使用安捷伦 7000A 三重串联四极杆 GC/MS 系统测定橡胶制品中亚硝胺的含量

Application Note

Consumer Products

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Abstract

亚硝胺是一大类强致癌物质。本文采用安捷伦 7000A 三重串联四极杆 GC/MS 系统和安捷伦 J&W DB-624 色谱柱对下列七种最常见的化合物进行了分析：N-亚硝基二甲胺（NDMA）、N-亚硝基二乙胺（NDEA）、N–亚硝基二丁胺（NDBA）、N-亚硝基哌啶（NPIP）、N-亚硝基吡咯烷（NPYR）、N–亚硝基吗啉（NMOR）和 N-亚硝基-N-乙基-N-苯胺（NEPhA）以及内标二丙基亚硝胺（NDPA）。得到的结果表现出低检测限、良好的线性以及较高的样品回收率，说明本应用摘要中所述的方法在用于检测和确定复杂样品基质中的痕量目标胺类时具有很高的选择性和灵敏度。

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Introduction

N-Nitrosamines are receiving special attention because they possess high mutagenic and carcinogenic potential. N-Nitrosamines and their precursors are used as additives to provide strength and elasticity during the manufacture of both natural and synthetic rubber accelerators, antioxidants, and reinforcing agent. Additional N-nitrosamines can be generated by interaction between the precursors and other additives during the vulcanization step or storage [1]. Among all the rubber products, emphasis was given to those that are in direct contact with food (such as food processing machines) or human beings (such as nipples and pacifiers). Since the 1980's, Holland, Germany, and the US have established limits for volatile N-nitrosamines. For example, the European Union (EU) imposed a maximum concentration of total N-nitrosamines at 10 ppb (µg/kg) and nitrosable materials at 200 µg/kg in children's products [2]. The US FDA (Food and Drug Administration) established a maximum level of 10 ppb of individual N-nitrosamines for nipples and pacifiers [3].

Due to the importance of monitoring N-nitrosamines, it is essential that the analytical results are reliable qualitatively and quantitatively. Various selective methods were developed for these compounds using GC-Thermal Energy Analysis (GC-TEA) [4], GC-NPD or GC-MS. The GC-TEA detector is not widely used because it is a not a universal detector, but specific for N-nitrosamines detection. As for GC-NPD, there may be interfering peaks from the sample matrices that affect the analytes of concern when analyzing real samples.

This application note demonstrates a triple quadrupole GC/MS multiple reaction monitoring (MRM) method for the analyses of N-nitrosamine compounds in rubber articles.

Experimental

Chemicals and standards

The standards used in the experiment are listed in Table 1.

Table1. Standard Chemicals

No.	Compounds	CAS No.	Purity	Supplier
1	N-nitrosodimethylamine (NDMA)	62-75-9	99%	ChemService.Inc (PA, USA)
2	N-nitrosodiethylamine (NDEA)	55-18-5	99%	ChemService.Inc (PA, USA)
3	N-nitrosodibutylamine (NDBA)	924-16-3	99.5%	ChemService.Inc (PA, USA)
4	N-nitrosopiperidine (NPIP)	100-75-4	99.4%	ChemService.Inc (PA, USA)
5	N-nitrosopyrrolidine (NPYR)	930-55-2	99.5%	ChemService.Inc (PA, USA)
6	N-nitrosomorpholine (NMOR)	59-89-2	99.9%	AccuStandard.Inc (New Haven, USA)
7	N-nitroso N-ethyl N-phenylamine (NEPhA)	612-64-6	98%	Toronto Research (Chemicals Inc. Ontario.Canada)
8	N-nitrosodipropylamine (NDPA) (ISTD)	621-64-7	99.5%	ChemService.Inc (PA, USA)

Six calibration solutions including NDMA, NDEA, NDBA, NPIP, NPYR, NMOR and NEPhA at 25, 50, 100, 200, 300 and 500 µg/L were prepared by dilution in n-hexane. Each standard solution contained 100 µg/L of NDPA as internal standard (ISTD).

Samples

Rubber articles were cut into pieces sized at less than 2 mm × 2 mm. Ten grams of cut pieces were Soxhlet-extracted in dichloromethane at 55 °C, followed by purification with distillation, and dilution to a volume of 2 mL with n-hexane for analysis.

