Extraction of Synthetic Cannabinoids (SPICE) and Metabolites from Urine, Plasma and Whole Blood using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

Introduction

This application note describes the extraction and quantitation of Cannabimimetic Naphthoylindoles (Synthetic Cannabinoids) and their metabolites (JWH Series) from various matrices using Supported Liquid Extraction (SLE).

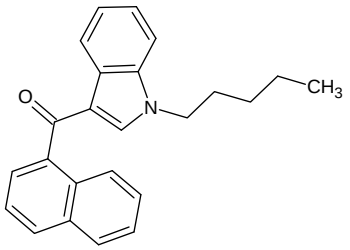

Figure 1. Structure of JWH-018

Synthetic Cannabinoids or SPICE as they are commonly known have become an increasing problem as one of the newest forms of illicit drugs being consumed today. These compounds bind to the cannabinoid receptors in mammals triggering similar euphoric symptoms as Tetrahydrocannabinoids (THC). Currently robust and fast analytical methods of analysis are required to aid in the screening and detection of this growing class of compounds. The recoveries obtained for the synthetic cannabinoids parent and metabolites ranged from 70-98 %.

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 time.

Analytes

JWH-018, JWH-073, JWH-200, JWH-250, JWH-250-N-(5-hydroxypentyl), JWH-018-N-5-(pentanoic acid), JWH-073 N-(3-hydroxybutyl), JWH 018 N-(4-hydroxypentyl)

Sample Preparation Procedure

Urine: ISOLUTE® SLE+ 1 mL Cartridge (820-0140-C) or 400 µL 96-well plate (820-0400-P01)

Urine Hydrolysis:	Add β-glucuronidase at a concentration of 5000 units/mL to urine and dilute sample 1:1 with 100 mM ammonium acetate (pH 5). Spike the matrix solution with internal standard. Incubate sample as per instructions with enzyme.
Sample loading:	Load pre-treated sample (1 mL onto **820-0140-C)** or (400 µL onto **820-0400-P01**). Apply a short pulse of vacuum or positive pressure and allow sample to flow under gravity for 5 minutes.
Analyte elution:	Choose **ONE** of the following elution strategies:
Urine (cartridge):	Apply Ethyl Acetate (2 x 3 mL) to 1 mL column. Apply short pulse of vacuum and collect eluent.
Urine (plate):	Apply Ethyl Acetate (3 x 500 µL) to 400 µL plate. Apply short pulse of vacuum and collect eluent.
Post extraction:	Evaporate to dryness and reconstitute sample in mobile phase.

Plasma or Whole Blood: ISOLUTE® SLE+ 1 mL Cartridge (820-0140-C) or 2 mL Cartridge (820-0290-D)

Sample pre-treatment:	Dilute plasma (1 mL), whole blood (2 mL) 1:1 (v/v) with HPLC grade water.
Sample loading:	Load pre-treated sample (1 mL onto **820-0140-C**) or (2 mL onto **820-0290-D**). Apply a short pulse of vacuum or positive pressure allow sample to flow under gravity for 5 minutes.
Analyte elution:	Apply Hexane (2 x 4 mL) to 2 mL column. Apply short pulse of vacuum and collect eluent.
Post extraction:	Evaporate to dryness and reconstitute sample in mobile phase.

HPLC Conditions

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

Column: Supelco Ascentis Express C_{18}, 2.7µm analytical column (100 x 2.1 mm id) (Supelco, Bellefonte, Pa.)

Mobile Phase: **Solvent A**: 0.1% Formic Acid in Water
Solvent B: 0.1% Formic Acid in Methanol

Isocratic: Initial solvent flow of 20% **A** and 80% **B** at a flow rate of 0.200mL/ min for 8.5 minutes minutes.

Injection Volume: 5 µL

Temperature: Ambient

Mass Spectrometry Conditions

Instrument: 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.

