

APPLICATION

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

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Overview

Marijuana is one of the most popularly used drugs in the world both for its medicinal and recreational use. Δ^9 -tetrahydrocannabinol (THC) is the most potent psychoactive compound in marijuana and is banned in many countries. In the human body THC is metabolized to form a hydroxylated compound and 11-nor-9-carboxy- Δ^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 μ L urine add 75 μ L of 300 mM ammonium acetate (pH 4.0) and 25 μ L of β -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 μ 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 μ 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 μ 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/Prolong 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_2 at room temperature.
- Reconstitute the dry residue in 200 μ L of initial mobile phase containing internal standard.

HPLC Conditions

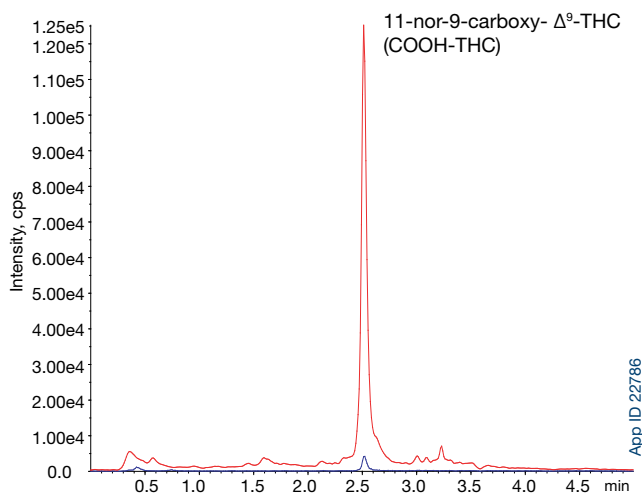
Column: Kinetex 2.6 μ m C8
Dimensions: 50 x 2.1 mm
Part No.: 00B-4497-AN
Mobile Phase: A: 0.1 % Formic acid in water
B: Methanol/Acetonitrile (50:50)
Gradient:

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

Flow Rate: 0.5 mL/min
Injection Volume: 20 μ L
Temperature: Ambient
Detection: MS/MS, API 5000™ (AB 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- Δ^9 -THC (COOH-THC)	2.51	345.2	327.0	29
		345.2	299.2	49
11-nor-9-carboxy- Δ^9 -THC-d3 (IS)	2.51	348.0	330.1	29

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



APPLICATION

Ordering Information

Novum™ Simplified Liquid Extraction (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

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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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Disclaimer

Novum is patent pending.

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