Advantages of GC-QQQ for the Analysis of Tobacco Alkaloids in Cigarette Filler

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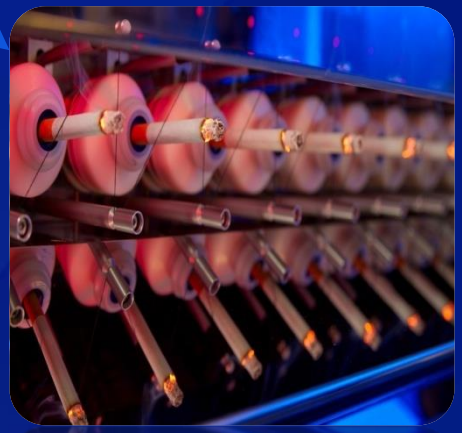
Tobacco Analysis Lab

Tobacco

- HPHCs (Harmful and Potentially Harmful Constituents) – compounds that are toxic and/or carcinogenic
- Nicotine, TSNA's, PAH's, Metals, Flavors, Volatiles, Ammonia, TNCO



Smoke

- All Types of Products: Cigarettes, Cigars, Cigarillos, Roll-Your-Own, Smokeless Product, e-Cigarettes, etc.

Exposure

- Topography: How people use the products

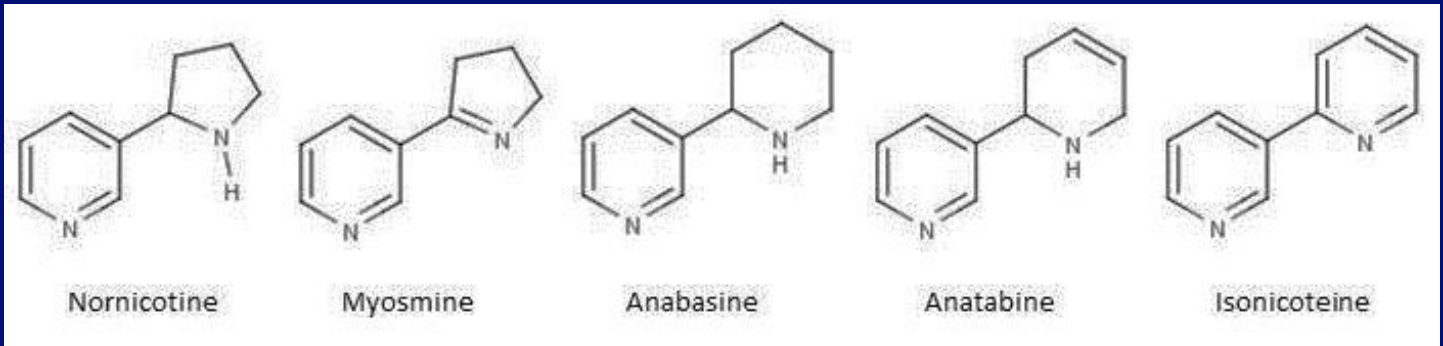
Tobacco and the Tobacco Matrix

- Tobacco use is the leading cause of preventable death worldwide – 5 million deaths annually

- The tobacco matrix is chemically complex and diverse.
- Approximately 4200 components have been found in tobacco – not including additives like flavors.
- Many factors affect the tobacco matrix – curing method, growing conditions, fertilizers, pesticides.
- Many classes of important compounds exist in the tobacco matrix: Metals, hydrocarbons, esters, lactones…..etc.

What are the minor tobacco alkaloids?

- **Naturally occurring compounds in tobacco**
- **Nicotine-like in structure**
- **Make up 5% of total alkaloids in *N. tabacum***
  - nicotine accounts for 95%
- **Biologically active in animals as well as humans**
- **Play a role in addiction and in carcinogenic TSNA formation**



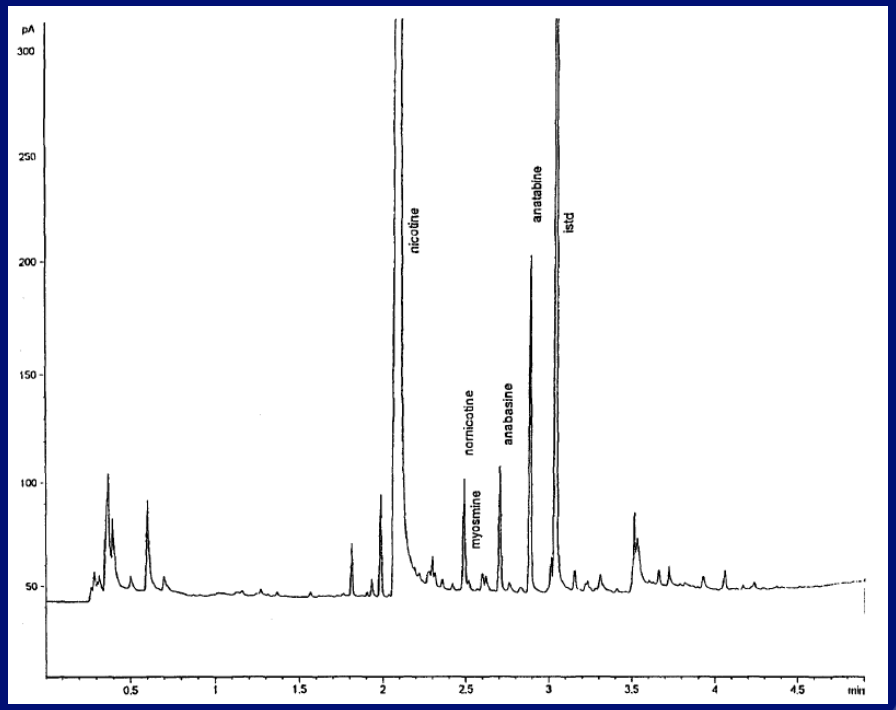
- **Literature reported methods included GC-NPD, GC-FID, GC-MS and HPLC-MS**

