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Automated Extraction of 11-nor-9-Carboxy- Δ^9 -THC from Hydrolyzed Urine Using ISOLUTE[®] SLE+ Prior to GC/MS Analysis

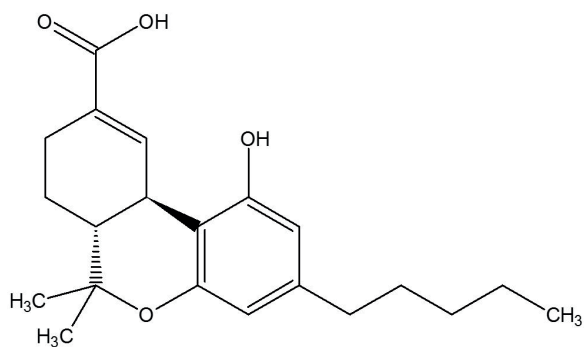


Figure 1. Structure of 11-nor-9-carboxy- Δ^9 -THC.

Introduction

This application note describes the fully automated extraction of carboxy-THC from urine, following base hydrolysis prior to GC/MS analysis. The method was automated using Biotage[®] Extrahera[™], configured for use with ISOLUTE SLE+ columns.

This application note describes an effective and efficient ISOLUTE SLE+ protocol optimized for extraction of 1 mL of pre-hydrolyzed urine. The simple sample preparation procedure delivers clean extracts and analyte recoveries greater than 80% with RSDs lower than 10% for carboxy-THC and its deuterated internal standard. Using Biotage Extrahera, 24 samples are extracted in approximately 35 minutes. Limit of quantitation is below the SAMHSA/EWDTS confirmation cut off of 15 ng/mL for workplace testing applications.

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

Carboxy-THC and carboxy-THC-D₉ as internal standard

Sample Preparation Procedure

Format

ISOLUTE[®] SLE+ 1 mL Sample Volume columns (Tables), part number 820-0140-CG.

Sample Pre-treatment

Apply 20 μ L of a 1 ng/ μ L aqueous internal standard solution to 1 mL of urine and allow to equilibrate for 1 hr at room temperature. Add 50 μ L sodium hydroxide (10N) to this urine sample and heat at 60 $^{\circ}$ C for 20 minutes. Allow to cool and add 60 μ L glacial acetic acid.

Sample Loading

Load 1 mL of the hydrolyzed sample 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 hexane/ethyl acetate (2.5 mL, 50/50, v/v) and allow to flow under gravity for 5 minutes. Apply a further aliquot of hexane/ethyl acetate (2.5 mL, 50/50, v/v) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure to pull through any remaining extraction solvent. (5–10 seconds).

Post Elution and Derivatization

Dry the extract in a stream of air or nitrogen using a Biotage[®] TurboVap[®] (1.2 bar at 40 $^{\circ}$ C for 25 mins).

Reconstitute the extracts with 250 μ L ethyl acetate and vortex for 10 seconds before transferring to high recovery GC vials. Dry the extract in a stream of air or nitrogen using a TurboVap (1.0 bar at 40 $^{\circ}$ C for 10 mins).

Upon dryness, reconstitute with 20 μ L ethyl acetate and 20 μ L BSTFA:TMCS 99:1 and vortex for 20 seconds. Place in a heating block set to 70 $^{\circ}$ C, for 25 minutes. Remove vial from the block and allow to cool.

GC Conditions

Instrument

Agilent 7890A with QuickSwap

Column

Restek Rxi-5ms, 30 m x 0.25 mm ID x 0.25 μ m

Carrier

Helium 1.2 mL/min (constant flow)

Inlet

280 °C, Splitless, purge flow: 50 mL/min at 1.0 min

Injection Volume

2 μ L

Wash Solvents

Methanol and ethyl acetate

Oven

Initial temperature 125 °C

Ramp 50 °C/min to 300 °C, hold for 2.5 minutes

Ramp 50 °C/min to 330 °C, hold for 1.4 minutes

Post Run

Backflush for 1.6 minutes (2 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 | 2nd Qual Ion |
|-----------|-------------------------|--------------------|--------------|--------------|
| 1 | THC-COOH-D ₉ | 380 | 479 | |
| 1 | THC-COOH | 371 | 488 | 473 |

Results

This optimized SLE+ protocol demonstrated analyte recoveries of 82% and 85% from urine for the carboxy-THC-D₉ and carboxy-THC respectively, as shown in Figure 2. RSDs were lower than 10% (n=7).

