Extraction of Synthetic Cannabinoids (SPICE) from Oral Fluid Using ISOLUTE® SLE+ prior to GC-MS Analysis

This application note describes the extraction of a range of synthetic cannabinoids and metabolites from oral fluid collected using the Quantisal™ Oral Fluid Collection Device prior to GC-MS analysis. An effective and efficient ISOLUTE® SLE+ protocol has been developed that is optimized for loading volumes of either 400 µL or 1 mL of matrix. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 80% with RSDs ‹10% for all analytes.

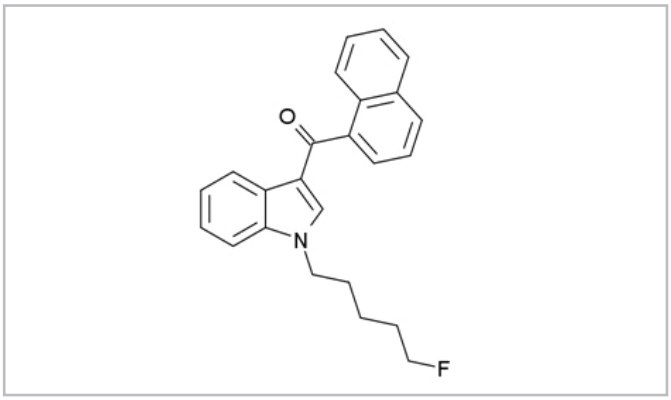

Figure 1. An example of a synthetic cannabinoid, AM-2201

Introduction

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE), 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 Collection Collect oral fluid samples as per collection device usage instructions. The Quantisal device uses a paddle to collect ~1 mL oral fluid, which is subsequently stored in a sealed tube containing proprietary buffer until required.

ISOLUTE SLE+ 400 µL Sample Volume columns, part number 820-0055-B

Sample Loading: Load 400 µL of the buffered sample direct from the collection device 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, 1 mL) and allow to flow under gravity for 5 minutes.

Apply a further aliquot of hexane /ethyl acetate (95/5, v/v, 1 mL) and allow to flow for another 5 minutes.

Apply vacuum or positive pressure to pull through any remaining extraction solvent.

ISOLUTE® SLE+ 1 mL Sample Volume columns, part number 820-0140-C

Sample Loading:	Load 1 mL of the buffered sample direct from the collection device 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 extract to dryness in a stream of air or nitrogen using a Biotage® 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 vial 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:	1 µL
Wash Solvent:	Ethyl acetate
Oven:	Initial Temperature 50 °C, hold for 1 minute Ramp 20 °C/min to 300 °C, hold for 2 minutes Ramp 20 °C/min to 310 °C, hold for 2 minutes Ramp 20 °C/min to 320 °C, hold for 2 minutes Ramp 20 °C/min to 330 °C, hold for 3 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	UR-144	214	296	311	N/A
2	JWH-073	327	200	310	N/A
3	JWH-018	341	214	324	N/A
3	5-hydroxypentyl-JWH-250	302	228	N/A	N/A
6	3-hydroxybutyl-JWH-073	285	270	415	N/A
7	AM-2201	359	284	342	N/A
8	4-hydroxypentyl-JWH-018	429	270	284	296
9	5-hydroxypentyl-JWH-018	270	284	414	429
10	JWH-200	100	384	N/A	N/A

Results

This optimized ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 80-110% in 4 different oral fluid donors. RSDs were below 10% for all. **Figure 2**. shows analyte recovery percentage from a single donor's oral fluid.

Sample volumes of either 400 µL or 1 mL can be extracted using the appropriate ISOLUTE SLE+ column format.

