POSTER NOTE

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Sensitive, Easy and Economical Method for Analysis of THC and THC-COOH in Oral Fluid Using Novel Solid Phase Extraction Technology for Sample Preparation

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INTRODUCTION

Cannabis is the most widely used illicit drug with over 170 million people using it at least once a year. Oral fluid testing for THC provides a convenient means of detecting recent cannabis use. Additional testing for a THC metabolite (THC-COOH) reduces the risk of a positive result due to passive exposure and extends drug detection window. However, concentrations of the metabolite are typically in the low pg/mL range and require sensitive analytical methods. Published methods capable of achieving the low detection limits utilize complicated instrumentation (2D-GC), added derivatization steps or time-consuming traditional SPE sample preparation. Here we developed a simpler analytical method that uses novel SPE technology to speed sample preparation while achieving the low limits of detection required.

OBJECTIVE

Develop a sensitive, easy and economical LC-MS method for analysis of THC and THC-COOH in oral fluid using novel fritless, low-elution volume solid phase extraction (SPE) plates for sample preparation.

MATERIALS AND METHODS

Sample Processing

1. Mix 250 µL oral fluid (OF), 500 µL preservation buffer, 25 µL spiking solution, 25 µL internal standard solution (1 ng/mL THC-COOH-D3, 10 ng/mL THC-d3), 200 µL acetonitrile and 50 µL ammonium hydroxide.
2. Load samples directly onto Thermo Scientific™ SOLAµ™ SAX SPE plate in 2 aliquots of ~500 µL each. No preconditioning is required.
3. Wash wells with 200 µL water:acetonitrile (50:50).
4. Elute with 2 separate aliquots of 30 µL of 5% formic acid in acetonitrile.
5. Add 40 µL of water for dilution.
6. Evaporation and reconstitution steps were not needed because of the low elution volume.

Liquid Chromatography

Mobile Phase A: 0.1% formic acid in water

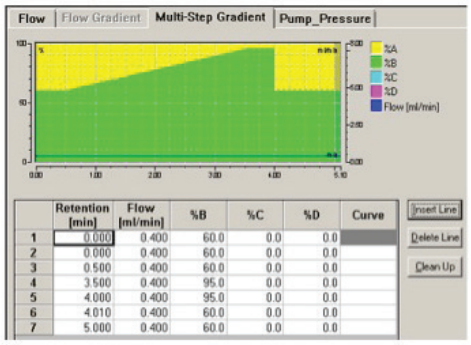
Mobile Phase B: 0.1% formic acid in acetonitrile

Column: Thermo Scientific™ Accucore™ RP-MS, 2.6 µm, 100 x 2.1 mm

Column Temperature: 40 °C

Injection Volume: 50 µL

Gradient:



	Retention [min]	Flow [ml/min]	%B	%C	%D	Curve
1	0.000	0.400	60.0	0.0	0.0	
2	0.000	0.400	60.0	0.0	0.0	
3	0.500	0.400	60.0	0.0	0.0	
4	3.500	0.400	95.0	0.0	0.0	
5	4.000	0.400	95.0	0.0	0.0	
6	4.010	0.400	60.0	0.0	0.0	
7	5.000	0.400	60.0	0.0	0.0	

Mass Spectrometry

- Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer with HESI ionization source
- 2 SRM transitions per analyte were monitored for quantitation and ion ratio confirmation, and one SRM transition for each stable-labeled analog internal standard (**Table 1**).

Table 1. Mass Transitions used for detection of THC-COOH and THC.

Compound	Retention Time (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)
THC-COOH	2.3	Negative	343.175	245.144	30
THC-COOH	2.3	Negative	343.175	191.115	33
THC-COOH-d3	2.3	Negative	346.275	302.28	22
THC	3.8	Positive	315.275	193.1	24
THC	3.8	Positive	315.275	123.1	33
THC-d3	3.8	Positive	318.275	196.1	25

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Table 2. Concentrations of evaluated calibrators, QCs and recovery samples for THC-COOH and THC.

Analyte	Calibrator Range	QC Concentrations	Recovery / Matrix Effect Samples
THC-COOH	5-1000 pg/mL	25, 100, 500 pg/mL	50 pg/mL
THC	0.5-100 ng/mL	2.5, 10, 50 ng/mL	5 ng/mL

RESULTS

Lower limits of quantitation (defined as the lowest concentrations that had back-calculated values within 20% of nominal, RSD for 5 replicates within 20%, and ion ratio within required range) were 10 pg/mL for THC-COOH (**Figure 1**) and 0.5 ng/mL for THC (**Figure 2**). The upper calibration range (equal to highest evaluated concentration) was 1000 pg/mL for THC-COOH and 100 ng/mL for THC.

Within-batch precision was better than 9.5% and 3.0% for THC-COOH and THC, respectively (**Table 3**). Between-batch precision was better than 8.4% and 3.2% for THC-COOH and THC, respectively (**Table 4**).

