## **HPLC Application**

ID No.: 22039



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



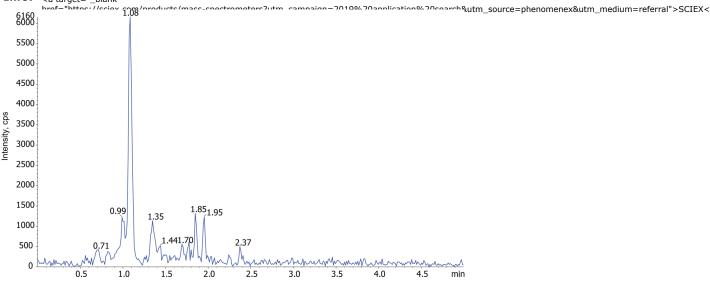
Products used in this application:



Flow Rate:  $500 \mu L/min$  Col. Temp.:  $25 \, ^{\circ}C$ 

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

**Detector Info:** <a target="\_blank"



### **ANALYTES:**

1 Nornicotine

Retention Time: 1.09 min 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

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# Sample Preparation Details for HPLC Application ID No.: 22039



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

### **PRODUCT DESCRIPTION:**

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

Order No.: 8B-S029-UBJ

### **SOLID PHASE EXTRACTION (SPE) PRODCEDURE:**

**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:	
Load:	
Wash:	
Dry:	
> 10" Hg for 5 min to remove residual water	
Elute:	
Final Prep and Analysis:	
Reconstitution Solvent: 500uL Acetonitrile/20mM Ammonium bicarbonate (10:90)	_
Inject: 10 ul. on HPI C 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				

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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