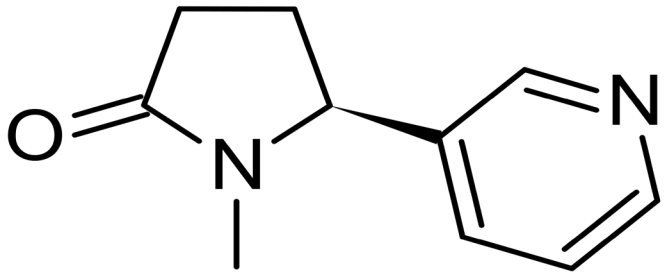
Extraction and Analysis of Nicotine, Cotinine and Anabasine from Urine using SPE and GC-MS



UCT Part Numbers

CSDAU206 Clean Screen DAU, 200mg / 6 mL tube	**SPHPHO6001-5** Select pH Buffer Pouch, phosphate buffer pH 6

Procedure:

1. Sample Preparation

a) Add 1mL of pH 6 phosphate buffer (0.1 M) into a clean sample tube and add internal standards.
b) Add 1 mL of urine and mix.
c) 2 mL of 0.1 M pH 6 phosphate buffer and mix.
d) Centrifuge for 10 minutes at 3000 rpm.

2. SPE Column Conditioning

a) 1 x 3 mL CH_3OH.
b) 1 x 3 mL D.I. H_2O.
c) 1 x 1 mL 0.1 M phosphate buffer (pH 6.0).

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

a) Load sample at 1 to 2 mL/minute.

4. Wash Column

a) 1 x 3 mL D.I. H_2O.
b) 1 x 3 mL 1 M Acetic Acid
c) 1 x 3 mL Methanol
d) Dry column (5 minutes at > 10 inches Hg or full flow on a positive pressure manifold).

5. Elute Nicotine, Cotinine and Anabasine

a) Add 1 x 3 mL Methylene chloride: Isopropanol: ammonium hydroxide (78:20:2).

Note: Prepare a fresh solution of the Methylene chloride: Isopropanol: ammonium hydroxide mixture by adding 2 mL of ammonium hydroxide to 20 mL of isopropanol. Mix and add 78 mL of methylene chloride.

b) Collect eluate at a rate of 1 to 2 mL / minute.

6. Dry Eluate

a) Evaporate to dryness under nitrogen < 35°C.
b) Reconstitute with 100 µL of methanol and vortex mix.
c) Transfer to an autosampler vial containing a low volume insert (150 µL)

FORENSICS

UCT

Instrument Conditions	
Instrumentation	ThermoScientific Trace 1300 GC TriPlus RSH autosampler with a 10 µL syringe
Column	TG-1MS 30 m x 0.25 mm (0.25 um df)
Carrier Gas	He, Ultrahigh purity
Flow Rate	1.2 mL/ minute
Split Flow	12 mL/ minute
Split Flow Ratio	10:1
Injector Temp	250°C
Septum Purge Flow	5.0 mL/ minute
Transfer Line Temp	280 °C
Injection Volume	1uL
Oven Run Program	
Initial Temp	50°C
Initial Hold Time	0.5 minute
Temperature Program	30°C / minute
Final Temperature	320°C
Final Hold Time	0.5 minute

Mass Spectrometry	
Detector	Thermo Scientific ISQ
Transfer Line	280°C
Ion Source Temperature	300°C
Ionization Mode	EI^+
Mode	Selective Ion Mode (SIM)
Data Analysis	Xcaliber version 2.2 SPI 2
Note: Quantification was performed on last saved autotune	

