LC-MS/MS-MS^3 for determination and quantification of Δ9-tetrahydrocannabinol and metabolites in blood samples

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59th Annual Meeting of TIAFT
Versailles, France
September 5th - 8th, 2022

INTRODUTION

Due to the high prevalence of cannabinoids in forensic toxicology, it is crucial to have an efficient method that allows the use of a small sample amount and that requires a minimal sample preparation, for the determination and quantification of low concentrations.

A simple, highly selective and high throughput LC-MS/MS-MS^3 method was developed for the determination and quantification of Δ9-tetrahydrocannabinol (THC), 11-hydroxy-Δ9-THC (THC-OH) and 11-nor-Δ9-THC-9-carboxylic acid (THC-COOH) in blood samples.

EXPERIMENTAL

Sample preparation

Blood (100 µL) with internal standard mix (30 µL) and 100 µL of H_2O:MeOH (1:1) were extracted by using a protein precipitation procedure with 900 µL of acetonitrile (-20 °C). After vortex, the samples were centrifuged for 10 min in a refrigerated centrifuge at 5 °C and 14.000 rpm. The supernatant was transferred out and evaporated to dryness under N_2 gas. The residues were reconstituted with 60 µL of methanol and 10 µL was injected into the LC-MS/MS system.

LC conditions

Equipment: SCIEX Exion LC

The separation was performed in an Acquity UPLC® HSS T3 (100x2.1 mm, 1.8 µm) column using a gradient elution with methanol/2 mM ammonium formate (formic acid 0.1%) (95:5, v/v) (A) and 2 mM ammonium formate (formic acid 0.1%)/methanol (95:5, v/v) (B) at a flow rate of 0.4 mL/min and a run time of 10 min.

MS/MS conditions

Equipment: SCIEX QTRAP 6500+

The optimized MS/MS parameters were as follows: Source temperature, 450°C; ion spray voltage (IS), +5500 V /-5500 V; ion source gas 1 (GS1) pressure, 40 psi; ion source gas 2 (GS2) pressure, 60 psi; curtain gas (CUR) pressure, 25 psi; collision gas (CAD), medium. Table I shows the MRM and MS^3 parameters used in the optimized survey scan.

Table I. Retention time, MRM and MS^3 parameters of cannabinoids.

		MRM			MS^3	
Analytes	Retention Time (RT)	Q1 mass (m/z)	Q3 mass (m/z)	Q1 mass (m/z)	Q3 mass (m/z)	MS^3 ion scan (m/z)
THC	8.39	315.1	193.1 123.0	315.1	193.0	123.0 137.0
THC-d3	8.40	318.1	196.0			
THC- OH	7.39	329.1	268.1 173.1	331.2	193.0	123.0 137.0
THC-OH-d3	7.38	332.2	314.4			
THC-COOH	7.50	343.1	299.1 245.1	343.1	299.0	245.0 191.0
THC-COOH-d3	7.49	346.1	302.2			

RESULTS AND DISCUSSION

Optimization MS^3 Scan

MS detection was carried out in two modes, MRM mode and MS^3 (linear ion trap) mode, in a single injection. In MS^3, negative polarity was applied monitoring transitions for THC-COOH and positive polarity for THC and THC-OH (Fig. 1) .

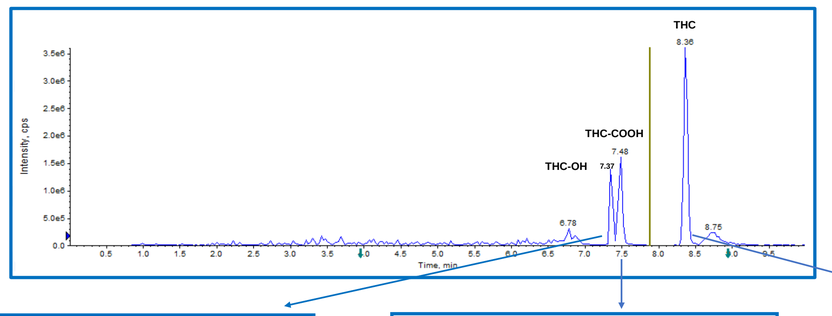

Figure 1. Total ion chromatogram of THC, THC-OH and THC-COOH in blood samples at 5 ng/mL.