Method 1: GC-NPD

- **Non-specific detection**
- **A matrix effect was observed despite using ITSD.**
- **Solid-Phase Microextraction (SPME) used for "pre-concentration" and injection**
  - Partition coefficients changed with organic content
  - The SPME fiber gave variable results as it aged
  - Precision (RSD%) was 5-8%
  - Recovery was >94% for nicotine (other analytes not tested)

	Alkaloids/Internal Standard (peak ratio)	
Alkaloids	standards	spiked tobacco
Nornicotine	0.047	0.041
Myosmine	0.142	0.137
Anabasine	0.084	0.089

	Alkaloids/Internal Standard (peak ratio)			
	new fiber		aged fiber	
(Concentration, μg mL^{-1})	10	20	10	20
Nornicotine	0.099	0.194	0.083	0.184
Myosmine	0.286	0.535	0.286	0.531
Anabasine	0.168	0.325	0.147	0.298

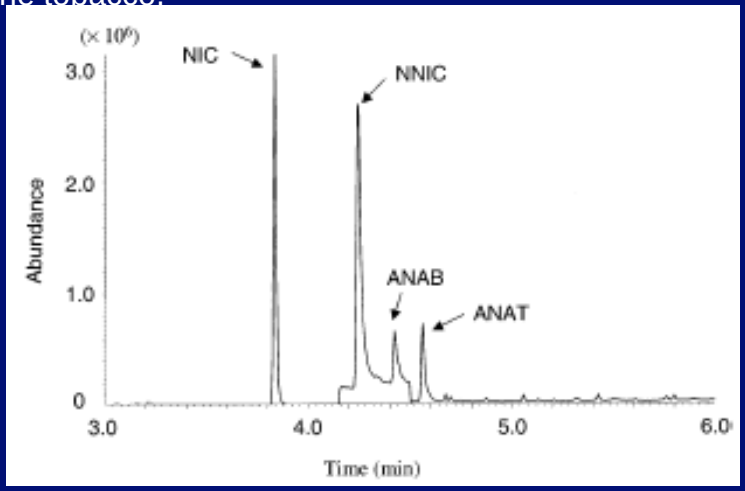


- Yang, S.S., Smetena, I. *Chromatographia*, **1998**, 47, 443

Method 2: GC-MS

- **SPME sampling used (headspace)**
- **Used retention times and confirmation/quantitation ion ratios for more selective and accurate identification of analytes.**
- **Single Ion Monitoring (SIM) mode was used for quantitation.**
- **Initially attempted to use a denicotinized tobacco matrix for isotope dilution calibration.**
  - Different relative response factors were observed between denicotinized tobacco and sample tobacco blends.
- **Standard addition was used for calibration curves to minimize matrix effects between tobacco types**
- **Relatively high RSD and LOD's for the alkaloids.**
- **The method was applied to domestic and foreign cigarette filler.**
  - Wide concentration ranges for all alkaloids were observed when comparing different international brands.
  - Most likely due to tobacco types used and/or quality of the tobacco.

Analyte	RSD (%)	Recovery (%)	LOD (µg/g)
nicotine	6.7	108	1.8
nornicotine	9.9	100	0.134
anabasine	17.6	103	0.475
anatabine	20.6	108	0.209



