

**Analysis of  
Tobacco Alkaloids  
using  
SOLID PHASE EXTRACTION**

**(UCT PART# CUBCX1HL2Z)**

*by*

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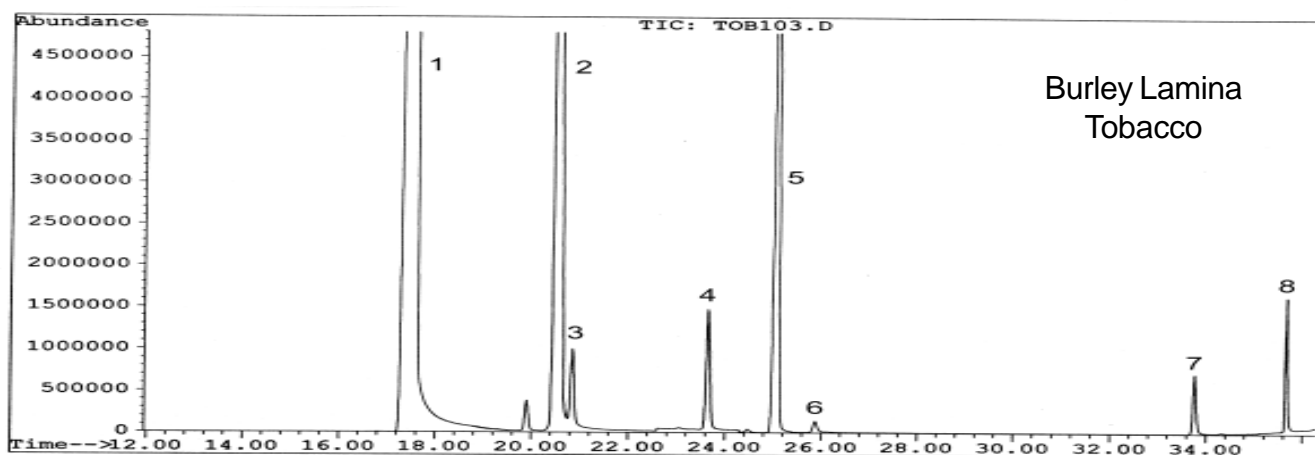
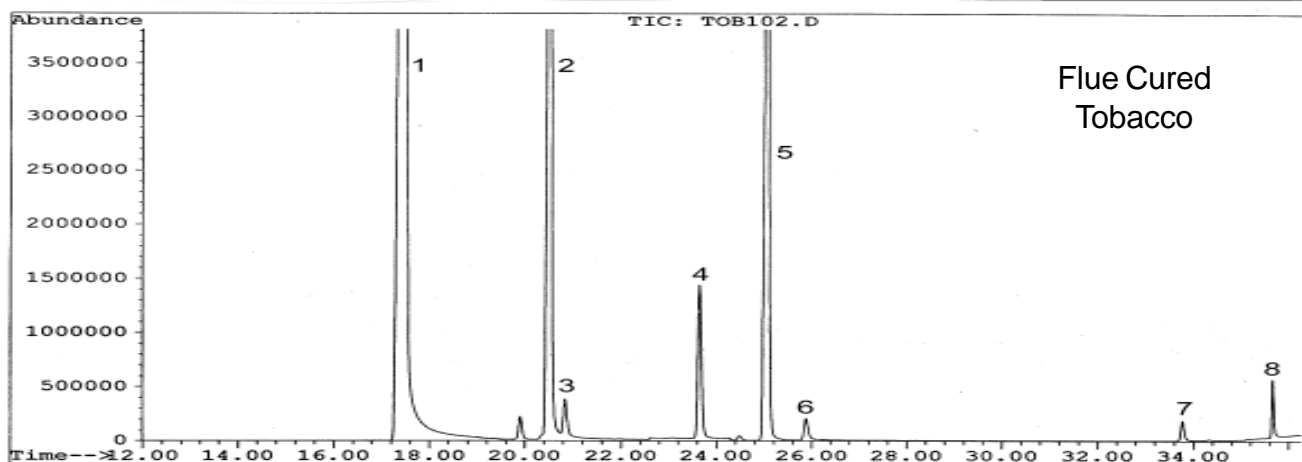
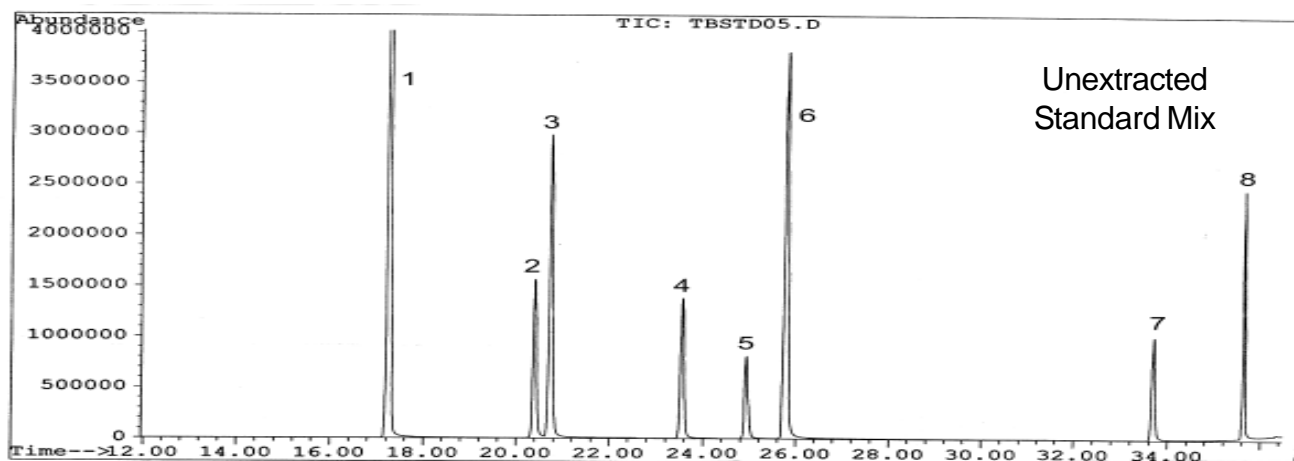
# INTRODUCTION

