Analysis of Carcinogenic Tobacco-Specific Nitrosamines in Mainstream Cigarette Smoke Using an Agilent J&W DB-35ms Ultra Inert GC Column

Application Note

Gas Chromatography/Mass Spectrometry

Authors

Da Wu
Shanghai Tobacco (Group) Corp.
717 Changyang Road
Shanghai 200082, P.R.China

Yun Zou and Chongtian Yu
Agilent Technologies (Shanghai) Co.Ltd
412 Ying Lun Road
Waigaoqiao Free Trade Zone
Shanghai 200131 P.R.China

Abstract

A simple and sensitive method for routine analysis of TSNAs in mainstream cigarette smoke has been developed. Cigarette smoke particulate matter was collected according to ISO 4387. The particulate matter was extracted with methylene chloride, cleaned up with an Agilent Bond Elut Alumina B SPE cartridge, and analyzed using an Agilent 7000A Triple Quadrupole GC/MS system with an Agilent J&W DB-35ms Ultra Inert GC column. The resulting low method detection limit, good linearity, and sample recovery were demonstrated, as was observed for NNK in this study.

Introduction

Tobacco-specific nitrosamines (TSNAs) are a group of carcinogens that are present only in tobacco and tobacco smoke. They are formed from nicotine and related alkaloids during the production and processing of tobacco and tobacco products [1]. NNN (N'-nitrosonornicotine), NNK ((4-methylnitrosamino)-1-(3-pyridyl)-1-butanone), NAB (N'-nitrosoanabasine) and NAT (N-nitrosoanatabine) are the most common TSNAs in tobacco and tobacco smoke [2]. NAB is a weak carcinogen, and NAT apparently lacks activity [1], but NNK and NNN have been evaluated by the International Agency for Research on Cancer as the most carcinogenic compounds to humans [3,4].

Conventional methods for TSNA analysis are based on gas chromatography with a thermal energy analyzer (GC-TEA) [5,6], or high-performance liquid chromatography (HPLC) with various detection techniques such as UV and mass spectrometry (MS) [7,8]. However, TEAs are nitro-specific, coeluting nitroso compounds cannot be differentiated with a TEA. The low level of TSNAs greatly challenges the sensitivity of the technique.

Agilent Technologies

Normally, a non-polarity GC column or mid-polarity GC column is used to analyze TSNAs, such as (5%-Phenyl)-methylpolysiloxane, (50%-Phenyl)-methylpolysiloxane and equivalent. TSNAs are active compounds. Trace level determination of TSNAs is required (low picogram level) in order to monitor exposure to tobacco smoke. It is very important to select low column bleed and a highly inert column for TSNAs analysis to ensure accurate quantitation.

This application note exhibits an Agilent 7000A Triple Quadrupole GC/MS method for the analyses of TSNA compounds in mainstream cigarette smoke using an Agilent J&W DB-35ms Ultra Inert GC capillary column. This method has been evaluated with respect to linearity, detection limits, method recovery, and accuracy. Improved inertness performance of the column makes it quite suitable for routine TSNAs analysis.

Experimental

Chemicals and standards

The standards in the experiment were purchased from Sigma-Aldrich (Shanghai, China).

Stock solutions of TSNAs including NNN, NAT, NAB, and NNK were prepared by weighing the appropriate standard and dissolving them in methylene chloride at concentrations 1,000 ng/mL. D4-NNK and D4-NNN were adopted as internal standards. The internal standard solutions were made from aliquots of pure compounds, diluted with methylene chloride to a concentration of 400 ng/mL, respectively. Stock solutions were used to prepare calibration standard solutions.

Calibration solutions were prepared in nine different concentrations ranging from 0.5 ng/mL to 200 ng/mL by dilution of TSNA stock solutions in methylene chloride. Each standard solution contained 20 ng/mL of internal standards.

