

A Simple and Rapid Solid Phase Extraction Method for Analysis of THC and THC-COOH in Oral Fluid Using LC-MS/MS

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ABSTRACT

Purpose: To develop a simple and fast LC-MS/MS method for quantification of THC and THC-COOH in oral fluid.

Methods: A solid phase extraction (SPE) method was developed on a Thermo Scientific™ SOLApu™ SAX 96-well plate to simultaneously extract THC and THC-COOH from oral fluid. Extracted compounds were separated on a reverse phase column chromatographically followed by analysis on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer.

Results: The entire SPE process takes less than 20 minutes, and no pre-conditioning, evaporation or reconstitution is required. Comparing to conventional SPE method which involves those steps, our method is simpler and faster.

The LOQ was **10 pg/mL for THC-COOH** and 0.1 ng/mL for THC with ion ratio confirmation in oral fluid. The linearity range was 10-1000 pg/mL for THC-COOH and 0.1-100 ng/mL for THC. The intra-assay precision was better than 9.5% RSD. The inter-assay precision was better than 8.4% RSD. The recovery rate ranged from 85% to 106% for THC-COOH and from 56% to 65% for THC. The matrix effect ranged from 80% to 126% for THC-COOH and from 95% to 99% for THC in oral fluid.

INTRODUCTION

11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH) quantification in oral fluid (OF) requires highly sensitive analytical methods as it is present in low pg/mL concentrations. Previous published GC-MS/MS or LC-MS/MS methods required derivatization or use of a time-consuming solid phase extraction (SPE) procedure to increase sensitivity. We developed a sensitive and efficient LC-MS/MS method for simultaneous determination of THC and THC-COOH in OF. Both analytes were extracted using a simple and rapid SPE method without pre-conditioning, evaporation and reconstitution.

MATERIALS AND METHODS

Sample Preparation

0.75 mL OF-buffer mixture (0.25 mL OF + 0.5 mL buffer) was combined with internal standard (IS) solution. Proteins were precipitated by addition of acetonitrile. The mixture was decanted onto a SOLApu™ SAX SPE plate. After wash, the elution was performed with two times of 30 µL formic acid/acetonitrile. Eluates were diluted with 60 µL water. 50 µL of the diluted eluate was injected for LC-MS/MS analysis.

Extracted compounds were separated on a reverse phase column chromatographically followed by analysis on a triple quadrupole mass spectrometer. Data were acquired in selected-reaction monitoring (SRM) mode. Two SRM transitions for each analyte were measured with polarity switching and ion ratios were calculated for confirmation.