SPE extraction efficiencies were in ranges of 85.4-106% and 55.8-65.1% for THC-COOH and THC respectively (**Table 5**). Carryover was not observed even at the highest calibrator concentration.

Limited matrix effects were observed and were corrected by internal standards. Absolute peak area recovery in spiked donor oral fluid samples compared to sample prepared in water were in ranges of 79.6-125% and 94.9-99.0% for THC-COOH and THC respectively (**Table 6**).

Figures 3 and **4** show chromatograms of quantifying and confirming ions for THC-COOH and THC along with their internal standards at their respective LOQs and in the lowest QC sample.

Table 3. Intra-assay precision and system robustness % RSD of QC samples containing each analyte and internal standard were processed and analyzed with 4 replicate injections in 3 batches. (n=4 per batch)

Analyte	%RSD		
	LQC	MQC	HQC
THC-COOH	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

Figure 1. Calibration curve and chromatogram of lowest calibrator for THC-COOH. LOQ (10 pg/mL) had back calculated concentration with in 20% of nominal and ion ratio tolerance of 20%.

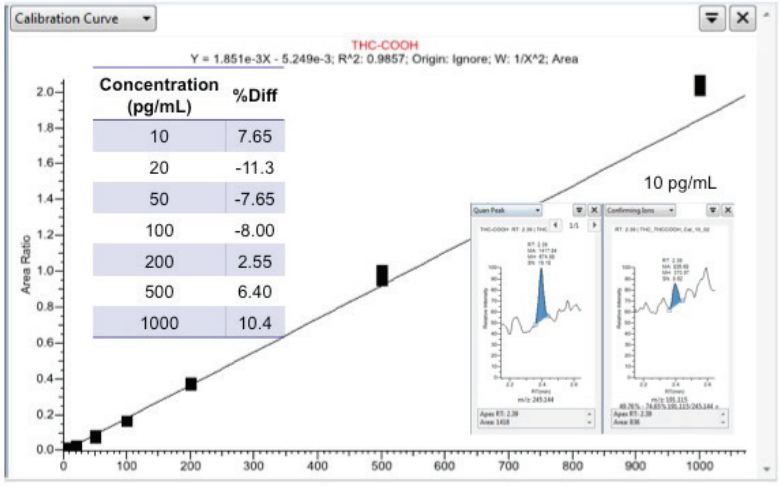


Figure 2. Calibration curve and chromatogram of lowest calibrator for THC. LOQ (0.5 ng/mL) had back calculated concentration with in 20% of nominal and ion ratio tolerance of 20%.

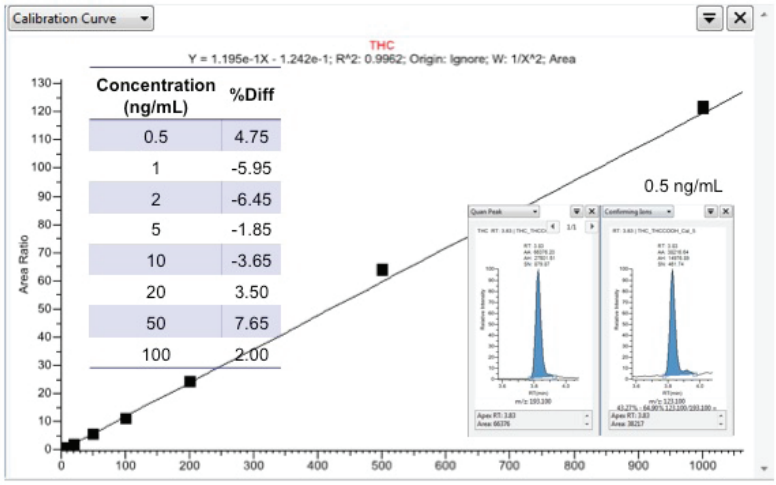
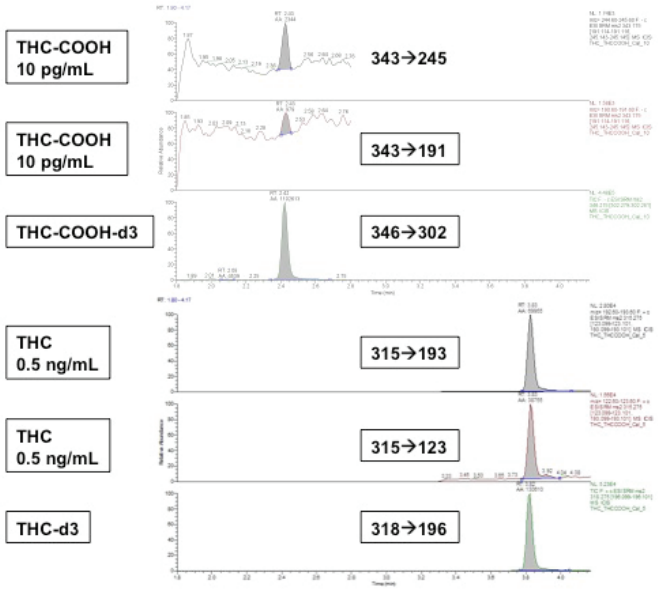


Table 4. Inter-assay precision % RSD of QC samples containing each analyte and internal standard were processed and analyzed with 4 replicate injections in 3 batches (n=12)

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

Figure 3. Chromatograms of THC-COOH and THC along with their internal standards at the limits of quantitation of 10 pg/mL and 0.5 ng/mL, respectively.



