Cannabis For Per Se Law Testing in Whole Blood using the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS System

Diana Tran[1], Xiang He[1], Kevin Kopp[2], Matt Kopp[2], Alexandre Wang[1] and Hua-Fen Liu[1]
[1]SCIEX, 1201 Radio Rd, Redwood City, CA 94065, USA; [2]UTAK, 25020 Ave Tibbitts, Valencia, CA 91355, USA..

Overview

A sensitive, robust and verified method with step by step instructions to quantify Cannabis in whole blood using Shimadzu HPLC system and the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system was developed (Figure 1). The method provides quantitation of -Δ9-tetrahydrocannabinol (THC) and its two major metabolites 11-Hydroxy-Δ9-tetrahydrocannabinol (THC-OH) and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) using each of their respective deuterated internal standards. The method also provides qualitative detection of Cannabinol (CBN), and Cannabidiol (CBD). Positive and negative polarity switching with two MRM transitions per compound and one MRM transition for each of the internal standards is utilized to optimize sensitivity and specificity. Chromatographic separation of interferences is achieved using a 4.25 minute LC gradient. The sample preparation methodology utilizes a mixed mode solid phase extraction (SPE) of C18 and strong anion exchange (C18/SAX).

Introduction

Tandem mass spectrometry using a triple quadrupole mass spectrometer coupled to a liquid chromatography instrument (LC-MS/MS) is a benchmark tool for both qualitative and quantitative forensic analysis. Multiple Reaction Monitoring (MRM) uses the first quadrupole as a mass filter for desired compounds, inducing fragmentation in the second and selectively monitoring respective product ions in the third enabling both specificity and sensitivity. Both specificity and sensitivity is critical for quantitative analysis in a complex matrix such as whole blood which contains interferences as well as matrix induced ion suppression.

Whole blood handling can be problematic because of its viscous nature which potentially could increase pipetting variance and reduce sample preparation reproducibility. In collaboration with UTAK Laboratories, a five calibrator and two quality control kit was designed for ease of use in creating a linear calibration curve for whole blood cannabis quantitation.

A robust technical application for quantitation of whole blood THC and its two major metabolites is presented using UTAK's THC kit followed by protein precipitation and a mixed mode ion exchange solid phase extraction. The following application is optimized for the SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS system coupled to a SCIEX ExionLC™ HPLC system and takes advantage of fast polarity switching to optimize peak signal intensity of each of the three analytes.

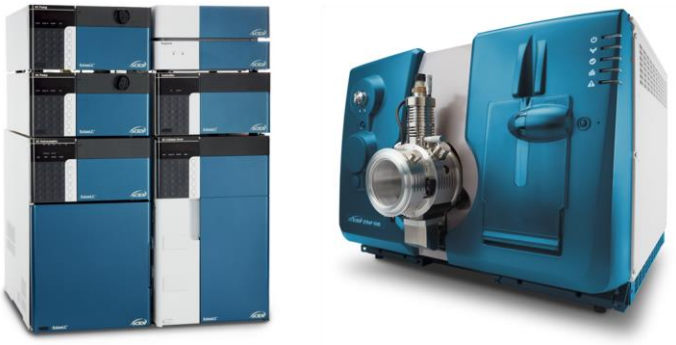

Figure 1: The SCIEX ExionLC™ AC HPLC system (left) and the SCIEX QTRAP® 4500 LC-MS/MS System (right).

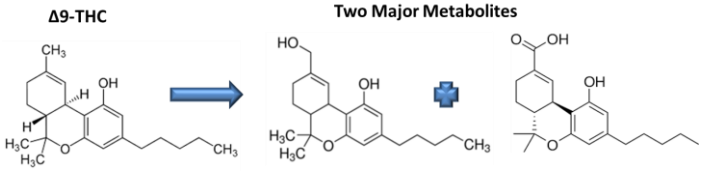

Figure 2: Structures of THC, THC-COOH and THC-OH monitored in the LCMS method.

Materials and Methods

Compound list and Standard Concentrations

Table 1 lists UTAK concentration levels for each calibrator and quality control sample for each of the compounds in the reagent kit (UTAK, SCIEX THC LCMS KIT). Only the whole blood matrix, methanolic spiking standards for calibrators and quality controls are included in the kit. Internal standards are purchased separately.

