A Sensitive and Efficient Method to Analyze THC and THCCOOH in Oral Fluid Using LC-MS/MS in Forensic Toxicology Laboratories

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Application Note 641

Key Words
THC, THCCOOH, SPE, SOLAμ, Cannabinoid, TSQ Quantiva MS, Oral Fluid, UltiMate 3400RS, Accucore

Goal
To develop a sensitive and simple LC-MS/MS method for quantitative analysis of THC and THCCOOH in oral fluid for forensic toxicology laboratories.

Application Benefits
- Simple and rapid SPE extraction method
- LOQ of THCCOOH: 10 pg/mL with ion ratio confirmation
- High recovery rate and limited matrix effect for THCCOOH

Introduction
The Substance Abuse and Mental Health Services Administration (SAMHSA) lists Δ9-tetrahydrocannabinol (THC) as the only target analyte for detection of cannabis use in oral fluid (OF) at 2 ng/mL confirmation cutoff concentration. However, only requiring THC in OF may yield interpretation issues. THC was detected in OF from non-smokers passively exposed for 3h to cannabis smoke.[1] Detection of 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) in OF provides clues of active cannabis smoking, as it is not present in cannabis smoke. However, THCCOOH quantification requires highly sensitive analytical methods as it is present in low pg/mL concentrations in OF.

Previously published methods for the quantification of THCCOOH included GC-MS/MS,[2] 2D GC-MS,[3] and LC-MS/MS technologies.[4,5] LC-MS/MS is an analytical technique of increasing interest in forensic toxicology laboratories, as simultaneous analysis of analytes with different polarities can be achieved with good sensitivity and without derivatization of analytes with vaporization, saving time and cost. However, existing LC-MS/MS methods require THCCOOH derivatization or use of a time-consuming solid phase extraction (SPE) procedure to achieve desired sensitivity levels of 10 or 15 pg/mL limit of quantification (LOQ).[4-6]

In this study, we developed a sensitive and efficient method for simultaneous determination of THC and THCCOOH in OF. Both analytes were extracted using a simple and fast SPE method without pre-conditioning, evaporation, or reconstitution. THCCOOH was detected with high sensitivity (LOQ 10 pg/mL), and no derivatization procedure was required.

Methods
Preparation of Calibrators and Controls
Calibrators' working solutions in the range of 0.05–10 ng/mL for THCCOOH and 5–1000 ng/mL for THC were prepared by appropriate dilution in methanol. The combined internal standard solution (THCCOOH-d3 at 1 ng/mL and THC-d3 at 10 ng/mL) was prepared in methanol. The low QC working solution (0.25 ng/mL for THCCOOH and 25 ng/mL for THC), medium QC working solution (1 ng/mL for THCCOOH and 100 ng/mL for THC) and high QC working solution (5 ng/mL for THCCOOH and 500 ng/mL for THC) were prepared in methanol.

The calibrators and controls were prepared by spiking 25 µL calibrators' and controls' working solutions in drug-free OF-buffer mixtures (0.25 mL OF + 0.5 mL buffer). The drug-free OF samples were collected from donors using the Salivette® saliva examination device (Sarstedt, P/N 51.1534).

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Sample Preparation

First, 0.75 mL OF-buffer mixture was combined with 25 µL internal standard solution (THCCOOH-d3 at 1 ng/mL and THC-d3 at 10 ng/mL). Proteins were precipitated by addition of 200 µL acetonitrile, followed by addition of 50 µL 1% ammonium hydroxide. The mixture was decanted onto a Thermo Scientific™ SOLAµ™ SAX SPE plate (P/N 60209-003), which requires no pre-conditioning. After washing with water/acetonitrile (50:50, v/v), the elution was performed with 2 x 30 µL formic acid/acetonitrile (5:95, v/v). Eluates were diluted with 60 µL water and vortexed gently. Then, 50 µL of the diluted eluate was injected for LC-MS/MS analysis.

Liquid Chromatography

A five-minute gradient elution was performed using a Thermo Scientific™ UltiMate™ 3000 UHPLC system with an LPG-3400XRS pump and an OAS-3300TXRS autosampler. Mobile phases were 0.1% formic acid in water and 0.1% formic acid in acetonitrile (Fisher Chemical Optima™ grade) for phase A and B, respectively. The analytical column was a Thermo Scientific™ Accucore™ RP-MS, 2.6 µm, 100 × 2.1 mm column (P/N 17626-102130).

