Extraction of Tetrahydrocannabinol (THC) and Metabolites from Whole Blood Using ISOLUTE® SLE+ Prior to LC-MS/MS

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This application note describes the extraction of tetrahydrocannabinol and its metabolites from spiked whole blood using ISOLUTE® SLE+ in a 96-well plate format.

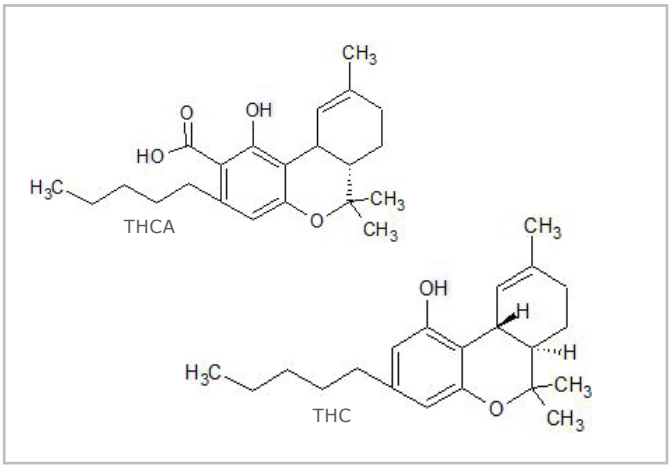

Figure 1. Tetrahydrocannabinol (THC) and Tetrahydrocannabinolic Acid (THCA)

Introduction

Toxicology screening for tetrahydrocannabinol and its metabolites is routinely conducted in various biological matrices. Whole blood (post mortem or ante mortem) is considered one of the most difficult to extract target analytes from with sufficient cleanliness to minimize ion suppression in an atmospheric pressure ionization source. The characteristics of whole blood (increased viscosity and cellular content) present challenges to sample preparation methodology that require a sorbent amenable to separating the target analytes from the endogenous interference. Here we demonstrate the extraction of THC and its metabolites using ISOLUTE SLE+ in a 96-well plate format. ISOLUTE SLE+ offers a fast and efficient alternative to traditional liquid-liquid extraction (LLE) and solid phase extraction (SPE) sample preparation methods.

Analytes

Δ9-tetrahydrocannabinol (THC), Δ9- tetrahydrocannabinol D_3 (THC-D_3), 11-nor- Δ9-carboxy tetrahydrocannabinol (THC-COOH), 11-nor-Δ9-carboxy tetrahydrocannabinol-D_3 (THC-COOH-D_3), 11-hydroxy- Δ9-tetrahydrocannabinol (THC-OH), 11-hydroxy-Δ9-tetrahydrocannabinol D_3 (THC-OH-D_3)

Sample Preparation Procedure

Format:	ISOLUTE SLE+ 400 µL Supported Liquid Extraction plate, part number 820-0400-P01
Matrix:	Pooled whole blood
Sample Pre-treatment:	1) Add 200 µL of spiked negative whole blood or sample whole blood to wells. 2) Add 100 µL of 0.1% Formic Acid to each well 3) Add analyte working standard and internal standard to each well (up to 30µL) **NOTE:** For whole blood the recommended total solution volume should be under-loaded by a minimum of 15% of the recommended loading capacity. The current method loading is 330 µL into each fixed well plate (max volume load is 400 µL).
Sample Loading:	Samples were prepared directly in designated wells. This was done to minimize loss of analytes due to sample transfer and binding of analytes to plastic transfer tubes. However, if an internal standard is used, off line sample pre-treatment and mixing is recommended. Apply a short pulse of vacuum (VacMaster-96 Sample Processing Manifold) or positive pressure (PRESSURE+ 96 Positive Pressure Manifold) to initiate flow and then allow sample to absorb on column for 5 minutes.

Analyte Elution: Apply 3 x 600 µL of methyl tert-butyl ether (MTBE) to each well and allow solvent to gravity flow. Apply positive pressure or pull slight vacuum as needed during collection process to facilitate a flow rate of 1 mL per minute.

Post Extraction: Evaporate sample and reconstitute in water: methanol (50:50, v/v, 500 µL).

Additional Information: Working standards and internal standards were prepared in 100% methanol. All standards were ordered from Cerilliant Corp.

HPLC Conditions

Instrument: Agilent 1200 Liquid Handling System (Agilent Technologies, Berkshire, UK)

Column: Phenomenex Gemini C18, 150 mm x 4.6 mm (5 µm)

Mobile Phase: A: 0.1% Formic Acid
B: Methanol with 0.1% Formic Acid

Isocratic: A 5% :B 95% (v/v), 5 minute run time

Mass Spectrometry Conditions

Applied Biosystems/MDS Sciex 4000 Q-Trap triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA.) equipped with a Turbo Ionspray® interface for mass analysis.

Ionization Source Temperature: 700 °C

Table 1. MRM transitions for cannabinoids in positive mode ESI-MS/MS.

