Extraction of THC and Metabolites Including 11-nor-9-carboxy-Δ⁹-THC Glucuronide from Urine Using ISOLUTE® SLE+ Prior to LC-MS/MS Analysis

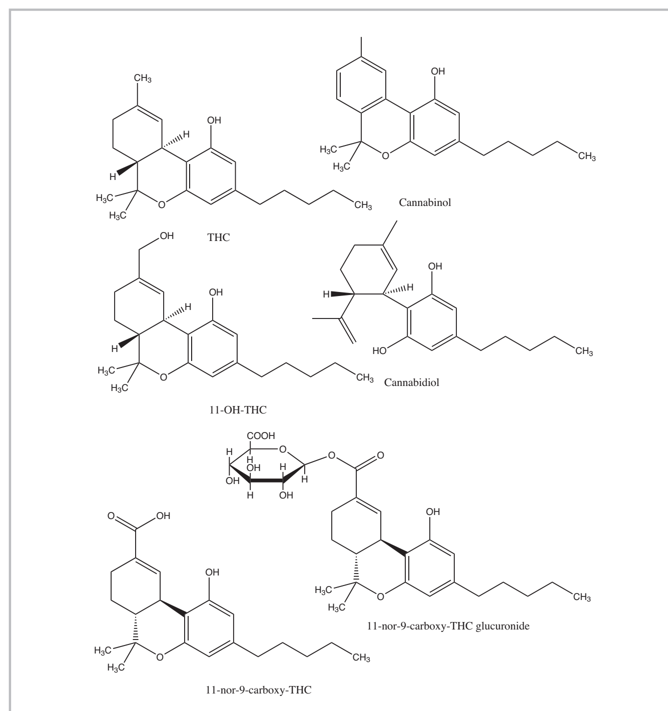

Figure 1. Structure of THC and major metabolites

This application note describes the simultaneous extraction of THC and its major metabolites, including 11-nor-9-carboxy-Δ⁹-THC glucuronide, from urine using supported liquid extraction (ISOLUTE® SLE+ in both plate and column formats) prior to analysis by LC-MS/MS.

Introduction

This application note describes effective and efficient ISOLUTE SLE+ protocols optimized for sample volumes of either 200 µL or 1 mL. Due to the pH sensitivity of glucuronidated metabolites and the necessity to avoid any hydrolysis back to parent analytes due to harsh pH conditions, sample pre-treatment using ion pair reagents was investigated. The simple sample preparation procedure delivers clean extracts and high analyte recoveries with RSDs of <10% for all analytes.

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

11-nor-9-carboxy-Δ⁹-THC, 11-nor-9-carboxy-Δ⁹-THC glucuronide, cannabinol, cannabidiol, Δ⁹-THC and 11-OH-Δ⁹-THC.

Sample Preparation Procedure

Sample Pre-treatment: Dilute urine with 25 mM dibutylammonium acetate (1:1, v/v). Vortex mix thoroughly.

Format: **ISOLUTE SLE+ 200 µL Supported Liquid Extraction Plate, part number 820-0200-P01**

Sample loading: Load diluted urine (200 µL total volume) onto each well and apply a pulse of vacuum or positive pressure to initiate flow. Allow the sample to adsorb for 5 minutes.

Analyte extraction: Apply ethyl acetate (1 mL) and allow to flow under gravity for 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

Format: **ISOLUTE SLE+ 1 mL Sample Volume columns, part number 820-0140-C**

Sample loading: Load the urine (1 mL 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 ethyl acetate (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a further aliquot of ethyl acetate (2.5 mL) and allow to flow for another 5 minutes. Apply vacuum or positive pressure to pull through any remaining extraction solvent.

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Post Elution & Reconstitution (200 µL and 1 mL protocols)

Evaporate to dryness using a SPE Dry (40°C, 20 to 40 L/min) or TurboVap (1.5 bar at 40°C for 1 hr) Reconstitute with 0.1% formic acid in water/acetonitrile (70/30, v/v, 200 µL). Cap with a sealing mat and vortex gently.

Buffer preparation: Dibutylammonium acetate (Sigma-Aldrich) supplied at a concentration of 0.5 M was diluted to 25 mM by adding 1 mL to 19 mL of H_2O.

HPLC Conditions

Instrument: Waters ACQUITY UPLC with 20 µL loop

Column: ACQUITY UPLC BEH C18 column (1.7 µ, 100 x 2.1 mm id)

Mobile Phase: Isocratic 20/80 0.1% formic acid (aq) and 0.1% formic acid/MeOH at a flow rate of 0.4 mL/min.

Injection Volume: 15 µL (partial loop with overfill)

Sample Temperature: 20 °C

Column Temperature: 40 °C

MS Conditions

Instrument: Premier XE triple quadrupole mass spectrometer equipped with an electrospray interface for mass analysis.

Desolvation Temperature: 450 °C

Ion Source Temperature: 150 °C

MRM Transitions

Analyte	Ionization Mode	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
THC-COOH-glucuronide	-	519.1 > 343.1	35	22
Cannabidiol	+	315.2 > 135.0	40	20
THC-OH	+	331.2 > 313.3	25	14
THC-COOH	+	345.1 > 327.2	35	16
Cannabinol	+	311.2 > 223.1	40	20
THC	+	315.2 > 193.1	30	21

Results

An LC-MS/MS method suitable for quantitation of THC and metabolites from urine was developed. **Figure 2** overleaf shows the MRM chromatogram for THC and metabolites extracted from urine, spiked at 40 ng/mL for each analyte.