Ion Source Temperature: 600 °C

Table 1. MRM Transitions for synthetic cannabinoids in positive mode Turbo Ionspray.

Scan Function	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
1	JWH-073	328>155	40	30	16
2	JWH-018	342>155	40	30	16
3	JWH-018 N- (4-hydroxypentyl)	358>155	40	30	16
4	JWH-018 5-pentanoic acid	372>155	40	30	16
5	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
6	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
7	JWH-200	385>155	40	30	16
8	JWH-250	336>121	40	30	16
9	d5-JWH-018 N- (4-hydroxypentyl	363.5> 155	40	35	16

Results

A typical extracted ion chromatorgram (XIC) for the target suite of synthetic cannabinoid parent and metabolites is shown in **Figure 2**. The recoveries obtained for the synthetic cannabinoids parent and metabolites ranged from 70-98%. Typical recoveries observed while using ISOLUTE SLE+ technology to extract the synthetic cannabinoids spiked into urine are shown in **Figure 3** for extraction of 1 mL samples, a concentration range of 1-1000 ng/mL was evaluated. For high throughput extraction of 400 µL samples, a concentration range of 0.1-50 ng/mL was evaluated. Similar results were achieved with plasma and whole blood.

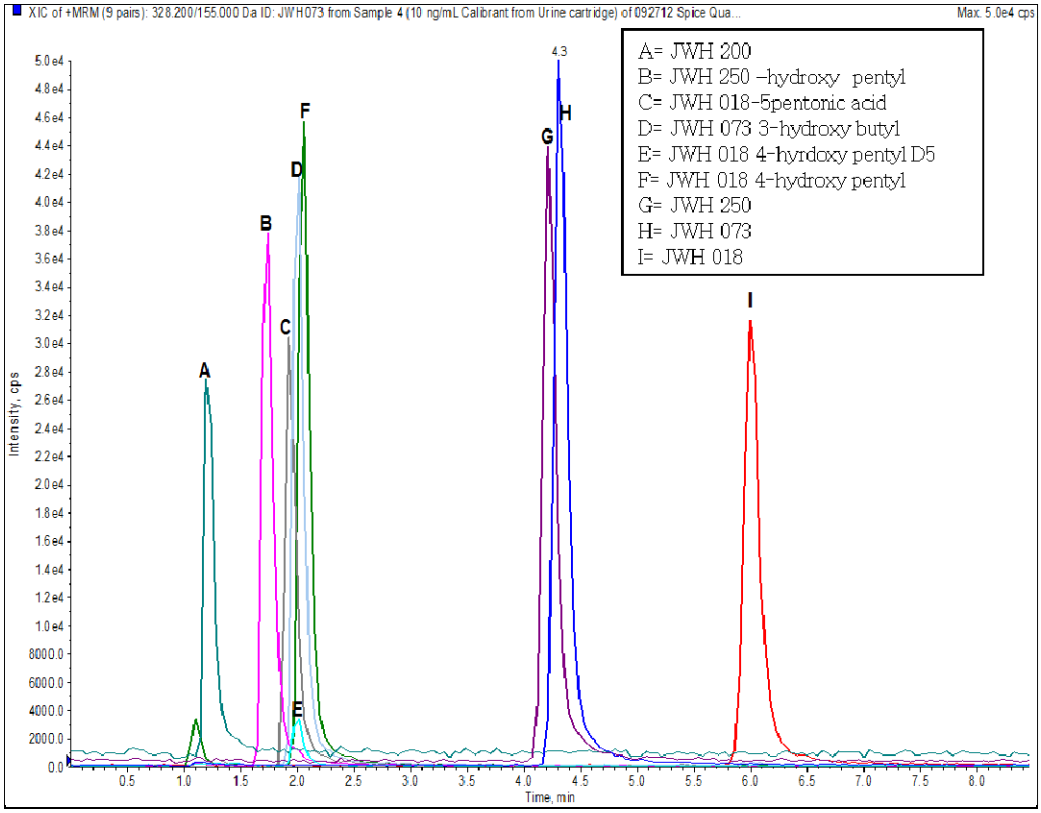

Figure 2. Typical extracted ion chromatogram for elution of urine extracted synthetic cannabinoids spiked at a concentration of 10 ng/mL.

