Extraction of Synthetic Cannabinoids (SPICE) from Oral Fluid Using ISOLUTE® SLE+ 96-well Plates and Columns Prior to LC-MS/MS

This application note describes the extraction of a range of SPICE drugs and metabolites from neat oral fluid and oral fluid from a commercial collection kit using ISOLUTE® SLE+ in both 96-well plate and column formats.

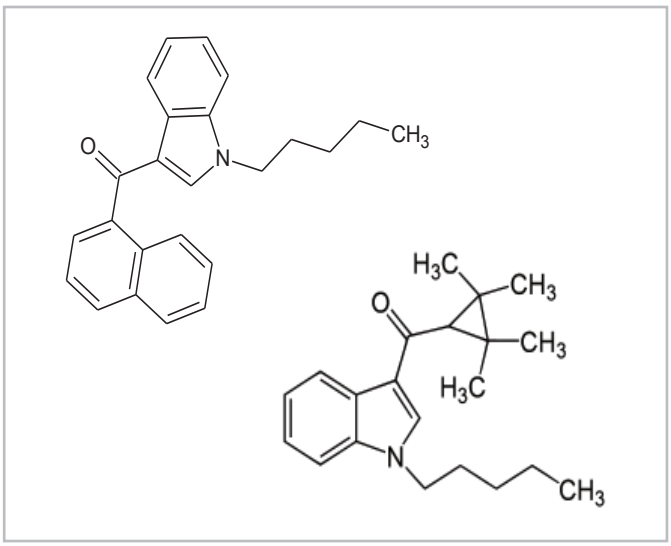

Figure 1. Structure of JWH 018 and UR-144

Introduction

Synthetic cannabinoids have become an increasing problem as an ever changing target for detection during drug screening. The need for quick, non-invasive sampling has been desired by law enforcement as a way of detecting illicit drug use. The collection of oral fluid addresses this need. A semi-automated extraction workflow process was developed for SPICE drugs fortified in oral fluid samples prior to LC-MS/MS. The method was facilitated using Biotage PRESSURE+ 96 and PRESSURE+ 48 Positive Pressure Manifolds for 400 µL sample capacity 96-well plates and columns, respectively. Oral fluid was collected both as a neat solution, and using a commercially available Intercept® (Orasure) kit.

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), XLR-11, UR-144, UR-144 (5-Chloropentyl), UR-144-(pentanoic acid), UR-144-(5-hydroxypentyl)

Sample Preparation Procedure

Format:	ISOLUTE SLE+ 400 µL Supported Liquid Extraction Plate, part number 820-0400-PO1 ISOLUTE SLE+ 400 µL Sample Volume Columns, part number 820-0055-BG
Oral Fluid Hydrolysis: (optional)	Add β-glucuronidase (5000 units/mL) to patient oral fluid, fortified calibration standards and/or QC standards (1 mL), in an appropriate container. Add ammonium acetate (100 mM, pH 5, 1 mL). Spike the solution with internal standard. Incubate sample as per enzyme instructions.
Sample Pre-treatment:	Mix oral fluid sample (neat or buffered, 200 µL) with ammonium acetate (100 mM, pH 5, 200 µL).
Sample Processing:	Load pre-treated oral fluid sample (400 µL) onto the ISOLUTE SLE+ 96-well plate or column. Apply a short pulse of positive pressure and allow samples to sit for 5 minutes.
Analyte Elution:	Apply ethyl acetate (2 x 700 µL). Apply short pulses of pressure and collect eluent.
Post Extraction:	Evaporate to dryness and reconstitute sample in mobile phase (500 µL).

HPLC Conditions

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

Column: Mac-MOD ACE Excel 2 C18-AR, 2.1 x 100 mm i.d. (Mac-MOD Analytical, Chadds Ford, PA.)