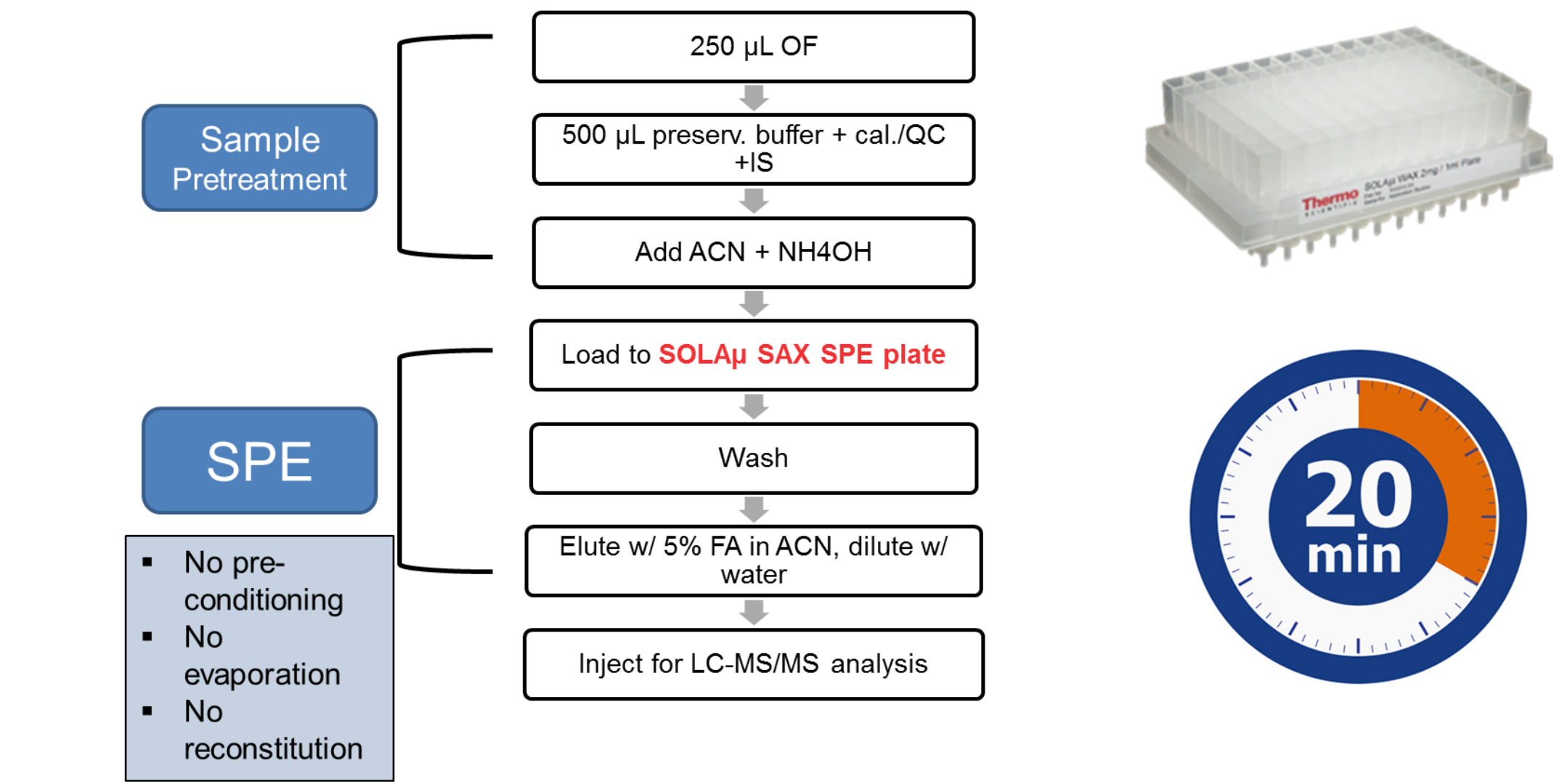