Sample preparation

Cigarettes in this study were provided by Shanghai Tobacco Corp. The smoke particulate matter from 20 cigarettes was collected in the CFPs according to ISO 4387:2000. The CFPs were transferred to a 250-mL Erlenmeyer flask. Then 20 mL of internal standard solution (methylene chloride solution of D4-NNK of 20 ng/mL) was added. The flask was shaken on a horizontal shaker for 40 minutes. A 3-mL extract was loaded on a Bond Elut Alumina B SPE cartridge (p/n 12102048, 500 mg, 3 mL), which had been preconditioned with 3 mL of methylene chloride. After 3-mL extract was passed through the SPE cartridge, the cartridge was washed with 2 mL of methylene chloride. The sample was eluted with 3 mL of 8% methanol in methylene chloride (v/v), eluent was collected and analyzed with an Agilent 7000A Triple Quadrupole GC/MS system.

Instrumentation

Analyses were performed on an Agilent 7890 GC system combined with a 7000A Triple Quadrupole GC/MS system. The split/splitless inlet was fitted with a long-lifetime septum (p/n 5183-4761) and splitless deactivated liner (p/n 5181-3316). The instrumental conditions are listed in Table 1. The 7000A Triple Quadrupole system was equipped with an inert electron impact (EI) source and was operated in MRM mode. Precursor ion and two transitions per target solute were selected (Table 2).

Table 1. Instrumentation and Analytical Conditions for the Agilent 7000A Triple Quadrupole GC/MS

GC	Agilent 7890A Series
Autosampler	Agilent 7683A Injector and sample tray
Inlet mode	Pulsed splitless
Carrier gas	Helium
Column flow	1.2 mL/min Constant Flow
Inlet temperature	250 °C
Injection volume	1 µL
Column 1	Agilent J&W DB-35ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm (p/n 122-3832UI)
Oven temperature program	50 °C (1 min), 30 °C/min to 170 °C, 5 °C/min to 250 °C, 30 °C/min to 300 °C (5 min)
Triple Quadrupole Mass Spectrometer	Agilent 7000A Series
Mode	Electron impact
Transfer line temperature	250 °C
Solvent delay	10 min
Source temperature	280 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
Tune file	Atunes.tune.xml
MRM Mode Conditions	
Resolution	Wide
Collision gas flows	Nitrogen at 1.0 mL/min, Helium at 2.25 mL/min
Detector gain	15

Table 2. MRM Parameters

	Quantitation transition			Confirmation transition		
Compounds	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**CE (eV)**	**Precursor ion (*m/z*)**	**Product ion (*m/z*)**	**CE (eV)**
NNN	177	147	5	105	104	10
D4-NNN	181	151	5	109	108	10
NAT	159	157	10	159	130	25
NAB	161	133	15	161	106	25
D4-NNK	181	150	5	181	122	15
NNK	177	146	5	177	118	15

Results and Discussion

Basic aluminum SPE cartridges are used in many regulations to further clean up smoke extracts. The type, origin, and storage conditions of tobacco can affect TSNA content. A basic aluminum SPE cartridge is suitable for most types of cigarette smoke analysis, but sometimes further enrichment or dilution is necessary.

Column selection

The tobacco smoke matrix contains trace levels of TSNAs. To achieve maximum sensitivity and optimize peak shape, different types of GC columns including (5%-Phenyl)-methylpolysiloxane and (50%-Phenyl)-methylpolysiloxane GC columns for the separation of TSNAs were evaluated, active nature of TSNAs leads to peak tailing on most of these GC columns when the columns are not inert or active sites are present. Figure 1 shows the 7000A Triple Quadrupole GC/MS system chromatograms of TSNAs standard solution (5 ng/mL, containing ISTDs at 20 ng/mL) and the real sample extracts. TSNA compounds can be baseline separated by an Agilent J&W DB-35ms Ultra Inert GC column with excellent peak shapes.

Two brands of tobacco cigarettes were prepared following the procedure previously described. Figure 1 shows that the impurities and matrix effects from tobacco smoke did not interfere with the quantitation of target compounds using a DB-35ms Ultra Inert GC column. The performance of the column ensures accurate quantitation for TSNAs analysis.

