Extraction of 11-nor-9-carboxy-tetrahydrocannabinol from Hydrolyzed Urine by ISOLUTE® SLE+ Prior to GC/MS Analysis

This application note describes the extraction of 11-nor-9-carboxy-THC from a urine matrix, prior to GC/MS analysis.

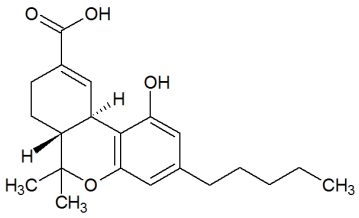

Figure 1. Structure of 11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC; THC-COOH)

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.

11-nor-9-carboxy-tetrahydrocannabinol (carboxy-THC; THC-COOH) is the primary metabolite of THC. In urine, 80% of THC-COOH is present in the form of its glucuronide metabolite. Therefore, to effectively quantitate the THC-COOH, urine is hydrolyzed before extraction. This application note describes optimized extraction of urine samples prepared by either enzymatic or base hydrolysis.

The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 87% with RSDs lower than 10% for all analytes, and is optimized for use with 1 mL sample capacity ISOLUTE SLE+ columns.

Analytes

THC-COOH and THC-COOH-D3 as the internal standard

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

Sample Preparation Procedure (enzyme-hydrolyzed urine)

Sample Pre-treatment

To 1 mL of urine, add 950 µL ammonium acetate 50 mM (pH5). Add 50 µL beta-glucuronidase enzyme. Mix. Heat sample for 2 hours at 37 °C.

Sample Loading

Load 1 mL of the pre-treated urine onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction

Apply MTBE (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of MTBE (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Sample Preparation Procedure (base-hydrolyzed urine)

Sample Pre-treatment

To 1.2 mL of urine, add 120 µL potassium hydroxide (10N). Mix. Heat sample for 25 minutes at 60 °C. Remove from heat and add 60 µL glacial acetic acid to modify the pH to approximately 5.3 prior to extraction.

Sample Loading

Load 1 mL of the pre-treated urine onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction

Apply MTBE (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of MTBE (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

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Sample Preparation Procedure (non-hydrolyzed urine)

In some circumstances, for example screening applications, the quantification of the level of non-glucuronidated carboxy-THC (THC-COOH) in non-hydrolysed urine may be sufficient. A method for extraction of carboxy-THC is shown below.

Note: this procedure will not extract the intact glucuronide form of THC-COOH. Biotage application note **AN809** describes method for simultaneous extraction of all major metabolites, including intact 11-nor-9-carboxy-Δ9-THC glucuronide, from non-hydrolysed urine.

Sample Pre-treatment

To 1 mL of urine, add 1 mL of HPLC-grade water

Sample Loading

Load 1 mL of the pre-treated urine onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.

Analyte Extraction

Apply MTBE (2.5 mL) and allow to flow under gravity for 5 minutes. Apply a second aliquot of MTBE (2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.

Post Elution & Reconstitution

Dry the extract in a stream of air or nitrogen using a SPE Dry (40 °C, 20 to 40 L/min) or TurboVap (1.0 bar at 40 °C for 40 mins).

Upon dryness, reconstitute with 40 µL ethyl acetate and 20 µL BSTFA:TMCS 99:1 and vortex for 20 seconds. Transfer to a high recovery glass vial. Place in a heating block set to 70 °C, for 25 minutes. Remove vial from the block and allow cooling.

GC Conditions

Instrument

Agilent 7890A with QuickSwap

Column

Agilent J&W DB-5, 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 1.0 min

Injection

2 µL

Wash Solvents

Acetone and Ethyl acetate

Oven

Initial temperature 60 °C
Ramp 25 °C/min to 350 °C, hold for 0.4 minutes

Post Run

Backflush for 2.4 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
1	THC-COOH-D3	374	491
1	THC-COOH	371	488

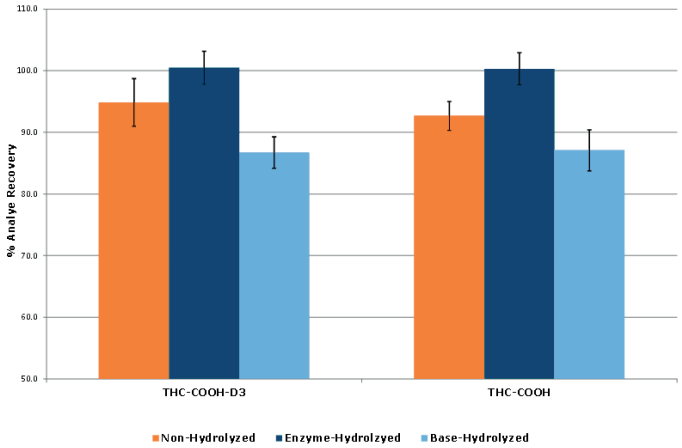

Figure 2. Typical extraction % recoveries (n=7) using the ISOLUTE® SLE+ protocols.