High analyte recoveries (>85%) were achieved when extracting either 100 µL (using the ISOLUTE SLE+ 200 µL plate) or 500 µL (using the ISOLUTE SLE+ 1 mL sample volume column) of urine spiked at 40 ng/mL. **Figure 3** overleaf shows average recoveries (n=7) of THC and metabolites from a 500 µL urine sample spiked at 40 ng/mL

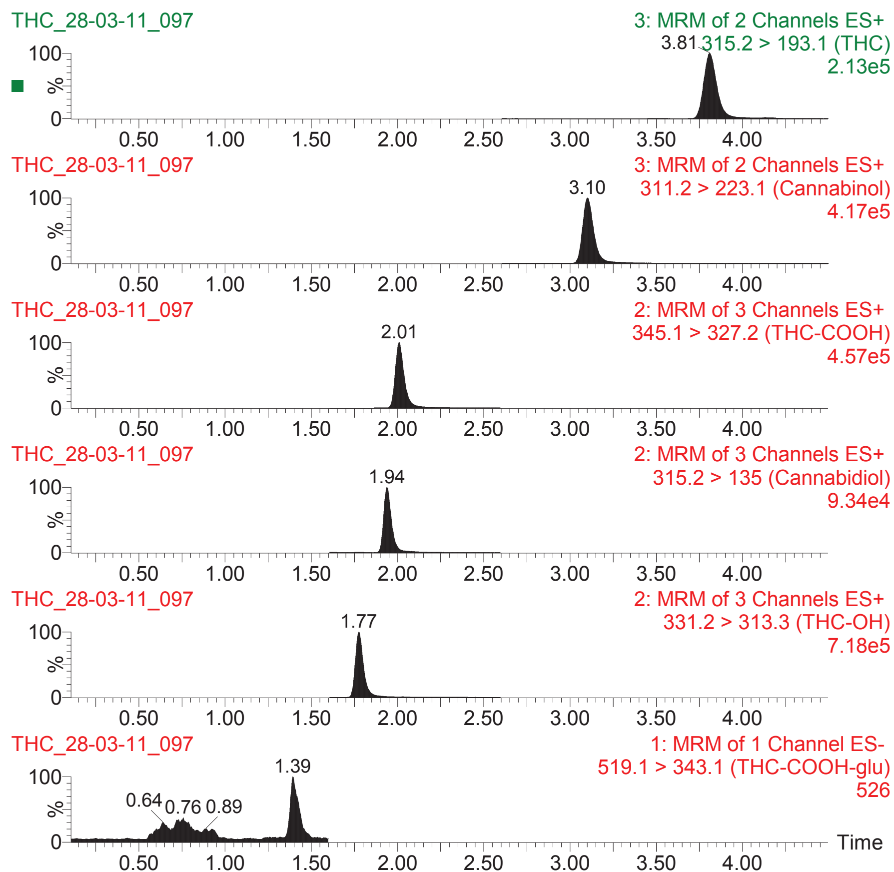

Figure 2. MRM chromatogram for THC and metabolites extracted from urine, spiked at 40 ng/mL for each analyte

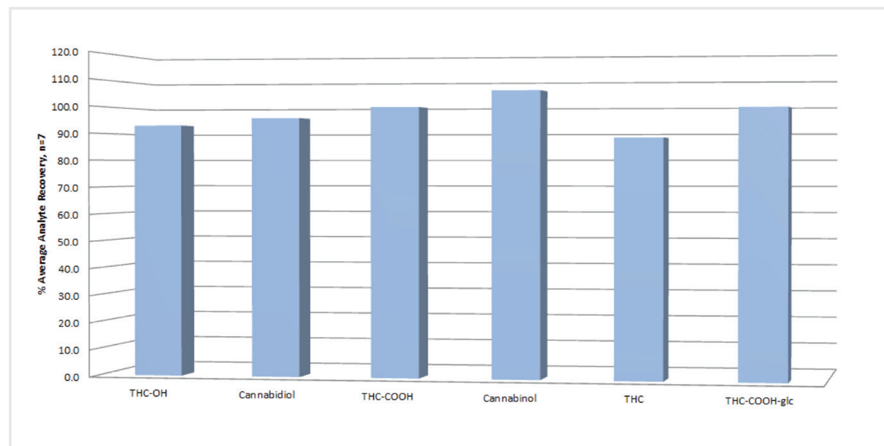

Figure 3. Average recoveries (n=7) of THC and metabolites from a 500 µL urine sample spiked at 40 ng/mL

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Ordering Information

Part Number	Description	Quantity
820-0200-P01	ISOLUTE® SLE+ 200 µL Supported Liquid Extraction Plate	1
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column	30
121-9600	Biotage® VacMaster™ -96 Sample Processing Manifold	1
PPM-96	Biotage® Positive Pressure Manifold 96 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
C103264	TurboVap® 96	1

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References

1. The data in this application note was originally presented in poster form at the 2011 combined TIAFT/SOFT annual conference in San Francisco.
2. Modification of this method was performed by NIH: Karl B. Scheidweiler, Nathalie A. Desrosiers, and Marilyn A. Huestis Clin Chim Acta. 2012 November 20; 413(23-24): 1839–1847. Published online 2012 July 6. doi: 10.1016/j.cca.2012.06.034

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