

For Research Use Only. NOT for Use in Diagnostic Procedures.

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

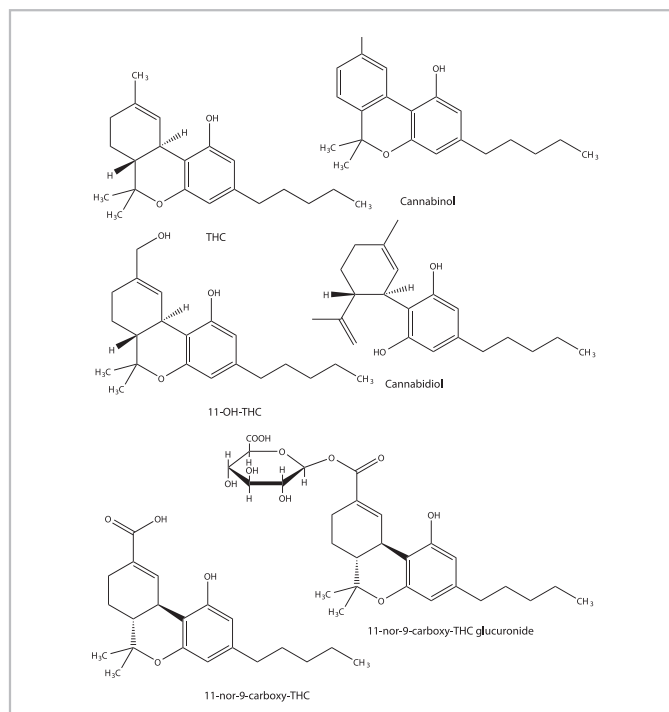


Figure 1. Structure of THC and major metabolites

This application note describes the simultaneous extraction of THC and its major metabolites, including 11-nor-9-carboxy- Δ^9 -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 μ L 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- Δ^9 -THC, 11-nor-9-carboxy- Δ^9 -THC glucuronide, cannabinol, cannabidiol, Δ^9 -THC and 11-OH- Δ^9 -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 μ L Supported Liquid Extraction Plate, part number 820-0200-P01**

Sample loading: Load diluted urine (200 μ L 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.

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 H₂O.

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

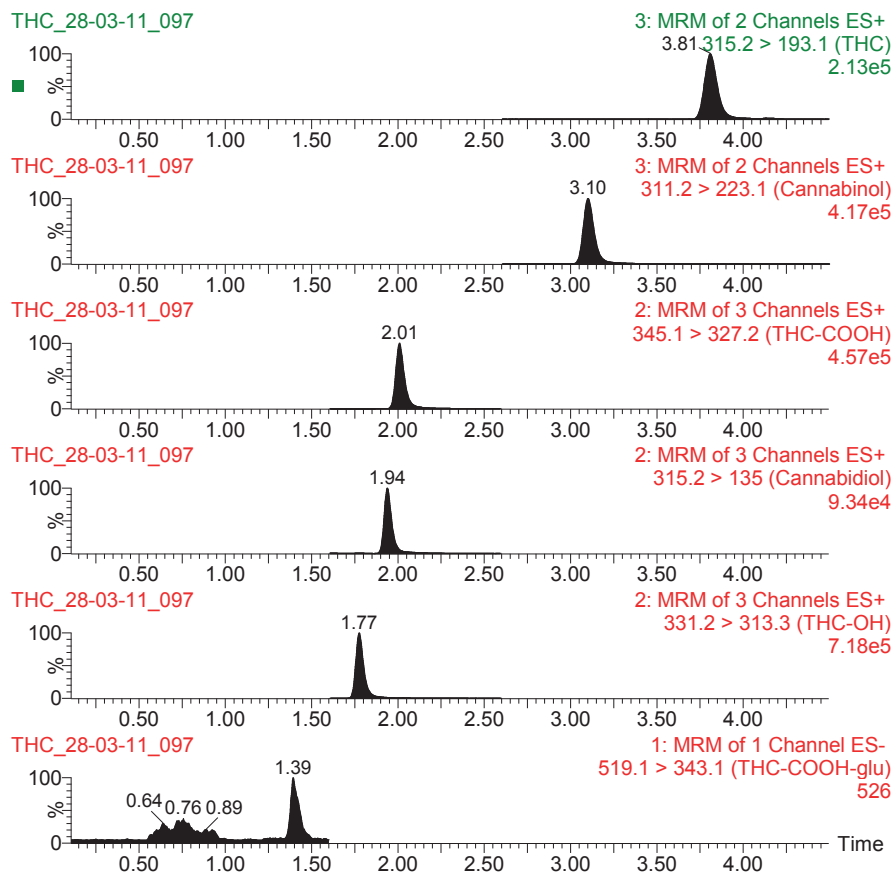


Figure 2. MRM chromatogram for THC and metabolites extracted from urine, spiked at 40 ng/mL for each analyte

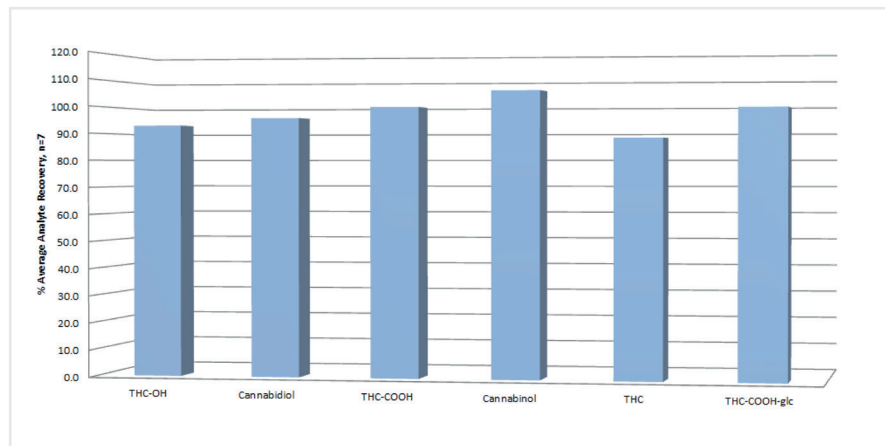


Figure 3. Average recoveries (n=7) of THC and metabolites from a 500 µL urine sample spiked at 40 ng/mL

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

For the latest application notes visit www.biotage.com

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

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

To locate a distributor,
 please visit our website at
www.biotage.com

Part Number: AN809.V.1

© 2014 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. Quantisal is a trademark of Immunalysis, Intercept is a registered trademark of OraSure Technologies. Other product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

For more information visit www.biotage.com.