HPLC Application

ID No.: **22037**

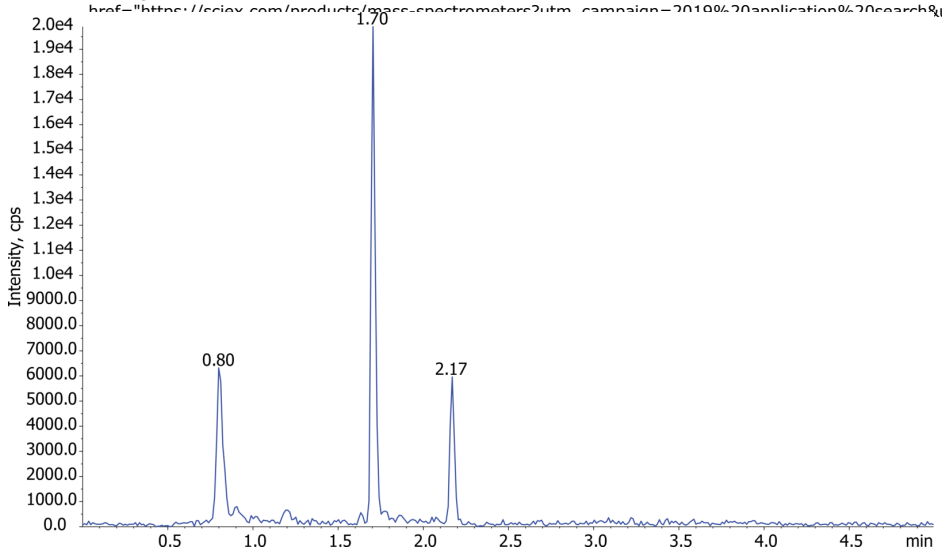
Cotinine using SPE with Gemini NX-C18, LC-MSMS (Fig.3b)

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<,



Products used in this application:

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

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)

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.*