The analysis of alkaloids from tobacco plant material is a widely performed procedure throughout the tobacco industry. Sample prep typically involves extraction with dichloromethane in the presence of acid or base. This procedure can be labor intensive and often requires large volumes of organic solvent. We have developed a solid phase extraction (SPE) method for tobacco alkaloids that utilizes a strong cation exchanger silica based sorbent (**UCT part# CUBCX1HL2Z**). The procedure is relatively quick, utilizes a minimal volume of reagents and results in extremely clean extracts. Several tobacco alkaloids have been successfully extracted including nicotine, nornicotine, myosmine, anabasine, anatabine, 2,3-dipyridyl, cotinine and formylornicotine.

## SAMPLE PREP

1. To 0.1 gram tobacco, add 6 mL 0.1M sodium acetate buffer (pH 4.5) and 100  $\mu$ L internal standard (d4-nornicotine, 1  $\mu$ g/ $\mu$ L).
2. Mix on rotating shaker for 10 minutes, then filter extract through 20 micron frit filter column.
3. Add 300  $\mu$ L glacial acetic acid, mix.
5. Condition SPE column, **part# CUBCX1HL2Z** with 3 mL of MeOH:1.0M acetic acid (80:20).
6. Pour sample onto column, aspirate at 1-2 mL/min by vacuum.
7. Wash column with 3 mL of MeOH:1.0M acetic acid (80:20).
8. Dry column for 5-10 min with full vacuum.
9. Elute alkaloids with 3 mL CH<sub>2</sub>Cl<sub>2</sub>/isopropanol/NH<sub>4</sub>OH (70:26:4) by gravity.
10. Evaporate eluant to dryness with nitrogen and low heat (< 40° C).
11. Reconstitute with 200  $\mu$ L ethyl acetate.
12. Analyze on GC/FID/NPD or GC/MSD.

# GC/MSD CHROMATOGRAMS



(1) nicotine, (2) nor nicotine, (3) myosmine, (4) anabasine,  
 (5) anatabine, (6) 2,3'-dipyridyl, (7) cotinine, (8) formyl nor nicotine

Instrument: Agilent 5890GC/5971MSD  
 GC column: Rtx-5 Amine, 30 m x 0.25 mm i.d. x 1.0  $\mu$ m film  
 Injector: 1  $\mu$ L sample at 10:1 split, 250 $^{\circ}$ C  
 Temp program: Initial 120 $^{\circ}$ C, hold 1 min, ramp 2.5 $^{\circ}$ C/min to 200 $^{\circ}$ C,  
 ramp 20 $^{\circ}$ C/min to 280 $^{\circ}$ C, hold 1 min.  
 MSD conditions: SIM monitoring, EI mode, 295 $^{\circ}$ C

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