

# APPLICATION

## Extraction of Urinary 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol by Novum® Simplified Liquid Extraction (SLE) and LC-MS/MS Analysis using a Kinetex® 2.6 $\mu$ m C8 HPLC/UHPLC Column

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### Overview

$\Delta^9$ -tetrahydrocannabinol (THC) is the most potent psychoactive compound in marijuana and in the human body THC is metabolized to form a hydroxylated compound and 11-nor-9-carboxy- $\Delta^9$ -THC (COOH-THC). The carboxylic form and the conjugated glucuronic acid are excreted in urine. 15-20% of a THC dose is eliminated as acidic urinary metabolites.

The aim of this application note is to introduce a fast and effective sample preparation technique using Novum Simplified Liquid Extraction (SLE) products that overcomes the challenges observed in a traditional liquid-liquid extraction (LLE).

### Sample Pretreatment Step Enzymatic Hydrolysis

- To 300  $\mu$ L urine add 75  $\mu$ L of 300 mM ammonium acetate (pH 4.0) and 25  $\mu$ L of  $\beta$ -Glucuronidase solution (100,000 units/mL, www.campbellscience.com, DR2100).
- Mix and vortex for 30 secs. Incubate at 37-40°C for 60 minutes. (Gentle shaking during this step is recommended). After incubation, bring samples to room temperature prior to extraction.

### Extraction Procedure Sample Loading

- Load the sample (~400  $\mu$ L) from pretreatment step onto the Novum SLE MAX plate (Part No. 8E-S138-5GA) and apply a short and gentle pulse of vacuum (~5-10 sec at 5" or less of Hg) until the sample has completely entered the media.

**NOTE: Avoid prolonged or excessive vacuum. The sample must not leave the Novum SLE bed.**

- Wait for 5-6 minutes.

**NOTE: Inadequate or excessive wait period will lead to variable recoveries and poor precision.**

### Elution

- Dispense 900  $\mu$ L of ethyl acetate onto the Novum SLE MAX plate and collect the solvent under force of gravity (approximately 5 min).
- Repeat with another 900  $\mu$ L addition of ethyl acetate and collect the solvent under gravity.
- Apply vacuum at 5" of Hg (or lower) for 20-30 secs to complete the extraction.

**NOTE: Excessive/prolonged application of the vacuum may force the elution of urine and cause dirty extraction**

### Dry Down

- Evaporate extracted samples to complete dryness under a slow stream of N<sub>2</sub> at room temperature.
- Reconstitute the dry residue in 200  $\mu$ L of initial mobile phase containing internal standard.

### HPLC Conditions

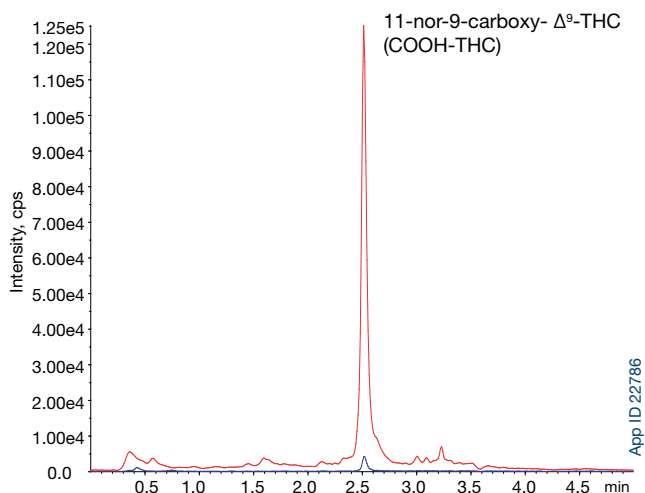
Column: Kinetex 2.6  $\mu$ m C8  
Dimensions: 50 x 2.1 mm  
Part No.: 00B-4497-AN  
Mobile Phase: A: 0.1 % Formic acid in water  
B: Methanol/Acetonitrile (50:50)  
Gradient: 

Time (min)	% B
0.00	50
2.5	95
3.5	95
5.0	50

  
Flow Rate: 0.5 mL/min  
Injection Volume: 20  $\mu$ L  
Temperature: Ambient  
Detection: MS/MS, API 5000™ (SCIEX), ESI+

### MS/MS Conditions

CUR: 25.00  
GS1: 50.00  
GS2: 50.00  
TEM: 600.00  
CAD: 7.00  
IS: 5500.00  
DP: 55.00



Analyte	RT, min	Q1, Da	Q3, Da	CE
11-nor-9-carboxy- $\Delta^9$ -THC (COOH-THC)	2.51	345.2	327.0	29
		345.2	299.2	49
11-nor-9-carboxy- $\Delta^9$ -THC-d3 (IS)	2.51	348.0	330.1	29

