Extraction of Synthetic Cannabinoids from Hydrolyzed Urine Using ISOLUTE® SLE+ Prior to GC-MS Analysis

This application note describes the extraction of a range of synthetic cannabinoids and metabolites from hydrolyzed human urine prior to GC-MS.

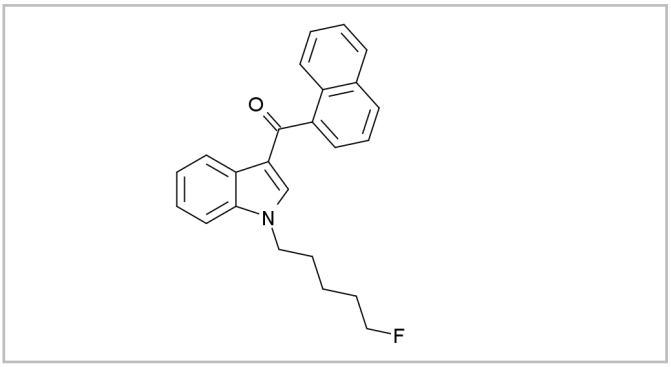

Figure 1. An example of a synthetic cannabinoid, AM2201

Introduction

ISOLUTE® Supported Liquid Extraction (SLE+) 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 effective and efficient ISOLUTE SLE+ protocols optimized for sample loading volumes of either 400 µL or 1 mL. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 83% with RSDs of <10% for all analytes.

Analytes

UR144, JWH073, JWH018, 5-hydroxypentyl-JWH250, 3-hydroxybutyl-JWH073, AM2201, 4-hydroxypentyl-JWH018, 5-hydroxypentyl-JWH018, JWH200

Sample Preparation Procedure

Sample Pre-treatment Dilute urine (1 mL) with 100 mM ammonium acetate buffer at pH 5 (950 µL) and β-Glucuronidase enzyme (50 µL, equivalent to approximately 4500 U/mL of urine). Hydrolyze with heat in a water bath at 60 °C for 2 hours.

ISOLUTE SLE+ 400 µL Sample Volume Columns, Part Number 820-0055-B

Sample Loading: Load the pre-treated hydrolyzed urine (400 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 (90 : 10, v/v, 1 mL) and allow to flow under gravity for 5 minutes. Apply a further 1 mL aliquot of hexane : ethyl acetate (90 : 10, v/v) 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 the pre-treated hydrolyzed urine (1 mL) 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 (90 : 10, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of hexane : ethyl acetate (90 : 10, v/v, 2.5 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure 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 (30°C, 20 to 40 L/min) or TurboVap (1.5 bar at 40 °C for 30 mins). Upon dryness, add 50 µL ethyl acetate and 50 µL BSTFA:TMCS 99:01. Vortex for 20 seconds and transfer to a high recovery glass vial and cap with a non-split cap. Heat vial in a heating block set to 70 °C, for 30 minutes. Remove vial from the block and allow to cool.

Biotage®

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 solvents: ethyl acetate
Oven:	Initial temperature 50 °C
	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 2.76 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	296	311	
2	JWH073	327	200	310	
3	JWH018	341	214	324	
3	5-hydroxypentyl-JWH250	302	228		
4	3-hydroxybutyl-JWH073	285	270	415	
5	AM2201	359	284	342	
6	4-hydroxypentyl-JWH018	429	270	284	296
7	5-hydroxypentyl-JWH018	270	284	414	429
8	JWH200	100	384		

Results

This ISOLUTE® SLE+ protocol demonstrated analyte recoveries ranging from 83-112% in 4 urine donors. The average analyte recovery, averaged in 4 urine sets of n=7, was 96%. **Figure 2** shows a single urine donor. RSDs were below 10% for all analytes for all donors.

