Application Note 657

Quantitation of THC and THC Metabolites in Blood Using SOLAµ SPE Plates and the TSQ Quantiva Triple Quadrupole Mass Spectrometer for Forensic Analysis

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Key Words

Marijuana, THC, THC-COOH, SOLAµ, TSQ Quantiva MS, LC-MS

Goal

To demonstrate a simple and economical quantitative method for the analysis of THC and THC metabolites in blood to address key forensic laboratory requirements.

Application Benefits

- Analysis of THC and four major metabolites, including glucuronides, to determine recency of cannabis intake
- Low limits of quantitation
- Simple, economical, easily automated sample preparation method
- Confident analyte identification with ion ratio confirmation
- Robust method with limited matrix effects corrected by internal standards

Introduction

Cannabis is the most frequently abused drug. THC (tetrahydrocannabinol) is the major psychoactive constituent of cannabis. THC is primarily metabolized to 11-hydroxy-THC (THC-OH), which has equipotent psychoactivity and is further metabolized to non-psychoactive 11-nor-9-carboxy-THC (THC-COOH). Second-phase metabolites, THC-glucuronide and THC-COOH-glucuronide, are also present in blood and can be used as markers to determine recency of cannabis intake and to improve interpretation of analytical results.[1] LC-MS analytical methods are widely used for analysis of THC and its metabolites in blood samples. LC-MS methods do not require sample derivatization, thus yielding savings over typical GC-MS procedures.

Methods

Calibrators and Quality Controls

Calibration standards and quality controls (LQC, MQC, and HQC) at concentrations specified in Table 1 and Table 2 were prepared in donor blood. Silanized labware was used to prepare standard spiking solutions to avoid adsorption of analytes to the glass surface.

Table 1. Analyte concentration in calibration standards.

Analyte	Cal 1	Cal 2	Cal 3	Cal 4
	Concentration (ng/mL)			
THC	0.2	0.5	1.0	2.0
THC-OH	0.2	0.5	1.0	2.0
THCCOOH	0.2	0.5	1.0	2.0
THC-glucuronide	0.2	0.5	1.0	2.0
THCCOOH-glucuronide	2.0	5.0	10	20
Analyte	**Cal 5**	**Cal 6**	**Cal 7**	**Cal 8**
	Concentration (ng/mL)			
THC	5.0	10	50	100
THC-OH	5.0	10	50	100
THCCOOH	5.0	10	50	100
THC-glucuronide	5.0	10	50	100
THCCOOH-glucuronide	50	100	500	1000

Table 2. Analyte concentrations in QC samples.

Analyte	LQC	MQC	HQC
	Concentration (ng/mL)		
THC	1.0	5.0	50
THC-OH	1.0	5.0	50
THCCOOH	1.0	5.0	50
THC-glucuronide	1.0	5.0	50
THCCOOH-glucuronide	10	50	500

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Sample Preparation

Blood samples, calibrators, and QCs (all 200 µL aliquots) spiked with internal standards (d_3-THC, d_3-THC-OH, d_3-THC-COOH, and d_3-THCCOOH-glucuronide) were processed with a protein precipitation procedure followed by solid phase extraction using Thermo Scientific™ SOLAµ™ SAX 96-well plates (P/N 60209-003).
The protein precipitation step was needed to release hydrophobic analytes from the sample matrix to ensure good SPE efficiency. Analytes were eluted from the extraction plate with 80 µL of 5% formic acid in acetonitrile directly into a Thermo Scientific™ WebSeal™ 96-Well Small Volume Microplate (P/N 60180-K101) and further diluted with 80 µL of water. In this cost-efficient approach, evaporation and reconstitution steps were not needed. Fifty microliters (50 µL) of processed sample were analyzed by LC-MS.

Liquid Chromatography

A 5-minute chromatographic elution through a Thermo Scientific™ Accucore™ RP-MS column (2.6 µm, 100 x 2.1 mm, P/N 17626-102130) at room temperature was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RS liquid chromatography pump with OAS autosampler. Mobile phases consisted of 0.1% formic acid in water and 0.1% formic acid in acetonitrile for phases A and B, respectively.

