HPLC Application

ID No.: **19734**



Nicotine and its Metabolites in Urine By LC/MS/MS Using Strata-X-C and Gemini-NX C18

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

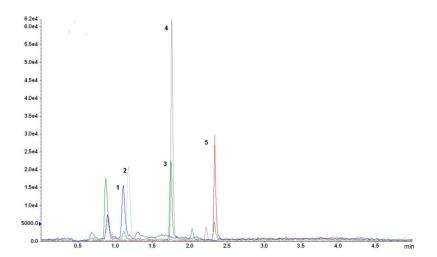
Flow Rate: 500 25 °C Col. Temp.:

Circular Dichroism @ 0.000000000 (550 °C) **Detection:** SecurityGuard™ Guard Cartridge System extends column lifetime. Analyst Note:

- SecurityGuard Cartridges, Gemini-NX C18 4 x 2.0mm ID, 10/Pk Part No.: AJ0-8367

- Holder Part No.: KJ0-4282

19734



ANALYTES:

Nornicotine

Retention Time: 1 min 3-Hydroxycotinine Retention Time: 1.16 min

Anabasine

Retention Time: 1.68 min

Cotinine

Retention Time: 1.73 min

Nicotine

Retention Time: 2.31 min



Products used in this application:



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Sample Preparation Details

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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:	
5min at full vacuum	
Elute:	
Final Prep and Analysis:	

Inject: 10 µL on HPLC Circular Dichroism @ 0.000000000 (550°C)

ANALYTES:		Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1	Nornicotine	4			101.5	
2	3-Hydroxycotinine	4			103.1	
3	Anabasine	4			97.03	
4	Cotinine	4			94.8	
5	Nicotine	4			96.13	

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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