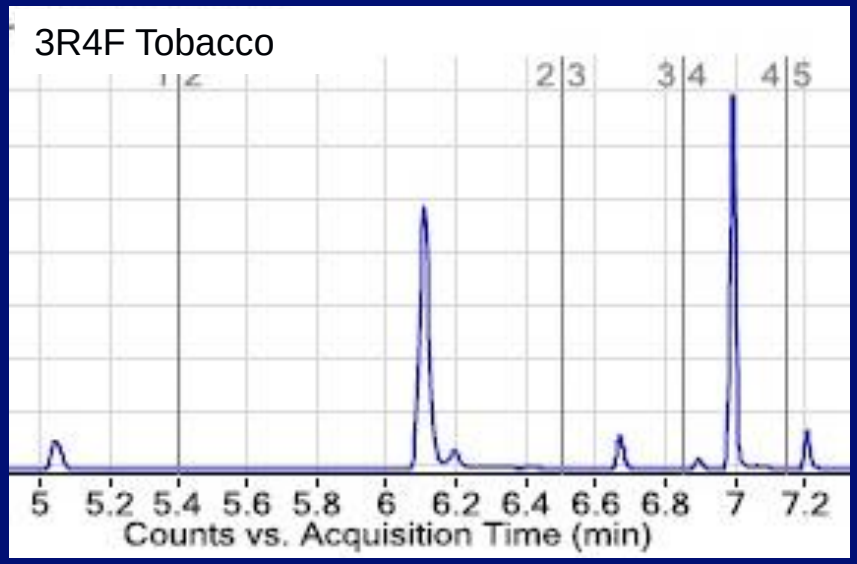
Wu, W., Ashley, D.L., Watson, C.H. *Anal. Chem.* **2002**, 74, 4878.

GC-MS/MS Method

- Method required for rapid processing of large sample loads without loss of data integrity.
  - Through a collaboration with the FDA-CTP, CDC analyzes numerous tobacco products.
  - QC validation and protocol is always required.

Sample Preparation Improvements

- Tomato-leaf matrix used as a blank to reduce background.
  - Originally looked at Quest 3 denicotinized tobacco.
- Liquid-Liquid extraction
  - SPME inherently has more sample variability – due to more factors to overcome i.e. solubility and partition coefficients



- Lisko, J.G., Stanfill, S.B., Duncan, B.W., Watson, C.H. *Anal. Chem*. **2013**, 85,

GC-MS/MS Method - Continued

- **MS/MS Method Optimization – Agilent 7890 GC/7000 QQQ**

- **Isotopically labeled internal standards used for quantification (d_3-nicotine and d_4-nornicotine.**
- **Multiple Reaction Mode (MRM) used to quantify analytes**
  - Virtually eliminates background interferences
- **Validation (Precision/Accuracy) was done by spiking five samples (n=5) at three concentration levels.**
- **Improved precision, accuracy and lower LOD's than prior methods.**

Analyte	Precision, RSD (%)	Accuracy, Recovery (%)	LOD (µg/g)
nornicotine	0.9 – 3.3	102 – 112	0.08
myosmine	0.4 – 2.0	100 – 105	0.04
anabasine	0.8 – 1.7	98 – 107	0.12
anatabine	1.1 – 1.8	99 – 108	0.12
isonicoteine	0.7 – 1.6	97 - 109	0.03

GC-MS/MS Method Application

- Analysis of cigarette filler from 50 popular US brands (n=7).

Analyte	Range (µg/g)	Mean (µg/g)	Median (µg/g)
Nornicotine	659 – 986	763	746
Myosmine	8.64 – 17.3	14.0	13.8
Anabasine	127 – 185	147	146
Anatabine	927 – 1390	1100	1090
Isonicoteine	23.4 – 45.5	34.1	33.7

- Separated into mentholated and non-mentholated brands

Analyte	Non-Menthol (38 brands)			Menthol (12 brands)		
	Range (µg/g)	Mean (µg/g)	Median (µg/g)	Range (µg/g)	Mean (µg/g)	Median (µg/g)
Nornicotine	659 – 986	758	754	709 – 922	779	738
Myosmine	8.64 – 17.3	13.9	13.8	12.8 – 17.1	14.4	13.9
Anabasine	127 – 185	147	147	131 – 161	144	145
Anatabine	933 – 1390	1100	1090	927 – 1200	1100	1100
Isonicoteine	23.4 – 45.5	33.8	33.7	30.0 – 45.5	35.0	33.4

Summary

Method	Number of Analytes	Sample Prep Method	QC (Y/N)	Validated (Y/N)	Internal Std (Y/N)	Precision (%)	Accuracy (%)	LOD (µg/g)
GC-NPD	4	SPME (in solution)	N	N	Y	5.0 – 8.0	>94*	ND
GC-MS	4	SPME (headspace)	Y	Y	Y#	6.7 – 20.6	100 - 108	0.134 – 1.8* (0.475)
GC-QQQ	5	Liquid-Liquid Extraction	Y	Y	Y#	0.4 – 3.3	97 - 112	0.03 – 0.12

ND = Not determined

* Nicotine

Isotopically labelled internal standards

Conclusions

- **GC-MS/MS approach provides a more efficient, more sensitive, and more robust method of alkaloids analysis**
  - LOD's were improved
  - Specificity was improved
  - Precision was improved
  - Sample throughput was increased
  - Tobacco matrix interferences were minimized

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Thank You!

For more information please contact Centers for Disease Control and Prevention

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