Mass Spectrometry

Compounds were detected on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer equipped with an Ion Max™ source and a heated electrospray (HESI) sprayer. Negative ionization mode was used in the detection of THC-COOH, THC-glucuronide, and THC-COOH-glucuronide (and corresponding internal standards), and positive ionization mode was used in the detection of THC and THC-OH (and corresponding internal standards). Two SRM transitions for each analyte and internal standard were monitored for quantitation and confirmation (Table 3).

Table 3. SRM transitions collected with mass spectrometry method.

Analyte	Polarity	Precursor (*m/z*)	Product (*m/z*)	Comments
THC	Positive	315.3	193.1	Quantifying ion
THC	Positive	315.3	123.1	Confirming ion
***d*3-THC**	Positive	318.3	196.1	Quantifying ion
***d*3-THC**	Positive	318.3	123.0	Confirming ion
THC-OH	Positive	331.3	313.2	Quantifying ion
THC-OH	Positive	331.3	193.1	Confirming ion
***d*3-THC-OH**	Positive	334.3	316.2	Quantifying ion
***d*3-THC-OH**	Positive	334.3	196.2	Confirming ion
THCCOOH	Negative	343.2	245.1	Quantifying ion
THCCOOH	Negative	343.2	191.1	Confirming ion
***d*3-THCCOOH**	Negative	346.3	302.3	Quantifying ion
***d*3-THCCOOH**	Negative	346.3	248.1	Confirming ion
THC-glucuronide	Negative	489.3	313.2	Quantifying ion
THC-glucuronide	Negative	489.3	245.1	Confirming ion
THCCOOH-glucuronide	Negative	519.2	343.2	Quantifying ion
THCCOOH-glucuronide	Negative	519.2	299.2	Confirming ion
***d*3-THCCOOH-glucuronide**	Negative	522.3	346.2	Quantifying ion
***d*3-THCCOOH-glucuronide**	Negative	522.3	302.2	Confirming ion

Method Performance Evaluation

SPE extraction recovery was obtained by spiking blood before and after SPE processing to the same concentrations as QC samples and comparing analyte peak areas.

Limits of quantitation (LOQ) and linearity ranges were evaluated by collecting calibration curve data. Method accuracy and precision were evaluated by processing and analyzing triplicates of QC samples on three different days. Matrix effects were evaluated by spiking analytes to the same concentrations as QC samples into SPE-processed pooled blood and calculating recovery against the same analyte amount spiked into SPE-processed water.

Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software. The average ion ratios calculated for analyte confirmation and required accuracies are presented in Table 4.

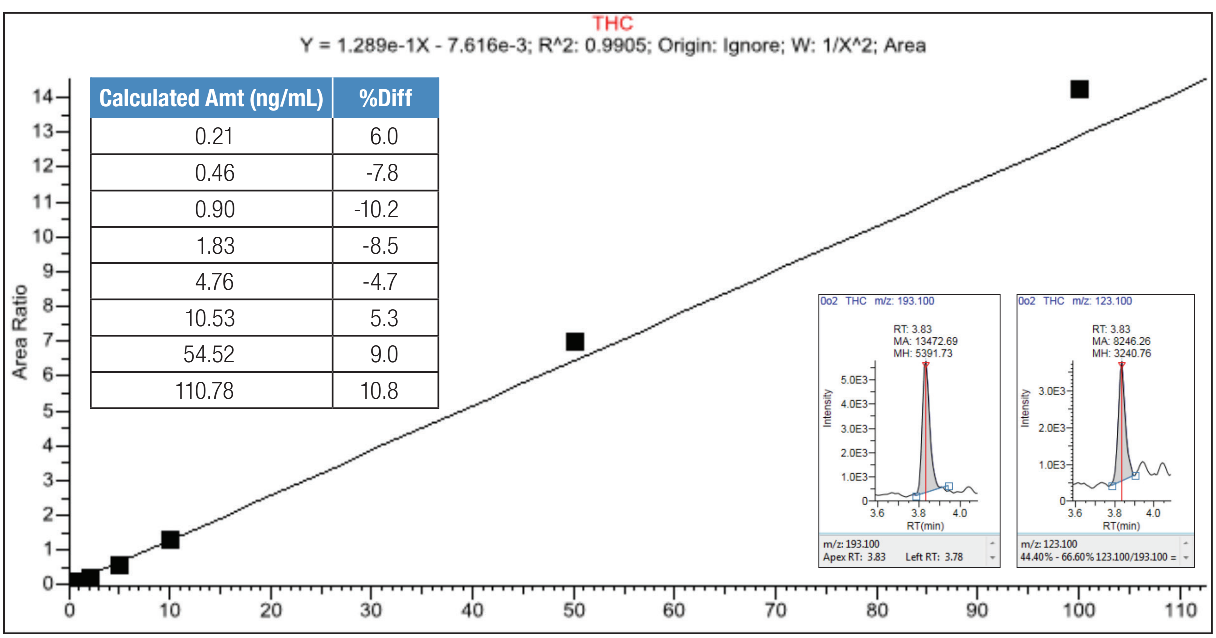
Table 4. Average ion ratios and allowed accuracy window.

