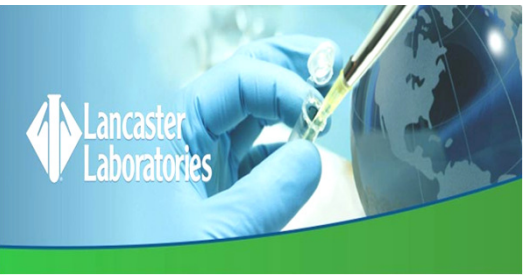


Quantitative Determination of Volatile Nitrosamines In Smokeless Tobacco Products

Mary Dennis, Darren Steelman, Robert Staches Lancaster Labs 2425 New Holland Pike Lancaster, PA 17605

INTRODUCTION

The analysis of volatile nitrosamines (VNAs) in smokeless tobacco products is routinely performed in our lab using gas chromatography thermal chemiluminesence detection (GC-TEA), and a GERSTEL large volume injector. The method is labor intensive and to achieve the needed sensitivity requires the use of a Gerstel large volume injector. The method is limited to the analysis of only one of the VNAs, N-nitrosodimethylamine (NDMA).

The objective of this work was to develop one method for the quantitative determination of six VNAs in smokeless tobacco products that provided the required sensitivity and analyte specificity. Two different GC columns were evaluated to obtain the best separation, yet maintaining an efficient and short analysis time. Different extraction volumes and different sample weights were also evaluated. This method was developed using a TSQ-Quantum GC triple quadrupole mass spectrometer (GC/MS/MS).

This paper will describe the final method of analysis for VNAs in smokeless tobacco products. The validation data obtained on smokeless tobacco products using this method are presented.

Procedure

Smokeless tobacco samples are air dried and ground, accurately weighed and extracted with 0.01 N aqueous potassium hydroxide containing the internal standards. An aliquot of the extract is placed on a Chem Elut™ extraction cartridge. The eluant is analyzed on a gas chromatograph equipped with a triple quadrupole mass spectrometer (GC/MS/MS). Chromatographic separation is accomplished using an Agilent DB-1701 30m X 0.25mm ID X 1.0 mm film thickness column. Detection is by selected reaction monitoring (SRM) of precursor ions to product ions specific to each compound.

Reagents

Certified standards were purchased from Absolute Standards. The certified standards were diluted and taken through the same processing as the samples.

Sample Preparation

Figure 1. Sample Preparation

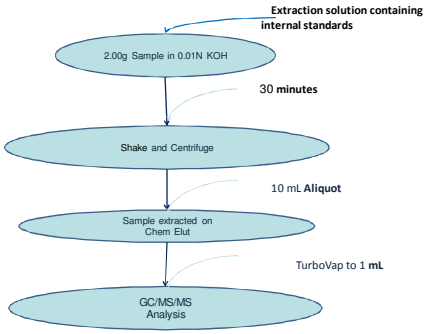


Table 1. GC Conditions

Rate ºC/min	Temperature ºC	Hold Time minutes
Initial	45	3
25	130	0
12	250	2
Constant Flow rate 1.5ml/minute	Carrier	Helium
Injection mode	Splitless surge	
Splitless time	2 minutes	
Injector temperature	140ºC	
Split flow	50 ml/min	
Surge	300 kPa	
Surge duration	2.0 minutes	
Injection volume	1 µL	

Mass Spectrometer Conditions

The TSQ Quantum mass spectrometer was used in the positive chemical ionization (PCI) mode using methane gas. PCI gives the molecular ion for each VNA. Listed below in Table 2 are the TSQ Quantum source parameters.

Table 2. TSQ Quantum Mass Spectrometer Conditions

Parameter	
Scan width	0.2 amu
Emission current	50 µA
Electron energy	-70ev
Collision gas pressure (Argon)	1.2 bar
CI gas flow	2.5 bar
Source temperature	270ºC

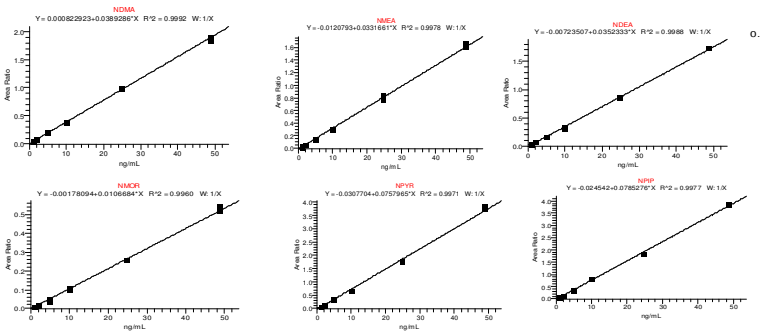
EZ Method selected reaction monitor (SRM) parameters are listed in Table 3. The SRM transitions were obtained using argon as the collision induced dissociation (CID) gas and transitions specific for each nitrosamine.

