Method for the Determination of Nicotine in Tobacco Products That Is Selective, Sensitive, and Suitable for Regulatory Reporting

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INTRODUCTION

- Annual nicotine reporting requirements in the United States for smokeless tobacco products (STPs) and cigarette filler require the use of the CDC nicotine method[1,2]
- The CDC method has limitations
  - Specifies a non-selective flame ionization detector (FID)
  - Has known matrix interferences that require the use of standards addition for the accurate determination of nicotine[1]
  - Certain flavor compounds co-elute with the internal standard quinoline[3]

OBJECTIVE

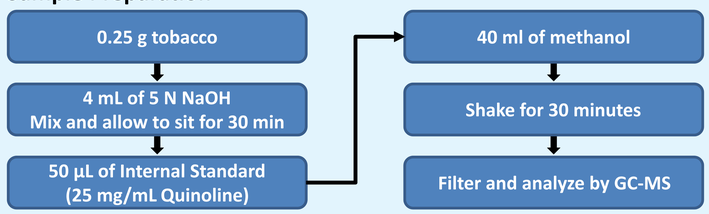
- Develop and validate a more rapid, selective, and sensitive method for the determination of nicotine in STPs and cigarette filler using GC-MS (adapted from CORESTA[4])
- Compare the results of the GC-MS method with those generated by the CDC nicotine method to determine if the GC-MS method is suitable for regulatory reporting purposes

METHOD

Instrument Conditions

GC Parameters	
Column	CAM 30 m x 0.25 mm ID x 0.25 µm df
Injection Temperature	250 °C
Injection Mode	Split (40:1)
Injection Volume	1 µL
Flow Rate	1.0 mL/min
Temperature Gradient	170 °C hold for 1 min 5 °C/min to 180°C 25 °C/min to 220°C
Run Time	7.6 min
MS Parameters	
Transfer Line Temperature	250 °C
Source Temperature	230 °C
Quadrupole Temperature	150 °C
Solvent Delay	3 min
Dwell Time	50 msec
Ions Monitored	m/z 162 and 84 for nicotine m/z 129 and 102 for quinoline

Sample Preparation



ABSTRACT

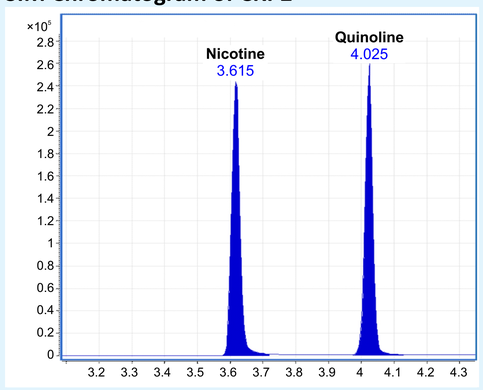
Smokeless tobacco manufacturers have been required to annually report the concentration of nicotine in smokeless tobacco products (STPs) sold in the United States to the Centers for Disease Control and Prevention (CDC) since 1999 using the CDC nicotine method.[1] Additionally, the Massachusetts Department of Public Health (MDPH) requires annual reporting of nicotine in cigarette filler for brand families with more than 3% national market share using the CDC nicotine method.[2] The CDC nicotine method uses gas chromatography with flame ionization detection (GC-FID) to measure nicotine. Since the method specifies a non-selective detector, new matrices, and matrices with know interferences, must be analyzed by the standards addition calibration technique to accurately determine nicotine.[1] The objective of this work was to develop and validate a more rapid, selective, and sensitive method for the determination of nicotine in STPs and cigarette filler using GC-MS (adapted from CORESTA[4]). Data were compared with those generated by the CDC nicotine method to confirm that this method is suitable for regulatory reporting purposes.