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	THC	315.2 → 193.1	30	27	16
2	THC-D3	318.2 → 196.1	30	25	16
3	THC-COOH	345.1 → 299.1	30	28	16
4	THC-COOH-D3	348.1 → 330.1	30	23	16
5	THC-OH	331.2 → 193.0	30	23	16
6	THC-OH-D3	334.2 → 316.0	30	25	16

Results and Discussion

The MRM transitions were identified for the THC and metabolites (see table 1). The deuterated internal standards were also extracted and analyzed. Extraction of the samples was carried out in each well utilizing the hydrophobic top frit which keeps aqueous blood sample from flowing onto the sorbent prior to addition of pretreatment solution, calibrants and internal standards. Once all components were added to the well, the samples were loaded onto the sorbent. The elution of the analytes was found to work best with methyl-tert-butyl ether (MTBE). A comparison of sample prior to elution and post elution is shown in **Figure 2**. The samples were reconstituted and injected onto the HPLC column following the conditions listed above. A typical extracted ion chromatogram is shown in **Figure 3** for the extracted analytes at 25 ng/mL concentration.

The averaged recoveries for the analytes ranged from 60-107% across a dynamic concentration range from 50 ng/mL to 12.5 ng/mL (**Figure 4**). The averaged recoveries were collected over seven replicates with %RSDs typically less than 10. The percent ion suppression/enhancement as a function of matrix effects post extraction from whole blood is detailed in **Figure 5**. The matrix effects accounted for less than 10% ion suppression.

Figure 2. Fortified whole blood sample and subsequent extracted cannabinoids in MTBE prior to dry down.

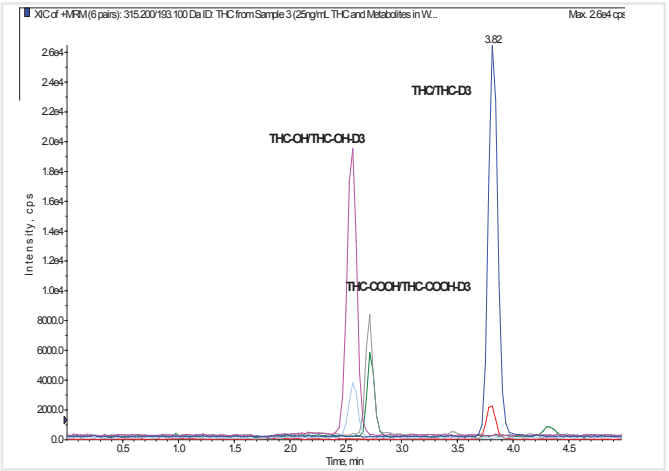

Figure 3. Typical Extracted Ion Chromatogram for 25 ng/mL spiked cannabinoid sample extracted from whole blood.

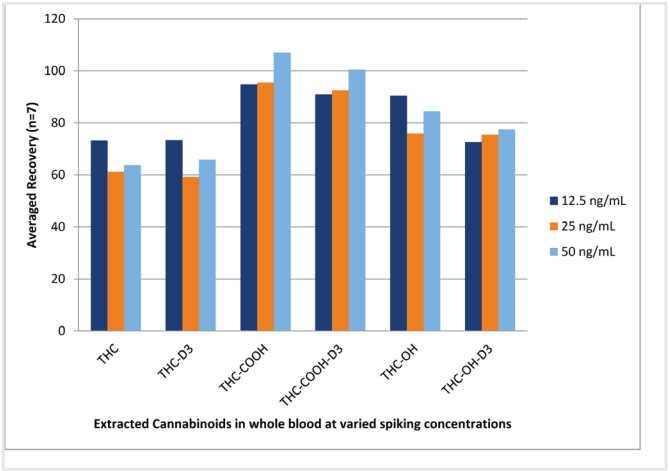

Figure 4. Plot of averaged recoveries across a concentration range for THC and metabolites fortified and extracted from whole blood.

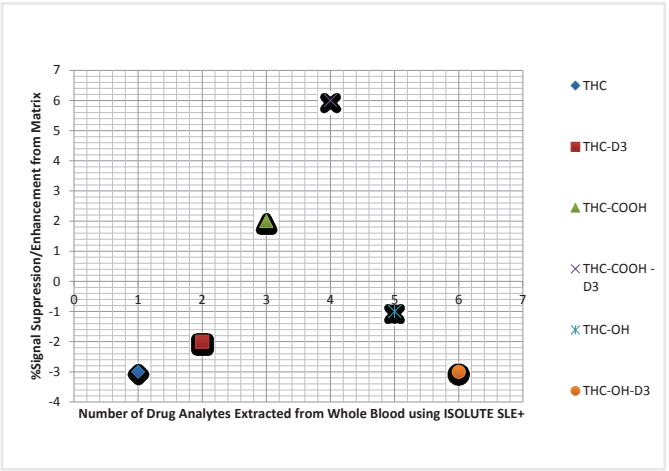

Figure 5. Plot of measured percent ion suppression/enhancement observed from matrix effects for 25 ng/mL analytes extracted from whole blood.

Ordering Information

Part Number	Description	Quantity
820-0400-P01	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
PPM-96	Biotage® Positive Pressure Manifold 96 Position	1

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