Extraction of Synthetic Cannabinoids (SPICE) from Whole Blood using ISOLUTE® SLE+ prior to GC-MS Analysis

This application note describes the extraction of a range of synthetic cannabinoids and metabolites from whole blood prior to GC-MS analysis. An effective and efficient ISOLUTE® SLE+ protocol has been developed that is optimized for extraction of 800 µL of pre-treated matrix. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 85% with RSDs lower than 10% for all analytes.

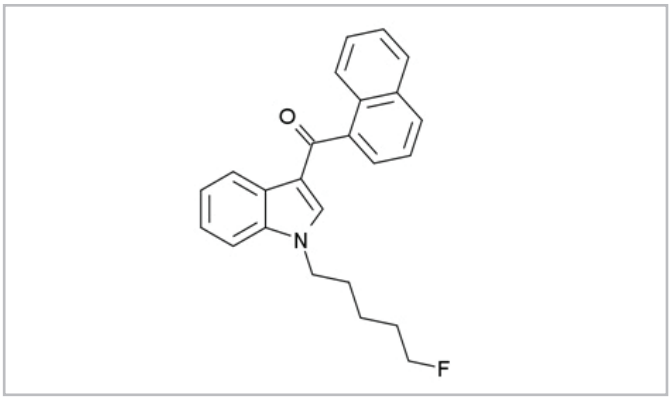

Figure 1. An example of a synthetic cannabinoid, AM-2201

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.

Analytes

UR-144, JWH-073, JWH-018, 5-hydroxypentyl-JWH250, 3-hydroxybutyl-JWH073, AM-2201, 4-hydroxypentyl-JWH-018, 5-hydroxypentyl-JWH-018, JWH-200

Sample Preparation Procedure

Sample Pre-treatment	Dilute whole blood (400 µL) with water (400 µL) and vortex for 10 seconds.
ISOLUTE SLE+ 1 mL Sample Volume columns, part number 820-0140-C	
Sample Loading:	Load the pre-treated whole blood (800 µL total volume) onto the column and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.
Analyte Extraction:	Apply hexane/ethyl acetate (95/5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of hexane/ethyl acetate (95/5, v/v, 2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent.
Post Elution and Derivatisation:	Evaporate to dryness in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap (1.5 bar at 40 °C for 40 mins). Reconstitute with ethyl acetate (250 µL) and vortex for 20 seconds. Transfer to a high recovery glass vial and evaporate to dryness. Add ethyl acetate (25 µL) and BSTFA:TMCS 99:01 (25 µL) and cap with a non-split cap . Vortex for 20 seconds and heat in a heating block set to 70 °C, for 30 minutes. Remove vial from the block and allow to cool.

GC Conditions

Instrument: Agilent 7890A with QuickSwap

Column: SGE capillary column; BPX5, 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 1.5 min, septum purge flow: 3 mL/min

Injection: 2 µL

Wash Solvent: Ethyl acetate

Oven: Initial Temperature 100 °C

Ramp 50 °C/min to 275 °C, hold for 4 minutes

Ramp 120 °C/min to 300 °C, hold for 8 minutes

Ramp 120 °C/min to 315 °C, hold for 1.5 minutes

Ramp 120 °C/min to 330 °C, hold for 1.5 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	3rd Qual Ion
1	UR144	214	311	N/A	N/A
2	RCS-4	320	263	N/A	N/A
3	JWH073	327	200	310	N/A
4	JWH018	341	214	324	N/A
5	5-hydroxypentyl-JWH250	302	228	N/A	N/A
6	3-hydroxybutyl-JWH073	285	270	415	N/A
7	AM2201	359	284	342	N/A
8	4-hydroxypentyl-JWH018	284	270	296	429
9	5-hydroxypentyl-JWH018	270	284	414	N/A
10	JWH200	100	384	N/A	N/A

Results

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 85-99% from whole blood, as shown in **Figure 2**. RSDs were below 10% for all analytes.