Analyte	Average Ion Ratio (%)	Accuracy Window (%)
THC	57.95	20%
*d*3-THC	53.84	20%
THC-OH	10.92	20%
*d*3-THC-OH	12.77	20%
THCCOOH	59.46	20%
*d*3-THCCOOH	24.94	20%
THC-glucuronide	14.55	20%
THCCOOH-glucuronide	85.57	20%
*d*3-THCCOOH-glucuronide	90.87	20%

Results and Discussion

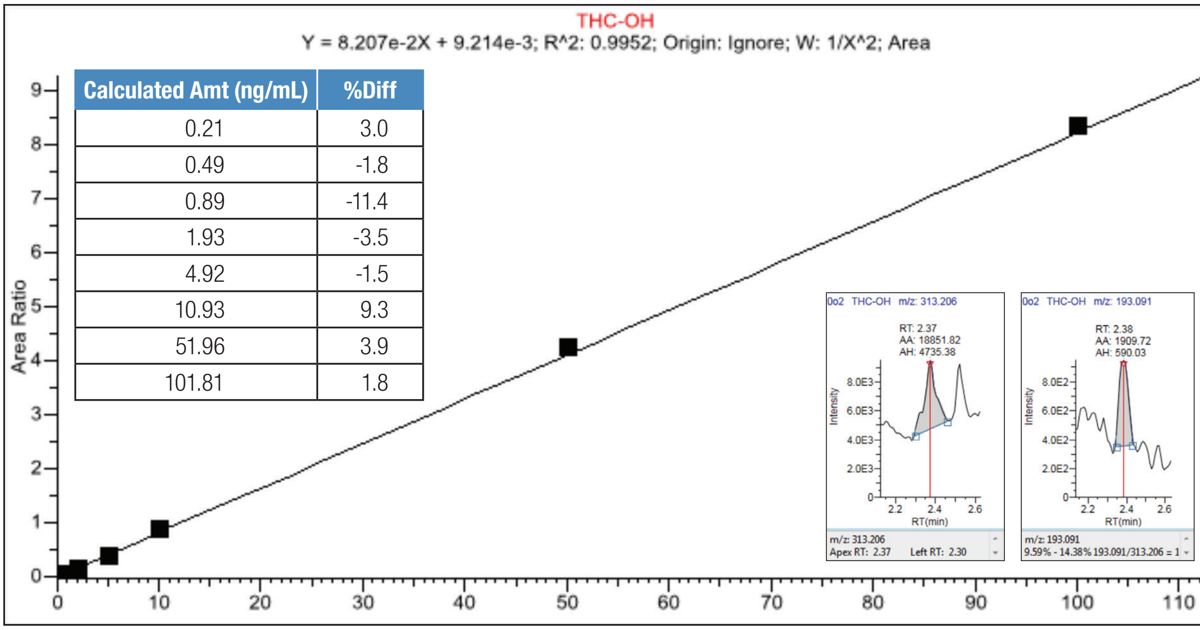
Limits of quantitation were defined as the lowest concentrations that had back-calculated values within 20% and ion ratios within the specified range. Using these criteria, the limits of quantitation were 0.2 ng/mL for THC, THC-OH, and THC-COOH; 0.5 ng/mL for THC-glucuronide; and 2 ng/mL for THC-COOH-glucuronide. The upper limit of the calibration curve was equal to the highest evaluated concentration, which was 100 ng/mL for THC, THC-OH, and THC-COOH; 50 ng/mL for THC-glucuronide; and 500 ng/mL for THC-COOH-glucuronide.