Sample preparation

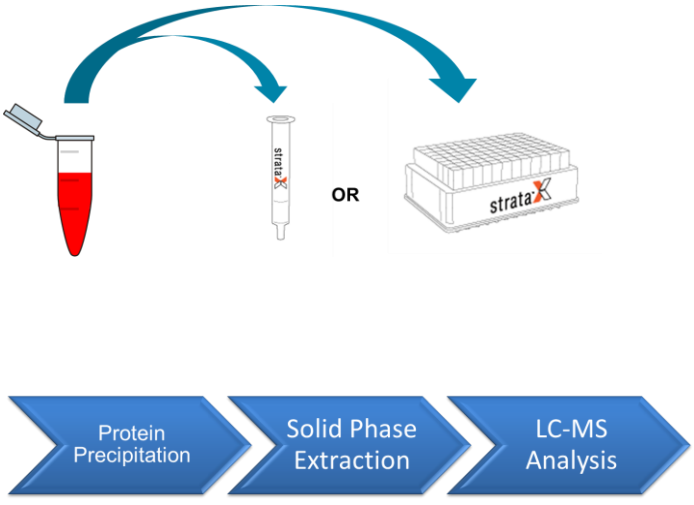

Figure 3: Simplified sample preparation overview.

Liquid Chromatography

Liquid Chromatography analysis was performed on the SCIEX ExionLC™ HPLC system at 40°C. Separation was achieved using a Phenomenex Kinetex column (50 × 4.6 mm, 2.6 µm,), with a Krudkatcher column guard (AF0-8497). Mobile phase A (MPA) was 0.01% acetic acid in water. Mobile phase B (MPB) was 0.01% acetic acid in methanol. The LC flow rate was 1 mL/min and the LC run-time was 4.25 minutes.

Suggested Injection Volumes:

SCIEX QTRAP®/Triple Quad™ 4500 LC-MS/MS System: 10 µL

SCIEX Triple Quad™ 3500 LC-MS/MS System: 30 µL

For the auto-sampler, the needle rinse solution was methanol: acetonitrile: isopropanol (1:1:3, v/v/v).

Results and Discussion

Protein precipitation prior to SPE was selected for this study for an additional sample clean up step that disregarded the variable hematocrit values in whole blood. The precipitation step also prevents sorbent bed saturation due to hemoglobin and albumin, allowing a faster and more efficient extraction.

Mixed mode C18 and strong anion exchange SPE was selected because ion exchange was proven to be more effective in recovery of carboxy-THC compared to a reverse-phase C18 chemistry alone. The SPE step provides salt removal, ensuring a robust method for forensic applications.

LC performance

A Phenomenex Kinetex column (50 × 4.6 mm, 2.6 µm) was coupled to a guard column. The phenyl-hexyl column provided chromatographic separation while the guard column provided column protection for extended column longevity. The method uses a 4.25 minute LC gradient to achieve separation of isobaric interferences at a flow rate of 1 mL/min for the entire gradient. The LC conditions maintains a narrow peak width up to 40 µL of 100% methanol:acetonitrile injections. Figure 4 shows the elution profile of THC-COOH, THC-OH and THC extracted from a whole blood matrix using the above, mentioned sample preparation method.

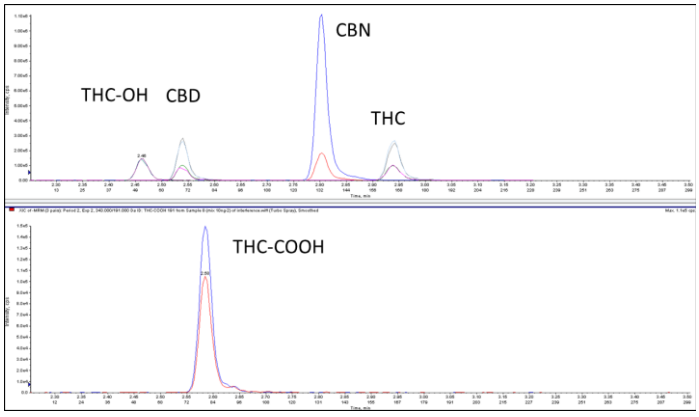

Figure 4: Elution profile of all analytes in positive polarity (THC-OH, CBD, CBN and THC) and THC-COOH in negative polarity during a one minute polarity switching acquisition period.

Analytical sensitivity

Extracted whole blood samples were analyzed in six biological replicates and sample preparation reproducibility was analyzed. The limit of quantitation (LOQ) was determined to be 1 ng/mL for THC-OH, THC and THC-COOH over 6 biological replicates whose %CV was in the ±20% range allowed by SWGTOX guidelines.

Figure 5 shows extracted ion chromatograms of THC-OH, THC and THC-COOH at 1 ng/mL extracted from human whole blood. All other calibrators, 5-200 ng/mL for THC-OH and THC, 5-500 ng/mL for THC-COOH had a %CV of ±15% for inter-day analysis.

