Application Note: 52117

Lower Detection Limits of Volatile Nitrosamines in Tobacco by Triple Quadrupole GC-MS/MS

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Key Words

- TSQ Quantum XLS GC-MS/MS
- Positive Chemical Ionization (PCI)
- Smokeless Tobacco
- Timed Selected Reaction Monitoring (t-SRM)
- Volatile Nitrosamines (VNA)

Introduction

Volatile nitrosamines (VNA) are a class of compounds that are known to pose significant health risks in tobacco. Measuring VNAs has traditionally been accomplished using gas chromatography (GC) and thermal energy analyzer (TEA) detection. This technique supports the detection of the VNAs, but poses many challenges. First, regulations continue to drive detection limits lower than what are achievable using GC-TEA. Second, TEA detection is not as specific as other detection techniques. Third, GC-TEA does not allow further analysis of the tobacco for other contaminants.

The use of GC is preferable due to the significant amount of chromatography information that is available using this technique. Coupling a gas chromatograph to a triple quadrupole mass spectrometer provides the chromatography benefits of separating the VNAs while decreasing detection limits, increasing specificity and allowing for the analysis of many other organic contaminants including pesticides.

This method demonstrates the use of the Thermo Scientific TSQ Quantum XLS triple quadrupole GC-MS/MS system and the use of timed selected reaction monitoring (t-SRM) for the analysis of smokeless tobacco for VNA. This unique feature allows the operator to easily setup methods and run samples while the instrument automatically determines the optimal time for SRM parameters, even with partial overlap of SRM transitions. This method demonstrates detection limits of 1 ng/mL and limits of quantitation of 2 ng/mL for six VNAs, using a 1 µL injection volume, 0.5 µg/kg and 1 µg/kg in sample respectively. Injecting small volumes is important to avoid contamination of the system. All method development and calibration curves were performed using spiked uncontaminated tobacco.

Method

Sample Preparation

The sample preparation followed a simple three-step process. A sample size of 2 g was placed in 0.01 N KOH solution. This was shaken for 30 minutes followed by centrifugation. A 10 mL aliquot of the sample was then extracted using solid phase extraction, going from an aqueous sample to methylene chloride. The final extract was then concentrated to 1 mL.

Autosampler Method

The Thermo Scientific TriPlus autosampler provided the 1 µL injection. The syringe was held in the injector for 4 seconds before and 3 seconds after the plunger was depressed.

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GC Method

The Thermo Scientific TRACE GC Ultra gas chromatograph used a two step oven ramp. The oven program started at 45 °C for 3 minutes, with a first ramp at 25 °C/min to 130 °C, and a second ramp at 12 °C/min to 250 °C, with a final hold of 2 minutes. A medium polarity column was used to provide better separations of the nitrosamines. The splitless injector temperature was set to 140 °C. A surge pressure was set to 300 kPa for 2 minutes. The column flow rate was held constant at 1.5 mL/min.

MS Method

The TSQ Quantum XLS™ mass spectrometer was optimized for positive chemical ionization (PCI) using methane as the reagent gas. The optimum methane flow rate was 2.5 mL/min. The source temperature was set to 210 °C. T-SRM provided increased precision and sensitivity by only performing SRM transitions near peak elution. This reduces the amount of transition overlap, which in turn increases dwell time and thus sensitivity and precision. The t-SRM parameters that were used for this method are shown in Table 1.

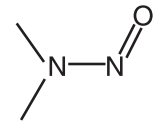
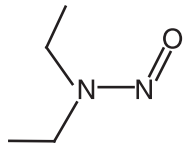
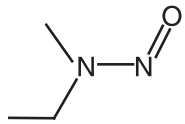
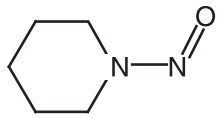
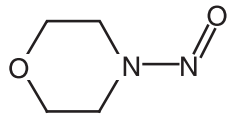
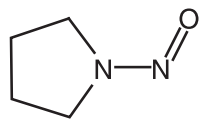
Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	Scan Time (s)	Start (min)	Stop (min)	Compound	Structure
81.30	46.20	15.00	0.20	6.29	7.09	NDMA-d6	
75.30	43.20	14.00	0.20	6.30	7.10	NDMA	
89.29	61.20	12.00	0.10	7.23	7.83	NMEA	
92.27	64.23	12.00	0.10	7.27	7.77	NMEA-d6	
113.30	81.20	15.00	0.05	7.85	8.45	NDEA-d10a	
113.30	49.20	15.00	0.05	7.89	8.49	NDEA-d10b	
103.30	47.20	15.00	0.05	7.90	8.50	NDEA-1	
103.30	75.00	15.00	0.05	7.90	8.50	NDEA-2	
125.24	49.20	15.00	0.05	10.08	10.68	NMOR-d8a	
125.24	93.20	15.00	0.05	10.08	10.68	NMOR-d8b	
117.24	86.44	15.00	0.05	10.10	10.70	NMOR	
109.40	62.20	15.00	0.05	10.29	10.89	NPYR-d8	
101.30	55.20	15.00	0.05	10.33	10.93	NPYR	
115.24	69.20	15.00	0.05	10.60	11.20	NPIP	

Table 1: t-SRM information for the VNAs and their internal standards

Results

The matrix calibration curves from 2-50 ng/mL, 1-25 µg/kg extracted from matrix, results are shown in Figures 1 through 6. All components showed a correlation coefficient of better than 0.995 r^2 value. The percent variance for the points of the matrix calibration curves is less than 25%.

