

Quantitative Analysis of THC and Main Metabolites in Whole Blood Using Tandem Mass Spectrometry and Automated Online Sample Preparation

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Overview

Purpose: Sensitive quantification of THC, 11-OH-THC and THC-COOH from whole blood with Thermo Scientific TurboFlow technology. For confirmation purposes, expected limit of quantification must be close to 0.5 ng/mL.

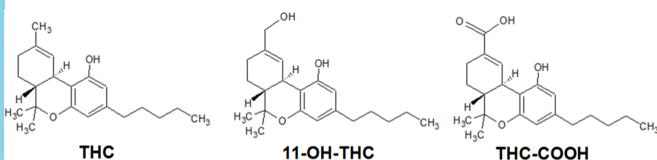
Methods: Blood samples were treated by protein precipitation followed by an online extraction and analysis by Reverse Phase Liquid Chromatography (RP-LC) coupled to mass spectrometry.

Results: This method was linear from 0.5-100 ng/mL for THC and its metabolites with good repeatability and sensitivity.

Introduction

Cannabis is the most highly used illicit substance around the world, and due to its psychoactive effects, it is of great importance to have analytical procedures for the assessment of the extent of its abuse. The major psychoactive constituent product of cannabis is Δ^9 -tetrahydrocannabinol (THC) that is rapidly metabolized mainly in 11-hydroxy- Δ^9 -tetrahydrocannabinol (11-OH-THC) and then in 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH).

FIGURE 1. Molecular structures of THC and main metabolites



To have a better understanding of the effects of cannabis abuse, blood analysis is recommended. Nevertheless, THC and 11-OH-THC have short windows of detection in this matrix, and therefore limits of detection for their analysis are often settled to concentrations as low as 0.5 ng/mL.

In recent years, LC-MS has gained ground to GC-MS as a reference method for the analysis and confirmation of drugs of abuse in biological matrices in clinical and forensic toxicology. In the case of cannabinoids, it is particularly interesting to attain high sensitivities without a need for derivatization, but one of the key parameters to achieve sensitivity requirements is the choice of an appropriate sample treatment prior to the LC-MS method.

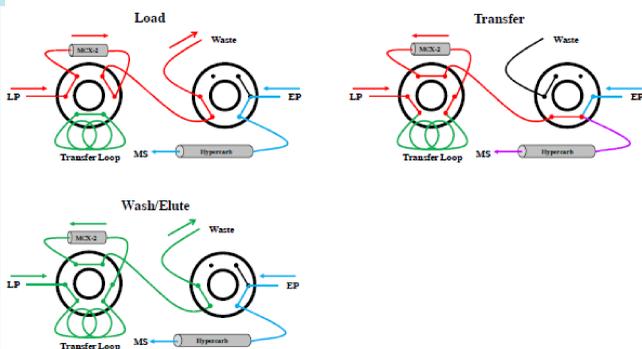
Thermo Scientific TurboFlow technology is an automated online sample preparation technique that has been coupled to LC-MS/MS for the quantitative analysis of biological samples. Our goal is to develop a method to measure THC and its metabolites by reducing method time while attaining good analytical performances.

Methods

Sample Preparation

A 0.2-mL sample (whole blood) was spiked with internal standards (IS) and then mixed with 0.4 mL of 0.1% formic acid in acetonitrile (v/v). The mixture was vortexed and stored at 0 °C for 10 min. After a 2 minutes sonication, the mixture was centrifuged at 10,000 rpm for 10 min, and 90 μ L of supernatant was injected for LC-MS/MS analysis.

FIGURE 2. "Focus Mode Technical" diagram of TurboFlow Technology.



The TurboFlow™ method was performed in Focus Technical mode (figure 2) with a Thermo Scientific TurboFlow Cyclone P column. Analytical separation was carried out on a Thermo Scientific Accucore C18 column (50x2.1 mm, 2.6- μ m particle size). The total LC runtime was 10.4 min (Table 1).

Table 1. TurboFlow and LC method conditions.

Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B	%C	%D
0.00	45	1.50	Step	80.0	-	20.0	-	=====	out	0.40	Step	-	-	80.0	20.0
0.75	75	0.10	Step	90.0	-	10.0	-	T	in	0.30	Step	-	-	80.0	20.0
2.00	119	1.50	Step	-	-	100.0	-	=====	out	0.40	Ramp	-	-	2.0	98.0
3.98	100	1.00	Step	-	-	100.0	-	=====	out	0.40	Step	-	-	2.0	98.0
5.65	15	0.50	Step	-	-	100.0	-	T	out	0.01	Step	-	-	2.0	98.0
5.90	30	1.50	Step	-	-	100.0	-	=====	out	0.40	Step	-	-	2.0	98.0
6.40	90	1.50	Step	20.0	-	80.0	-	=====	in	0.40	Step	-	-	80.0	20.0
7.90	150	1.00	Step	80.0	-	20.0	-	=====	out	0.40	Step	-	-	80.0	20.0

Loading : A: 0.1% formic acid in water; C: 0.1% formic acid in acetonitrile; D: mixture of isopropanol, acetonitrile, and acetone (40/40/20 v/v/v)
Eluting : C: 10mM ammonium formate + 0.1% formic acid in water; D: 0.1% formic acid in methanol

A Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer was operated with a heated electrospray ionization (HESI-II) source in positive ionization mode for THC and 11-OH-THC and in negative ionization mode for THC-COOH. Data were acquired in the selected reaction monitoring (SRM) mode (Table 2).

Table 2. MS source parameters and SRM transitions.

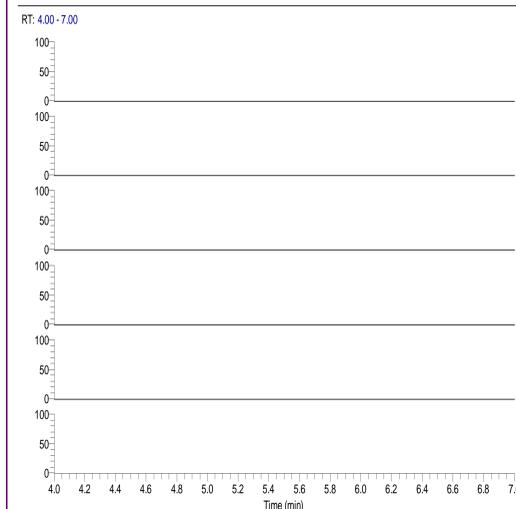
Ionization	HESI-II	Compound	Parent Ion (m/z)	Product Ion (m/z)	S-Lens	CE	Polarity
Spray voltage (V)	3500 (+) 2700 (-)						
Vaporizer Temp (°C)	330	THC	315.2	193.2	89	20	+
Capillary Temp (°C)	270	THC-D3	318.2	196.2	89	23	+
Sheath gas (AU)	35	11-OH-THC	331.1	193.1	83	24	+
Auxiliary gas (AU)	25	11-OH-THC-D3	334.2	316.3	83	15	+
Ion sweep gas (AU)	5	THC-COOH	343.1	245.2	118	28	-
Collision gas pressure (mTorr)	25	THC-COOH-D3	346.2	302.2	119	21	-
Q1 (FWMH)	0.4						
Q3 (FWMH)	0.7						

Results

Method Development

Different Turboflow columns (Cyclone, Cyclone P, Fluoro, Phenyl-Hexyl) were evaluated with different loading conditions. Also different separation columns were evaluated (Accucore C18, Hypersil Gold C18, Accucore PFP and Accucore aQ) with different gradients. And finally, transfer optimization was also studied. The final chromatogram is shown in Figure 3.

FIGURE 3. SRM chromatograms of THC, 11-OH-THC and THC-COOH as well as deuterated standards (D3) from a blood sample spiked at 0.5 ng/mL.



Recovery and matrix effects

Recovery was evaluated by comparing a direct injection of a standard solution to the analytical column against an injection to the TurboFlow column. Matrix Effects were evaluated by comparing an injection of standard solution to the Turboflow column against an injection of blood spiked at the same concentration. Overall recovery was obtained considering both recovery and matrix effects. Results are presented on table 3.

Compound	Recovery	Matrix effects	Overall recovery
THC	61%	+ 50%	92%
11-OH-THC	82%	+ 36%	112%
THC-COOH	76%	- 5%	73%