Figures 6 to 8 show representative calibration curves extracted from whole blood. Tables 4 to 6 summarize the %CV,

Table 1: List of Analytes and Internal Standards and their Concentration in UTAK Reagent Kit (ng/mL)

Compound	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	QC 1	QC 2
THC-OH	*1*	*5*	*25*	*100*	*200*	*2*	*80*
THC-OH -d3 IS*	-	-	-	-	-	-	-
THC	*1*	*5*	*25*	*100*	*200*	*2*	*80*
THC-d3 IS*	-	-	-	-	-	-	-
THC-COOH	*1*	*5*	*50*	*200*	*500*	*2*	*400*
THC-COOH -d9 IS*	-	-	-	-	-	-	-

***: Internal Standards**

%Accuracy across 3 biological replicates for all calibrators and quality controls for intra-assay precision.

Table 2: MRM Transitions (Positive Polarity)

Compound	Q1	Q3	RT (min)
THC-OH 1	*331*	*193.1*	*2.45*
THC-OH 2	*331*	*201.1*	*2.45*
THC-OH -d3 IS*	*334*	*196.1*	*2.45*
THC 1	*315*	*193.1*	*2.95*
THC 2	*315*	*135*	*2.95*
THC-d3 IS*	*318.2*	*196.2*	*2.95*
CBD 1	*315*	*259*	*2.55*
CBD 2	*315*	*193*	*2.55*
CBN 1	*311.2*	*223*	*2.80*
CBN 2	*311.2*	*214*	*2.80*

***: Internal Standards**

Table 3: MRM Transitions (Negative Polarity)

Compound	Q1	Q3	RT (min)
THC-COOH 1	343	245	2.60
THC-COOH	343	191	2.60
THC-COOH -d9 IS*	352.2	308.2	2.60

***: Internal Standards**

Table 4: For Analyte THC-OH, all Concentrations are Reported in ng/mL

Compounds	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	QC 1	QC 2
Replicate 1	1.06	5.03	25.25	105.08	203.62	1.91	78.19
Replicate 2	0.97	4.86	22.89	95.50	190.85	2.15	81.40
Replicate 3	1.13	4.62	25.92	93.35	212.86	1.60	74.57
Target Concentration	1.00	5.00	25.00	100.00	200.00	2.00	80.00
MEAN	1.05	4.84	24.69	97.98	202.44	1.89	78.05
SD	0.08	0.21	1.59	6.24	11.05	0.28	3.42
%CV	**7.61%**	**4.26%**	**6.45%**	**6.37%**	**5.46%**	**14.62%**	**4.38%**
%Accuracy	**105.33%**	**96.73%**	**98.75%**	**97.98%**	**101.22%**	**94.33%**	**97.57%**

Table 5: For Analyte THC, all Concentrations are Reported in ng/mL

Compounds	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	QC 1	QC 2
Replicate 1	1.07	5.80	25.79	102.07	205.68	1.90	80.40
Replicate 2	1.00	4.49	23.43	93.59	187.99	2.41	86.59
Replicate 3	1.00	4.73	25.10	94.26	217.01	2.02	83.66
Target Concentration	1.00	5.00	25.00	100.00	200.00	2.00	80.00
MEAN	1.02	5.01	24.77	96.64	203.56	2.11	83.55
SD	0.04	0.70	1.21	4.71	14.63	0.27	3.10
%CV	**3.95%**	**13.93%**	**4.90%**	**4.88%**	**7.18%**	**12.64%**	**3.71%**
%Accuracy	**102.33%**	**100.13%**	**99.09%**	**96.64%**	**101.78%**	**105.50%**	**104.44%**

Table 6: For Analyte THC-COOH, all Concentrations are Reported in ng/mL

Compounds	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	QC 1	QC 2
Replicate 1	0.84	5.90	56.11	216.07	494.32	2.42	436.09
Replicate 2	0.84	4.91	50.79	207.28	466.72	2.20	435.71
Replicate 3	0.81	4.95	56.6	215.33	486.54	1.80	366.33
Target Concentration	1.00	5.00	50.00	200.00	500.00	2.00	400.00
MEAN	0.83	5.25	54.50	212.89	482.53	2.14	412.71
SD	0.02	0.56	3.22	4.88	14.23	0.31	40.17
%CV	**2.09%**	**10.67%**	**5.91%**	**2.29%**	**2.95%**	**14.69%**	**9.73%**
%Accuracy	**83.00%**	**105.07%**	**109.00%**	**106.45%**	**96.51%**	**107.00%**	**103.18%**

Unknown Sample Analysis

Figure 9 shows chromatograms of an unknown blood donor who tested positive for THC-COOH and THC-OH. The donor tested negative for parent THC, and below the 1 ng/mL cut off for THC-OH. The donor did, however possess a concentration of 150 ng/mL of THC-COOH at the time of analysis.