Validation Summary

	Parameters	Results
Calibration	r^2	>0.995
	Range (mg/g)	0.8 to 50.0
Recovery	Laboratory fortified matrix (%)	93.1 to 103.4
Precision	Instrument Precision (n=10, %RSD) 3R4F	<1.4
	Intra-day Precision (n=24, %RSD) 3R4F, CRP1, CRP2, CRP3, CRP4	0.8 to 7.1
	Inter-day Precision (n=96, %RSD) 3R4F, CRP1, CRP2, CRP3, CRP4	0.4 to 9.7
LOD	Limit of Detection (mg/g)	0.04

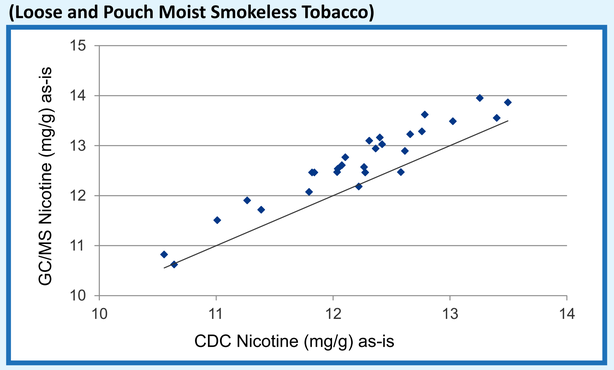
CRP = CORESTA reference product

SIM Chromatogram of CRP2

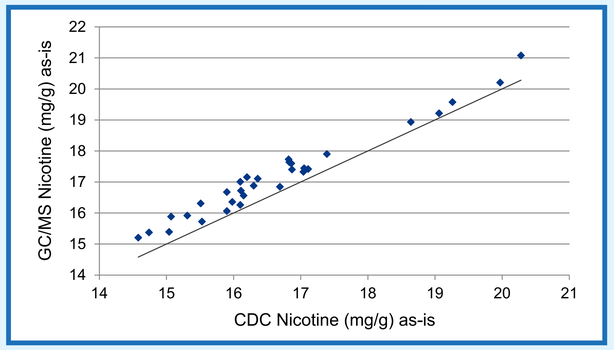


RESULTS

GC-MS vs. CDC Method for Nicotine in STPs
(Loose and Pouch Moist Smokeless Tobacco)



GC-MS vs. CDC Method for Nicotine in Cigarette Filler



Relative Comparison of the Methodologies
The tabulated values are (GC/MS - CDC)/CDC

	STP Method Comparison	Cigarette Filler Method Comparison
Average	3.5%	3.2%
Std dev	2.0%	1.6%
Lower Confidence Limit (95%)	2.8%	2.6%
Upper Confidence Limit (95%)	4.3%	3.8%

SUMMARY

- Statistical equivalence testing demonstrates the two methods are equivalent for the quantitation of nicotine in STPs and cigarette filler and are thus both suitable for regulatory reporting (<5% difference)
- The GC-MS method has no known matrix interferences; therefore, it does not require standards addition testing
- This method was validated for the quantitative analysis of nicotine as well as 3 additional alkaloids (nornicotine, anabasine, anatabine) in STPs and cigarette filler

REFERENCES

1. Federal Register, Notice regarding requirements for annual submission of the quantity of nicotine contained in smokeless tobacco products manufactured, imported, or packaged in the United States FR Doc. 99-7022, March 22, 1999, p. 14085-14096.
2. The Commonwealth of Massachusetts General Laws, Part I Administration of the Government, Title XV Regulation of Trade, Chapter 94 Inspection and Sale of Food, Drugs and Various Articles, Section 307B Manufacture of tobacco products; annual reports including added constituents and nicotine yield ratings; disclosure; exclusions. (1997).
3. Stanfill S, Jia L, Ashley D, Watson C. (2009) Rapid and chemically selective nicotine quantification in smokeless tobacco products using GC-MS. J Chromatog Sci 47: 902-909.
4. CORESTA Routine Analytical Chemistry Subgroup Study Protocol and Test Method Collaborative Test for Alkaloids in Tobacco, October 16, 2013, not published.

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