Rubber sample spiked with seven target N-nitrosamines at the 10 µg/kg level were treated according to the procedure described above.

Instrumentation

Analyses were performed on an Agilent 7890A GC system combined with an Agilent 7000A Triple Quadrupole GC/MS system. The instrumental conditions are listed in Table 2. The Agilent 7000A Triple Quadrupole GC/MS was equipped with an inert electron impact (EI) source and operated in MRM mode. Precursor ion and two transitions per target solute were selected and are listed in Table 3.

Table 2. Instrumentation and Analytical Conditions for the Triple Quadrupole System

GC	Agilent 7890A GC System
Autosampler	Agilent 7683A Injector and sample tray
Inlet Mode	splitless
Carrier gas	Helium
Column flow	2.1 mL/min Constant Flow
Inlet temperature	250 °C
Injection volume	1 µL
Purge flow to split vent	50 mL/min at 0.75 min
Gas saver	On (20 mL/min at 2.0 min)
Column (p/n 122-1334)	Agilent J&W DB-624 30 m × 0.25 nm × 1.40 µm
Oven temperature program	60 °C (5 min), 20 °C/min to 120 °C (5 min), 3 °C/min to 140 °C (3 min), 20 °C/min to 160 °C (5 min), 40 °C/min to 240 °C (8 min) Postrun 250 °C (2 min)

Triple Quadrupole Mass Spectrometer	Agilent 7000A Triple Quadrupole GC/MS System
Mode	Electron impact
Transfer line temperature	250 °C
Solvent delay	4 min
Source temperature	230 °C
Quadrupole temperature	Q1 and Q2 = 150 °C
Tune file	atunes.tune.xml
MRM Mode Conditions	
MS1 resolution	1.2 u
MS2 resolution	1.2 u
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.25 mL/min
Detector Gain	15

Results and Discussion

Figure 1 shows the total ion chromatogram (TIC) in MRM mode for the compounds of interest. N-nitrosamines are derivatives of amines with nitroso group (N=O) bonded to the nitrogen of the corresponding amine. Active nature of these compounds leads to peak tailing on most GC columns when the columns are not inert or active sites are present. As shown in Figure 1, all target N-nitrosamine compounds can be baseline separated by an Agilent J&W DB-624 GC column with excellent peak shapes, except for NDMA.

