



Analysis of Tobacco Alkaloids

Part #: **EUBCX1HL2Z** (200 mg benzenesulfonic acid high load, 10 ml)

October 2012

Tobacco alkaloids are extracted using an acidic buffer, filtered and further acidified. Alkaloids are then extracted with a strong cation exchange sorbent while 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- nor nicotine, 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

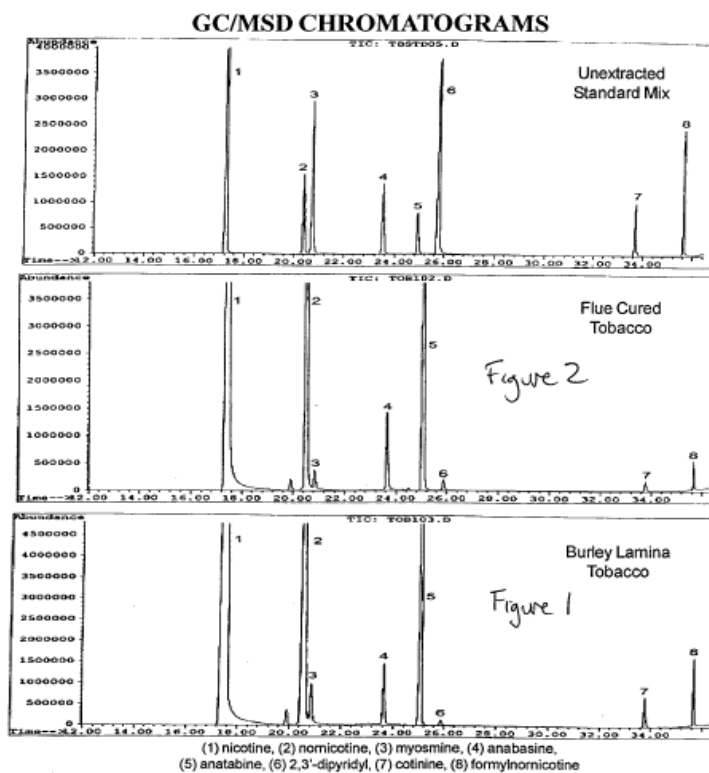
3. Elution

- 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	Burley Lamina Tobacco
	n=15/mean	mg/gram/CV	mg/gram/CV
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
Formylnornicotine	NA	31/11.9	145/12.4

The average of 15 separate runs show excellent reproducibility



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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