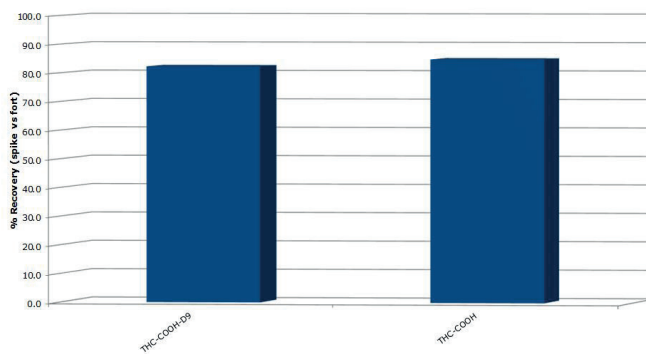


Figure 2. Analyte Recoveries.

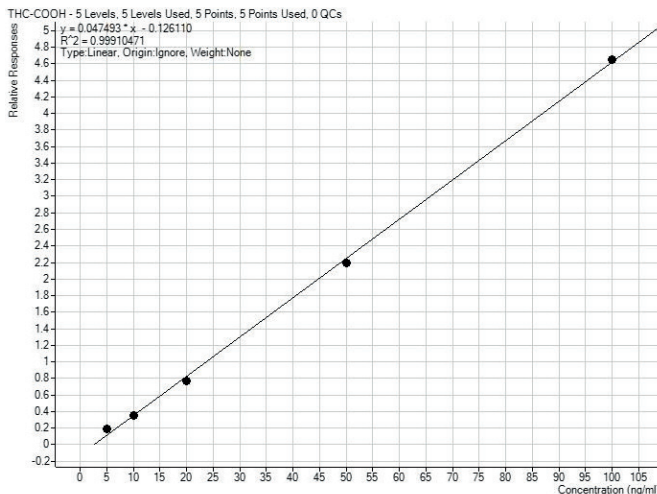


Figure 3. A calibration curve of carboxy-THC constructed following extractions using ISOLUTE SLE+ with the optimal protocol. Analyte concentrations shown here are 5, 10, 20, 50 and 100 ng/mL with r^2 values of greater than 0.999. The carboxy-D₉ deuterated internal standard concentrations is at 20 ng/mL.

The analyte signal allows an approximate inferred limit of quantitation of between 5 and 10 ng/mL.



Additional information

- » **Sodium Hydroxide 10N** is prepared with 40 g of pellets in 100 mL deionized water. Prepare this solution with extreme care. As an added precaution, place the beaker on ice prior to gradual pellet addition.
- » **Glacial Acetic Acid** was purchased from Sigma-Aldrich at $\geq 99.85\%$
- » The use of MTBE is an acceptable alternative to hexane/ethyl acetate (50/50, v/v)
- » For manual extraction protocols, ISOLUTE® SLE+ 1 mL Sample Volume columns are available in tabbed format (p/n 820-0140-C) and can be used as an alternative to the tabless version used in this Biotage® Extrahera™ automated protocol.

Ordering Information

| Part Number | Description | Quantity |
|--------------------|---|----------|
| 820-0140-CG | ISOLUTE® SLE+ 1 mL Sample Volume Column (Tabless) | 30 |
| 414001 | Biotage® Extrahera™ | 1 |
| 415041 | Configuration Kit 24 Positions, Dual Flow | 1 |
| 415000 | TurboVap® LV Evaporator | 1 |

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