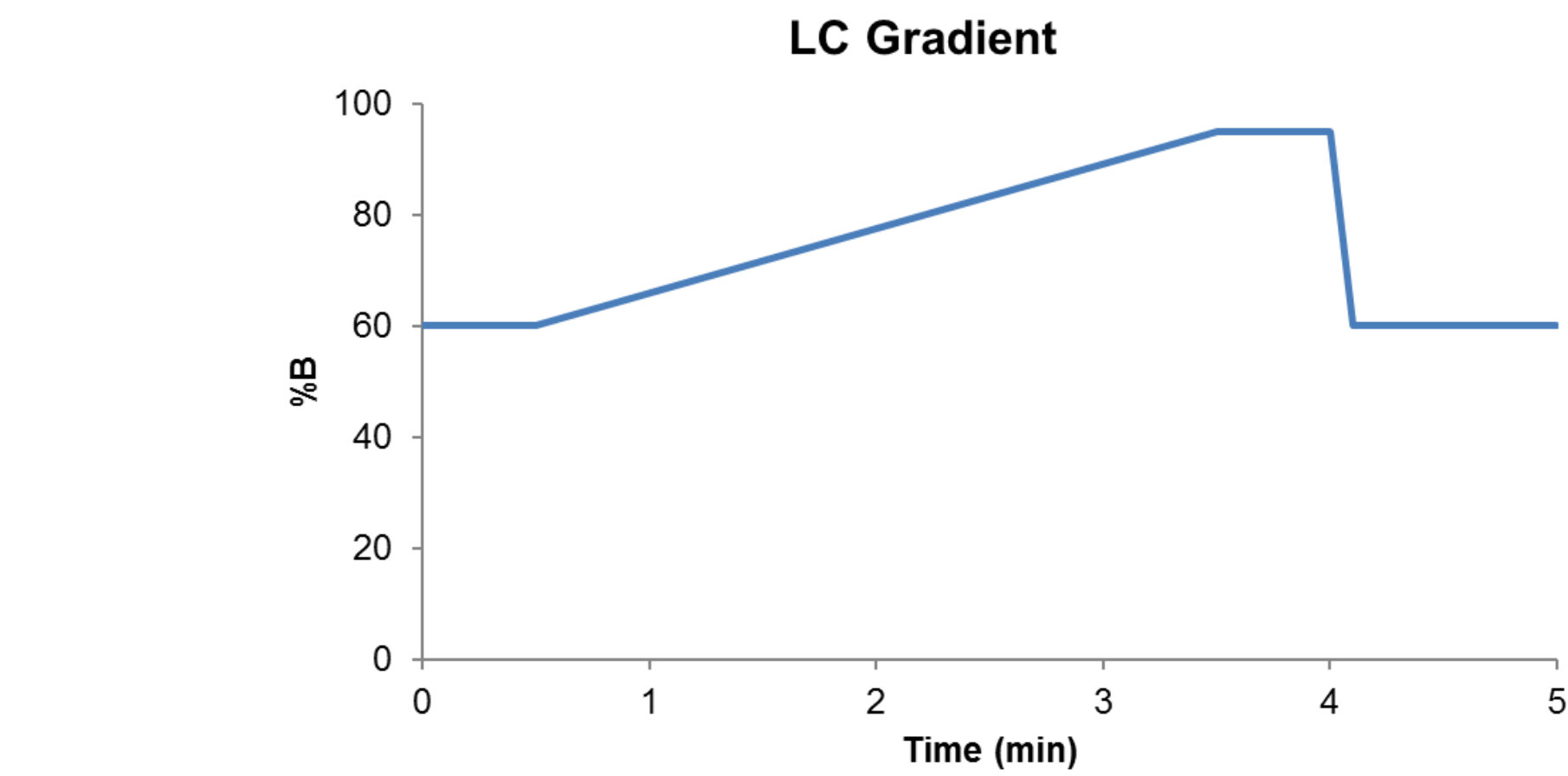


