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

July 2014

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 0.1M 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) 1x 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)

Instrument Conditions

Instrumentation	ThermoScientific Trace 1300 GC TriPlus RSH autosampler with a 10 µL syringe	
Column	TG-1MS 30m x 0.25mm (0.25um df)	
Carrier Gas	He, Ultrahigh purity	
Flow Rate	1.2mL/ 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	0.5minute
	Temperature Program	30°C/minute
	Final Temperature	320°C
	Final Hold Time	0.5minute

Mass Spectrometry

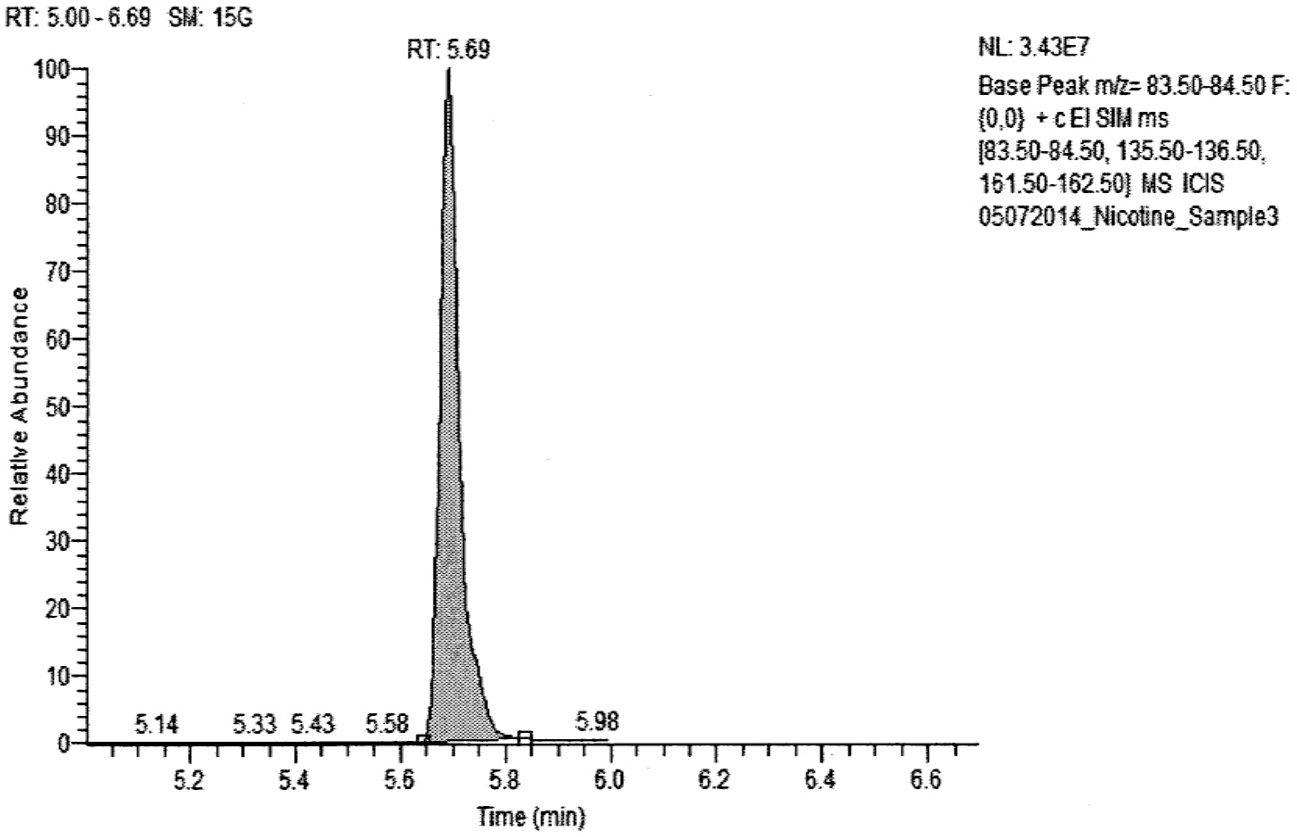
Detector	Thermo Scientific ISQ
Transfer Line	280°C
Ion Source Temperature	300°C
Ionization Mode	EI+
Mode	Selective Ion Mode (SIM)
Data Analysis	Xcaliber version 2.2 SPI 2
Note: Quantification was performed on last saved autotune	