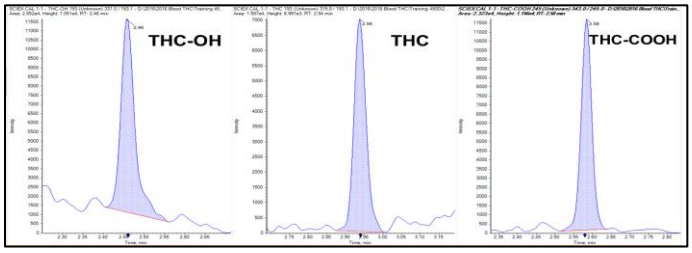

Figure 5: Chromatograms of THC-OH, THC and THC-COOH extracted from whole blood at 1 ng/mL using a 10µL injection volume of a QTRAP/Triple Quad™ 4500.

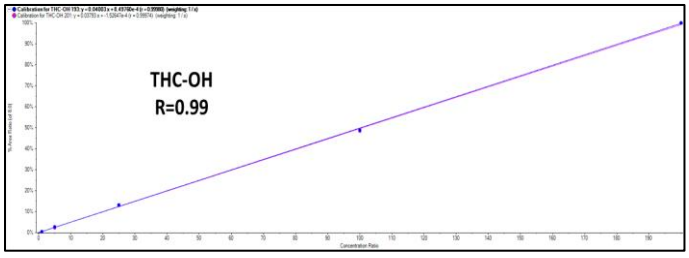

Figure 6: Calibration curve of both quantifier and qualifier ions for THC-OH from 1 – 200 ng/mL with a linear regression of 1/x fitting.

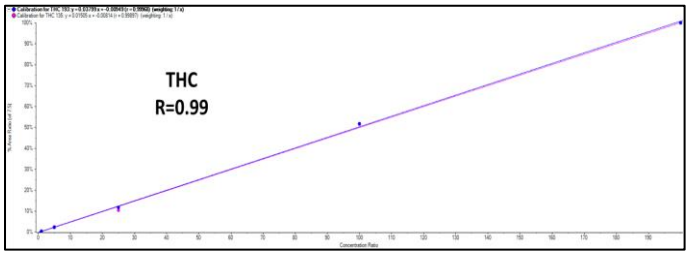

Figure 7: Calibration curve of both quantifier and qualifier ions for THC from 1 – 200 ng/mL with a linear regression of 1/x fitting.

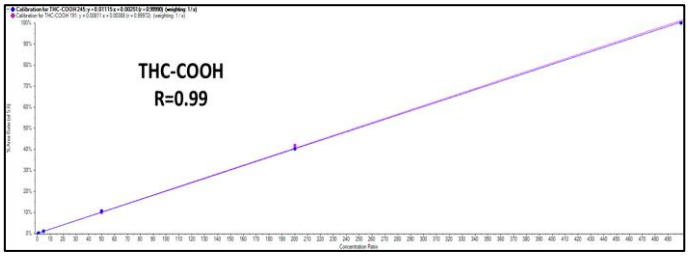

Figure 8: Calibration curve of both quantifier and qualifier ions for THC-COOH from 1 – 500 ng/mL with a linear regression of 1/x fitting

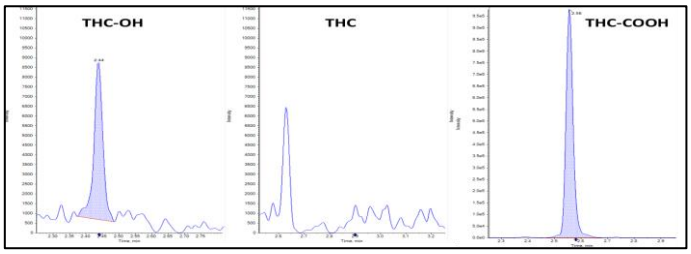

Figure 9: Chromatograms of THC-OH, THC and THC-COOH extracted from an unknown blood donor which tested positive for THC metabolites, but had a concentration of THC below LOQ 1 ng/mL of the method.

Conclusion

A rapid and sensitive LC-MS/MS method for the analysis of THC and its two major metabolites in whole blood was established. The collaboration with UTAK yielded a reagent kit that would reduce variability in both creating calibrators and quality controls in a difficult sample matrix. The method also falls within SWGTOX guidelines for an acceptable LCMS assay because of the method's precision and accuracy of sample preparation.

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Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
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