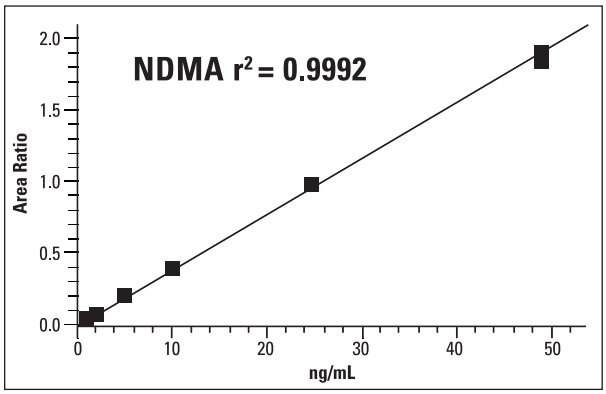


Figure 1: Calibration curve for NDMA

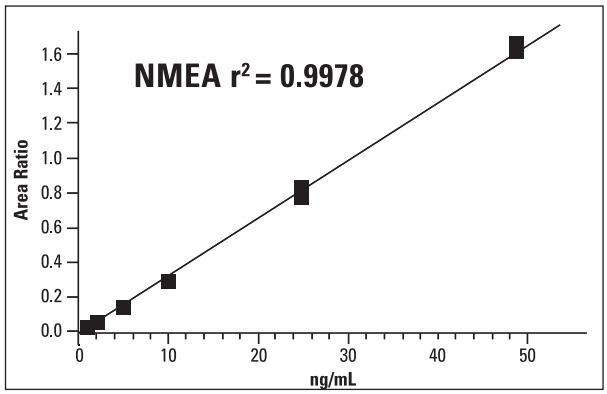


Figure 2: Calibration curve for NMEA

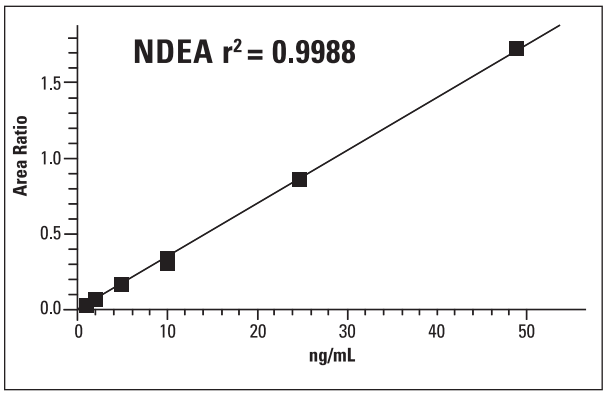


Figure 3: Calibration curve for NDEA

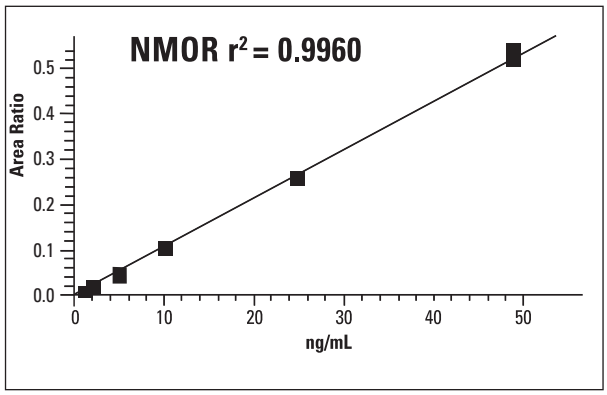


Figure 4: Calibration curve for NMOR

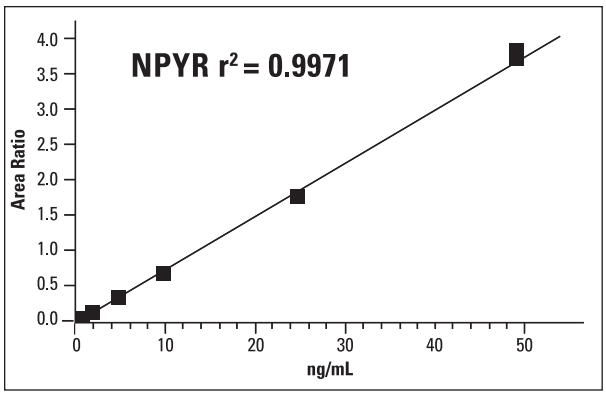


Figure 5: Calibration curve for NPYR

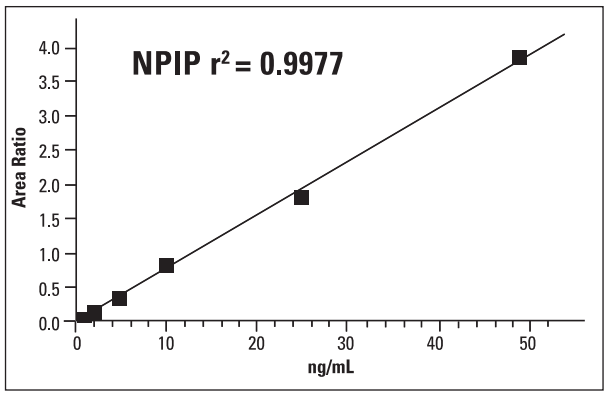


Figure 6: Calibration curve for NPIP

Initially, a minimum detection limit was found to be 1 ng/mL with a 1 µL injection volume. A known uncontaminated sample was spiked and run at that level. The result is seen in Figure 7. The results indicate that detection lower than 1 ng/mL, or 0.5 µg/kg in sample, is easily achieved.

