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

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## Overview

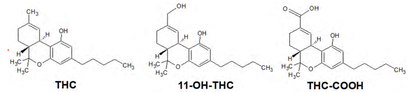
**Purpose:** Sensitive quantification of THC, 11-OH-THC and THC-COOH from whole blood with Thermo Scientific TurboFlow technology. For confirmation purposes, expected limit of quantification must be close to 0.5 ng/mL.
**Methods:** Blood samples were treated by protein precipitation followed by an online 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 $\Delta^9$-tetrahydrocannabinol (THC) that is rapidly metabolized mainly in11-hydroxy-$\Delta^9$-tetrahydrocannabinol (11-OH-THC) and then in 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), chemical structures are presented on figure 1.

**FIGURE 1. Molecular structures of $\Delta^9$-tetrahydrocannabinol (THC) and main metabolites,11-hydroxy-$\Delta^9$-tetrahydrocannabinol (11-OH-THC) and 11-nor-$\Delta^9$-tetrahydrocannabinol-9-carboxylic acid (THC-COOH).**



THC 11-OH-THC THC-COOH

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 µL of supernatant was injected for LC-MS/MS analysis.

### TurboFlow and LC method

The TurboFlow™ method was performed in Focus mode (figure 2) with a Thermo Scientific TurboFlow Cyclone-P column. Analytical separation was carried out on a Thermo Scientific Accucore C18 column (50×2.1 mm, 2.6-µm particle size) . The mobile phases were as follows: loading A : 0.1% formic acid in water; loading C: 0.1% formic acid in acetonitrile; loading D : mixture of isopropanol, acetonitrile, and acetone (40/40/20 v/v/v) ; elutingC : 10mM ammonium formate + 0.1% formic acid in water; elutingD : 0.1% formic acid in methanol. The total LC runtime was 10.4 min (Figure 3).

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

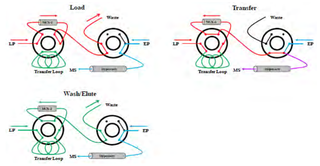



**FIGURE 3. TurboFlow and LC method conditions.**

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

| Flow | Grad | %A | %B | %C | %D |
|---|---|---|---|---|---|
| 0.40 | Step | - | - | 80.0 | 20.0 |
| 0.30 | Step | - | - | 80.0 | 20.0 |
| 0.40 | Ramp | - | - | 2.0 | 98.0 |
| 0.40 | Step | - | - | 2.0 | 98.0 |
| 0.01 | Step | - | - | 2.0 | 98.0 |
| 0.40 | Step | - | - | 2.0 | 98.0 |
| 0.40 | Step | - | - | 80.0 | 20.0 |
| 0.40 | Step | - | - | 80.0 | 20.0 |

TurboFlow method conditions (Loading Pump)

LC gradient conditions (Eluting Pump)

### Mass Spectrometry

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 (Figure 4).

**FIGURE 4. MS source parameters and SRM transitions.**

| Ionization | HESI-II |
|---|---|
| Spray voltage (V) | 3500 (+) 2700 (-) |
| Vaporizer Temp (°C) | 330 |
| Capillary Temp (°C) | 270 |
| Sheath gas (AU) | 35 |
| Auxilliary gas (AU) | 25 |
| Ion sweep gas (AU) | 5 |
| Collision gas pressure (mTorr) | 25 |
| Q1 (FWMH) | 0.4 |
| Q3 (FWMH) | 0.7 |

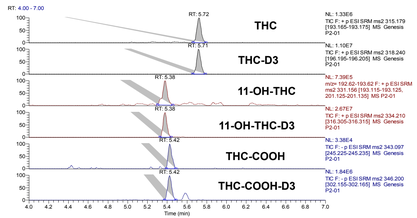
| Compound | Parent Ion (m/z) | Product Ion (m/z) | S-Lens | CE | Polarity |
|---|---|---|---|---|---|
| THC | 315.2 | 193.2 | 89 | 20 | + |
| THC-D3 | 318.2 | 196.2 | 89 | 23 | + |
| 11-OH-THC | 331.1 | 193.1 | 83 | 24 | + |
| 11-OH-THC-D3 | 334.2 | 316.3 | 83 | 15 | + |
| THC-COOH | 343.1 | 245.2 | 118 | 28 | - |
| THC-COOH-D3 | 346.2 | 302.2 | 119 | 21 | - |

## 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 5.

**FIGURE 5. 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

*Precipitation Recovery* was obtained by comparing an injection of whole blood spiked with the analytes and then crashed, against whole blood crashed first and then spiked.

*On-line extraction 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 figure 6.