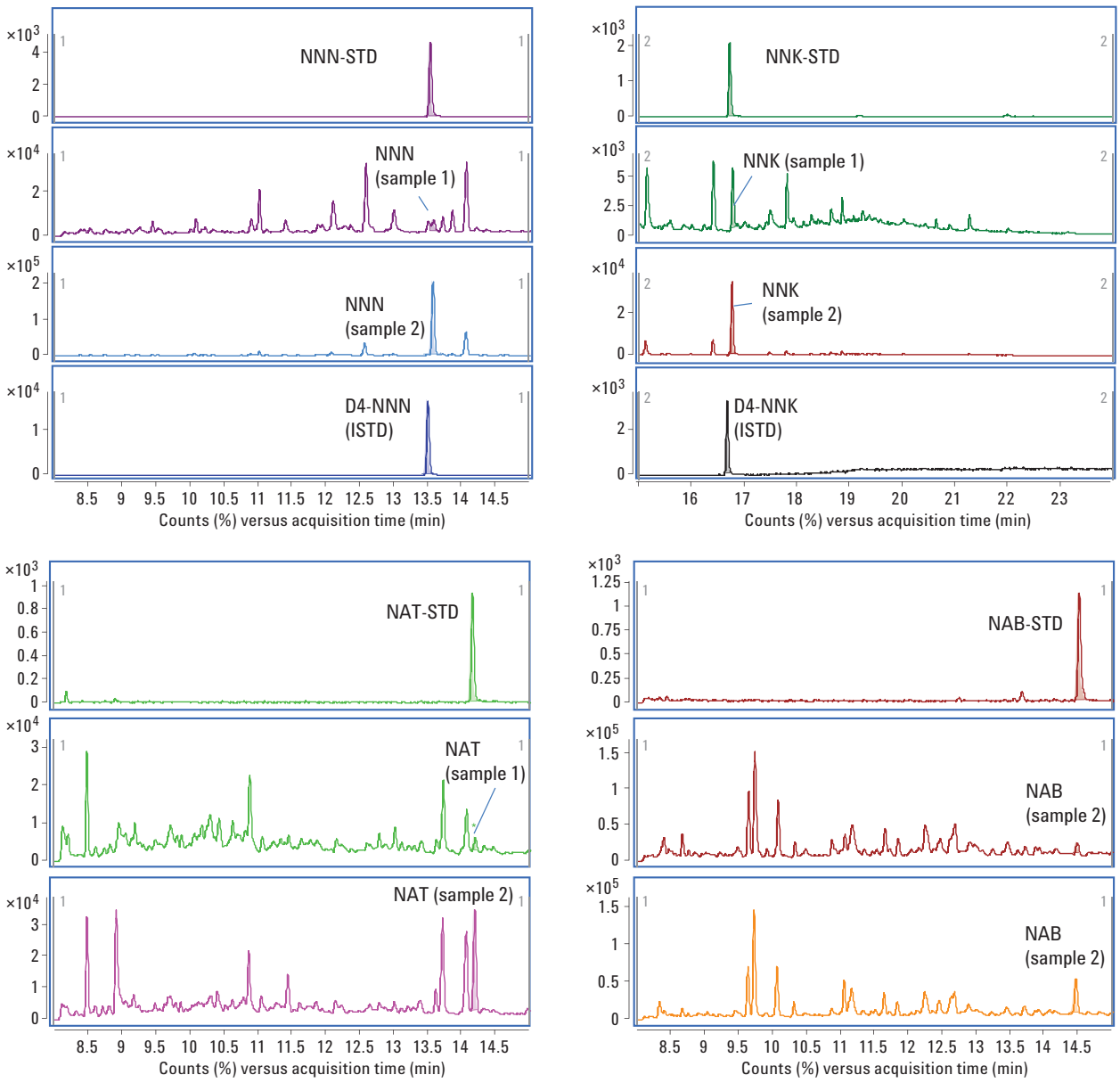

Figure 1. MRM chromatograms of TSNA standard solution and the real samples using an Agilent 7000A Triple Quadrupole GC/MS system and an Agilent J&W DB-35ms Ultra Inert 0.25 mm × 30 m, 0.25 µm column.