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

Isocratic: 15% A: 85% B at 300 µL/min; 9 minute run time

Injection Volume: 10 µL

Temperature: Ambient

MS Conditions

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: 500 °C

Retention Time (minutes)	Analyte	MRM Transition	Declustering Potential (DP)	Collision Energy (CE)	Cell Exit Potential (CXP)
6.36	JWH-073	328>155	40	30	16
8.14	JWH-018	342>155	40	30	16
3.14	JWH-018 N- (4-hydroxypentyl)	358>155	40	30	16
3.34	JWH-018 5-pentanoic acid	372>155	40	30	16
2.99	JWH-073 N-(3-hydroxybutyl)	344>155	40	30	16
2.55	JWH-250 N-(5-hydroxypentyl)	352>120.9	40	30	16
3.98	JWH-200	385>155	40	30	16
5.32	JWH-250	336>121	40	30	16
3.14	d5-JWH-018 N- (4-hydroxypentyl	363.5> 155	40	35	16
4.69	XLR-11	330>125	30	35	16
6.55	UR-144	312.5>125	30	35	16
6.37	UR-144 5-Chloro-pentyl	346.9>125	30	35	16
3.03	UR-144 Pentanoic Acid	342.5>125	30	35	16
3.00	UR-144 5-Hydroxy-pentyl	328.5>125	30	35	16

Table 1. Retention times and MRM transitions for SPICE drugs in positive mode Turbo Ionspray.

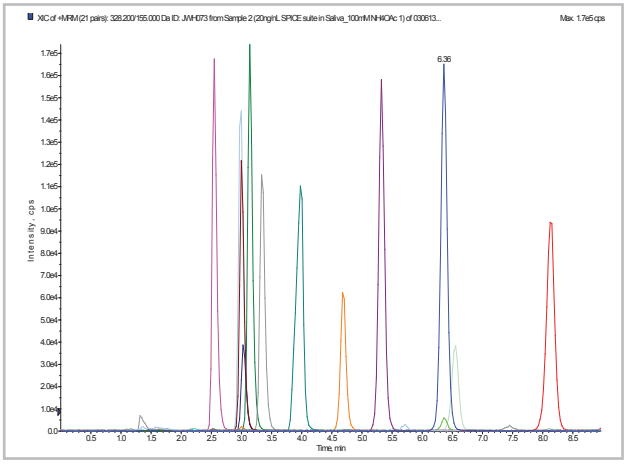

Figure 2. Typical extracted ion chromatogram for SPICE analytes fortified in neat oral fluid at 20 ng/mL and extracted on the ISOLUTE® SLE+ 400 µL sample capacity column or 96-well plate format.

Results

Blank oral fluid was collected as a neat solution from subjects who expectorated into a cup. Blank oral fluid was also collected using an Orasure Intercept kit which includes a buffering solution for preserving the sample. These two types of oral fluid matrix blanks were fortified with a SPICE working standard solution. The oral fluid was fortified with SPICE standards to final concentrations ranging from 50 ng/mL to 1.0 ng/mL. The analytes were extracted from the oral fluid samples using both ISOLUTE® SLE+ 400 µL sample capacity supported liquid extraction column and 96-well plate formats.

The cleanliness of the extracted samples was verified by conducting matrix effect studies to determine the level of ion suppression or enhancement as a function of matrix effects. The two matrix blanks were pre-treated, loaded and extracted on ISOLUTE SLE+. The extracted blanks were then fortified with SPICE standard at a final concentration of 10 ng/mL. The samples were dried down and reconstituted in mobile phase. A sample of mobile phase fortified with the same amount of SPICE standard added to the extracted blanks was also prepared. Both samples were analyzed via LC-MS/MS. Figure 2 shows a typical extracted ion chromatogram for the SPICE analytes. Figure 3 shows the plot of the ratio of the peak area response for fortified extracted blank and fortified mobile phase. The degree of matrix effect can be evaluated as ion suppression (‹100% recovery) or ion enhancement (› 100% recovery). The matrix effect for the extracted blanks (neat or buffered) was observed as ±16%.

An evaluation of the recovery of SPICE analytes using ISOLUTE SLE+ 400 µL sample capacity columns and plates was conducted. Figure 4 shows the plot of typical recoveries observed for neat oral fluid fortified at 10 ng/mL and extracted on each SLE+ format. The averaged recoveries ranged from 65-87% for the analytes across each format with % RSDs ‹10. Typical recoveries for SPICE analytes fortified into oral fluid collected using the Orasure Intercept kit (Figure 5) was observed ranging from 68-110% with %RSD‹10. Samples were prepared at low nanogram per milliliter concentrations to determine recoveries and establish a lower limit of detection. Figure 6 shows the plot of typical recoveries observed for SPICE analytes fortified at a concentration of 1.0 ng/mL and extracted from neat and buffered oral solutions. The recoveries range from 65-110% with %RSDs ‹10.