Figure 1 shows representative calibration curves of all analytes, along with quantifying and confirming ion chromatograms for the lowest calibration standard.



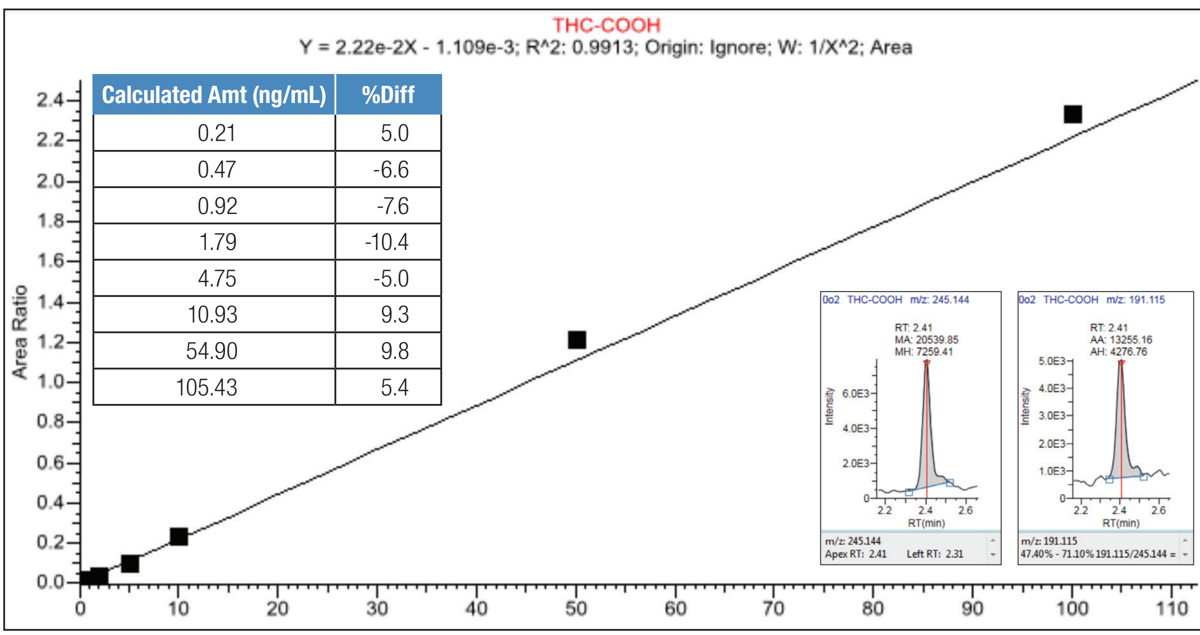
Calculated Amt (ng/mL)	%Diff
0.21	6.0
0.46	-7.8
0.90	-10.2
1.83	-8.5
4.76	-4.7
10.53	5.3
54.52	9.0
110.78	10.8

Figure 1a. THC representative calibration curve and the chromatogram for the lowest calibration standard (0.2 ng/mL).



Calculated Amt (ng/mL)	%Diff
0.21	3.0
0.49	-1.8
0.89	-11.4
1.93	-3.5
4.92	-1.5
10.93	9.3
51.96	3.9
101.81	1.8

Figure 1b. THC-OH representative calibration curve and the chromatogram for the lowest calibration standard (0.2 ng/mL).



Calculated Amt (ng/mL)	%Diff
0.21	5.0
0.47	-6.6
0.92	-7.6
1.79	-10.4
4.75	-5.0
10.93	9.3
54.90	9.8
105.43	5.4

Figure 1c. THCOOH representative calibration curve and the chromatogram for the lowest calibration standard (0.2 ng/mL).

