

# Quantitative Analysis of Cannabinoids in Whole Blood Using UPLC-MS/MS for Forensic Laboratories

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## Abstract

This study reports a quantitative method based on SPE, following protein precipitation and UPLC-MS/MS. The method has been verified and its performance evaluated using authentic samples. Data were compared to results obtained with a GC-MS/MS method.

Cannabinoids should be monitored in both forensic and roadside drug testing laboratories, thus requiring an accurate, reliable, and robust method to quantify these compounds in biological samples. The developed approach meets these requirements, and demonstrates excellent correlation with an alternative GC-MS/MS method for the analysis of cannabinoids in human whole blood samples.

The method offers a number of noteworthy benefits over the GC-MS/MS approach including the following: utilization of UPLC rather than GC separation means that the lengthy post-extraction derivatization, used by the latter technique, can be eliminated with the analytical run time reduced from 20 minutes to 6.5 minutes, a three-fold reduction. The combination of these factors allows for significantly higher sample throughput. Furthermore, the superior sensitivity of the Xevo TQ-S permits detection of the required low levels of cannabinoids even with much smaller blood sample volumes, for example 0.2 mL compared with 1 mL required for other reported methods, even without the need of a post-extraction concentration step. This can be particularly advantageous as the volumes of whole blood available for testing can be small and must be sufficient for testing a number of drug classes.

### Benefits

- Reduced sample volume
- Elimination of derivatization step prior to analysis
- Shorter analytical run times
- Improved sample throughput

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## Introduction

Cannabis is the most widely used illicit substance in the world, and long-term use can lead to dependency. Consequently, the cannabinoids are one of the most commonly detected classes of illegal drugs, and their analysis plays a key role in both forensic and roadside drug testing.

$\Delta$ -9 tetrahydrocannabinol (THC) is the main psychoactive element present in the plant *Cannabis sativa*.<sup>1</sup> THC

produces a number of metabolites, including the active hydroxy-THC (THC-OH), and inactive carboxy-THC (THC-COOH), which can be detected circulating in blood after smoking or ingestion of cannabis.<sup>2,3</sup> Quantitative analysis of the psychoactive constituents in whole blood is an indicator of cannabis consumption and may provide information relating to the individual's state of impairment at the time of sample collection.

Previous publications have described the use of GC-MS, after solid-phase extraction (SPE),<sup>4</sup> or liquid-liquid extraction,<sup>5</sup> and pre-column derivatization for the determination of cannabinoids in whole blood. Recently a publication described the use of pre-column derivatization in conjunction with HPLC-MS/MS for this analysis.<sup>6</sup> This study reports a quantitative method based on a previously reported SPE method following protein precipitation and UPLC-MS/MS.<sup>8</sup> The method has been verified and its performance evaluated using authentic samples. Data were compared to results obtained with a GC-MS/MS method.

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## Experimental

### Sample description

THC, THC-OH and THC-COOH (1 mg/mL), and their deuterated (d-3) analogues for use as internal standards (ISTD) at 0.1 mg/mL were purchased from LGC Standards (Teddington, UK). A mixture of pooled ISTDs at 50 ng/mL in methanol was prepared and stored at -20 °C.

Whole blood calibrators were prepared by spiking blank whole blood samples with known amounts of cannabinoids.

Forty-five anonymized samples containing pre-analyzed cannabinoids were obtained from J Monod Hospital, Le Havre, France. The samples were collected in the presence of either sodium fluoride or lithium heparin as anticoagulant.

### Sample preparation

Twenty microlitres ISTD were added to 0.2 mL whole blood (either sample or calibrator), which was then precipitated by drop-wise addition of 0.4 mL acetonitrile while vortex-mixing. The sample was then centrifuged at 4000 g for 10 minutes at 4 °C. Supernatant (0.4 mL) was then added to 0.6 mL 1% ammonium hydroxide, and the resulting solution loaded onto the Oasis MAX SPE Cartridge (p/n 186000366).

### Solid-phase extraction with Oasis MAX

Condition: 1 mL methanol followed by 1 mL 1% ammonium

	hydroxide
Load:	prepared 1 mL sample
Wash:	0.5 mL 50% acetonitrile
Dry:	10 minutes under full vacuum
Elute:	1.5 mL hexane/ethyl acetate/acetic acid (49:49:2 v/v/v)
Evaporate:	under nitrogen at 40 °C

The sample was reconstituted in 0.133 mL 70% aqueous methanol, vortex-mixed, then transferred to a Waters Total Recovery Vial.

## UPLC conditions

Column:	ACQUITY UPLC BEH C <sub>18</sub> , 1.7 µm, 2.1 x 100 mm, (p/n 186002352)
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	15 µL (PLNO)
Strong wash:	methanol/acetonitrile/ propan-2-ol (1:1:1 v/v/v)
Weak wash:	50% aqueous methanol
Flow rate:	400 µL/min
Mobile phase A:	0.1% formic acid
Mobile phase B:	acetonitrile

Gradient: Linear from 60% B to 90% B over four minutes

## MS conditions

Mass spectrometer: Xevo TQ-S

Ionization mode: ESI positive

Capillary voltage: 2.5 kV

Cone voltage: 25 V

Cone offset: 50 V

Desolvation temp.: 550 °C

Desolvation gas: 900 L/h

Cone gas: 150 L/h

Acquisition mode: multiple reaction monitoring (MRM), see Table 1.