Analyte	% Absolute Recovery	%CV (N=8)
11-nor-9-carboxy- $\Delta^9$ -THC	83 %	3.5



# APPLICATION

## Ordering Information

### Novum<sup>®</sup> Simplified Liquid center (SLE)

Part No.	Description	Unit/Box
8E-S138-FGA	Novum SLE MINI 96-Well Plate	1/Box
8E-S138-5GA	Novum SLE MAX 96-Well Plate	1/Box

#### Australia

t: +61 (0)2-9428-6444  
 auinfo@phenomenex.com

#### Austria

t: +43 (0)1-319-1301  
 anfrage@phenomenex.com

#### Belgium

t: +32 (0)2 503 4015 (French)  
 t: +32 (0)2 511 8666 (Dutch)  
 beinfo@phenomenex.com

#### Canada

t: +1 (800) 543-3681  
 info@phenomenex.com

#### China

t: +86 400-606-8099  
 cninfo@phenomenex.com

#### Denmark

t: +45 4824 8048  
 nordicinfo@phenomenex.com

#### Finland

t: +358 (0)9 4789 0063  
 nordicinfo@phenomenex.com

#### France

t: +33 (0)1 30 09 21 10  
 franceinfo@phenomenex.com

#### Germany

t: +49 (0)6021-58830-0  
 anfrage@phenomenex.com

#### India

t: +91 (0)40-3012 2400  
 indiainfo@phenomenex.com

#### Ireland

t: +353 (0)1 247 5405  
 eireinfo@phenomenex.com

#### Italy

t: +39 051 6327511  
 italiainfo@phenomenex.com

#### Luxembourg

t: +31 (0)30-2418700  
 nlinfo@phenomenex.com

#### Mexico

t: 01-800-844-5226  
 tecnicomx@phenomenex.com

#### The Netherlands

t: +31 (0)30-2418700  
 nlinfo@phenomenex.com

#### New Zealand

t: +64 (0)9-4780951  
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#### Norway

t: +47 810 02 005  
 nordicinfo@phenomenex.com

#### Poland

t: +48 (12) 881 0121  
 pl-info@phenomenex.com

#### Portugal

t: +351 221 450 488  
 ptinfo@phenomenex.com

#### Singapore

t: +65 800-852-3944  
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#### Spain

t: +34 91-413-8613  
 espinfo@phenomenex.com

#### Sweden

t: +46 (0)8 611 6950  
 nordicinfo@phenomenex.com

#### Switzerland

t: +41 (0)61 692 20 20  
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#### Taiwan

t: +886 (0) 0801-49-1246  
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#### United Kingdom

t: +44 (0)1625-501367  
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#### USA

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 info@phenomenex.com

#### ☎ All other countries/regions Corporate Office USA

t: +1 (310) 212-0555  
 info@phenomenex.com

## Accessories

### Collection Plates (deep well, polypropylene)

		Unit
AHO-7192	96-Well Collection Plate, 350 µL/well	50/pk
AHO-7193	96-Well Collection Plate, 1 mL/well	50/pk
AHO-7194	96-Well Collection Plate, 2 mL/well	50/pk
AHO-8635	96-Well Collection Plate, 2 mL Square/Round-Conical	50/pk
AHO-8636	96-Well Collection Plate, 2 mL Round/Round, 8 mm	50/pk
AHO-7279	96-Well Collection Plate, 1 mL/well Round, 7 mm	50/pk

### Sealing Mats

AHO-8597	Sealing Mats, Pierceable, 96-Square Well, Silicone	50/pk
AHO-8598	Sealing Mats, Pre-Slit, 96-Square Well, Silicone	50/pk
AHO-8631	Sealing Mats, Pierceable, 96-Round Well 7 mm, Silicone	50/pk
AHO-8632	Sealing Mats, Pre-Slit, 96-Round Well 7 mm, Silicone	50/pk
AHO-8633	Sealing Mats, Pierceable, 96-Round Well 8 mm, Silicone	50/pk
AHO-8634	Sealing Mats, Pre-Slit, 96-Round Well 8 mm, Silicone	50/pk
AHO-7362	Sealing Tape Pad	10/pk

### Vacuum Manifold

AHO-8950	96-Well Plate Manifold, Universal with Vacuum Gauge	ea
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