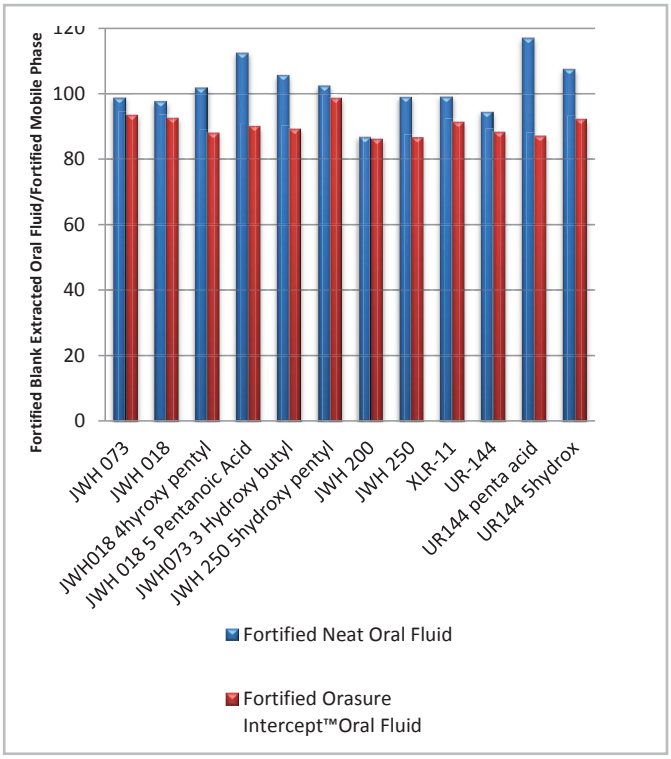

Figure 3. Matrix effects plot: ratio of peak area response of extracted blank oral fluid (fortified post extraction) and fortified mobile phase for SPICE drugs. The extracted blank matrix and mobile phase were fortified at 10 ng/mL.

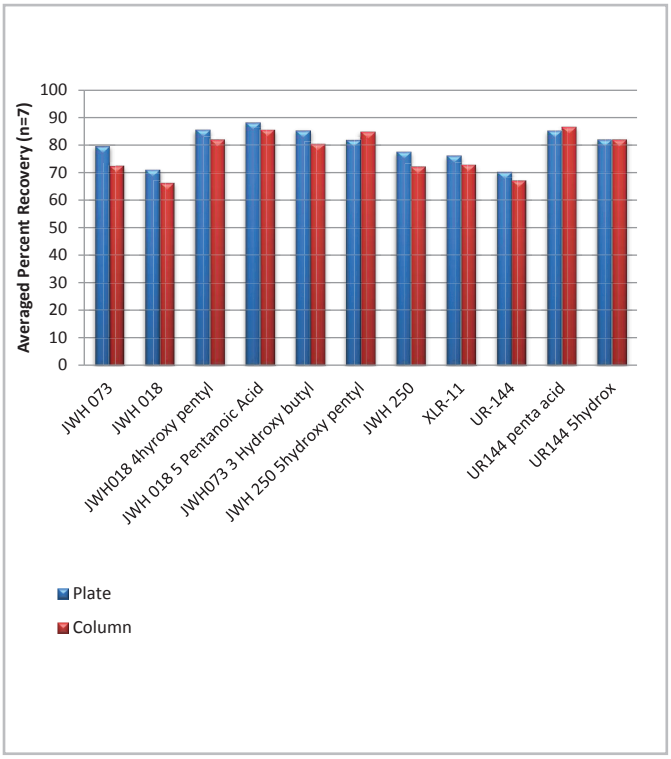

Figure 4. Plot of average recoveries (n=7) for SPICE drugs fortified into neat oral fluid at 10 ng/mL