Figure 1. SPE process to extract THC and THC-COOH from oral fluid

Test Method(s)



Figure 2. Thermo Scientific™ UltiMate™ 3000 HPLC and TSQ Quantiva MS with heated ESI source



A: 0.1% FA in water
B: 0.1% FA in ACN
Column: Thermo Scientific™ Accucore™ RP-MS,
100X2.1mm, 2.6µm
Temperature: 40°C

Figure 3. LC gradient

Compound	Retention Time (min)	RT Window (min)	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (v)	Comment
THC-COOH*	2.3	1	Negative	343.2	245.1	30	87	Quantifying ion
THC-COOH	2.3	1	Negative	343.2	191.1	33	87	Confirming ion
THC-COOH-d3	2.3	1	Negative	346.2	302.3	22	85	
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	

Figure 4. SRM transitions (two for each analyte)

RESULTS

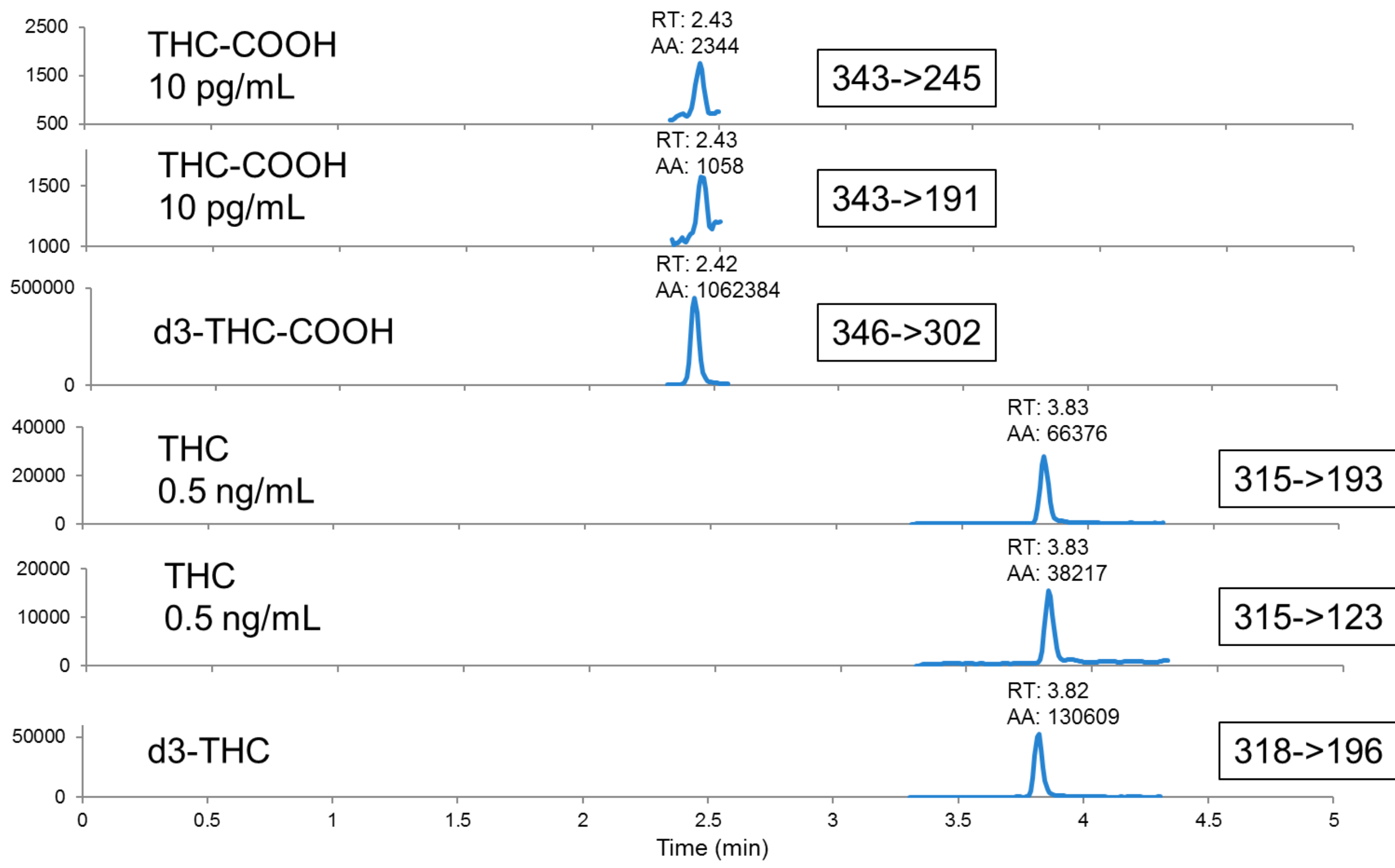


Figure 5. Chromatograms on the lower limit of quantitation level in neat solution

