Service of Forensic Chemistry and Toxicology

QUANTIFICATION OF Δ9-THC, 11-OH-THC AND THCCOOH BY SPE AND GC-MS-MS IN WHOLE BLOOD SAMPLES FOR FORENSIC PURPOSES

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AIMS & INTRODUCTION

Marijuana and its variations (hashish, weed, …) is a product obtained from *Cannabis sativa* (figure 1), and is the most consumed illicit drug worldwide. Due to the existence of a wide range of different compounds, marijuana metabolism is complex and is dependable of various aspects, such as route of administration and consumed dose. However, the most active substance, in terms of psychoactive action, is the Δ9-tetrahydrocannabinol (Δ9-THC) (figure 2). Its metabolism lead to the production of an active metabolite, named 11-hydroxy- Δ9-tetrahydrocannabinol (11-OH-THC) (figure 3), and a further inactive metabolite 11-nor-9-carboxy- Δ9-tetrahydrocannabinol (THCCOOH) (Figure 4).

Its abuse represents a major issue in terms of public health, not only due to its physical and psychological effects in terms of individual behaviour, but also due to consequences in terms of impairment, mainly as to driving capabilities is concerned.

Nevertheless, these compounds are used, controversially, in pain therapeutics procedures in terminal condition patients.

Detection and accurate quantification at low levels, namely for Δ9-THC and 11-OH-THC, seems crucial, in terms of response of a forensic toxicology laboratory to answer to legal requirements. The aim of this work considers the analytical validation for detection and quantification of Δ9-THC, 11-OH-THC and THCCOOH in whole blood samples, in vivo and post-mortem, for forensic purposes.

Figure 1. *Cannabis sativa* leaf

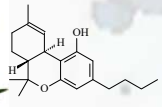


Figure 2. Δ9-THC

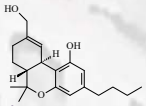


Figure 3. 11-OH-THC

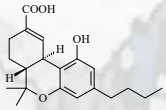


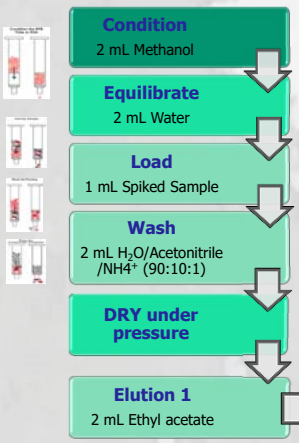
Figure 4. THCCOOH

MATERIAL & METHODS

The applied methodology included:

✓ a solid phase extraction (SPE) procedure with Oasis HLB® 3cc columns from WATERS (Figure 5).

✓ an analytical technique using a gas chromatograph GC-450, coupled to a mass spectrometer MS-300 with a triple quadrupole detector from BRUKER (Figure 6 and Tables 1 and 2).



✓ Derivatization with BSTFA / TMCS (99:1) for 30' at 65ºC.

Figure 5. Sample preparation and extraction scheme, based on Simões *et al.* (1).

Figure 6. GC-MS-MS triple quadrupole

TABLE 1. Analytical parameters of GC-MS-MS system

Injector	Temperature	260ºC
	Sample Volume	2.0 µL
	Injection Mode	Splitless
Column	Agilent J & W HP-5MS	0.25 mm / 30 m / 0.25 µm
	Constant Flow	1.3 mL/min
Column Oven	Initial Temperature	100ºC (0,5')
	Temperature "Ramp" 1	40ºC/min (4')
	Final Temperature	290ºC
	Total Run Time	7,21 min
Mass Spectrometer	Transfer Line	280ºC
	Quadrupole Temperature	40ºC
	Ionization Source Temperature	260ºC
	Ionization	EI (*Electron Impact*)
	Solvent Delay	4 min
	Acquisition Parameters	Quad1: SIM Mode (*Selected Ion Monitoring*) Quad3: SIM Mode (*Selected Ion Monitoring*)

TABLE 2. Mass Spectrometer Acquisition parameters. The quantitation ion is underlined.

Compound Name	Precursor-ion (m/z)	Product-ion (m/z)	Collision Energy	Retention time (min)
THC	371	289, 305	16 V	4,74
THC-d_3 (IS)	374	292	16 V	4,72
11-OH-THC	371	289, 305	16 V	5,63
11-OH-THC-d_3 (IS)	374	292	16 V	5,61
THCCOOH	371	289, 305	16 V	6,35
THCCOOH-d_3 (IS)	374	292	16 V	6,33

RESULTS

This analytical methodology validation was accomplished with the assessment of some parameters. In the specificity/selectivity and identification capacity, 0% false positive and false negative results were achieved, whereby confirming the specificity and the selectivity of this method (Figures 7 and 8).

To test the LOD and LOQ value, blank whole blood samples were spiked with the compounds at 1 ng/mL concentration (n= 3). All the samples were positive for the three compounds (Figure 9). Table 3 shows the analytical validation results for the target-compounds.

TABLE 3. Analytical validation parameters results.

Compound Name	LOD	LOQ	Work range	Intra-run precision (n=3)	Inter-run precision (n=3)
THC	1 ng/mL	1 ng/mL	1 – 100 ng/mL	8,96 %	4,11 %
11-OH-THC	1 ng/mL	1 ng/mL	1 – 100 ng/mL	3,03 %	10,29 %
THCCOOH	1 ng/mL	1 ng/mL	1 – 100 ng/mL	4,35 %	2,73 %

As to carryover is concerned, it has not been noticed, even with samples positive over 100 ng/mL. Intra-run precision was studied at 1 ng/mL, 5 ng/mL and 10 ng/mL for each compound. Inter-run precision was studied at 5 ng/mL for each compound. A maximum of 10% of CV was found for both parameters.

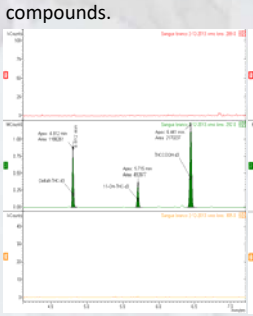


Figure 7. Blank whole blood sample

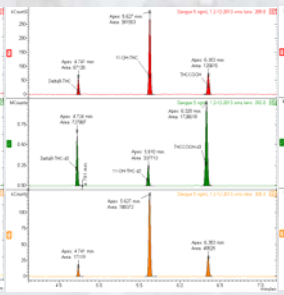


Figure 8. Whole blood sample with compounds at 5 ng/mL

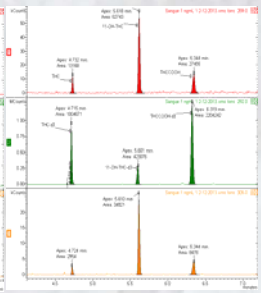


Figure 9. Whole blood sample with compounds at 1 ng/mL

DISCUSSION

The developed method has shown to be fit to purpose, with good results in terms of increased sensibility and detection limits, due to an optimal response from the analytical equipment, based on tandem MS technology. The LOD and LOQ are compatible with reference values for positive real samples, namely applied to traffic roadside testing. This method has been applied to the laboratory routine, representing an effective improvement in terms of the lab response to legal requirements.

Bibliography:

(1) Simões S.S., Ajenjo A.C., Dias M.J.; Qualitative and quantitative analysis of THC, 11-hydroxy-THC and 11-nor-9-carboxy-THC in whole blood by ultra-performance liquid chromatography /tandem mass spectrometry; *Rapid Communications in Mass Spectrometry*; 2011; **25**; 2603-2610.