Cannabinoid	Retention time (min)	Precursor ion ( <i>m/z</i> )	Product ion ( <i>m/z</i> )	Collision energy (eV)
THC	4.1	<b>315.3</b>	<b>193.1</b>	<b>22</b>
		315.3	123.0	32
THC-d3	4.1	318.3	196.1	22
THC-OH	2.3	<b>331.3</b>	<b>193.1</b>	<b>24</b>
		331.3	313.2	14
THC-OH-d3	2.3	334.3	196.1	24
THC-COOH	2.4	<b>345.2</b>	<b>193.1</b>	<b>26</b>
		345.2	299.2	18
THC-COOH-d3	2.4	348.2	196.1	26

Table 1. Retention times and MRM transitions for analytes and ISTDs, the quantifier transitions are in bold.

## Data management

MassLynx Software incorporating TargetLynx Application Manager

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## Results and Discussion

### Method verification

The MRM transitions for all of the cannabinoids and ISTDs are shown in Table 1. The analytes were monitored using two transitions (quantifier and qualifier). The acceptance criteria for a positive identification of analytes include retention time within 0.2 minutes of predicted, and the quantifier/qualifier ion ratio within 20% of the predicted ratio, which was based on the average of the ratios across the entire calibrator range. The ISTD was monitored using a single transition. Figure 1 shows a chromatogram of a whole blood calibrator spiked at 0.5 ng/mL.

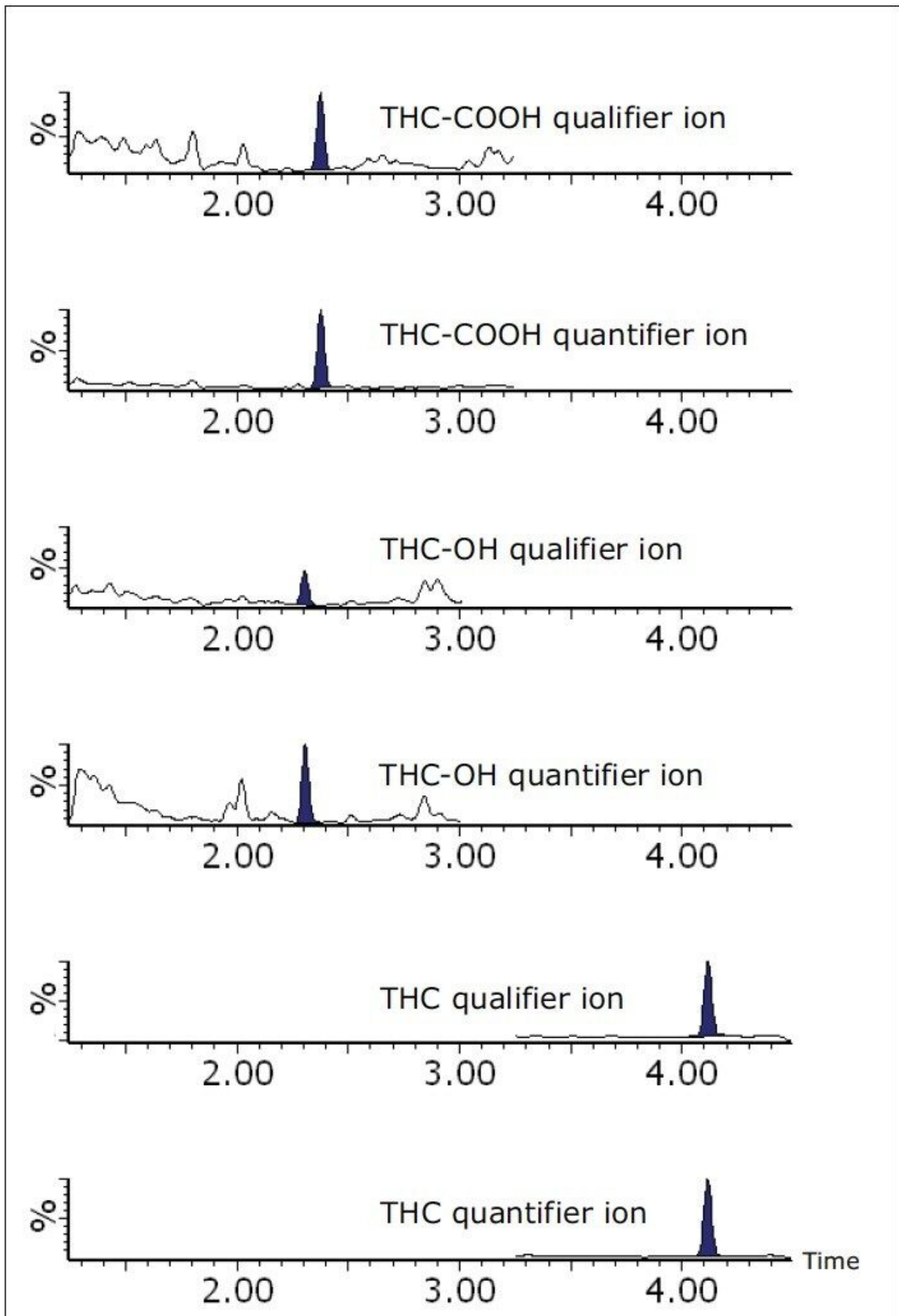


Figure 1. Chromatogram showing cannabinoids spiked into whole blood at 0.5 ng/mL (ISTDs not shown).

To investigate linearity for all cannabinoids, spiked whole blood calibrators were prepared at 0.0, 0.5, 1.0,

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## Acknowledgement

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## Featured Products

ACQUITY UPLC System <<https://www.waters.com/514207>>

Xevo TQ-S <<https://www.waters.com/10160596>>

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Oasis MAX 1 cc Vac Cartridge, 30 mg Sorbent per Cartridge, 30 µm Particle Size, 100/pk <

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ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 2.1 mm X 100 mm, 1/pkg <

<https://www.waters.com/waters/partDetail.htm?partNumber=186002352>>

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