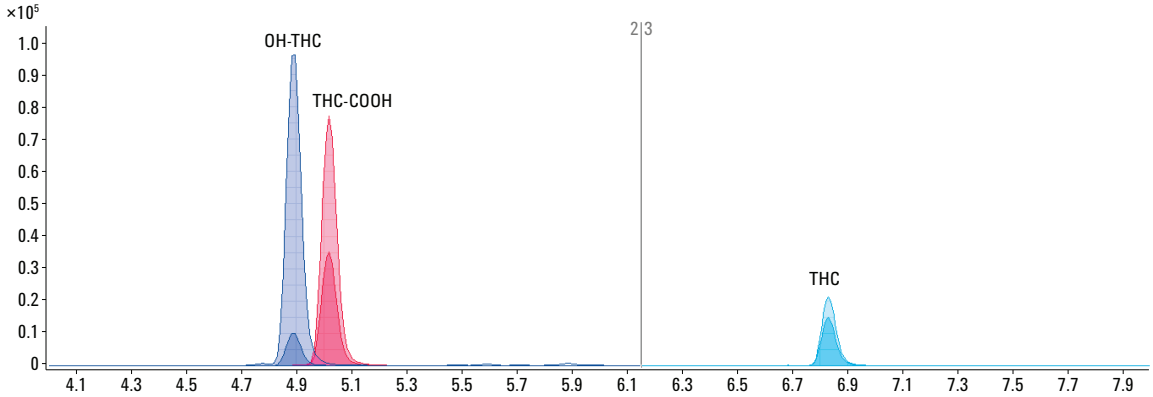

Figure 2. Qualitative chromatographic retention of analytes.

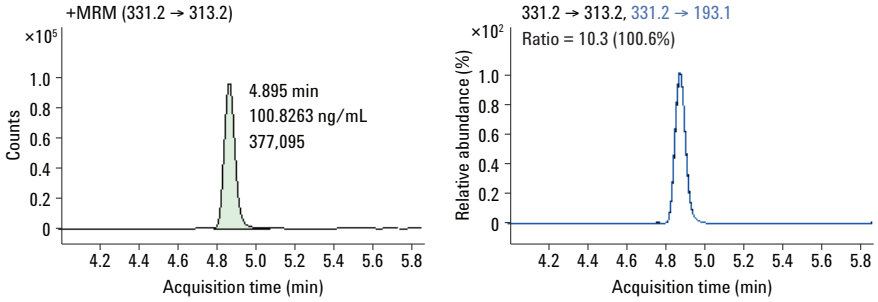

Figure 3. MRM of OH-THC.

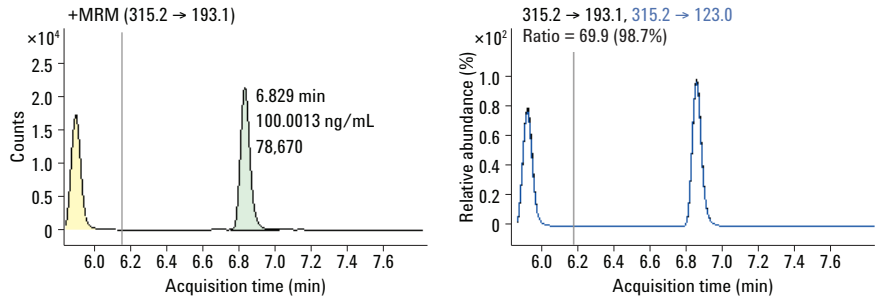

Figure 4. MRM of THC.

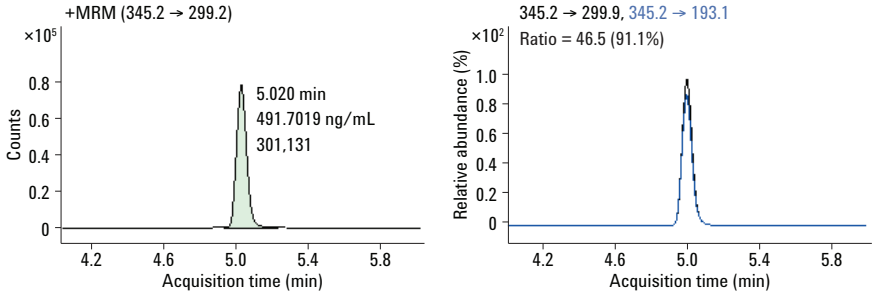

Figure 5. MRM of Carboxy-THC.

