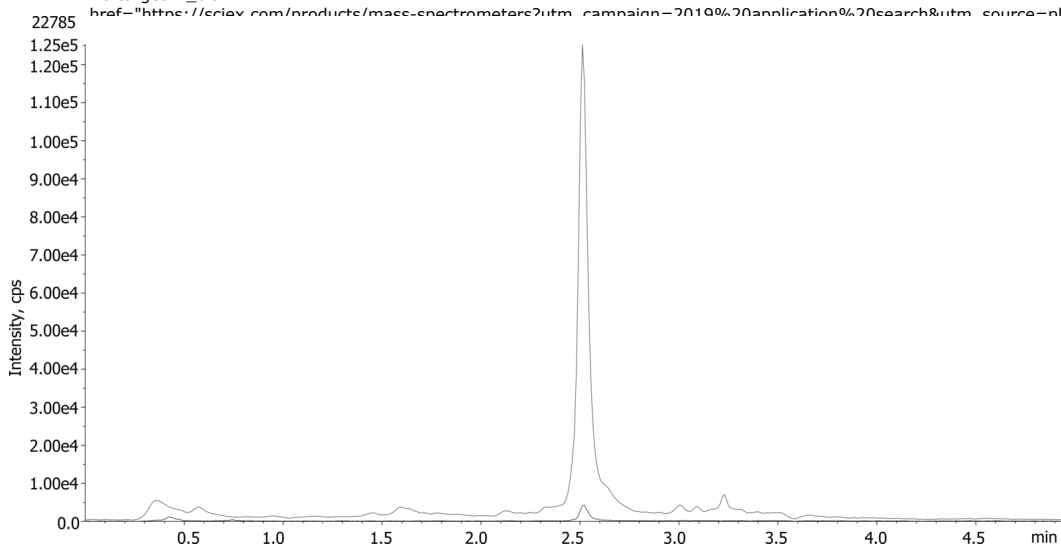


Extraction of COOH-THC from Urine Using Novum SLE & Kinetex 2.6u C8**Column:** Kinetex® 2.6 µm C8 100 Å, LC Column 50 x 2.1 mm, Ea**Dimensions:** 50 x 2.1 mm ID**Order No:** 00B-4497-AN**Elution Type:** Gradient**Eluent A:** 0.1% formic acid**Eluent B:** Methanol/acetonitrile(50:50)

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	50	50
	2	2.5	5	95
	3	3.5	5	95
	4	5	50	50

Flow Rate: 0.5 mL/min**Col. Temp.:** 25 °C**Detection:** Tandem Mass Spec (MS-MS) @ (ambient)**Detector Info:** <a target="_blank"**Products used in this application:****ANALYTES:**

- 1 11-nor-9-carboxy-delta9-THC
Retention Time: 2.51 min
- 2 11-nor-9-carboxy-delta9-THC-d3 (IS)
Retention Time: 2.51 min



Sample Preparation Details

for HPLC Application ID No.: 22786

Extraction of COOH-THC from Urine Using Novum SLE & Kinetex 2.6u C8

PRODUCT DESCRIPTION:

Novum SLE MAX 96-Well Plate, 1/Pk

Order No.: 8E-S138-5GA

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 400 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Enzymatic Hydrolysis: To 300 uL urine add 75 uL of 300 mM ammonium acetate (pH 4.0) and 25 uL of -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.

Wash:

Dry:

Elute:

Final Prep and Analysis:

Sample Pretreatment Step

Enzymatic Hydrolysis: To 300 uL urine add 75 uL of 300 mM ammonium acetate (pH 4.0) and 25

Inject: 20 µL on HPLC Tandem Mass Spec (MS-MS) @ (ambient)

ANALYTES:		Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1	11-nor-9-carboxy-delta9-THC	20			83	
2	11-nor-9-carboxy-delta9-THC-d3 (IS)					

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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