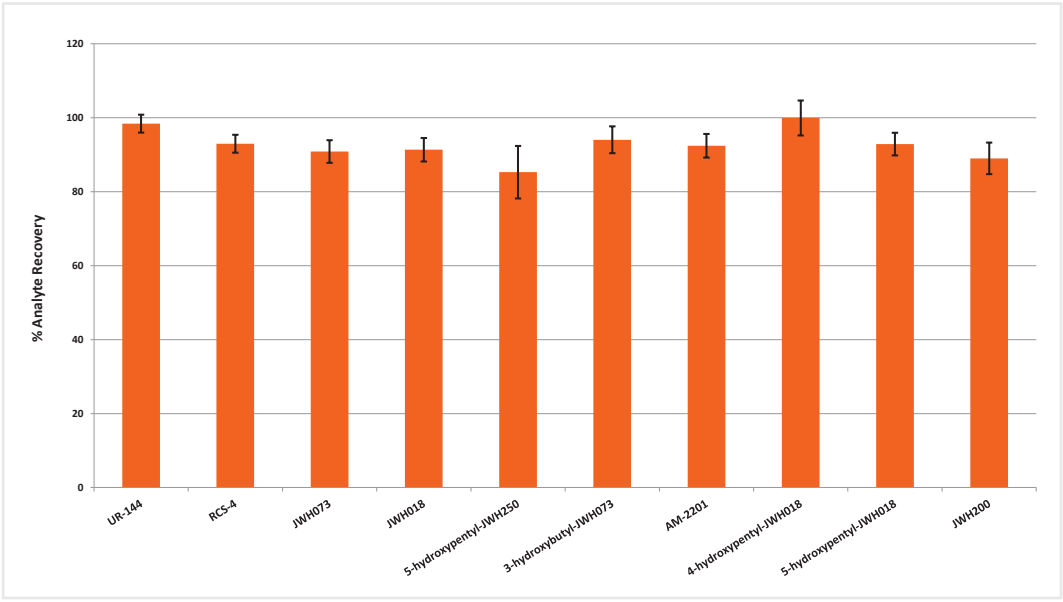

Figure 2. Typical analyte % recoveries for extracted synthetic cannabinoids and metabolites (n=7) using the ISOLUTE® SLE+ protocol.