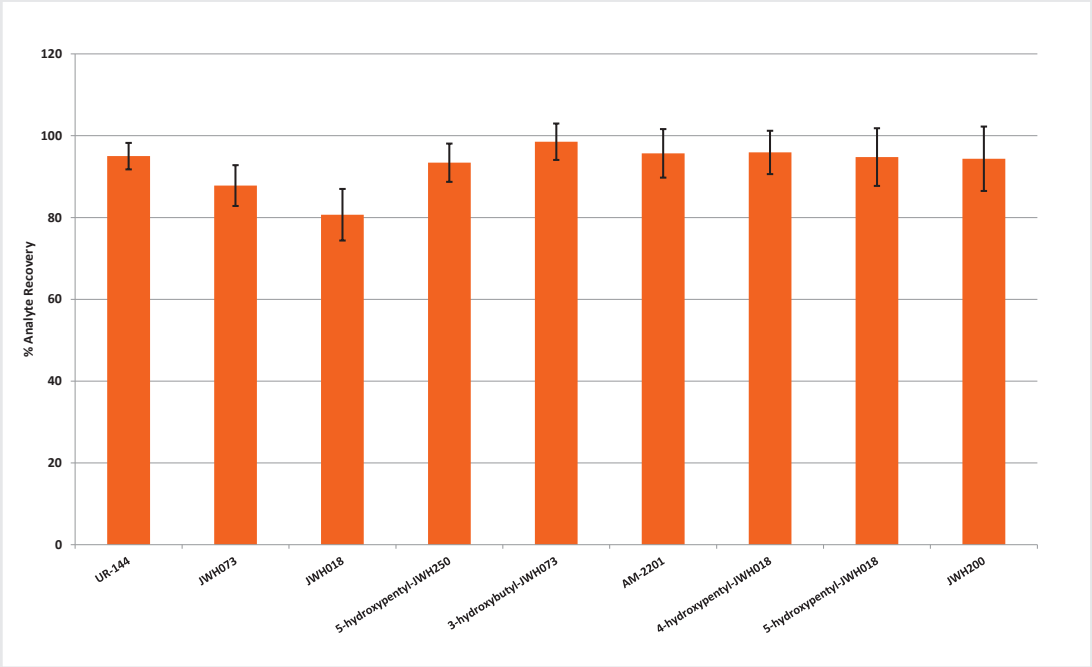

Figure 2. Typical analyte % recoveries for extracted synthetic cannabinoids and metabolites from oral fluid (n=7) using the ISOLUTE® SLE+ protocol.