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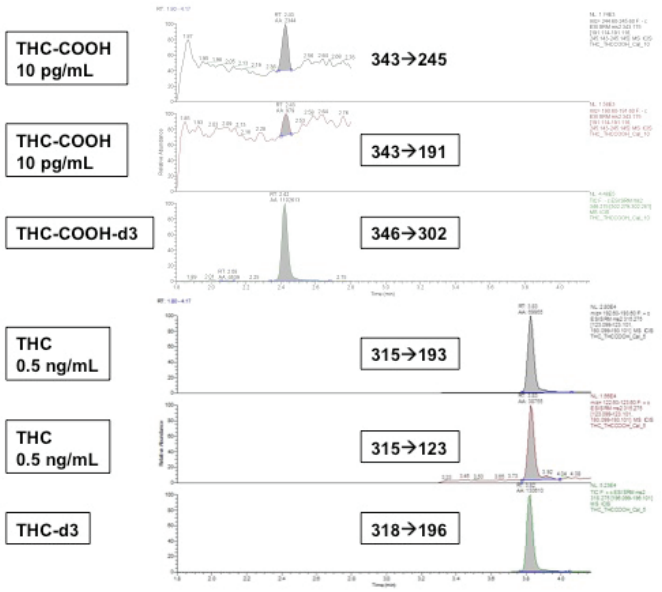

Figure 4. Chromatograms of THC-COOH and THC along with their internal standards in the Low QC at 25 pg/mL and 2.5 ng/mL, respectively.

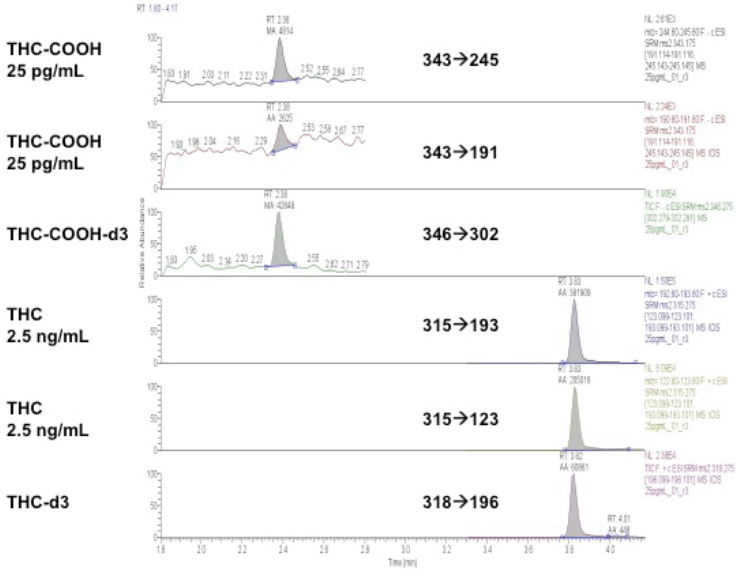

Table 5. Recovery 50 pg/mL of THC-COOH and 5 ng/mL of THC and internal standards was spiked into 5 donor oral fluid samples before and after SPE. Duplicate injections were performed. (%recovery = Response ratio before SPE / after SPE)

Analyte	%Recovery				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THC-COOH	85.4	98.5	105.6	96.0	104.8
THC	65.1	61.3	61.4	55.8	64.2

Table 6. Matrix effects THC-COOH, THC and internal standards was spiked into 5 donor oral fluid samples and water. Duplicate injections were performed.
% Absolute Matrix effect = peak area of donor / water;
% Relative Matrix Effect = peak area ratio against IS of donor/water).
Addition of IS corrects matrix effects to some extent for both analytes.

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

CONCLUSIONS

- We developed a sensitive and easy method for analysis of THC and metabolite in oral fluid using novel SPE technology for sample preparation. This technology eliminates the evaporation and reconstitution steps, making sample preparation simple, fast and economical.
- An efficient SPE method (no pre-conditioning, no evaporation and reconstitution) was developed to extract THC-COOH and THC from oral fluid.
- LOQ for THC-COOH is 10 pg/mL with ion ratio confirmation. The linear range for THC-COOH is 10-1000 pg/mL and for THC is 0.5-100 ng/mL.
- High recovery for THC-COOH and low matrix effects were observed.
- This chromatography method can be run on an LX-2 multi-channel LC to improve throughput by 2-fold.

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