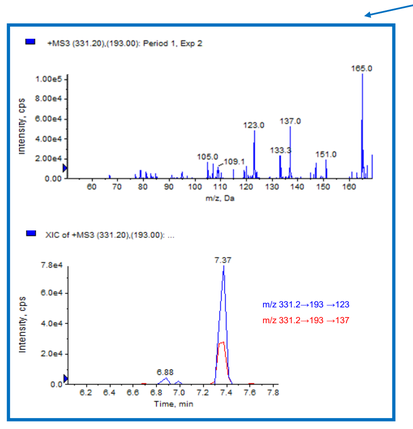

Figure 2. MS^3 scan and extracted ion chromatogram of THC-OH.

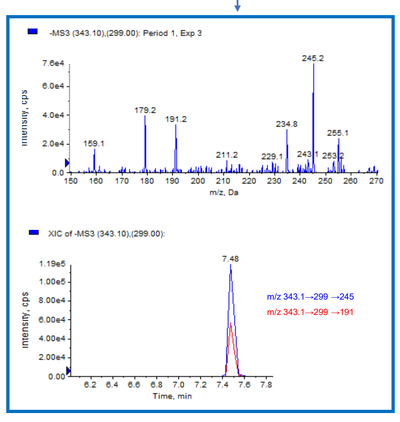

Figure 3. MS^3 scan and extracted ion chromatogram of THC-COOH.

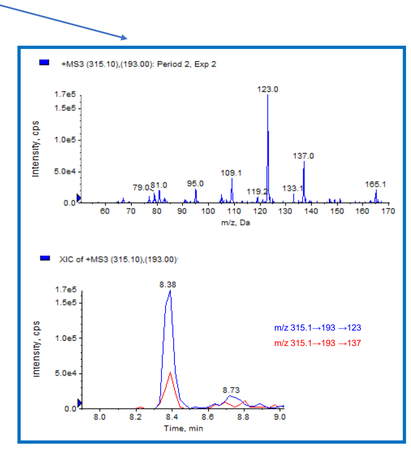

Figure 4. MS^3 scan and extracted ion chromatogram of THC.

MRM vs. MS^3

- ✓ MS^3 exhibits a higher signal to noise ratio (Fig. 5)
- ✓ Large reduction of background interferences in MS^3
- ✓ MS^3 improve the performance of identification and confirmation of cannabinoids
- ✓ MS^3 provide much higher sensitivity and selectivity