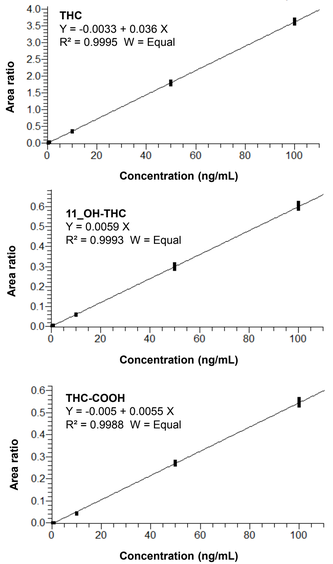
**FIGURE 6. Method recovery and matrix effects.**

*The concentration was 7.5 ng/mL in standard , crashed whole blood and whole blood samples. Injection volume was set to 20µL in all cases and 5 injections were performed in each condition.*

| Compound | Precipitation recovery | On-line extraction Recovery | Matrix effects | Overall recovery |
|---|---|---|---|---|
| THC | 97% | 61% | + 50% | 92% |
| 11-OH-THC | 94% | 82% | + 36% | 112% |
| THC-COOH | 88% | 76% | - 5% | 73% |

Calibration curves were generated with LCQuan 2.7 SP1 software by injecting whole blood samples spiked with THC, 11-OH-THC and THC-COOH. And crashed before injection Their deuterated (D3) compounds were used as internal standards. With a concentration of 17ng/mL The calibration model was linear with an equal weighting. In these conditions, curves were linear through the calibration range, from 0.5ng/mL to 100ng/mL. The calibration curves are presented in figure 7.

**FIGURE 7. Calibration curves for THC, 11-OH-THC and THC-COOH from spiked and crashed whole blood. Calibration ranges goes from 0.5ng/mL to 100ng/mL.**




Each calibration point was injected 10 times. The mean calculated concentration, the accuracy (%Diff) and the repeatability (%RSD) for each calibration point are presented in figure 8.

**FIGURE 8. Accuracy (%Diff) and repeatability (%RSD) obtained for each calibrator (n=10)**

| Conc (ng/mL) | | 0.5 | 1 | 10 | 50 | 100 |
|---|---|---|---|---|---|---|
| THC | Mean | 0.49 | 1.02 | 10.03 | 49.5 | 94.8 |
| | %Diff | -2 | +2 | +0.3 | -1 | -5.2 |
| | %RSD | 5 | 3 | 2 | 2 | 2 |
| 11-OH-THC | Mean | 0.50 | 1.00 | 9.97 | 49.7 | 94.6 |
| | %Diff | 0 | 0 | -0.3 | -0.6 | -5.4 |
| | %RSD | 5 | 4 | 3 | 3 | 2 |
| THC-COOH | Mean | 0.50 | 1.00 | 9.95 | 50.4 | 94.2 |
| | %Diff | 0 | 0 | -0.5 | +0.8 | -5.8 |
| | %RSD | 9 | 7 | 2 | 2 | 2 |

Limits of quantification were determined as the lowest concentration for which a 20% RSD is obtained as well as a bias inferior to 20%. The results are presented on figure9.

**FIGURE 9. Limits of quantification for THC, OH-THC and THC-COOH in spiked and crashed whole blood samples.**

| Compound | Concentration (ng/mL) | % RSD (n=10) | Bias (Mean +/- RSD) |
|---|---|---|---|
| THC | 0.5 | 5 | 0.49 +/- 0.02 |
| 11-OH-THC | 0.5 | 5 | 0.50 +/- 0.03 |
| THC-COOH | 0.5 | 9 | 0.50 +/- 0.04 |

The limits of quantification satisfy the requirements for cannabis analysis in whole blood, considering that the limits of detection are expected to be close to 0.5 ng/mL.

## Conclusion

- A fast, automated, and analytically sensitive LC-MS/MS method was developed to quantify THC and its metabolites in crashed whole blood.
- The total online extraction and analytical LC runtime was 10.4 minutes. This throughput could be increased by multiplexing this method on a Thermo Scientific Transcend TLX system.
- This method was linear from 0.5 to 100 ng/mL.
- The lower limit of quantitation was at least of 0.5 ng/mL for THC and its metabolites. Good repeatability was obtained for the different calibration levels with %RSD inferior to 10%.
- Correlation between GC-MS and this analytical method is being performed by Analysis – Expertise laboratory.

## References

1. Musshoff F., Burkhard M. – Therapeutic Drug Monitoring, 28 (2006) 155-163
2. Fabritius M., Staub C., Giroud C. – Annales de Toxicologie Analytique 23 (2011) 21-35
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## Acknowledgements

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