Conclusion

The TSQ Quantum XLS system was able to provide low-level analysis of VNA in smokeless tobacco with a 1 µL injection using t-SRM. The sensitivity of the method is seen in the 1 ng/mL, 0.5 µg/kg in sample, demonstrating the possible detection limits. The calibration curves show good linearity from 2 ng/mL to 50 ng/mL, 1 µg/kg and 25 µg/kg respectively.

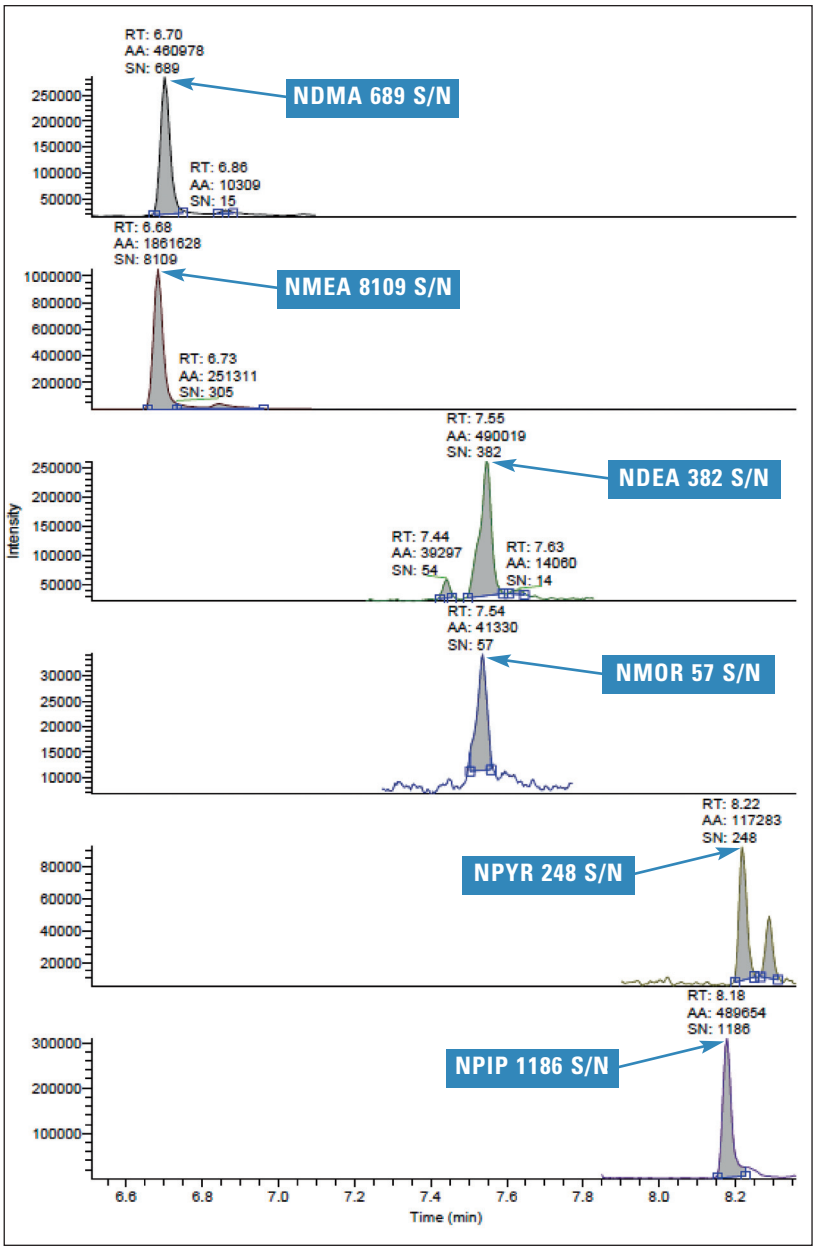


Figure 7: Injection of 1 ng/mL VNA in an uncontaminated clean tobacco sample, demonstrating the signal-to-noise ratio

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