Method performance

The most carcinogenic of the commonly occurring TSNAs is NNK [9]. The monitoring of NNK is the most important part for routine analysis. Because of the limit of the sensitivity for the TEA method, NNK in the mainstream cigarette smoke of some Virginia-type cigarettes could not be detected [10]. A simple and highly sensitive Agilent 7000A Triple Quadrupole GC/MS method using an Agilent J&W DB-35ms Ultra Inert GC column for trace-level NNK in mainstream cigarette smoke analysis was demonstrated as follows.

Internal standard quantitation was performed using isotope labeled D4-NNK (ISTD). Calibration standards were prepared at nine levels from 0.5 ng/mL to 200 ng/mL. Triplicate injections were performed to generate calibration curves, and excellent linearity was achieved for NNK with $R^2 = 0.9993$, suggesting that the use of isotope-labeled analogs as internal standards could provide better quantitation accuracy and precision. The calibration curve is shown in Figure 2.

Method detection limits (MDLs) were statistically calculated using the equation MDL = S × t (99%, n = 10) where S is the standard deviation, and t is the student's t at 99% confidence interval. Ten replicate injections of a standard solution at 2.5 ng/mL were performed, and used for MDL calculations. The estimated MDLs for NNK is 0.03 ng/cig. The MDL for this method is much lower than for the GC/TEA method, (MDL for NNK was 0.42 ng/cig)[10], which illustrates the high sensitivity provided by the Agilent 7000A Triple Quadrupole GC/MS system and DB-35ms ultra inert GC column.

Lower limits of quantitation (LLOQ) value was determined by measuring the signal-to-noise ratio (SNR) for NNK response. LLOQ is 0.087ng/cig (SNR=10). Normally, the level of NNK in Virginia-type cigarette smoke is several times lower than that in blend-type cigarette smoke, ranging from 1–10 ng/cig. Due to excellent sensitivity of the 7000A Triple Quadrupole, the content of NNK can be monitored for almost all type cigarettes without further concentration.

An estimation of method recovery was performed using blank matrix spikes. A blank CFP was spiked with a known amount of NNK. The CFP was extracted and cleaned up following the sample preparation process described previously. The results are listed in Table 3. The recovery was determined by calculating the mean of the experimentally determined amount and dividing by the nominal amount. The results were excellent and ranged from 98.8% to 100.2%.

Two types of cigarettes, blended and Virginia type, were used for accurate determination of NNK in two smoke samples. Accuracy of the method was evaluated by spiking a known amount of the NNK on CFPs containing the two smoke samples. NNK was added at three concentrations: approximately half the amount, approximately the same amount, and approximately double the amount of the NNK. The spiked samples were treated according to the procedure described in the sample preparation. The accuracy data for spiked samples are listed in Table 4. All data were based on six replicates of matrix spikes at each level. Good recoveries were achieved for NNK, ranged from 100.0% to 116.8%, and repeatability with RSDs was less than 5%.

