

## 3-Hydroxycotinine using SPE with Gemini NX-C18, LC-MSMS (Fig.3d)

**Column:** Gemini® 3 µm NX-C18 110 Å, LC Column 50 x 2 mm, Ea

**Dimensions:** 50 x 2 mm ID

**Order No:** 00B-4453-B0

**Elution Type:** Gradient

**Eluent A:** 20mM Ammonium Bicarbonate

**Eluent B:** 100% Acetonitrile

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	90	10
	2	3	25	75
	3	3.1	90	10
	4	5	90	10

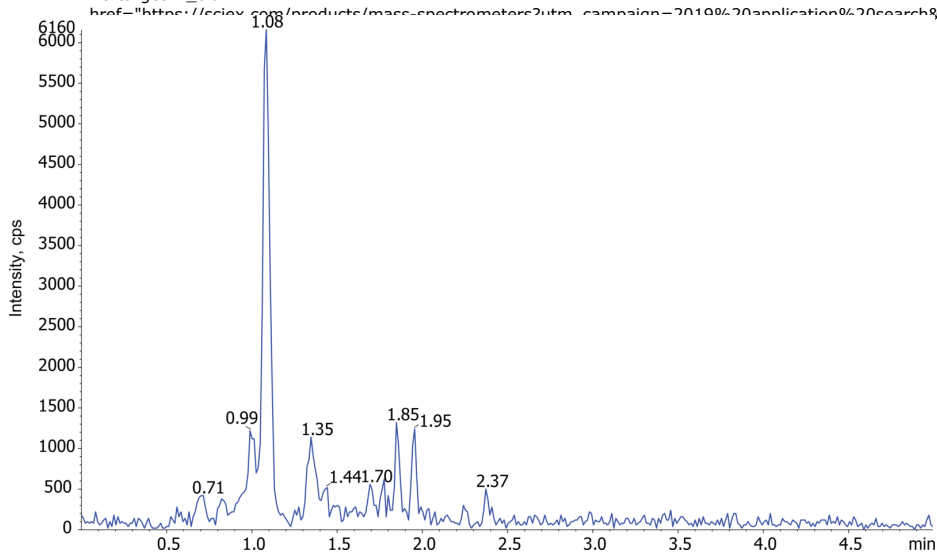
**Flow Rate:** 500 µL/min

**Col. Temp.:** 25 °C

**Detection:** Tandem Mass Spec (MS-MS) @ (ambient)

**Detector Info:**

<a target="\_blank" href="https://sciex.com/products/mass-spectrometers?utm\_campaign=2019%20application%20search&utm\_source=phenomenex&utm\_medium=referral">SCIEX</a>



### ANALYTES:

- 1 Nornicotine  
Retention Time: 1.09 min
- 2 3-OH-Cotinine  
Retention Time: 1.16 min
- 3 Anabasine  
Retention Time: 1.71 min
- 4 Cotinine  
Retention Time: 1.73 min
- 5 Nicotine  
Retention Time: 2.31 min



Products used in this application:



# Sample Preparation Details

for HPLC Application ID No.: 22039

## 3-Hydroxycotinine using SPE with Gemini NX-C18, LC-MSMS (Fig.3d)

### PRODUCT DESCRIPTION:

Strata™-X-C 33 µm Polymeric Strong Cation, 60 mg / 3 mL, Tubes , 50/Pk

Order No.: 8B-S029-UBJ

### SOLID PHASE EXTRACTION (SPE) PROCEDURE:

**Note:** The solvent volumes shown below are for a 60 mg bed mass.

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

#### Condition:

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#### Load:

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#### Wash:

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#### Dry:

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> 10" Hg for 5 min to remove residual water

#### Elute:

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#### Final Prep and Analysis:

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Reconstitution Solvent: 500µL Acetonitrile/20mM Ammonium bicarbonate (10:90)

Inject: 10 µL on HPLC Tandem Mass Spec (MS-MS) @ (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Nornicotine	0				
2 3-OH-Cotinine	0				
3 Anabasine	0				
4 Cotinine	0				
5 Nicotine	0				

**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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