Table 3. EZ Method

Precursor m/z	Product m/z	Collision Energy (v)	Scan Time (sec)	Retention Time Minutes	Compound
81.3	46.2	15	0.2	6.80	NDMA-d_6
75.3	43.2	14	0.2	6.81	NDMA
89.29	61.2	12	0.1	7.62	NMEA
92.27	64.23	12	0.1	7.60	NMEA-d_3
113.3	81.2	15	0.05	8.25	NDEA-d_{10}a
113.3	49.2	15	0.05	8.25	NDEA-d_{10}b
103.3	47.2	15	0.05	8.27	NDEA-a
103.3	75	15	0.05	8.27	NDEA-b
125.24	49.2	15	0.05	10.49	NMOR-d_8a
125.24	93.2	15	0.05	10.49	NMOR-d_8b
117.24	86.44	15	0.05	10.50	NMOR
109.4	62.2	15	0.05	10.70	NPYR-d_8
101.3	55.2	15	0.05	10.73	NPYR
115.24	69.2	15	0.05	10.99	NPIP

The calibration curves are shown in Figure 2. Calibration was from 5.0 ng/mL to 50.0 ng/mL. The Minimum detection limit (MDL) was 2.00 ng/mL with a S/N ≥5 and the Limit of Quantitation (LOQ) was 5.00 ng/mL with a S/N ≥ 10. The r^2 for each calibration curve is ≥ 0.998.

Figure 2. Calibration curves for VNAs



VNAs were not detected at levels ≥ 2 ng/g (MDL) in smokeless tobacco products. A spiked Snus was used to determine the method precision and accuracy. Shown in Figure 3 are the ion chromatograms of each VNA in a spiked Snus. The recoveries of the VNAs in Snus are shown in the bar graph (see Figure 4). NPIP does show loss over time. We feel this is due to adsorption in the system. Cleaning the injector and source, changing the column will resolve this issue.

Figure 3. Ion chromatograms of VNAs Snus Spiked at 10 ng/mL

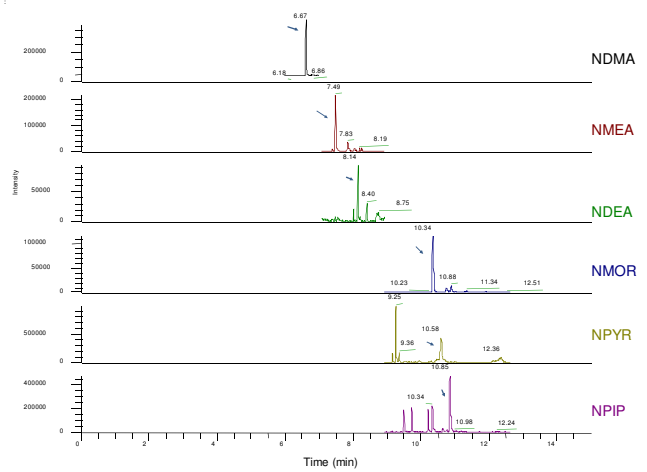
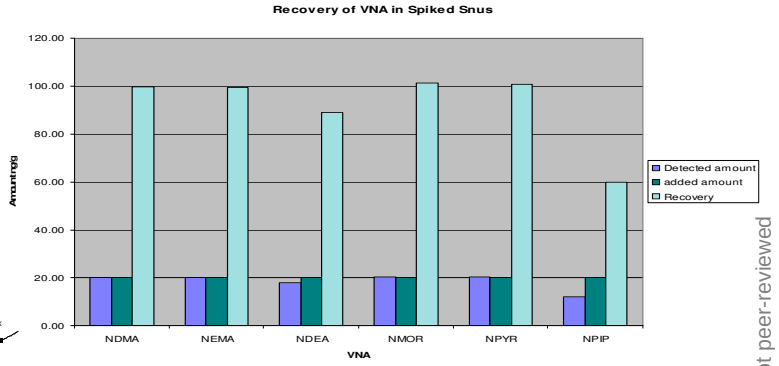


Figure 4. Recovery of VNAs in a spiked Snus



Conclusion

A method for the quantitative determination of six VNAs has been developed and validated. The method is linear over the range of 5.00 to 50.0 ng/mL. The MDL is 2.00 ng/mL and the LOQ is 5.00 ng/mL. VNA were not detected in smokeless tobacco products at levels >2.00 ng/g. The inter-day precision is 3 % and the intra-day precision 6 %.

References

1. EPA Method 521: 'Determination of Nitrosamines in Drinking Water by Solid Phase Extraction and Capillary column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS)'. EPA Document # EPA/600/R-05/054
2. Varian Application Note 01400, 'Determination of Nitrosamines in Drinking Water by Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass spectrometry (CI/MS/MS)' .