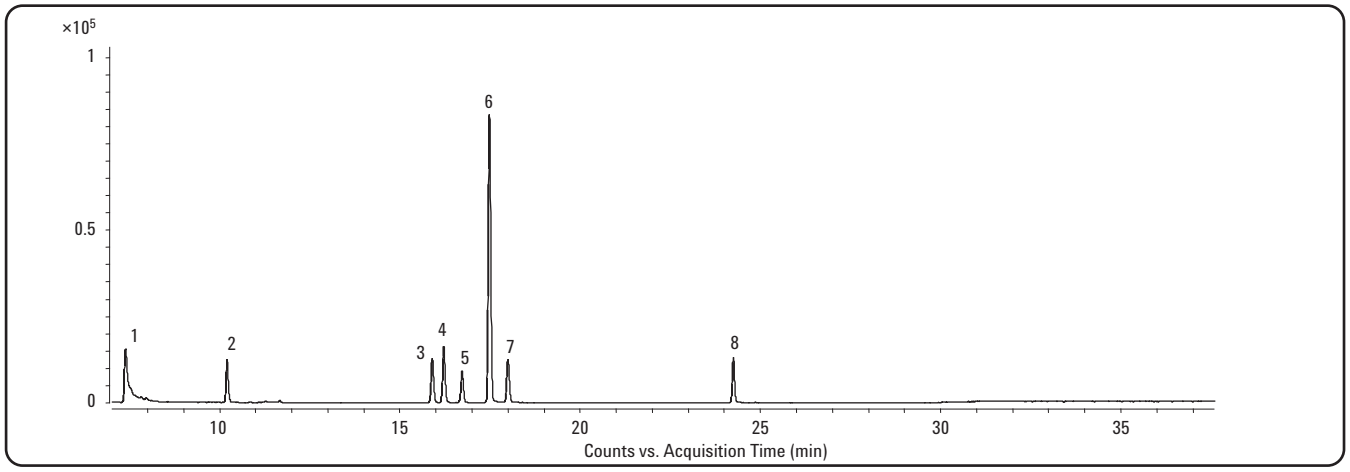

Figure 1. TIC of N-nitrosamine standard solution using Agilent 7000A Triple Quadrupole GC/MS system in MRM mode and Agilent J&W DB-624 30 m × 0.25 mm × 1.4 um column. 1.NDMA 2.NDEA 3.NDPA 4.NMOR 5.NPYR 6.NEPhA 7.NPIP 8.NDBA.

Table 3. MRM Parameters

Compound	RT(min)	Precursor ion	Product ion	Collision energy	Precursor ion	Product ion	Collision energy
N-nitrosodimethylamine (NDMA)	7.421	74	44	0	74	42	20
N-nitrosodiethylamine (NDEA)	10.211	102	85	0	102	44	15
N-nitrosomorpholine (NMOR)	16.223	116	86	0	116	56	10
N-nitrosopyrrolidine (NPYR)	16.728	100	43	10	100	55	5
N-nitroso N-ethyl N-phenylamine (NEPhA)	17.486	106	77	15	106	51	30
N-nitrosopiperidine (NPIP)	17.997	114	84	5	114	41	10
N-nitrosodibutylamine (NDBA)	24.259	116	99	0	84	56	15
N-nitrosodipropylamine (NDPA) (ISTD)	15.899	70	43	5	130	113	0

Calibration curves were constructed from data obtained by 1-µL injections of standards. Figure 2 shows the MRM transitions for NEPhA. The calibration curve for NEPhA ranging from 25 ppb to 500 ppb is shown in Figure 3. All the N-nitrosamines have excellent linearity with calibration coefficients greater than 0.998.

Figure 4 shows the TIC in MRM mode for the rubber article extract and matrix spiked extract. The recovery data for spiked samples and the method detection limits are listed in Table 4. All data were based on seven replicates of matrix spikes with seven target N-nitrosamines at the 10 µg/kg level, which is considered a low detection limit compared to those specified in many regulations. Good recoveries were achieved for all the compounds with relative standard deviation (RSD) less than 7.6%.