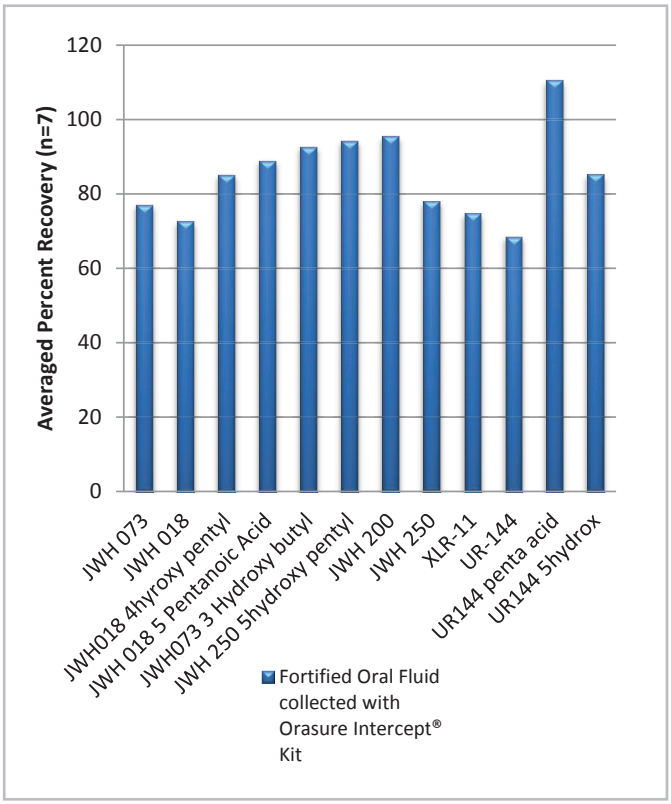

Figure 5. Plot of average recoveries (n=7) for SPICE drugs fortified in Orasure Intercept oral fluid at 10 ng/mL

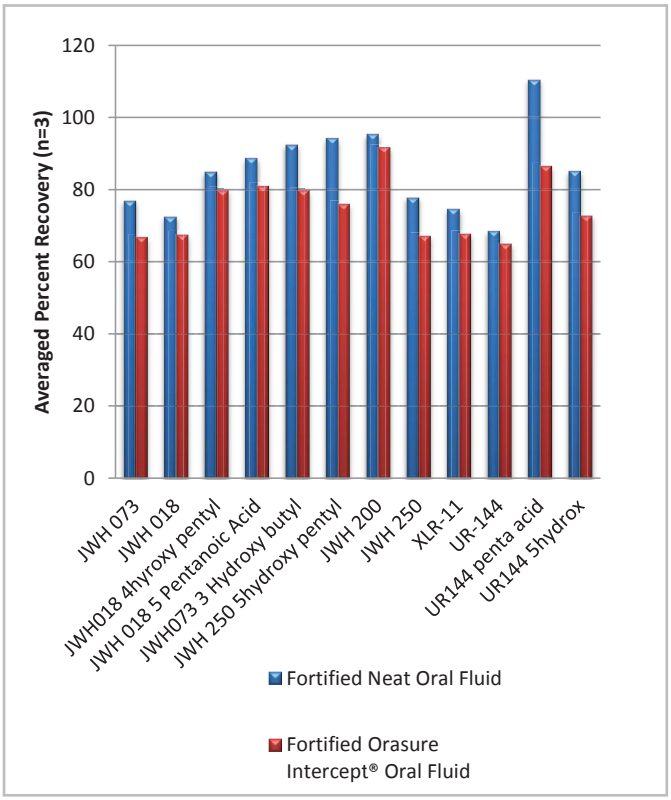

Figure 6. Plot of average recoveries (n=3) for SPICE drugs fortified in neat oral fluid and Orasure Intercept oral fluid at 1.0 ng/mL

Ordering Information

Part Number	Description	Quantity
820-0400-P01	ISOLUTE SLE+ 400 µL Supported Liquid Extraction Plate	1
820-0055-B	ISOLUTE SLE+ 400 µL Sample Volume Columns	50
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
SD2-9600-DHS-NA	Biotage® SPE Dry Dual Sample Concentrator System, 110V	1
SD2-9600-DHS-EU	Biotage® SPE Dry Dual Sample Concentrator System, 220V	1

For the latest application notes and more information about ISOLUTE® SLE+ visit www.biotage.com

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