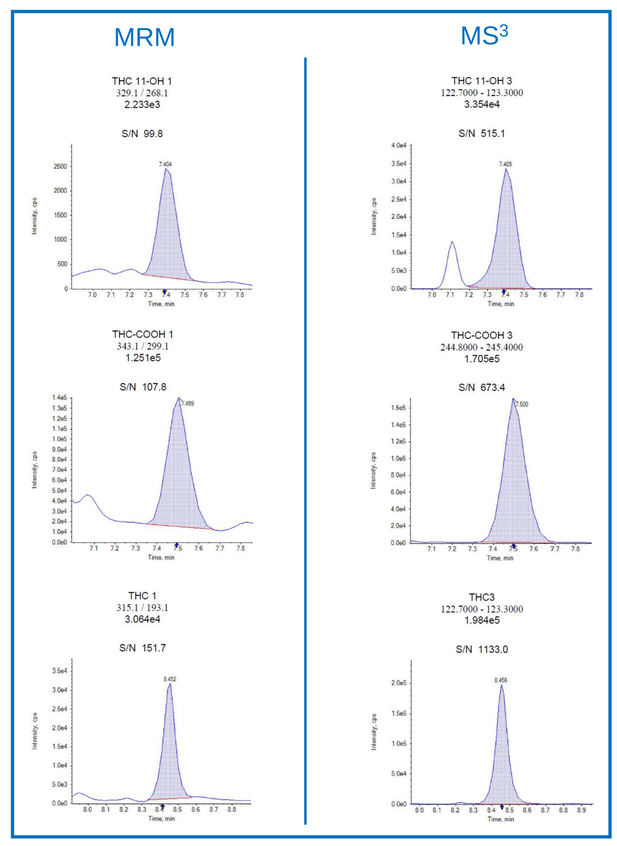

Figure 5. Chromatograms of cannabinoids in blood samples at LLOQ (0.5 ng/mL) in MRM and MS^3 mode.

Method validation

- ✓ LLOQ was determined to be 0.5 ng/mL for all three compounds
- ✓ Linear calibration range used for THC, THC-OH and THC-COOH was 0.5-100 ng/mL in MRM and MS^3 modes (Fig. 6)
- ✓ Linearity: $r^2 > 0.996$
- ✓ Repeatability (n=5) and intermediate precision (5 day) 3 QC (1, 5, 50 ng/mL) CV <15%, accuracy > 88%
- ✓ Recovery: from 88.2 to 117.2 %

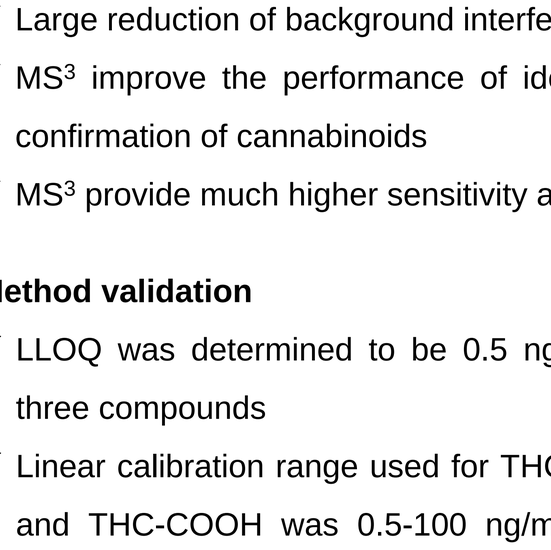

Figure 6. Calibration curves in MRM and MS^3 mode.

Table III. Repeatability, intermediate precision, accuracy and recovery for three levels of concentration (1, 5 and 50 ng/mL).

Analyte	QC sample (ng/mL)	Repeatability CV (%)	Intermediate precision CV (%)	Accuracy (%)	Extraction recovery (%)
THC (MRM)	1	7.6	14.9	114.9	94.3
	5	7.2	8.0	97.6	98.0
	50	3.1	8.2	92.1	101.3
THC (MS^3)	1	11.7	14.7	130.0	98.5
	5	4.2	6.4	96.6	88.0
	50	3.3	7.0	94.1	98.9
THC-OH (MRM)	1	9.3	14.8	105.1	117.2
	5	3.0	6.9	92.7	93.2
	50	6.4	11.0	88.0	97.6
THC-OH (MS^3)	1	9.8	12.8	118.6	98.8
	5	14.3	13.2	100.7	88.2
	50	12.8	14.2	92.4	104.2
THC-COOH (MRM)	1	4.0	14.8	107.0	100.1
	5	8.5	8.7	101.3	98.5
	50	4.9	8.8	90.3	101.8
THC-COOH (MS^3)	1	12.4	14.8	92.1	104.7
	5	7.4	8.2	110.7	96.6
	50	5.0	10.6	89.2	99.1

CONCLUSION

In this work, a LC-MS/MS analysis in two different acquisition modes (MRM and MS^3) were compared after a protein precipitation procedure with a sample volume of 100 µL in order to develop a fast and selective method for the determination of cannabinoids. The validated method provides a sensitive, efficient and robust procedure for the quantification of cannabinoids suitable for DUID cases and *postmortem* analysis.

Acknowledgements

Our thanks go to Carlos Bueno, SCIEX Spain, for the technical support in the development of LC-MS^3 technique.