HPLC Application

ID No.: 22037



Cotinine using SPE with Gemini NX-C18, LC-MSMS (Fig.3b)

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

50 x 2 mm ID **Dimensions:** 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

Flow Rate: 500 μL/min 25 °C Col. Temp.:

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

Detector Info:



Products used in this application:



SCIEX< 2.0e4 1.9e4 1.8e4 1.7e4 1.6e4 1.5e4 1.4e4 1.3e4 1.2e4 1.1e4 1.0e4 9000.0 8000.0 7000.0 0.80 6000.0 5000.0 4000.0 3000.0 2000.0 1000.0 0.0 0.5 1.0 2.0 3.0 4.0 3.5 4.5 min

ANALYTES:

Nornicotine

Retention Time: 1.09 min

3-OH-Cotinine

Retention Time: 1.16 min

Anabasine

Retention Time: 1.71 min

Cotinine

Retention Time: 1.73 min

Nicotine

Retention Time: 2.31 min

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Sample Preparation Details

for HPLC Application ID No.: 22037



Cotinine using SPE with Gemini NX-C18, LC-MSMS (Fig.3b)

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

ANAL	YTES:	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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