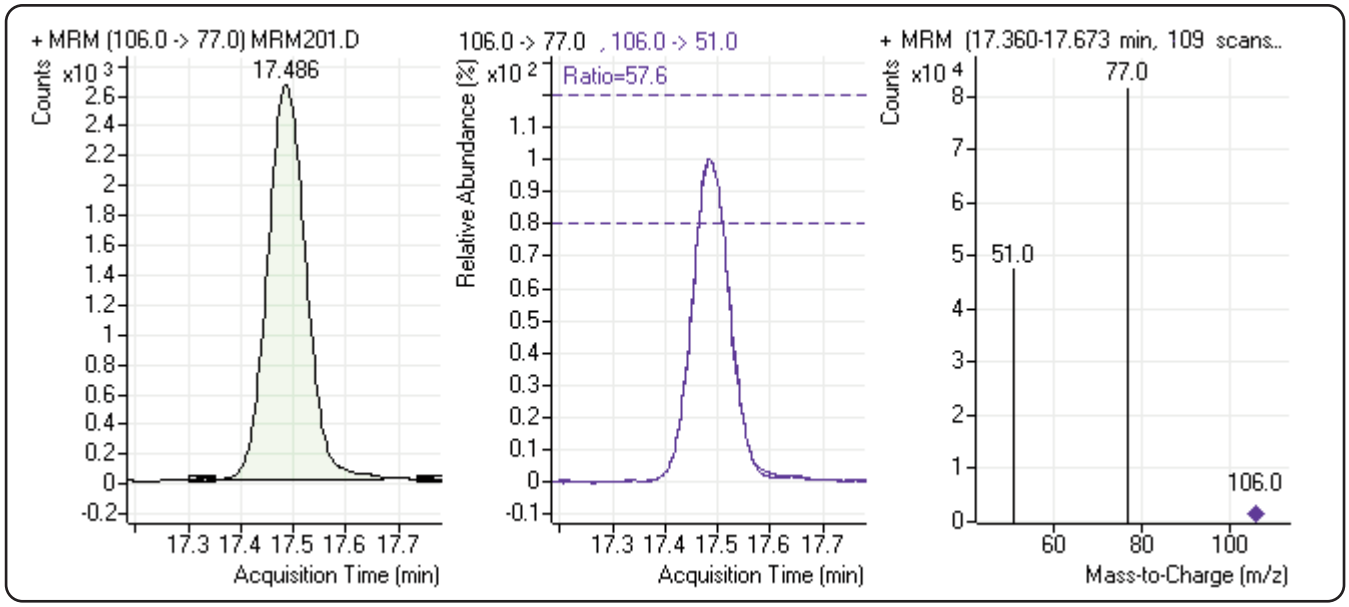

Figure 2. MRM transitions identifying NEPhA at 25 ppb.

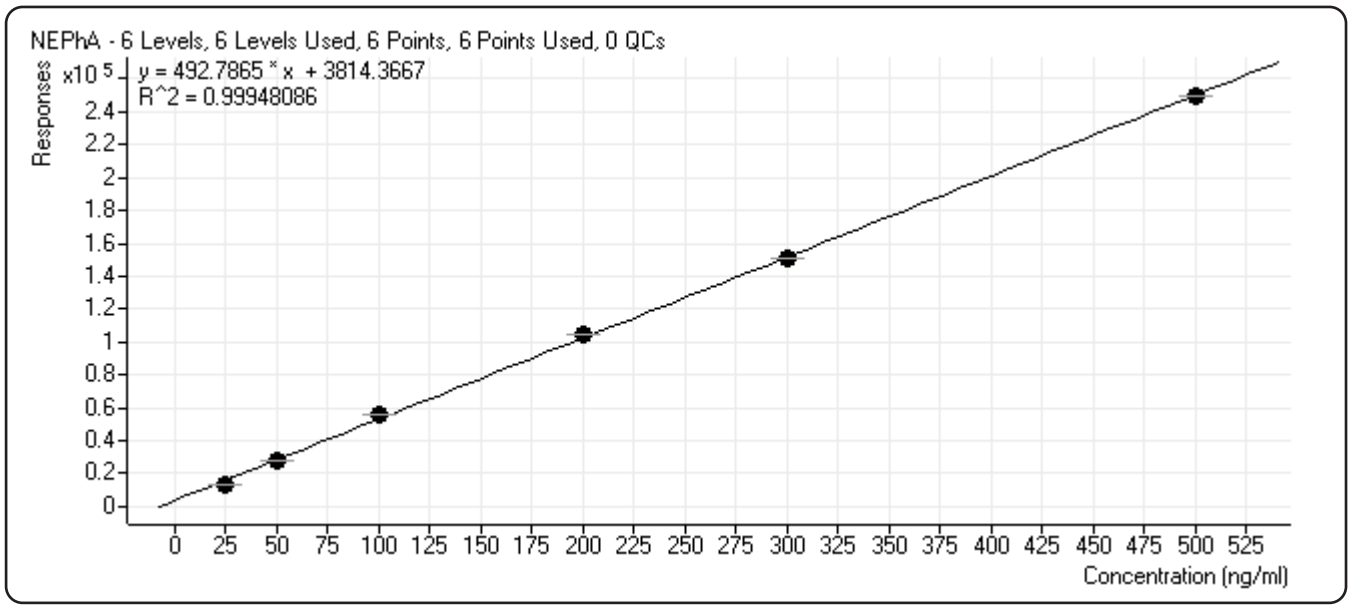

Figure 3. A calibration curve for NEPhA from 25 ppb to 500 ppb with a quadratic curve fit > 0.998.