Results

The optimized ISOLUTE® SLE+ protocols for the three approaches are demonstrated in **Figure 2**. THC-COOH-D3 and THC-COOH recoveries from enzyme-hydrolyzed urine were 101% and 100% respectively; from base-hydrolyzed, they were 87% for each. From non-hydrolyzed urine, recoveries were 95% and 93% respectively. For all procedures, RSD values were below 4%.

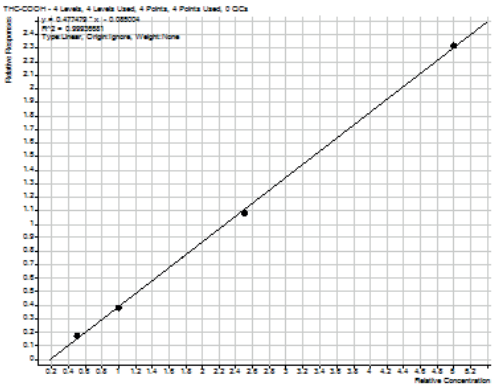

Figure 3. Calibration curves for extracted levels of spiked enzyme-hydrolyzed urine using 1 mL ISOLUTE® SLE+ columns from concentrations of 15, 30, 75 and 150 ng/mL showing r^2 greater than 0.999.

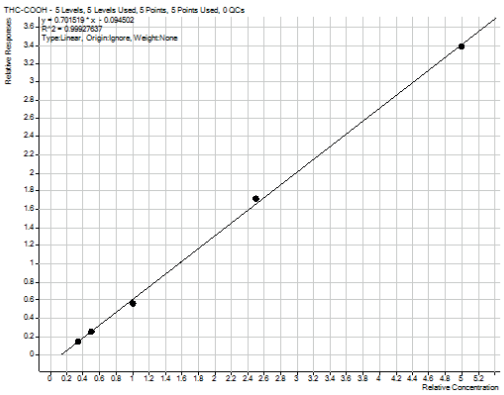

Figure 5. Calibration curves for extracted levels of spiked base-hydrolyzed urine using 1 mL ISOLUTE® SLE+ columns from 10, 15, 30, 75 and 150 ng/mL showing r^2 greater than 0.999.

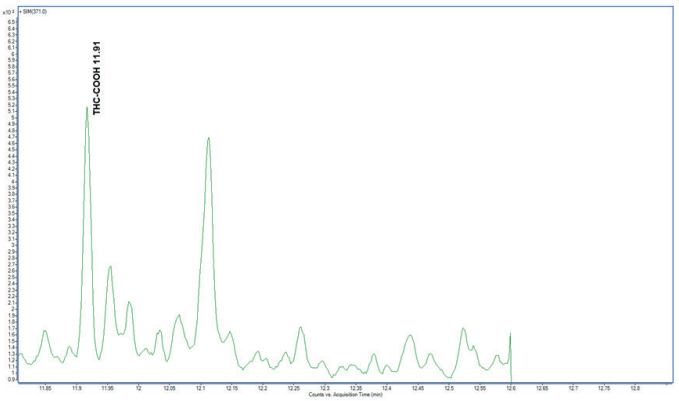

Figure 4. GC/MS chromatography of THC-COOH from enzyme-hydrolyzed urine at 15 ng/mL.

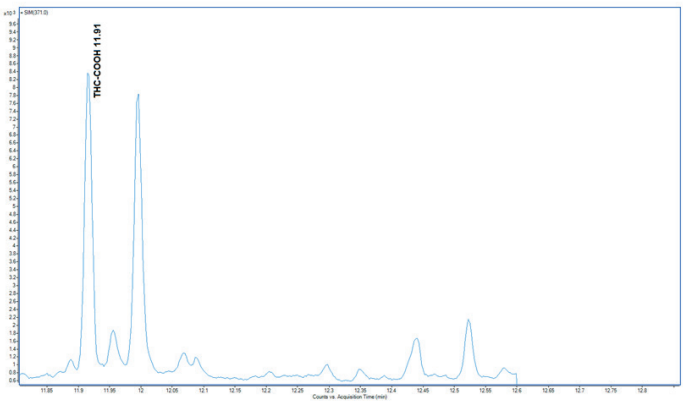

Figure 6. GC/MS chromatography of THC-COOH from base-hydrolyzed urine at 15 ng/mL.

