Extraction of 11-nor-9-carboxy-Δ^9-tetrahydrocannanbinol from Urine Using ISOLUTE® SLE+

Application Note

Introduction

This application note describes the extraction of THC and metabolites from urine using ISOLUTE SLE+ columns or plates.

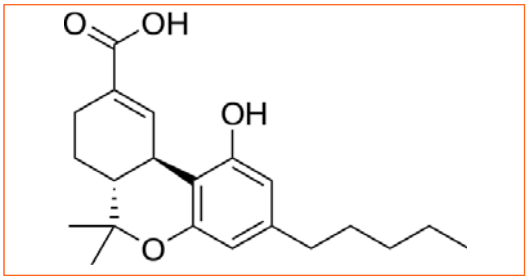

Figure 1. 11-nor-9-carboxy-Δ9-THC (THC-COOH)

Globally, cannabis is one of the most widely used illicit drugs. The naturally occurring cannabinoids found in hemp species bind to receptors in the brain and cause sensations of relaxation and calm. Widespread legislation against the use of cannabis has led to the necessity for rapid and reliable methods for the analysis and quantitation of cannabinoids and metabolites. The most prevalent marker in biological samples taken from cannabis abusers is 11-nor-9-carboxy-Δ^9-THC. Here we demonstrate a supported liquid extraction procedure for 11-nor-9-carboxy-Δ^9-THC.

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 time.

Analytes

11-nor-9-carboxy- Δ^9-THC (THC-COOH).

ISOLUTE SLE+ Procedure

ISOLUTE SLE+ configuration:	ISOLUTE SLE+ 1 mL sample volume, part number 820-0140-C.
Sample loading:	Load hydrolyzed urine* (750 µL) onto the column. Apply a short pulse of vacuum and then allow to flow under gravity for 5 minutes.
Analyte elution:	Elution 1: Apply 2 mL of dichloromethane and wait for 5 minutes. Elution 2: Apply 2 mL of methyl tert-butyl ether (MTBE), wait 5 minutes and apply a short pulse of vacuum.
Post extraction:	Evaporate eluate to dryness in stream of air without heat and reconstitute in 500 µL of acidified acetonitrile: acidified water (v/v, 70:30).
Reagents:	Acidified acetonitrile: Add 100 µL of concentrated formic acid to 100 mL acetonitrile. Acidified water: Add 100 µL of concentrated formic acid to 100 mL water.

*Hydrolyse 1 mL of urine by adding 500 µL of ammonium acetate buffer (100 mM) followed by 50 µL of β-glucuronidase (4500 U/mL). Heat to 37° C for 60 minutes. Other hydrolysis methods are available and suitable for this extraction process as long as the sample is loaded at sample ≤ pH 5.

For high throughput assays, methodology for use with 96-well ISOLUTE SLE+ plates is described below. Options are available for 100 µL or 200 µL sample sizes.

ISOLUTE SLE+ Procedure	Solution	ISOLUTE SLE+ 200 µL 96-well-plate part # 820-0200-P01	ISOLUTE SLE+ 400 µL 96-well-plate part # 820-0400-P01	ISOLUTE SLE+ 1 mL column part # 820-0140-C
Load sample	Hydrolyzed urine	150 µL	300 µL	750 µL
Leave for 5 minutes, followed by a short pulse of vacuum				
Analyte elution 1	Dichloromethane	500 µL	900 µL	2 mL
Leave for 5 minutes				
Analyte elution 2	MTBE	500 µL	900 µL	2 mL
Leave for 5 minutes, followed by a short pulse of vacuum				
Evaporate and reconstitute	Acidified ACN : Acidified Water (v/v, 70/30)	100 µL	100 µL	500 µL

HPLC Conditions

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).

Column: Acquity UPLC BEH C18 column (1.7µ, 100 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).

Flow rate: 0.3 mL/min

Mobile Phase: A: 0.1% formic acid aq and B: 0.1% formic acid in Methanol.

Gradient conditions:

Time	% A	% B
0	95	5
4	5	95
5.4	5	95
5.41	95	5
6.9	95	5

Injection Volume: 10 µL.

Column Temperature: 30 °C.

Mass Spectrometry Conditions

Instrument: Quattro Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. *Table 1.* shows the positive ions acquired in the multiple reaction monitoring (MRM) mode.

Desolvation Temperature: 450 °C.

Ion Source Temperature: 150 °C .

Collision Gas Pressure: 3.46 x 10^{-3} mbar.

Scan Function	MRM Transition	Cone Voltage (V)	Collision Energy (eV)
1	343.2 -> 299.1	40	21

Table 1. MRM transitions for 11-nor-9-carboxy- Δ^9-THC (THC-COOH).

Results

Recoveries above 85% (RSDs < 10 %, n=7) for THC-COOH were obtained using ISOLUTE SLE + 1 mL column as shown in Table 2. LOQ values of 2 ng/mL were obtained. Figure 1. shows an example chromatogram of extracted 11-nor-9-carboxy- Δ^9-THC (THC-COOH) at a concentration of 20 ng/mL.

Analyte	Mean % recovery	% RSD
11-nor-9-carboxy- Δ^9-THC (THC-COOH)	85.1	2.08

Table1. Mean % recovery of 11-nor-9-carboxy- Δ^9-THC (THC-COOH) using ISOLUTE SLE +

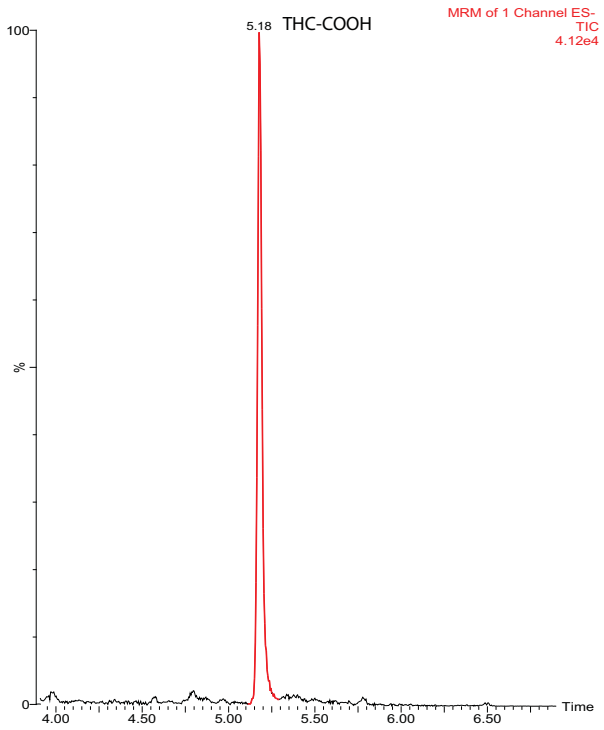

Figure 1. Example chromatogram of THC-COOH from urine at 20 ng/mL

References

Williams, L., et al, 2010, 'Extraction of THC and metabolites from Urine and Plasma using Supported Liquid Extraction (SLE) prior to UPLC-MS/MS Analysis', SOFT 2010, Richmond, VA.

Ordering information

Part number	Description	Quantity
820-0200-P01	ISOLUTE SLE+ 200 96-well-plate	1
820-0400-P01	ISOLUTE SLE+ 400 96-well-plate	1
820-0140-C	ISOLUTE SLE+ 1 mL column	30

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