



Analysis of Tobacco Alkaloids

UCT Part Number:

EUBCX1HL2Z (200 mg benzenesulfonic acid high load, 10 ml)

October 2012

Tobacco alkaloids are extracted with a strong cation exchange sorbent using an acidic buffer, filtered and further acidified. Extraneous compounds are removed by washing the sorbent, yielding clean chromatography without loss of target analytes.

Procedure

1. Sample Preparation

- a) Weigh 100 mg of finely ground tobacco in a screw cap vial
- b) Add 6 mL 0.1M sodium acetate buffer (pH 4.5) and 100 μ L IS (d4- nornicotine, 1 μ g/ μ L)
- c) Mix on rotating shaker for 10 minutes
- d) Filter extract through 20 micron frit filter column
- e) Add 300 μ L glacial acetic acid and mix

2. Sample Extraction

- a) Condition column **EUBCX1H2Z** with 3 mL of MeOH:1.0M acetic acid (80:20)
- b) Pour sample onto column, draw through at 1-2 mL/min
- c) Wash column with 3 mL of MeOH:1.0M acetic acid (80:20)
- d) Dry column for 5-10 min using full vacuum

3. Elution

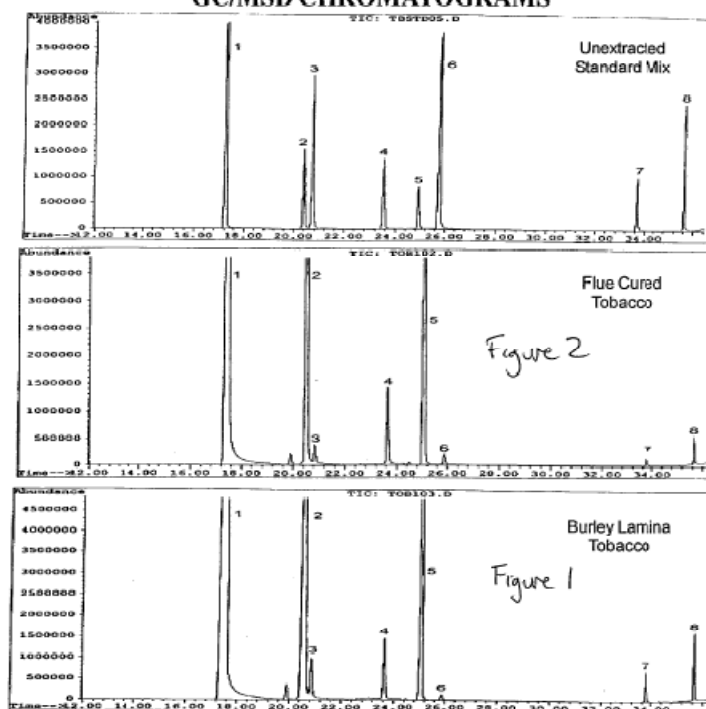
- a) Elute alkaloids with 3 mL CH_2Cl_2 /isopropanol/ NH_4OH (70:26:4) by gravity
- b) Evaporate eluant to dryness with N_2 and low heat ($< 40^\circ \text{C}$)
- c) Reconstitute with 200 μ L ethyl acetate
- d) Analyze on GC/FID/NPD or GC/MSD

Results

Alkaloid	pKa	Flue Cured Tobacco mg/gram/CV	Burley Lamina Tobacco mg/gram/CV
	n=15/mean		
Mysomine	NA	48/6.2	189/7.9
Nicotine	7.94	39406/6.2	39119/8.8
Nornicotine	9.46	1381/3.5	5429/5.2
Anatasine	9.20	229/5.2	183/8.7
Anatabine	8.23	1932/2.3	1774/2.3
2,3'-dipyridyl	4.25	54/8.9	30/11.2
Cotinine	4.88	20/11.2	52/12.4
Formylornicotine	NA	31/11.9	145/12.4

The average of 15 separate runs show excellent reproducibility

GC/MSD CHROMATOGRAMS



(1) nicotine, (2) nornicotine, (3) mysomine, (4) anabasine,
(5) anatabine, (6) 2,3'-dipyridyl, (7) cotinine, (8) formylornicotine

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

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