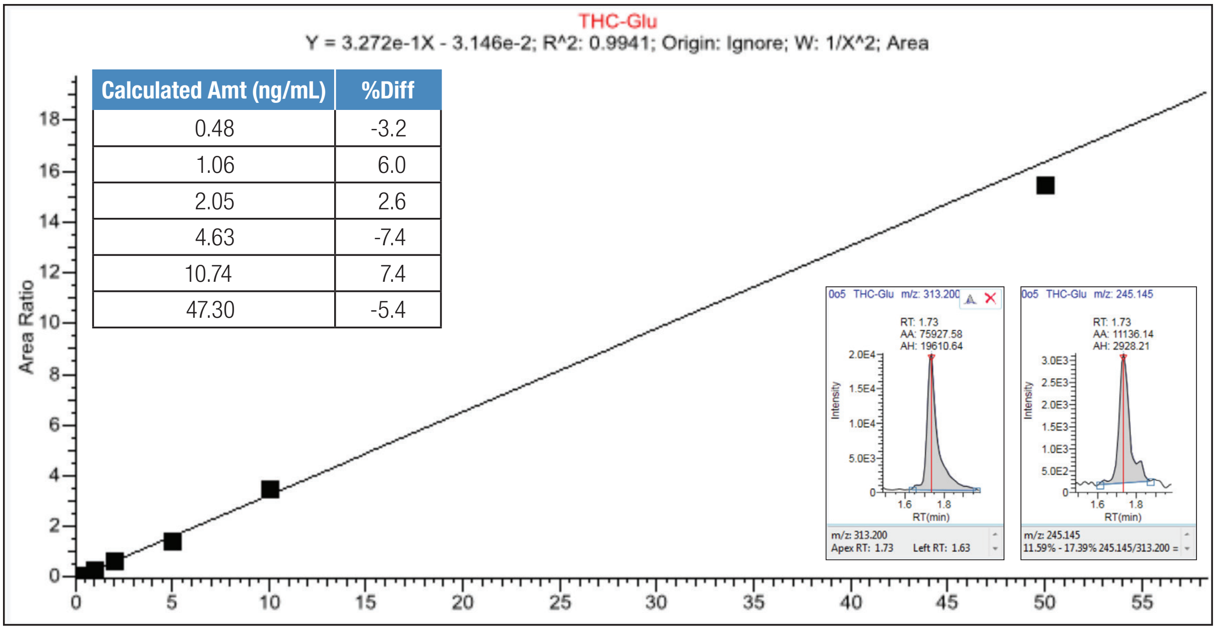


Figure 1d. THC-glucuronide representative calibration curve and the chromatogram for the lowest calibration standard (0.5 ng/mL).

