

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

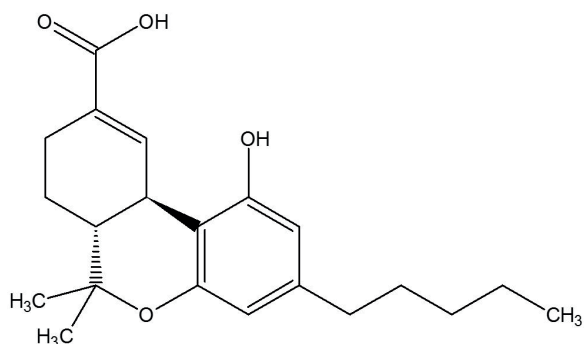


Figure 1. Structure of 11-nor-9-carboxy- Δ^9 -THC.

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 EVOLUTE EXPRESS ABN columns.

The method delivers clean extracts and analyte recovery of 90% with RSD lower than 10% for carboxy-THC and its deuterated internal standard. Using the Biotage[®] Extrahera[™], 24 samples were extracted in approximately 38 minutes. The limit of quantitation of 2 ng/mL is below the SAMHSA/EWDTs confirmation cut off of 15 ng/mL for workplace testing applications.

The EVOLUTE[®] EXPRESS range of SPE columns and 96-well plates combine powerful EVOLUTE sorbent chemistry with innovative features that provide robust, reliable SPE, giving ultra-pure extracts and high analyte recoveries.

With improved flow-through characteristics and by eliminating the need for conditioning and equilibration, samples can be prepared much faster using the simplified Load-Wash-Elute procedure.

Analytes

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

Sample Preparation Procedure

Format

EVOLUTE[®] EXPRESS ABN 60 mg/3 mL tabless SPE columns, part number 610-0006-BXG.

Sample Pre-treatment

Apply 40 μ L of a 1 ng/ μ L aqueous internal standard solution to 2 mL of urine, and allow to equilibrate for 1 hr at room temperature. Add 100 μ L sodium hydroxide (10N) to this urine sample and heat at 60 $^{\circ}$ C for 20 minutes. Allow to cool and add 1 mL glacial acetic acid.

Sample Loading

Load the full sample volume onto the column and apply positive pressure to achieve approximately 3 mL/minute. Gravity or very low pressure may be sufficient for this step.

Wash 1

Apply 2 mL of HPLC water and apply pressure to elute.

Wash 2

Apply a further 2 mL of HPLC water and apply pressure to elute.

Pre-Elution Step

Dry the column for 10 mins using the Biotage[®] Extrahera[™] dry plate function, or at 10 psi using other processing systems.

Elution

Elute direct into GC vials with hexane/ethyl acetate (1 mL, 50/50, v/v)

Post Elution and Derivatization

Dry the extract in a stream of air or nitrogen using a TurboVap (1.2 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 EVOLUTE® EXPRESS ABN protocol demonstrated analyte recoveries of 90% and 92% from urine for the carboxy-THC-D₉ and carboxy-THC respectively, as shown in Figure 2. RSDs were lower than 10% (n=7).

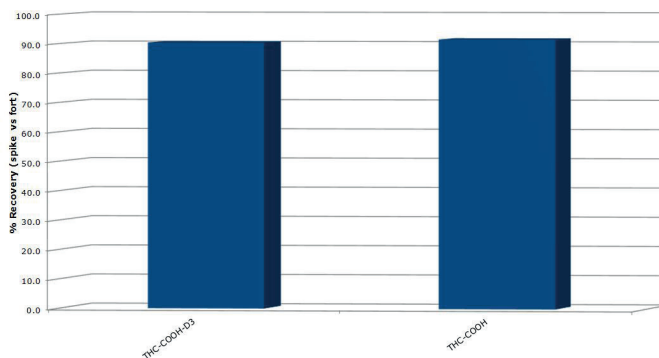


Figure 2. Analyte Recoveries.

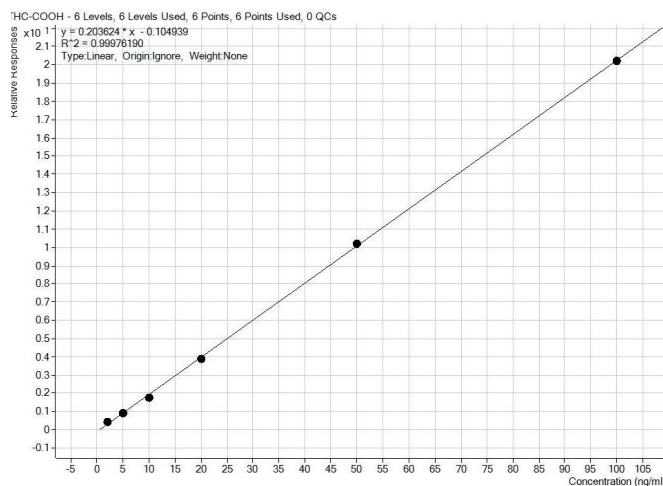


Figure 3. A calibration curve of carboxy-THC constructed following extractions using EVOLUTE® EXPRESS ABN with the optimal protocol. Analyte concentrations visible here are 2, 5, 10, 20, 50 and 100 ng/mL showing r^2 values of greater than 0.999. The carboxy-d₉ deuterated internal standard concentrations is at 20 ng/mL.

Signal to noise measurement shows LOQ to be 2 ng/mL.



Additional information

- » Sodium Hydroxide 10N is prepared with 40 g of pellets in 100 mL deionised 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 $\geq 99.85\%$

Ordering Information

Part Number	Description	Quantity
610-0006-BXG	EVOLUTE® EXPRESS ABN 60 mg/3 mL column	50
414001	Biotage® Extrahera™	1
415041	Configuration Kit 24 Positions, Dual Flow	1
415000	TurboVap® LV Evaporator	1

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