Extraction of THC, Hydroxy-THC and Carboxy-THC from Whole Blood Using ISOLUTE® SLE+ Prior to GC/MS Analysis

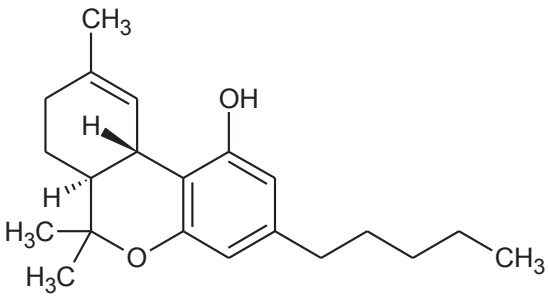

Figure 1. Structure of Δ^9 -THC (tetrahydrocannabinol)

This application note describes the extraction of Δ^9-THC, 11-hydroxy- Δ^9-THC and 11-nor-9-carboxy-THC from whole blood matrix, prior to GC/MS analysis

Introduction

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

This application note describes an effective and efficient ISOLUTE SLE+ protocol optimized for 1 mL capacity ISOLUTE SLE+ columns. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 74% with RSDs lower than 9% for all analytes.

Analytes

THC, THC-OH, THC-COOH with THC-D3, THC-OH-D3 and THC-COOH-D3 as internal standards

Sample Preparation Procedure

Sample Pre-treatment:	To 1.2 mL of whole blood, add 0.4 mL of 0.1% formic acid (aq), and mix thoroughly.
Format:	**ISOLUTE® SLE+ 1 mL Sample volume columns, part number 820-0140-C**
Sample loading:	Load 800 µL of the pre-treated whole blood onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
Analyte extraction:	Apply MTBE (3 mL) and allow to flow under gravity for 5 minutes. Apply Hexane (3 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.
Post elution and reconstitution:	Dry the extract in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap® (1.0 bar at 40 °C for 40 mins). Upon dryness, reconstitute with 40 µL ethyl acetate and 20 µL BSTFA:TMCS 99:1 and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

Biotage®

GC Conditions

Instrument: Agilent 7890A with QuickSwap

Column: Agilent J&W DB-5,
30 m x 0.25 mm ID x 0.25 µm

Carrier: Helium 1.2 mL/min (constant flow)

Inlet: 250 °C, Splitless, purge flow:
50 mL/min at 1.0 min

Injection: 2 µL

Wash solvents: Acetone and Ethyl acetate

Oven: Initial temperature 60 °C

Ramp 25 °C/min to 350 °C,
hold for 0.4 minutes

Post run: Backflush for 2.4 minutes
(3 void volumes)

Transfer Line: 280 °C

MS Conditions

Instrument: Agilent 5975C

Source: 230 °C

Quadrupole: 150 °C

MSD mode: SIM

SIM Parameters

Table 1. Ions acquired in the Selected Ion Monitoring (SIM) mode

SIM Group	Analyte	Target (Quant) Ion	1st Qual Ion	2nd Qual Ion
1	THC-D3	374	306	315
1	THC	317	343	386
2	THC-OH-D3	374		
2	THC-OH	371		
3	THC-COOH-D3	374	491	
3	THC-COOH	371	488	

Results

The optimized SLE+ protocol demonstrated analyte recoveries ranging from 72-83% as shown in **Figure 2**. RSD's were below 10% for all analytes.

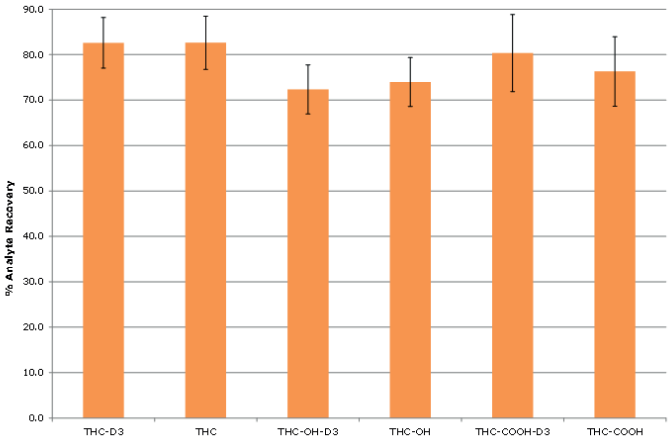

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocol.

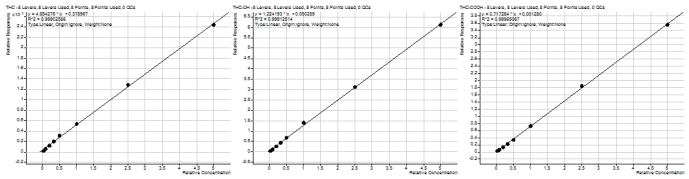

Figure 3. Calibration curves for extracted levels of spiked whole blood using 1 mL capacity ISOLUTE® SLE+ columns from 1 ng/mL to 150 ng/mL showing r^2 values of 0.999 or greater.

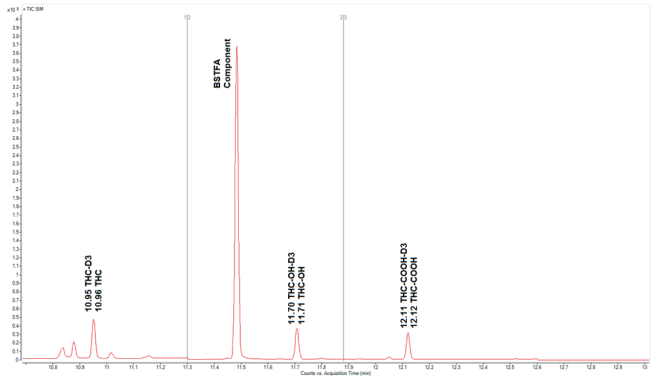

Figure 4. GC/MS chromatography of whole blood. All analytes spiked at 1 ng/mL.

Table 2.
Lower Limits of Quantitation (LLOQ) using this ISOLUTE® SLE+ procedure

Analyte	Lower Limit Of Quantitation
THC	1 ng/mL
THC-OH	3 ng/mL
THC-COOH	3 ng/mL

Additional Information

1. All solvents were HPLC grade.
2. 0.1% formic acid in water was prepared by adding 50 µL concentrated formic acid to 49,950 µL HPLC grade water.
3. Columns are slightly underloaded (800 µL of pre-treated sample on a 1 mL total capacity column) to ensure clean extracts from whole blood.

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column	30
820-0140-CG	ISOLUTE® SLE+ 1 mL Sample Volume Column (tabless) for use on Biotage® Extrahera	30
820-0140-C-1000	ISOLUTE® SLE+ 1 mL Sample Volume Column (Bulk pack)	1000
820-0140-CG-1000	ISOLUTE® SLE+ 1 mL Sample Volume Column (tabless) (Bulk Pack) for use on Biotage® Extrahera™	1000
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold for Columns	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
C103198	TurboVap® LV, Evaporator 100/120V	1
C103199	TurboVap® LV, Evaporator 220/240V	1

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