Mass Spectrometry

Compounds were detected on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer equipped with a heated electrospray ionization source (HESI II). Data were acquired in selected-reaction monitoring (SRM) mode. Two SRM transitions for each analyte were measured with polarity switching (negative mode for THCCOOH and positive mode for THC) and ion ratios were calculated for confirmation (Table 1).

Data Analysis

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software version 3.2.

Method Performance Evaluation

The limits of quantitation (LOQ) and linearity ranges were evaluated by collecting calibration curve data in duplicate. Method precision was evaluated by running quadruplicate replicates of QCs on three different days. Sample preparation recovery was evaluated by spiking THCCOOH at 50 pg/mL and THC at 5 ng/mL into five different donor samples before and after the SPE procedure. Recovery rate, %, was expressed as the analyte peak area of samples added before SPE, divided by the analyte peak area of samples added after SPE. Matrix effects were evaluated by spiking THCCOOH at 50 pg/mL and THC at 5 ng/mL into five different donor samples and water. Absolute matrix effect was computed by dividing the analyte peak area of donor samples by peak area of water, expressed as percent. Relative matrix effect was computed by dividing the analyte peak area ratio against internal standard of donor samples by peak area ratio of water, expressed as percent.

Results and Discussion

Limits of quantitation (LOQs) were defined as the lowest concentrations that had back-calculated values within 20%, RSD for five QC replicates within 20%, and the ion ratio between quantifying ion and confirmation ion within 20%. Using these criteria, the limit of quantitation was 10 pg/mL for THCCOOH and 0.5 ng/mL for THC in oral fluid.

Figure 1 shows representative calibration curves for both analytes, collected in duplicate, along with chromatograms for the lowest calibration standard. Calibration standards' accuracy was within 15%. Figure 2 presents chromatograms of donor oral fluid sample spiked with THCCOOH at 25 pg/mL and THC at 2.5 ng/mL.

Table 1. SRM transitions monitored for THC and THCCOOH and their internal standards.

Compound	Retention Time (min)	RT Window (min)	Polarity	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF Lens (V)	Comment
THCCOOH	2.3	1	Negative	343.2	245.1	30	87	Quantifying ion
THCCOOH	2.3	1	Negative	343.2	191.1	33	87	Confirming ion
THCCOOH-d3	2.3	1	Negative	346.2	302.2	22	85	Quantifying ion
THCCOOH-d3	2.3	1	Negative	346.2	248.1	31	85	Confirming ion
THC	3.8	1	Positive	315.3	193.1	24	58	Quantifying ion
THC	3.8	1	Positive	315.3	123.1	33	58	Confirming ion
THC-d3	3.8	1	Positive	318.3	196.1	25	59	Quantifying ion
THC-d3	3.8	1	Positive	318.3	123.1	33	59	Confirming ion