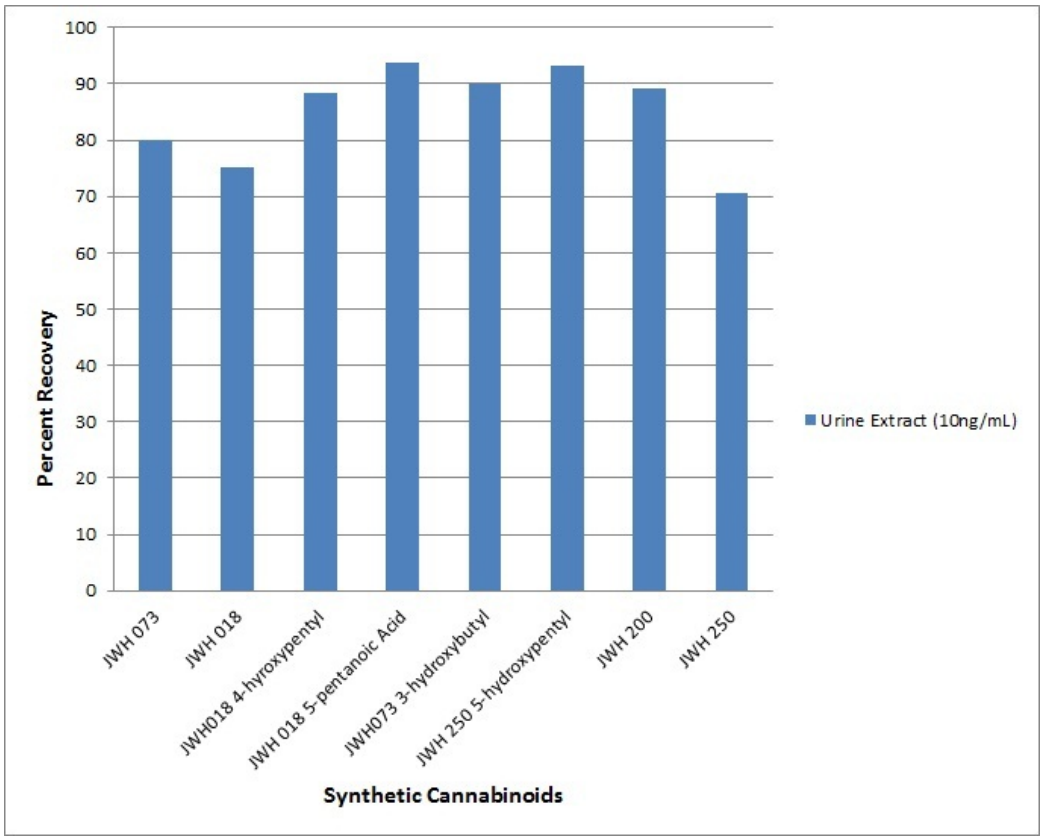

Figure 3. Plot of recoveries for synthetic cannabinoids in urine at 10 ng/mL using SLE+ 1 mL cartridge.

Ordering information

Part number	Description	Quantity
820-0140-C	ISOLUTE SLE 1mL Cartridge	1
820-0290-D	ISOLUTE SLE 2mL Cartridge	1
820-0400-P01	ISOLUTE SLE 400uL 96-well plate	1
121-2068	VacMaster 20 vacuum manifold	1
PPM-96	Positive Pressure Manifold-96	1
PPM-48	Positive Pressure Manifold-48	1
SD-9600-DHS-NA	SpeDry-96 Sample Concentrator System 110 V USA	1
C103198	TurboVap LV 120 V	1

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