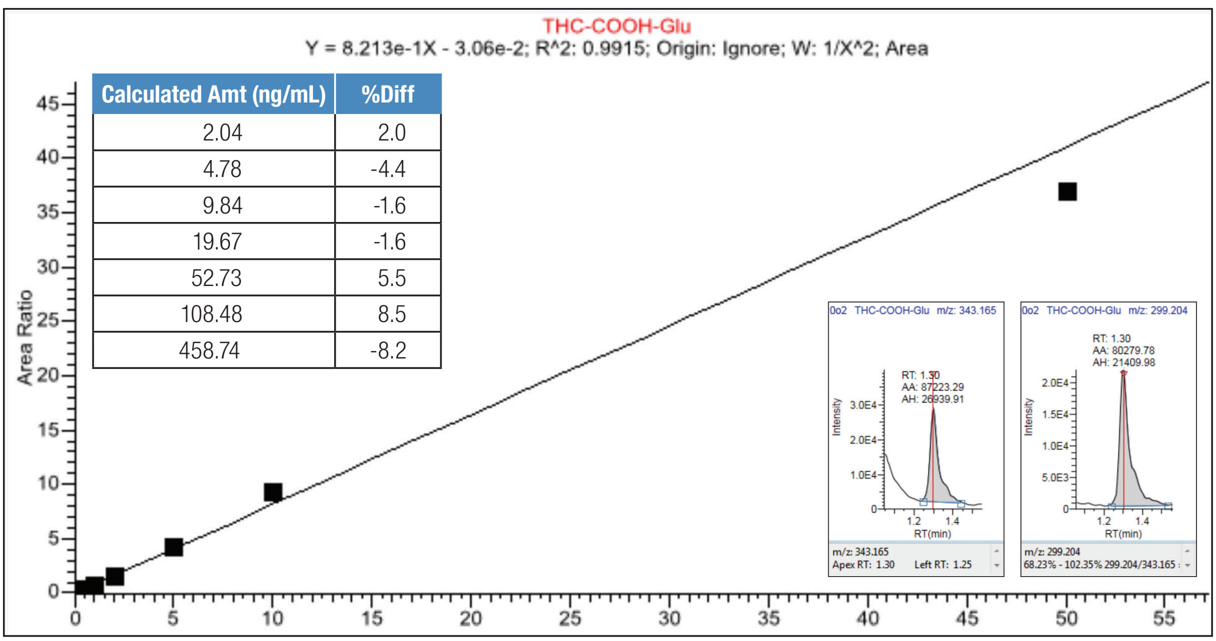


Figure 1e. THCOOH-glucuronide representative calibration curve and the chromatogram for the lowest calibration standard (2 ng/mL).

Method accuracy calculated as % recovery of QC samples ranged from 90.1% to 107% (Table 5). Intra-assay precision for all analytes in all QC levels was better than 9.4% and inter-assay precision was better than 8.8% (Table 5). SPE extraction efficacy was compound-dependent and was between 25% and 82% (Table 6).

Matrix effects were observed (absolute recoveries were 50–140%) and were corrected by deuterated internal standards as proved by relative recoveries, which were 82.6–120% (Table 6).

Table 5. Intra- and Inter-assay precision and method accuracy.

	Intra-assay Precision (% RSD, n=3)			Inter-assay Precision (% RSD, n=9)			Accuracy (% Recovery, n=9)		
Analyte	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
THC	3.8-6.1	5.2-6.8	2.4-4.4	7.4	5.3	3.4	93.8	93.7	94.0
THC-OH	0.8-6.4	3.0-5.5	2.4-3.5	5.9	2.7	4.3	92.9	95.2	98.5
THCCOOH	2.9-5.9	4.9-6.0	1.5-3.1	4.5	4.7	2.3	91.4	95.3	96.6
THC-glucuronide	3.5-9.4	1.7-6.6	5.9-9.2	9.9	5.3	8.8	92.5	92.5	97.4
THCCOOH-glucuronide	2.5-4.3	5.9-6.5	2.6-5.0	3.1	5.4	3.4	96.3	94.6	90.1

Table 6. Extraction recovery of sample preparation method and matrix effects obtained for blood samples spiked to concentrations of low, medium, and high QC samples.

	Recovery (%)			Absolute Matrix Effect (% Recovery)			Relative Matrix Effect (% Recovery)		
Analyte	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**	**LQC**	**MQC**	**HQC**
THC	58.3	52.5	49.9	81.7	53.3	46.1	111	111	107
THC-OH	29.0	30.0	27.1	61.8	64.1	56.5	88.1	106	100
THCCOOH	81.7	67.6	63.6	63.6	50.2	45.5	98.6	83.5	88.6
THC-glucuronide	69.9	55.7	53.8	58.6	49.7	52.1	89.5	82.6	90.4
THCCOOH-glucuronide	25.6	26.2	28.7	131	140	110	120	107	105

Conclusion

We demonstrated a simple and economical quantitative method for analysis of THC and metabolites in blood for forensics. Method performance meets forensic lab requirements. Analysis of glucuronides allows for better data interpretation to determine recent cannabis intake. To improve laboratory throughput by 30%, this method can be implemented on a 2-channel Thermo Scientific™ Transcend™ II LC system to provide data for 17 samples per hour.

References

1. Schwope, D. M.; Scheidweiler, K. B.; Huestis, M. *Anal Bioanal Chem* **2011** Sep, *401*(4), 1273-1283.

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