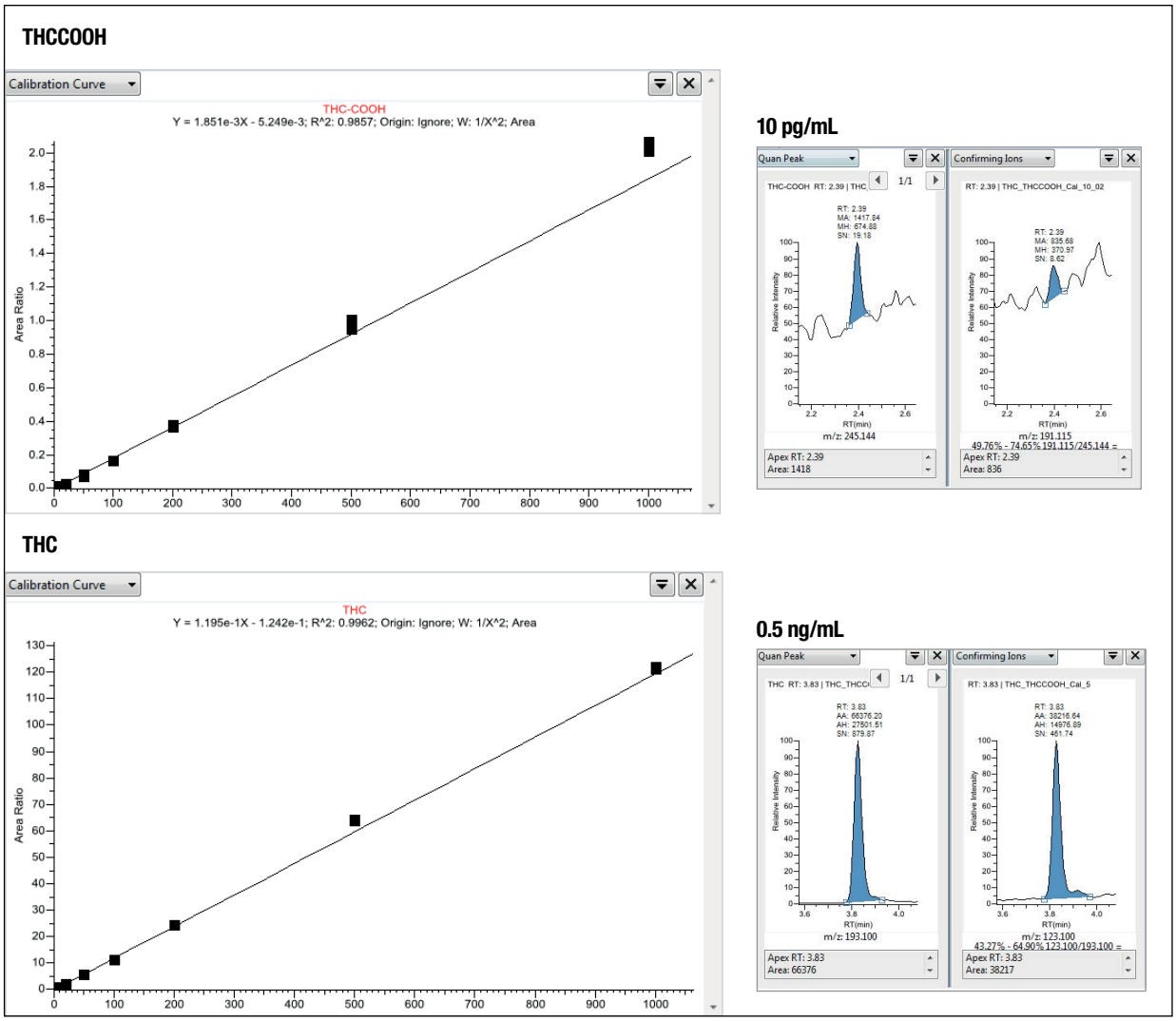

Figure 1. Representative calibration curves for THC and THCCOOH, collected in duplicate, along with chromatograms for the lowest calibration standard.