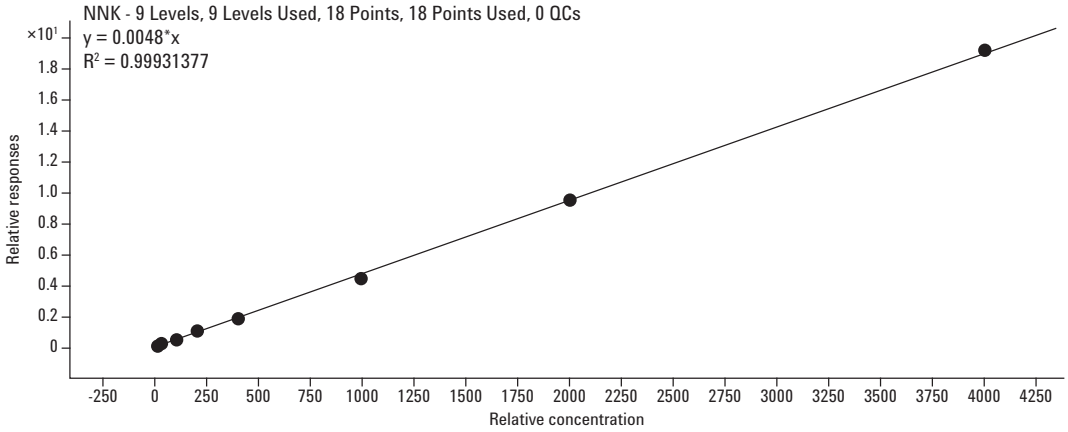

Figure 2. A calibration curve for NNK from 0.5 ng/mL to200 ng/mL with a quadratic curve fit > 0.999.

Table 3. Recovery of NNK Spikes in Blank CFP at Three Spike Concentrations

Analyte	Spiked (ng)	Calculated (ng)	Recovery (%)
NNK	90.88	89.92	101.1
	373.18	377.83	98.8
	2000.07	2004.97	100.2

Table 4. Method Accuracy for Laboratory-Fortified Matrix Spikes in Smoke Extracts at Three Spike Concentrations

Analyte	Sample	Content of NNK (ng/20 cig)	Spiked (ng)	Calculated (ng)	Accuracy (%)
NNK	Virginia-type	102.4	50	50	100
	cigarette	102.4	100	109	109
		102.4	200	204	102
NNK	Blend-type	1124	500	501	100
	cigarette	1124	1000	1078	107.8
		1124	2000	2337	116.8

Conclusions

This application demonstrates a highly sensitive Agilent 7000A Triple Quadrupole GC/MS method for TSNA compounds analysis in mainstream cigarette smoke using an Agilent J&W DB-35 Ultra Inert GC column. TSNA compounds can be baseline separated with excellent peak shapes. Excellent performance of the column ensures accurate quantitation. The system allows for low method detection limit, exceeding the requirements of regulatory methods. Good linearity and recoveries were achieved, as was observed for NNK in this study. The described method is very suitable for the routine detection of TSNAs in mainstream cigarette smoke.

References

1. S.S. Hecht, D. Hoffmann, "Carcinogenesis" 1988, 9, 875–884.
2. K.D. Brunnemann, L. Genoble, D. Hoffmann, "Carcinogenesis" 1987; 8: 465.
3. S.S. Hecht, *J. Natl. Cancer Inst.* 91 (1999), p. 1194.
4. "IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Smokeless Tobacco and Some Tobacco-specific Nitrosamines," vol. 89, International Agency for Research on Cancer (IARC), Lyon (2007).
5. J.D. Adams, K.D. Brunnemann, D.J. Hoffmann, *Chromatogr., A* 1983, 256, 347–351.
6. I.S. Krull, M. Swartz, R. Hilliard, K.H. Xie, J.N. Driscoll, J. *Chromatogr., A* 1983, 260, 347–362.
7. K.A. Wagner, N.H. Finkel, J.E. Fossett, I.G. Gillman, *Anal. Chem*. 2005, 77, 1001–1006.
8. J. Wu, P. Joza, M. Sharifi, W.S. Rickert, J.H. Lauterbach, *Anal. Chem.* 2008, 80, 1341–1345.
9. S.S. Hecht, "Biochemistry, Biology, and Carcinogenicity of Tobacco Specific N-nitrosamines," *Chem Res Toxicol* 1998;11:559-603
10. F. Xie, Y. Jin, M. Zhao, S. Wang, K. Liu, Q. Su, *Acta Tabacaria Sinica* 2004; 10: 8.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
September 8, 2011
5990-8894EN

Agilent Technologies