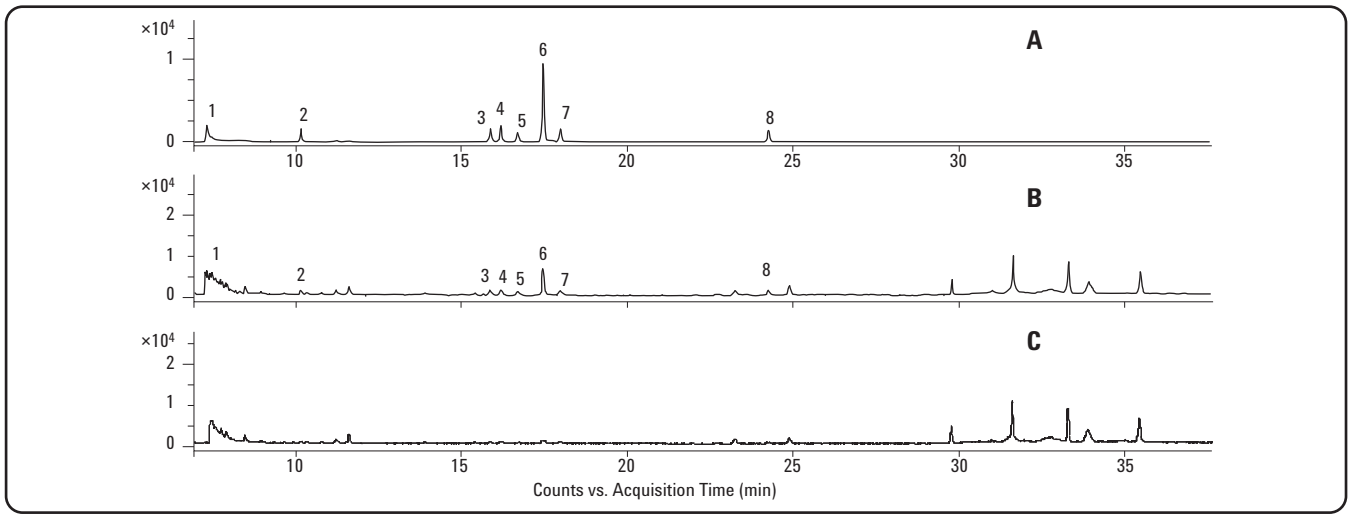

Figure 4. TIC of N-nitrosamine standards (A), matrix spiked extract (B) and the rubber article extract (C) using Agilent 7000A Triple Quadrupole system in MRM mode and Agilent J&W DB-624 30 m × 0.25 mm × 1.4 µm column 1.NDMA 2.NDEA 3.NDPA 4.NMOR 5.NPYR 6.NEPhA 7.NPIP 8.NDBA.

The method detection limit (MDL) is based on samples that have gone through the entire sample preparation scheme prior to analysis. The EPA defines MDL as "the minimum concentration that can be determined with 99% confidence that the true concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte." This procedure is outlined in 40 CFR 136. The method detection limit is calculated according to the formula:

$$MDL = t_{(n-1,1-\alpha = 0.99)} \times S$$

where:

MDL = the method detection limit

$t_{(n-1,1-\alpha = 0.99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

S = standard deviation of the replicate analyses.

MDLs of all target N-nitrosamines are well below the regulatory limits (for example, FDA, 10 µg/kg), illustrating the high sensitivity provided by the Agilent Triple Quadrupole GC/MS/MS.

Table 4. Recovery Data of N-nitrosamines Spikes in Sample and MDL

Compound Name	Spiked amount µg/kg	No. of replicates	Recovery mean, %	RSD %	*MDL (µg/kg)
N-nitrosodimethylamine (NDMA)	10	7	76.62	4.49	1.05
N-nitrosodiethylamine (NDEA)	10	7	74.38	4.90	1.12
N-nitrosomorpholine (NMOR)	10	7	74.97	3.78	0.87
N-nitrosopyrrolidine (NPYR)	10	7	81.91	7.48	1.86
N-nitroso N-ethyl N-phenylamine (NEPhA)	10	7	70.10	1.80	0.39
N-nitrosopiperidine (NPIP)	10	7	78.26	7.54	1.79
N-nitrosodibutylamine (NDBA)	10	7	80.36	3.88	0.96

*EPA defined MDL

Conclusion

This application demonstrates a highly sensitive triple quadrupole GC/MS method for N-nitrosamine compound analysis in rubber products using an Agilent J&W DB-624 GC column. The system allows for trace-level detection of the N-nitrosamines in rubber articles, exceeding the requirements of regulatory methods. Good linearity and recoveries were achieved for all targeted compounds.

References

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