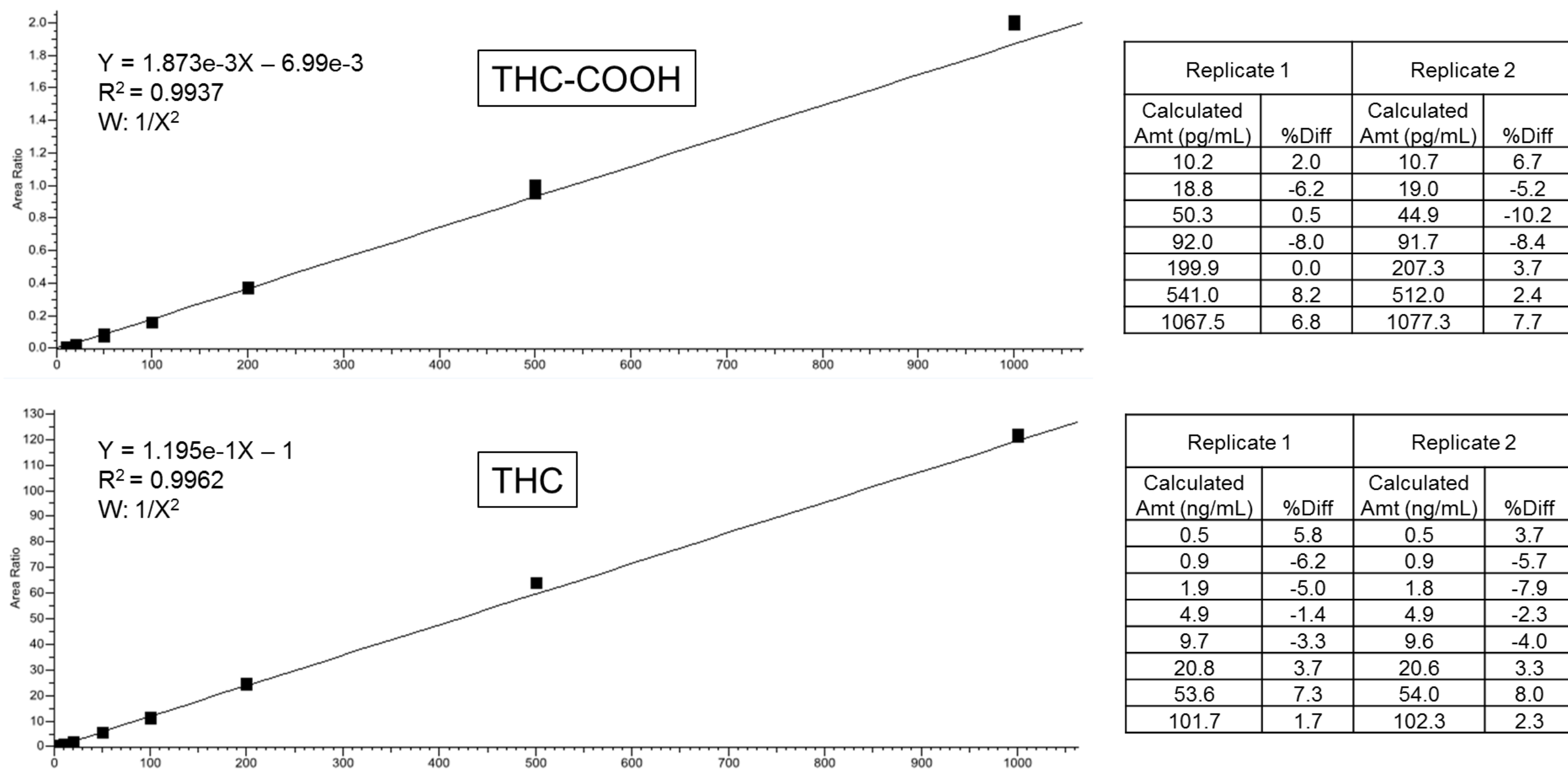


Figure 6. Calibration curve

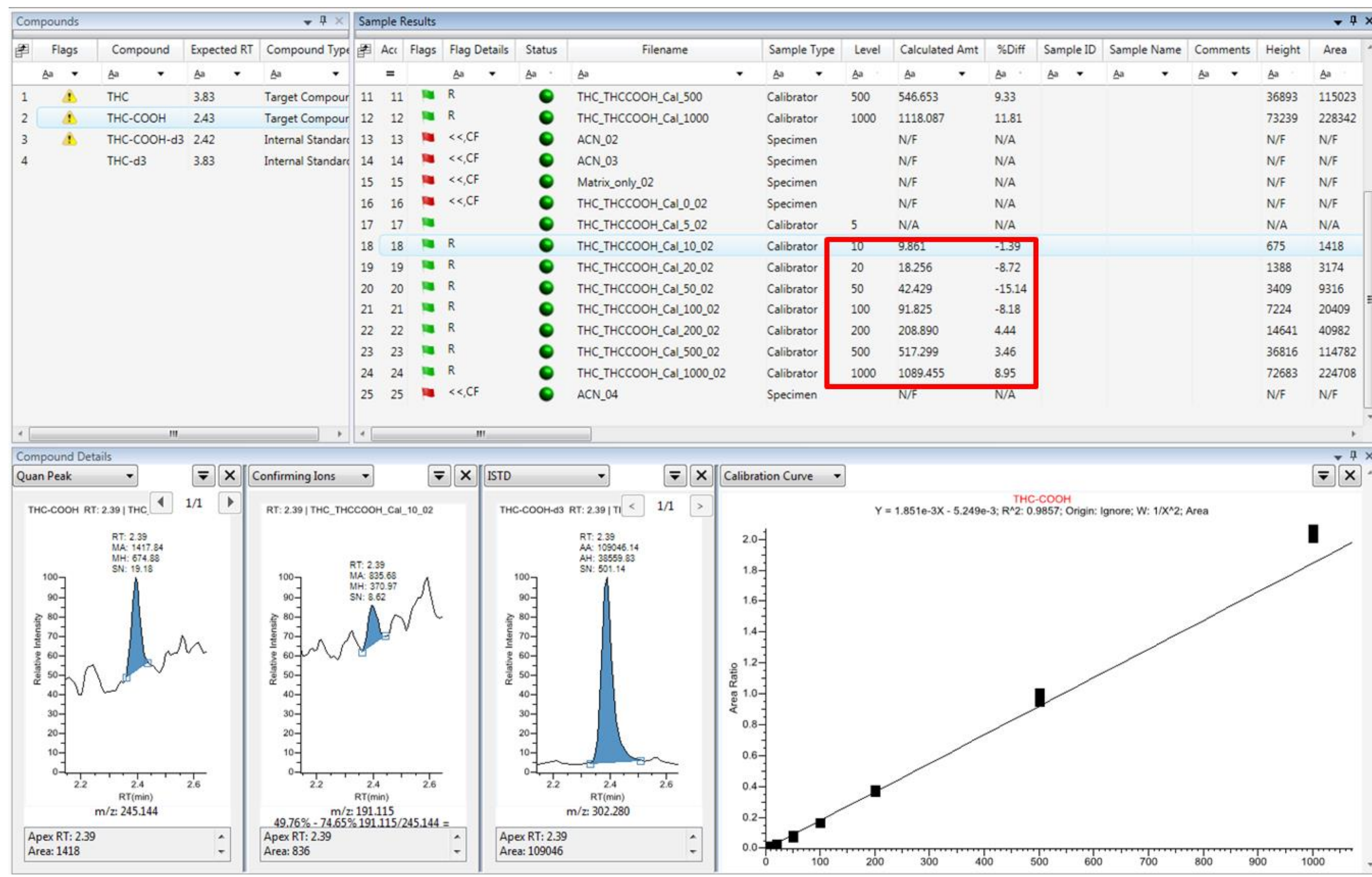


Figure 7. Calibration Curve and the Lowest Calibration Standard Peak (THC-COOH, 10 pg/mL) in TraceFinder software.

