

## Overview

- ▶ **Purpose** – Develop an extraction method and GC-MS/MS method to quantify cotinine in human urine and plasma.
- ▶ **Methods** – SLE extraction and GC-MS/MS Analysis.
- ▶ **Results** – Range from 2.00 – 500 ng/mL with accuracies and precision better than  $\pm 15\%$  using GC-MS/MS.

## Introduction

Cotinine is the most abundant metabolite of nicotine and is used as the primary indicator for tobacco exposure. Cotinine is typically quantified in human biological matrices using HPLC-MS/MS and GC-MS. However, due to selectivity problems inherent with biological fluid analysis HPLC-MS/MS extraction methods usually require solid phase extraction in order to remove interfering compounds. Published GC-MS methods lack the sensitivity and selectivity requirements necessary to detect cotinine at low ng/mL levels present in those exposed to tobacco smoke. In this work we report a novel method to quantify cotinine in human plasma and urine utilizing a selective and sensitive GC-MS/MS method following a simple supported liquid-liquid exchange extraction (SLE). The method can be used to precisely track the level of tobacco smoke exposure for clinical research applications.

## Methods

### Urine Extraction

- ▶ Sample volume: 100  $\mu$ L
- ▶ Add 25  $\mu$ L Internal Standard (Cotinine-D<sub>3</sub>)
- ▶ Add 10  $\mu$ L 10% Ammonium Hydroxide
- ▶ Entire Volume is added to SLE Versatubes (Hydromatrix 260 mg, Agilent)
- ▶ Elute with 500  $\mu$ L of Ethyl Acetate/MTBE 1/1 Twice
- ▶ Evaporate under Nitrogen
- ▶ Reconstitute with 100  $\mu$ L of Methanol

### Plasma Extraction

- ▶ Sample volume: 100  $\mu$ L
- ▶ Add 25  $\mu$ L Internal Standard
- ▶ Add 10  $\mu$ L 10% Ammonium Hydroxide
- ▶ Precipitate with 500  $\mu$ L of Methanol
- ▶ Supernatant is added to SLE Versatubes (Hydromatrix 260 mg, Agilent)
- ▶ Elute with 500  $\mu$ L of Ethyl Acetate/MTBE 1/1 Twice
- ▶ Evaporate under Nitrogen
- ▶ Reconstitute with 100  $\mu$ L of Methanol

### Thermo Scientific Trace 1310 GC

- ▶ Carrier Gas: Helium 1.2 mL/min
- ▶ Oven: Initial 50°C ramped to 300°C at 42°C/min
- ▶ Splitless PTV Injector: Initial 65°C ramped to 295°C
- ▶ GC Column: TR-5MS (30m, 0.25mm ID and 0.25 $\mu$ m Film)

### Thermo Scientific MS

- ▶ TSQ Quantum XLS Ultra operating in SRM mode
- ▶ Electron Impact Ionization
- ▶ Positive Ion Mode
- ▶ SRM transitions:  
Cotinine: 176  $\rightarrow$  98  
Cotinine-D<sub>3</sub>: 179  $\rightarrow$  101

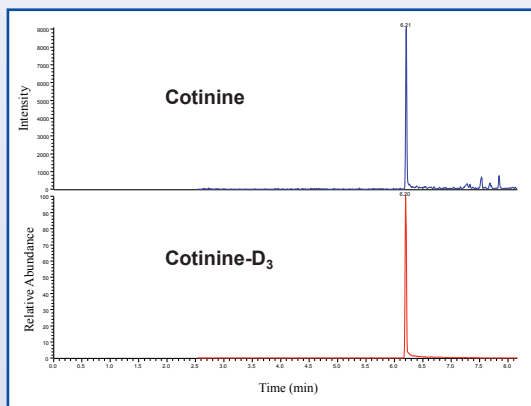


Figure 1: Figure 1: Extracted Human Urine (2.00 ng/mL)