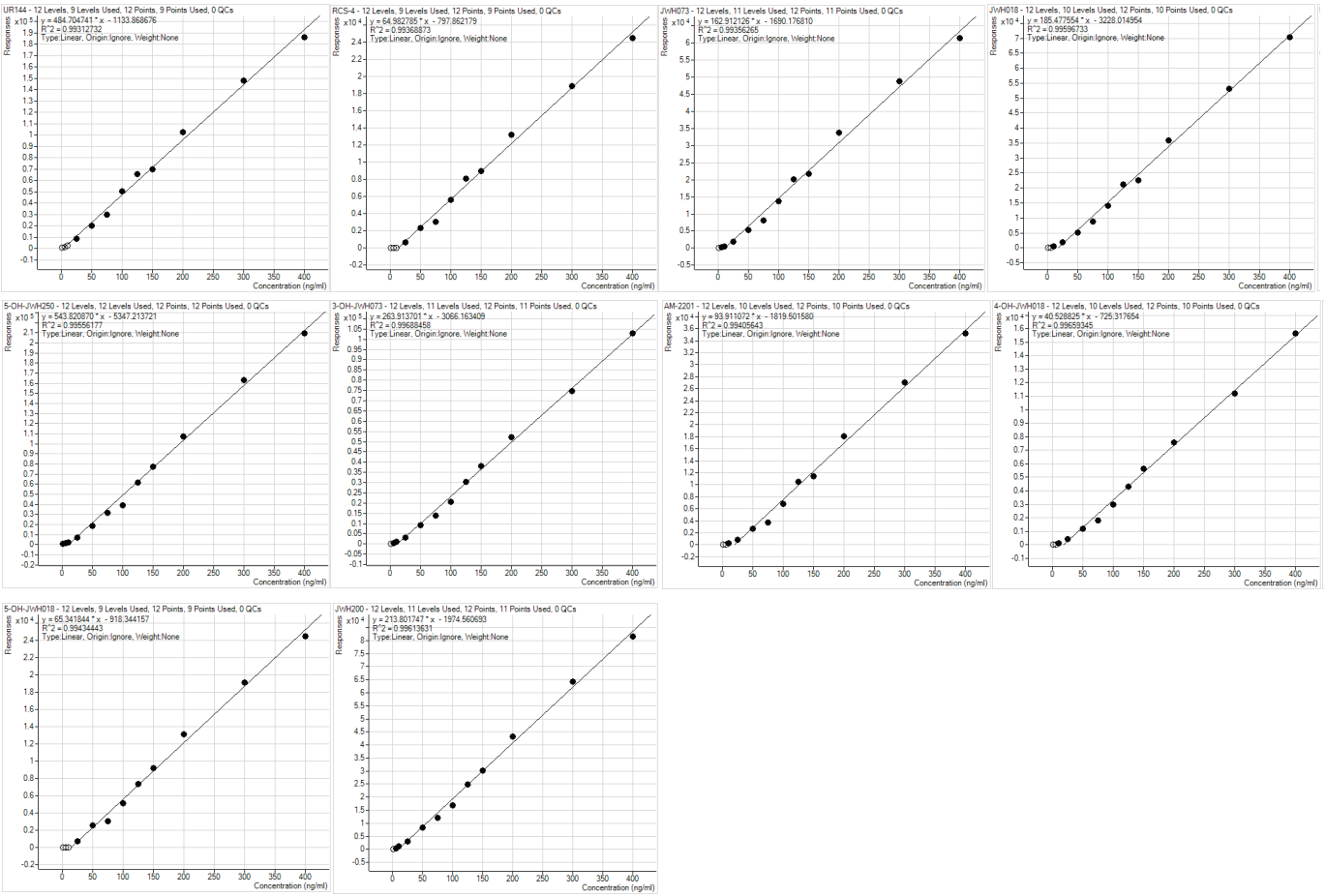

Figure 2. Calibration curves for extracted levels of spiked whole blood from 1 ng/mL to 400 ng/mL using 1 mL capacity ISOLUTE SLE+ columns showing r^2 values ranging from 0.9931 to 0.9968.

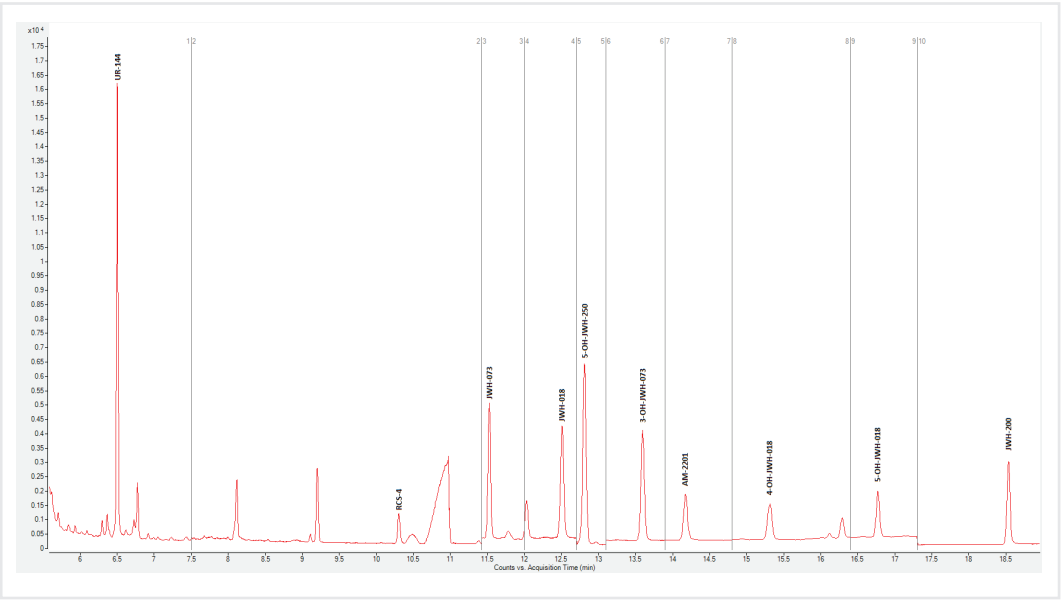

Figure 4. SIM chromatogram for whole blood spiked at 50 ng/mL

Table 3. Lower Limits of Quantitation (LLOQ) using the ISOLUTE SLE+ procedure

Analyte	Lower Limit Of Quantitation
UR-144	10 ng/mL
RCS-4	25 ng/mL
JWH-073	10 ng/mL
JWH-018	10 ng/mL
5-hydroxypentyl-JWH-250	25 ng/mL
3-hydroxybutyl-JWH-073	5 ng/mL
AM-2201	10 ng/mL
4-hydroxypentyl-JWH-018	10 ng/mL
5-hydroxypentyl-JWH-018	25 ng/mL
JWH-200	5 ng/mL

Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Columns	30
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold 48 Position	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® 96 without racks 100/120 VAC	1
C103199	TurboVap® LV without racks 220/240 VAC	1

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