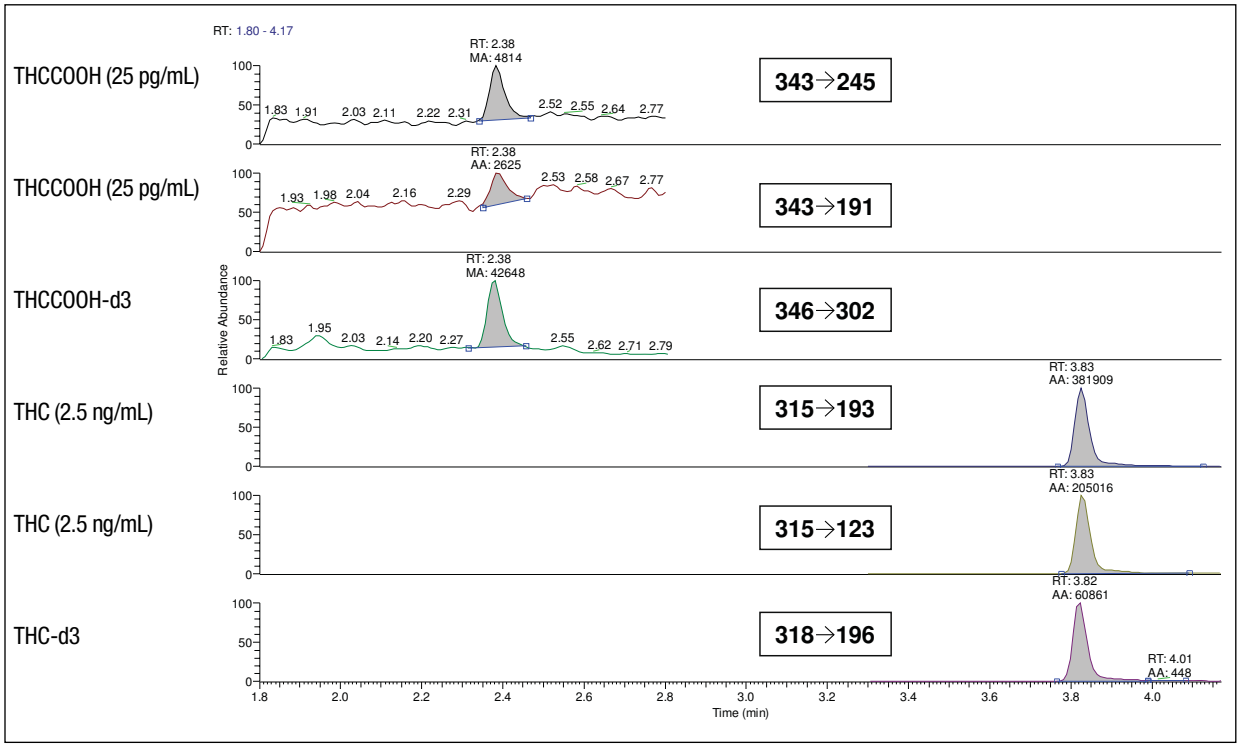

Figure 2. Chromatograms of donor oral fluid sample spiked with THCCOOH at 25 pg/mL and THC at 2.5 ng/mL.

Intra-assay precision was better than 10% (Table 2), and inter-assay precision was better than 10% (Table 3) for both analytes.

Table 2. Intra-assay precision for QC samples (n=4).

Analyte	%RSD		
	LQC	MQC	HQC
THCCOOH	5.1–9.5	5.6–9.4	5.3–8.3
THC	1.2–3.0	0.9–1.8	0.7–2.3

Table 3. Inter-assay precision for QC samples (n=12).

Analyte	%RSD		
	LQC	MQC	HQC
THCCOOH	8.4	7.7	6.3
THC	3.2	2.4	2.2

A high sample preparation recovery rate was observed for THCCOOH. The recovery rate ranged from 70% to 97% for THCCOOH and from 33% to 42% for THC (Table 4).

Table 4. Sample preparation recovery rate in five donor samples.

Analyte	%Recovery				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THCCOOH	96.8	70.0	78.9	75.9	83.7
THC	32.8	37.1	38.2	36.0	41.6

Limited matrix effect was observed for THCCOOH. The absolute matrix effect ranged from 86% to 113% and the relative matrix effect ranged from 84% to 105% for THCCOOH. For THC, ion suppression produced by matrix effect was observed. The absolute matrix effect ranged from 64% to 71%. However, the ion suppression was corrected by addition of internal standard. The relative matrix effect ranged from 98% to 103% (Table 5).

Table 5. Matrix effect in five donor samples.

Analyte	Absolute Matrix Effect (%)				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THCCOOH	113	86.8	97.0	102	86.5
THC	68.0	71.1	65.8	63.7	67.1

Analyte	Relative Matrix Effect (%)				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THCCOOH	105	84.8	84.3	97.0	88.9
THC	103	99.1	102	101	98.0

Conclusion

We demonstrated a sensitive and efficient LC-MS/MS method for the simultaneous quantification of THCCOOH and THC in forensic toxicology laboratories. Both analytes were extracted from oral fluid using a simple and fast SPE method (no pre-conditioning, no evaporation, or reconstitution). The LOQ for THCCOOH was 10 pg/mL with ion ratio confirmation. Quantification of both analytes provides the opportunity to improve interpretation of cannabinoid OF results by eliminating the possibility of passive inhalation and providing markers of recent cannabis smoking.

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References

1. Moore, C., et al., Cannabinoids in oral fluid following passive exposure to marijuana smoke. *Forensic Sci Int*, 2011, *212*(1-3), 227-30.
2. Day, D., et al., Detection of THCA in oral fluid by GC-MS-MS. *J Anal Toxicol*, **2006**, *30*(9), 645-50.
3. Moore, C., et al., Analytical procedure for the determination of the marijuana metabolite 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid specimens. *J Anal Toxicol*, **2006**, *30*(7), 409-12.
4. Coulter, C.; Garnier, M; Moore, C. Analysis of tetrahydrocannabinol and its metabolite, 11-nor-Delta(9)-tetrahydrocannabinol-9-carboxylic acid, in oral fluid using liquid chromatography with tandem mass spectrometry. *J Anal Toxicol*, **2012**, *36*(6), 413-7.
5. Lee, P.D., et al., Simultaneous determination of Delta9-tetrahydrocannabinol and 11-nor-9-carboxy-Delta9-tetrahydrocannabinol in oral fluid using isotope dilution liquid chromatography tandem mass spectrometry. *Anal Bioanal Chem*, **2012**, *402*(2), 851-9.
6. Concheiro, M., et al., Simultaneous quantification of Delta(9)-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography-high resolution mass spectrometry. *J Chromatogr A*, **2013**, *1297*, 123-30.

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