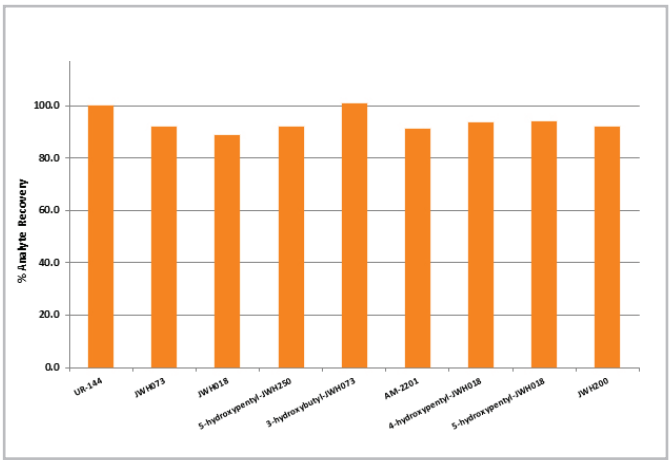

Figure 2. Typical analyte % recoveries for the application analytes (n=7) using the ISOLUTE® SLE+ protocol

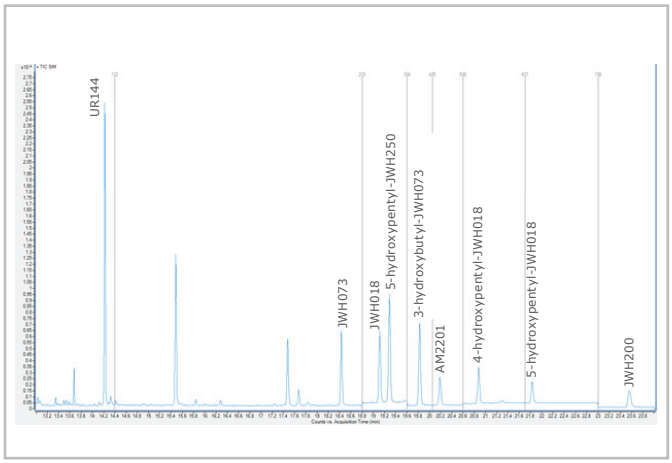

Figure 3. GC-MS chromatography for the application analytes from hydrolyzed urine spiked at 100 ng/mL