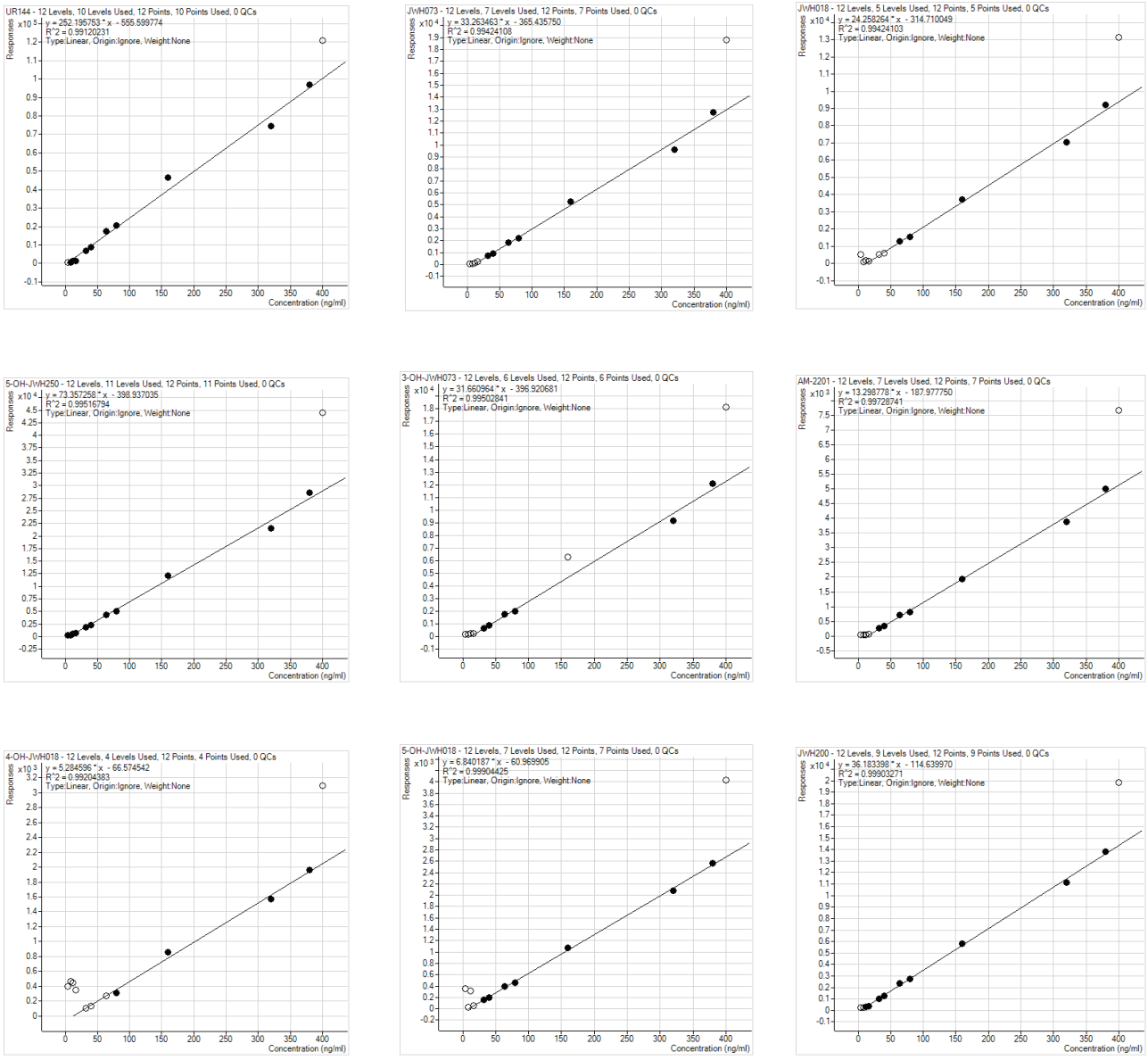

Figure 3. Calibration curves for extracted levels of spiked oral fluid from 4 ng/mL to 400 ng/mL using the 1 mL sample volume ISOLUTE® SLE+ column format showing r^2 values ranging from 0.9912 to 0.9990.

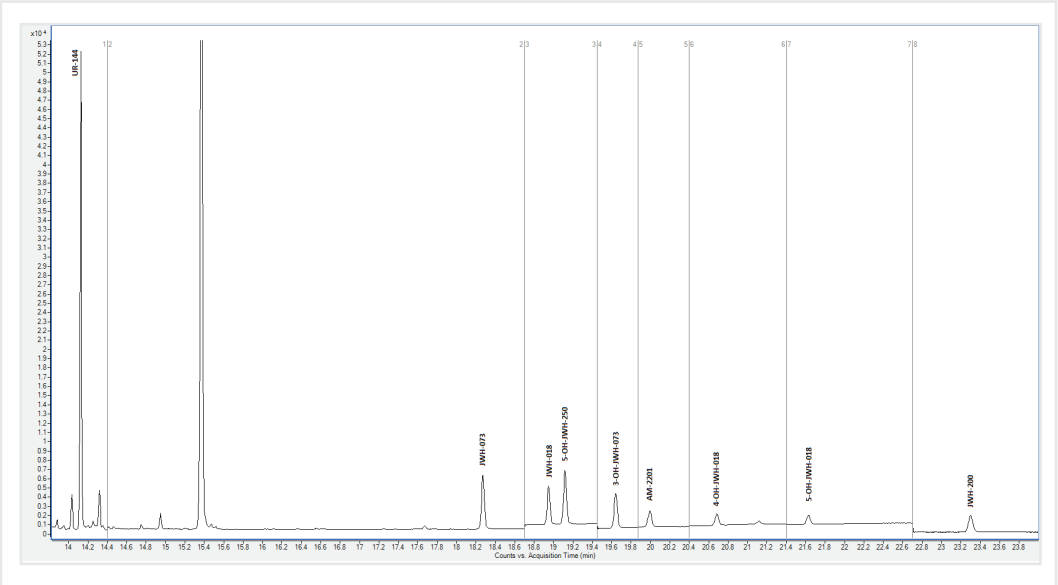

Figure 4. SIM chromatogram for oral fluid spiked at 160 ng/mL.

Table 2. Lower Limits of Quantitation (LLOQ) using the 400 µL sample volume procedure.

Analyte	Lower Limit Of Quantitation
UR-144	20 ng/mL
JWH-073	100 ng/mL
JWH-018	200 ng/mL
5-hydroxypentyl-JWH-250	20 ng/mL
3-hydroxybutyl-JWH-073	80 ng/mL
AM-2201	80 ng/mL
4-hydroxypentyl-JWH-018	200 ng/mL
5-hydroxypentyl-JWH-018	100 ng/mL
JWH-200	40 ng/mL

Table 3. Lower Limits of Quantitation (LLOQ) using the 1 mL sample volume procedure

Analyte	Lower Limit Of Quantitation
UR-144	8 ng/mL
JWH-073	32 ng/mL
JWH-018	64 ng/mL
5-hydroxypentyl-JWH-250	4 ng/mL
3-hydroxybutyl-JWH-073	32 ng/mL
AM-2201	32 ng/mL
4-hydroxypentyl-JWH-018	80 ng/mL
5-hydroxypentyl-JWH-018	32 ng/mL
JWH-200	12 ng/mL

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Sample Volume Columns	50
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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EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 2898 6655
Fax: +86 21 2898 6153
cn_order@biotage.com
cn-1-pointsupport@biotage.com

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