Mass Spec Table

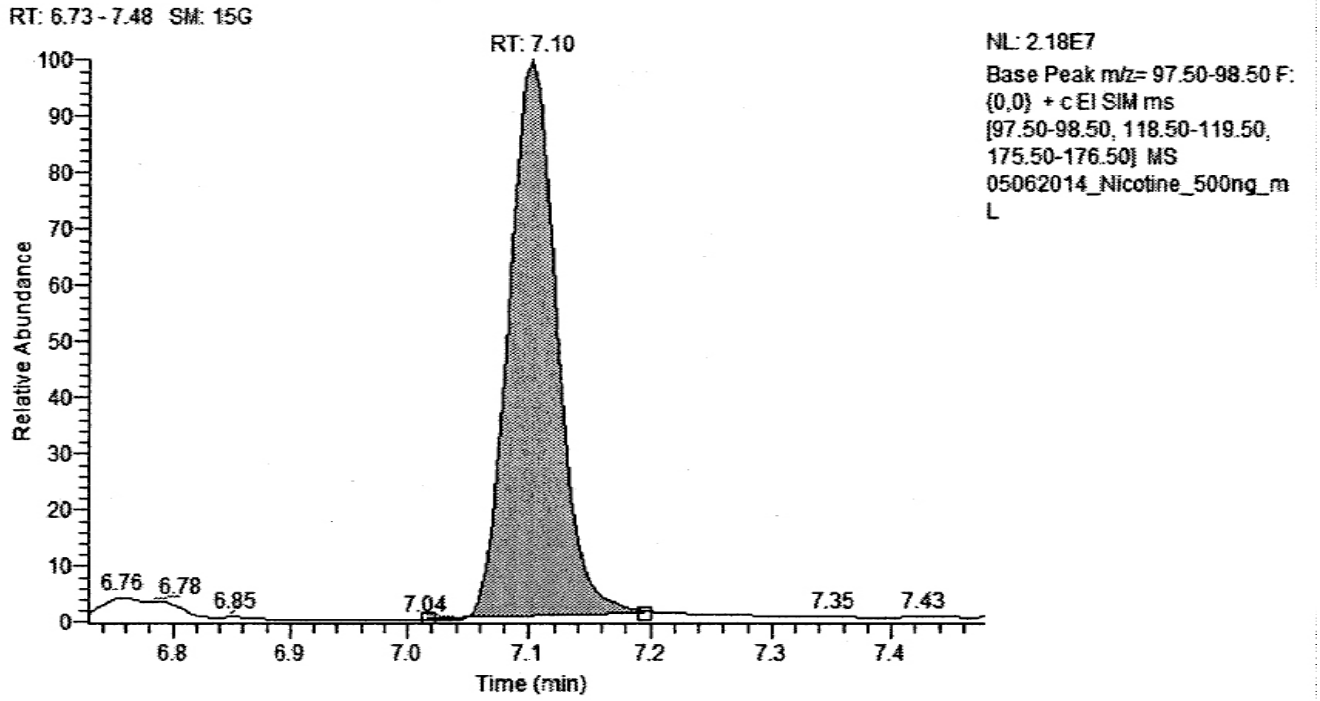
Compound	Retention Time (minute)	Primary Ion	Secondary Ion	Tertiary Ion
Nicotine:	5.69	84*	136	162
Nicotine-d4:	5.60	136	166*	
Cotinine:	7.10	98*	119	176
Cotinine-d3:	7.10	101*	122	
Anabasine:	6.34	84*	105	133

*Selected monitoring ion

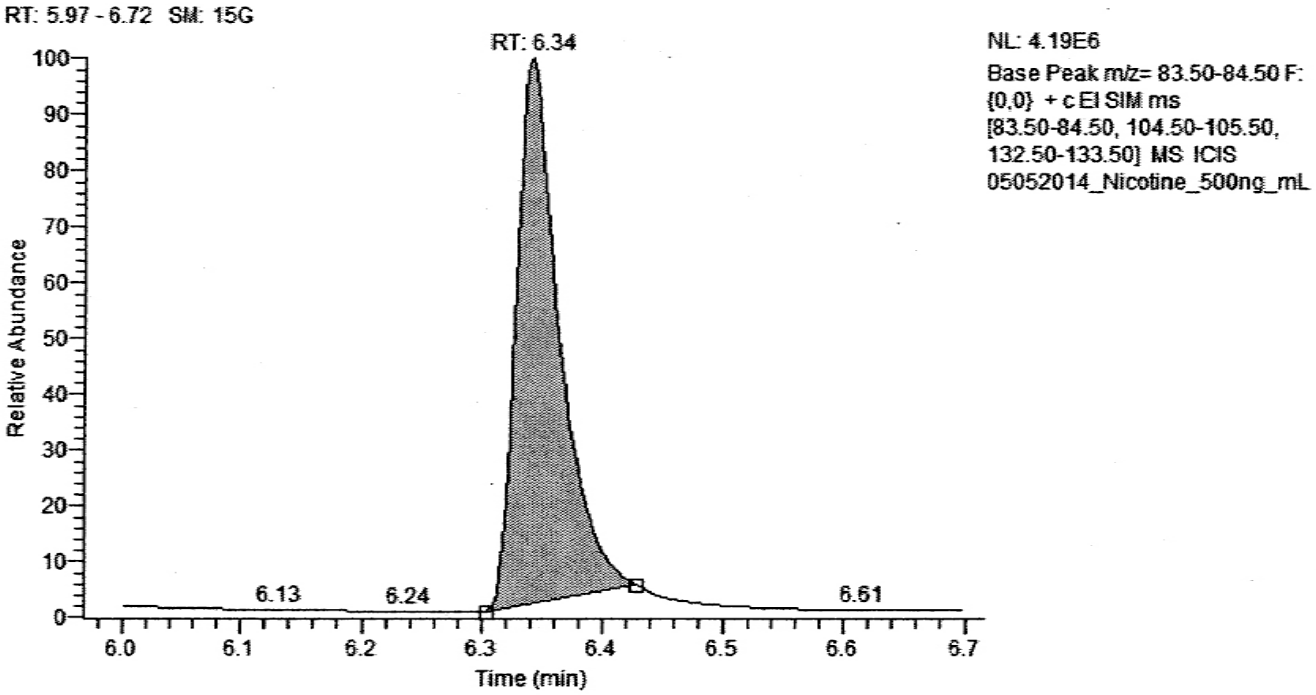
Chromatogram of Nicotine (500 ng / mL)



Chromatogram of Cotinine (500 ng / mL)



Chromatogram of Anabasine (500 ng / mL)



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