Seven calibrators were analyzed with every batch and used to assess the linearity of the instrumental response. Figures 6–8 show sample calibration curves derived from the method. Calibrations were linear from 1–100 ng/mL for THC and OH-THC, and 5–500 ng/mL for carboxy-THC. The R^2 coefficients in this study were greater than 0.992 ± 0.007 for all target compounds.

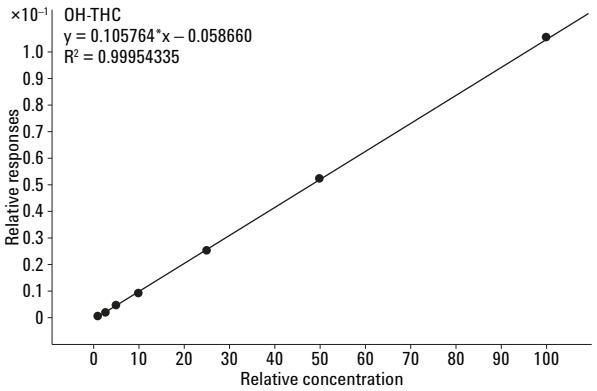

Figure 6. Calibration Curve for OH-THC.

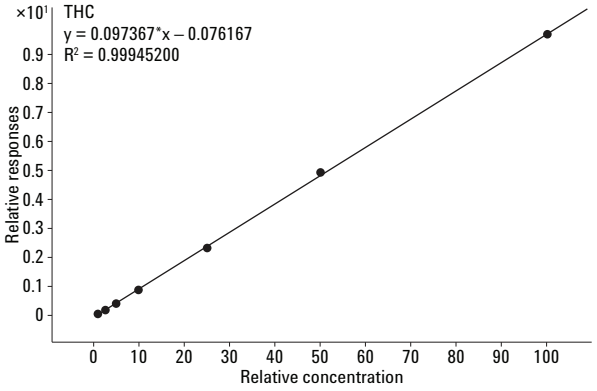

Figure 7. Calibration Curve for THC.

Validation studies were conducted using the SWGTOX guidelines in conjunction with the Virginia Department of Forensic Science validation guidelines [7]. This method was determined to meet all criteria for the qualitative and quantitative analysis of cannabinoids [8]. Items assessed during this validation study included linearity and calibration model fit, precision and accuracy using pooled and spiked whole blood samples, sensitivity (Limit of Detection (LOD) and Limit of Quantitation (LOQ)) interferences, robustness, carryover, dilution integrity, stability, and ion suppression/enhancement. As a result of this validation study, it was determined that matrix effects of this method were minimal. For THC, carboxy-THC and OH-THC LODs were 1, 2.5, and 1 ng/mL, and the LOQs were 1, 5, and 1 ng/mL, within acceptable ranges for these compounds. At the default collision cell accelerator voltage of 7 V, there was no evidence of cross talk between MRM transitions for target compounds and isotopically-labeled ISTDs. A more detailed explanation and results of this validation study can be found in "Validation of a Cannabinoid Quantitation Method Using an Agilent 6430 LC/MS/MS" [7].

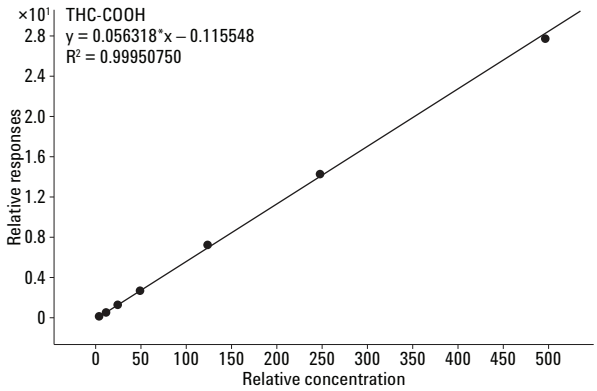

Figure 8. Calibration Curve for THC-COOH.

Conclusion

This method development provides a rapid and sensitive technique for the detection and quantitation of cannabinoids by LC/MS/MS. Sample preparation for LC/MS/MS analysis is more streamlined and does not require derivitazation as opposed to traditional GC/MS analysis. Validation studies detailed in "Validation of a Cannabinoid Quantitation Method Using an Agilent 6430 LC/MS/MS" indicate that the method meets all criteria required for the routine analysis of cannabinoids in whole blood [7].

References

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