Mass Spec Table

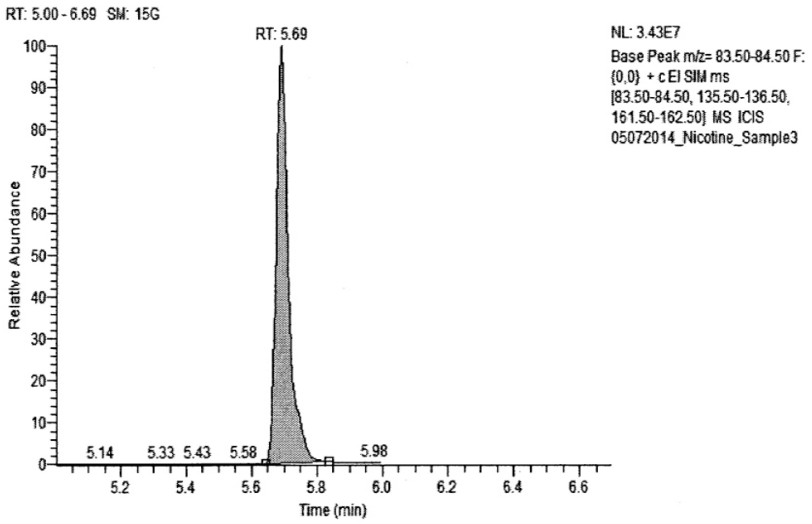
Compound	Retention Time (minute)	Primary Ion	Secondary Ion	Tertiary Ion
Nicotine	5.69	84*	136	162
Nicotine-d4	5.60	136	166*	
Cotinine	7.10	98*	119	176
Cotinine-d3	7.10	101*	122	
Anabasine	6.34	84*	105	133

*Selected monitoring ion

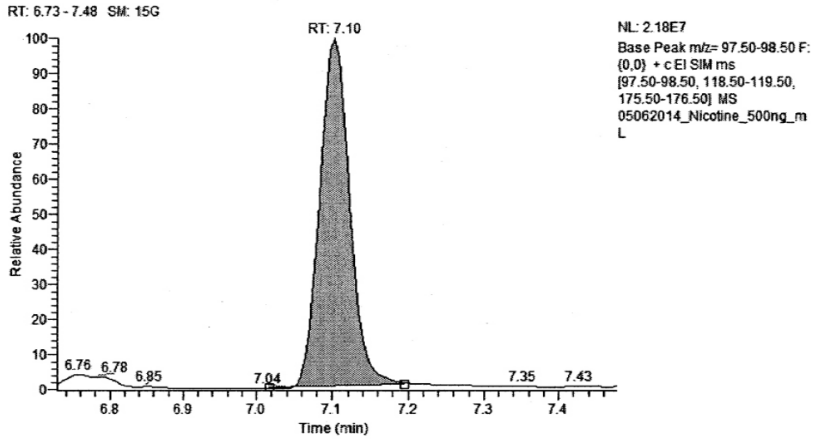
FORENSICS

UCT

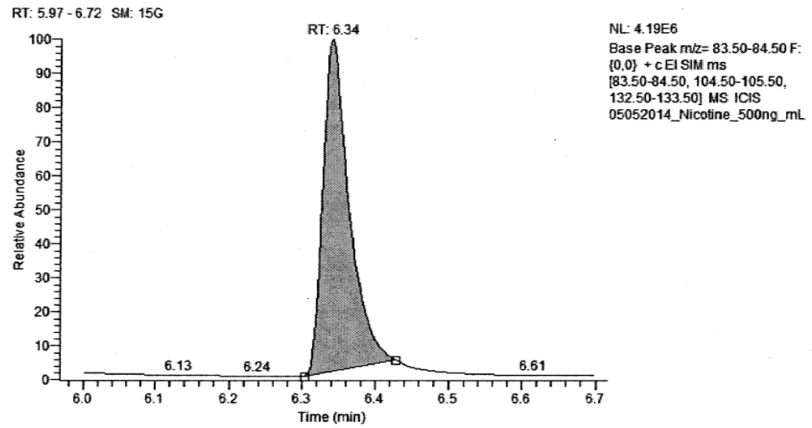
Chromatogram of Nicotine (500 ng / mL)



Chromatogram of Cotinine (500 ng / mL)



Chromatogram of Anabasine (500 ng / mL)



4107-01-01

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSICS

UCT