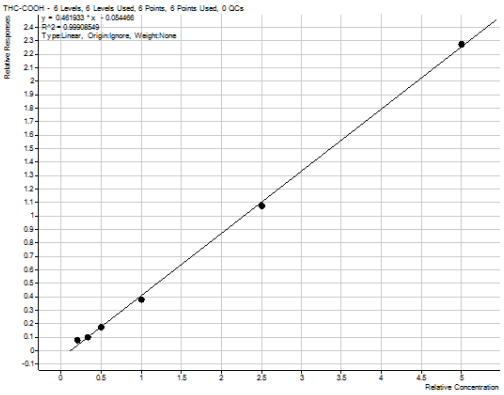

Figure 7. Calibration curves for extracted levels of spiked non-hydrolyzed urine using 1 mL ISOLUTE® SLE+ columns from concentrations of 6, 10, 15, 30, 75 and 150 ng/mL showing r^2 greater than 0.999.

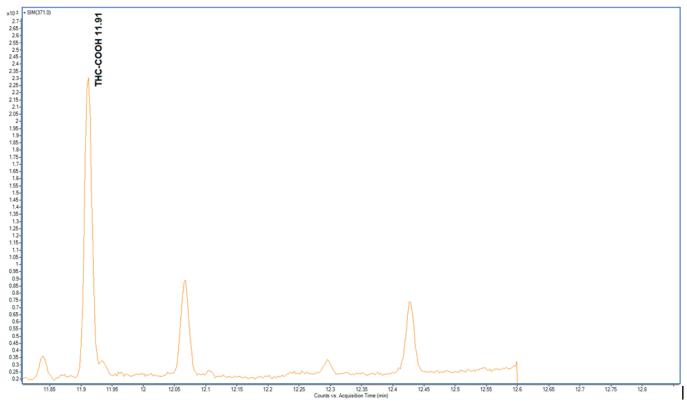

Figure 8. GC/MS chromatography of THC-COOH from non-hydrolyzed urine at 10 ng/mL.

Table 2. Lower Limits of THC-COOH Quantitation (LLOQ) using ISOLUTE® SLE+ procedure

Analyte	Lower Limit Of Quantitation (ng/mL)
Enzyme-Hydrolyzed LLOQ	15-30*
Base-Hydrolyzed LLOQ	10-15*
Non-Hydrolyzed LLOQ	6

*estimated LLOQ of 10:1 signal :noise falls in between extracted calibrator concentrations.

Hydrolysis Strategy

Glucuronide-conjugated metabolites require hydrolysis to capture total concentration of the analyte from urine. The two options described in this application note are widely accepted in forensic science and both have the merits. As shown in **Table. 2**, the enzymatic hydrolysis approach shows improved recoveries compared to those from the base-hydrolyzed samples. However due to the lower baseline noise in the basic hydrolyzed sample extracts, the LLOQ is superior to the enzyme approach. It is also less time demanding, taking 20 minutes for basic hydrolysis compared to 2 hours for the enzymatic hydrolysis. These steps need to be considered when choosing which strategy to use for the lab workflow.

Additional Information

1. All solvents were HPLC grade.
2. 10 N potassium hydroxide was prepared by adding 56.11g of potassium hydroxide pellets to 100 mL of HPLC grade water.
3. 50 mM ammonium acetate was prepared by adding 1.927 g of ammonium acetate to 500 mL of HPLC grade water. pH was modified to 5 with formic acid.
4. β-Glucuronidase: Type H-3 from Helix Pomatia, ~4500 units/mL of urine

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Ordering Information

Part Number	Description	Quantity
820-0140-C	ISOLUTE® SLE+ 1 mL Sample Volume Column	30
820-0140-CG	ISOLUTE® SLE+ 1 mL Sample Volume Column (tabless) for use on Biotage® Extrahera	30
820-0140-C-1000	ISOLUTE® SLE+ 1 mL Sample Volume Column (Bulk pack)	1000
820-0140-CG-1000	ISOLUTE® SLE+ 1 mL Sample Volume Column (tabless) (Bulk Pack) for use on Biotage® Extrahera™	1000
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold for Columns	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
C103198	TurboVap® LV, Evaporator 100/120V	1
C103199	TurboVap® LV, Evaporator 220/240V	1

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