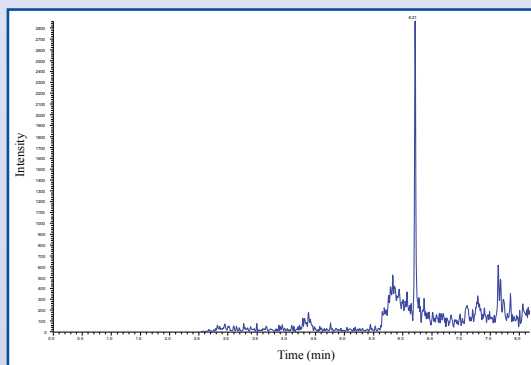


Figure 2: Cotinine in Extracted Human Plasma (2.00 ng/mL)

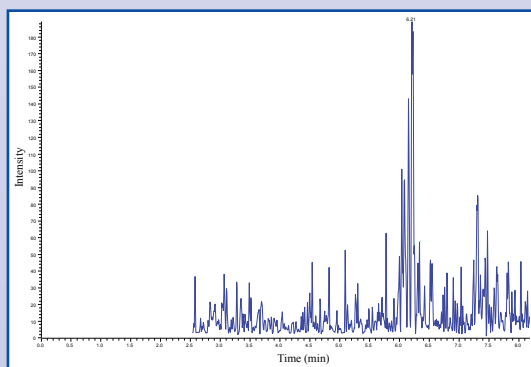


Figure 3: Cotinine in Extracted Blank Human Urine

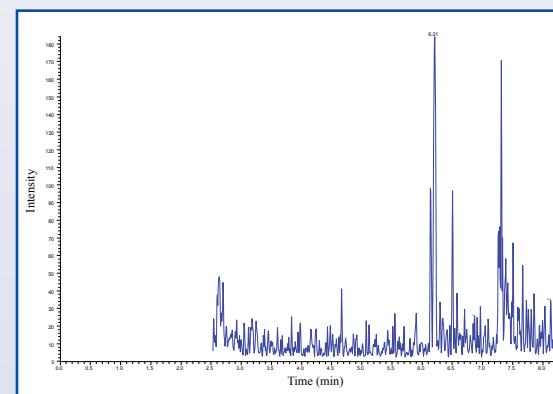


Figure 4: Cotinine in Extracted Blank Human Plasma

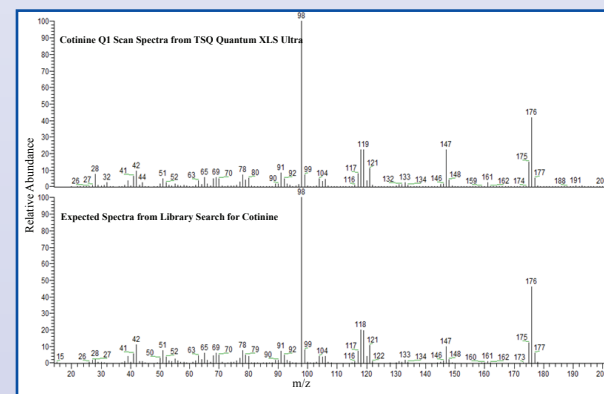


Figure 5: Cotinine Q1 Full Scan Comparison to Library

Table 1: Cotinine in Human Urine and Plasma Accuracy and Precision Data.

	LQC (6.00 ng/mL)	MQC (30.0 ng/mL)	HQC (400 ng/mL)
Accuracy/Precision Urine (%)	4.1 $\pm$ 6.8	2.2 $\pm$ 4.6	2.8 $\pm$ 3.9
Accuracy/Precision Plasma (%)	5.2 $\pm$ 2.9	2.6 $\pm$ 0.3	-3.1 $\pm$ 3.6

## Conclusions

- ▶ Developed an extraction and GC-MS/MS method for the determination of Cotinine in human urine and plasma.
- ▶ Method is accurate/precise and can be used to quantify cotinine down to 2.00 ng/mL.