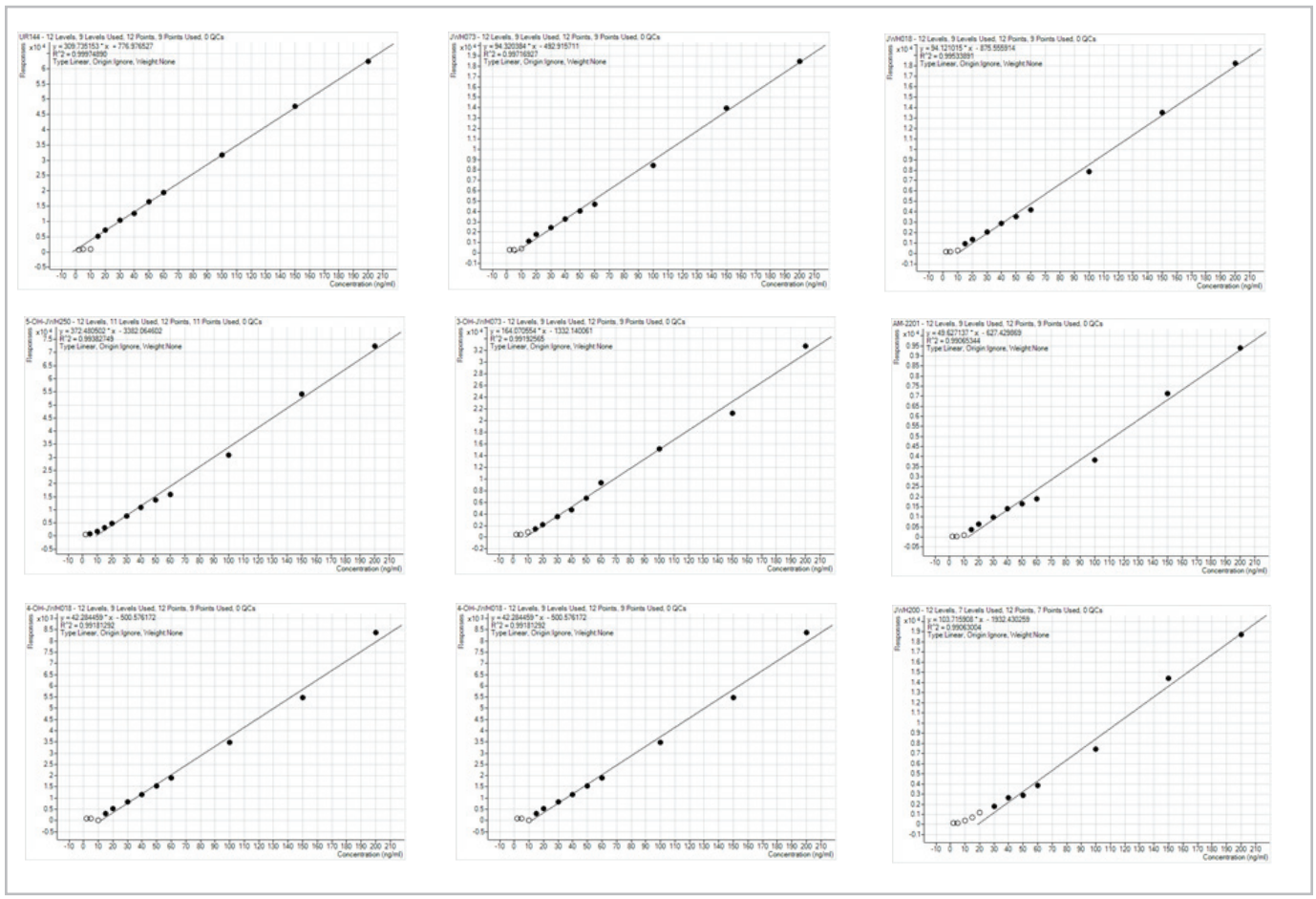

Figure 4. Calibration curves for extracted levels of spiked urine using 1 mL ISOLUTE® SLE+ format from 5 ng/mL to 200 ng/mL showing r^2 values ranging from 0.9906 to 0.9997.

Table 2. Limits of Quantitation using the ISOLUTE® SLE+ 400 µL format (200 µL urine)

Analyte	Extracted LOQ
UR144	37.5 ng/mL
JWH073	37.5 ng/mL
JWH018	37.5 ng/mL
5-hydroxypentyl-JWH250	12.5 ng/mL
3-hydroxybutyl-JWH073	37.5 ng/mL
AM2201	37.5 ng/mL
4-hydroxypentyl-JWH018	37.5 ng/mL
5-hydroxypentyl-JWH018	37.5 ng/mL
JWH200	75 ng/mL due to qualifier ratios

Table 3. Limits of Quantitation using the ISOLUTE® SLE+ 1 mL format (500 µL urine)

Analyte	Extracted LOQ
UR144	15 ng/mL
JWH073	15 ng/mL
JWH018	15 ng/mL
5-hydroxypentyl-JWH250	5 ng/mL
3-hydroxybutyl-JWH073	15 ng/mL
AM2201	15 ng/mL
4-hydroxypentyl-JWH018	15 ng/mL
5-hydroxypentyl-JWH018	15 ng/mL
JWH200	30 ng/mL due to qualifier ratios

Ordering Information

Part Number	Description	Quantity
820-0055-B	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Column	50
820-0140-C	ISOLUTE® SLE+ 1 mL Supported Liquid Extraction Column	30
121-9600	Biotage® VacMaster™-96 Sample Processing Manifold	1
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
C103263	TurboVap® 96 100/120 VAC	1
C103264	TurboVap® 96 1220/240 VAC	1

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