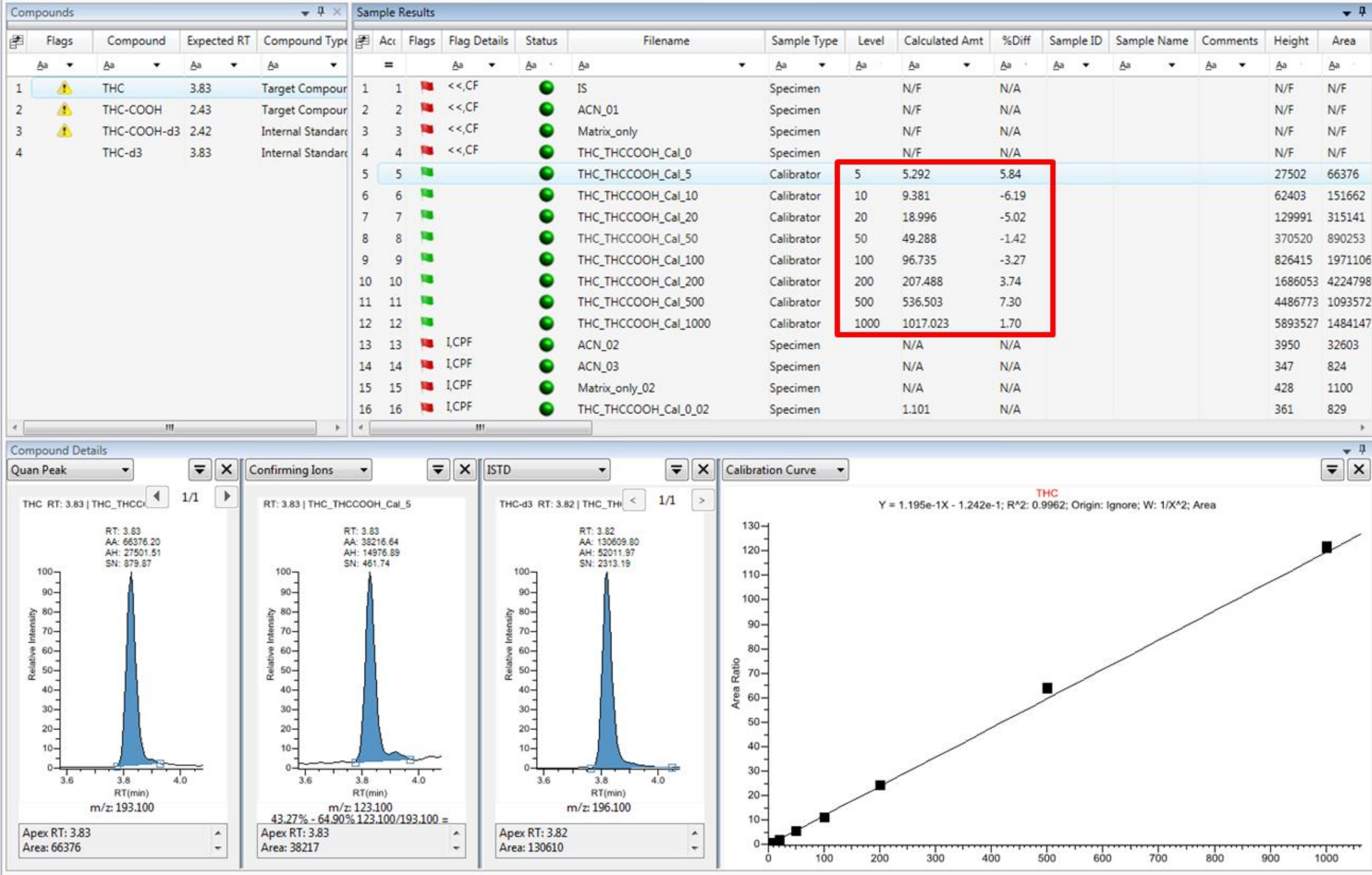


Figure 8. Calibration Curve and the Lowest Calibration Standard Peak (THC, 0.5 ng/mL) in TraceFinder software.

Analyte	QC intra-assay precision (% RSD, n=4)			QC inter-assay precision (% RSD, n=12)		
	Low	Med	High	Low	Med	High
THC-COOH	5.1 – 9.5	5.6 – 9.4	5.3 – 8.3	8.4	7.7	6.3
THC	1.2 – 3.0	0.9 – 1.8	0.7 – 2.3	3.2	2.4	2.2

Figure 9. Method precision

Analyte	Recovery (%)				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THC-COOH	96.8	70.0	78.9	75.9	83.7
THC	65.1	61.3	61.4	55.8	64.2

Figure 10. Recovery

Analyte	Absolute matrix effect (%Recovery)					Relative matrix effect (%Recovery)				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
THC-COOH	113	86.8	97.0	102	86.5	105	84.8	84.3	97.0	88.9
THC	68.0	71.1	65.8	63.7	67.1	103	99.1	102	101	98.0

Figure 11. Matrix effect

CONCLUSIONS

- Simple and rapid SPE extraction method (in 20 min)
- LOQ of THC-COOH: 10 pg/mL with ion ratio confirmation
- High recovery rate and limited matrix effect for THC-COOH

For forensic toxicology use only

TRADEMARKS/LICENSING

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