Application Note

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Analysis of the Release of N-Nitrosamines from Rubber Teats and Soothers

Using the Agilent 8890 Gas Chromatography System and 8255 Nitrogen Chemiluminescence Detector

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Abstract

N-nitrosamines from rubber teats and soothers are detected using an Agilent 8890 gas chromatography system configured with an Agilent 8255 nitrogen chemiluminescence detector (NCD). The resulting low detection limit, good linearity and repeatability demonstrate a simple and reliable method for N-nitrosamine analysis without hydrocarbon interference.

Introduction

It has been shown that the use of teats and soothers (also known as bottle nipples and pacifiers) made of elastomer or rubber may release N-nitrosamines, which are a large group of chemical substances. To ensure the safety of child use, some countries have issued a series of regulations which provide basic rules for determining the release of N-nitrosamines, and criteria for the method of analysis to be adopted. Various methods were developed for these compounds using GC-thermal energy analysis (GC/TEA), GC/NCD, GC/NPD or GC/MS. The European Standard BS EN 12868:2017[1] demonstrates the method for determining the release of N-nitrosamines and N-nitrosatable substances from elastomer or rubber teats and soothers by using GC/TEA. The China standard GB 28482-2012[2] details the safety requirements of soothers for babies and young children, and the reference method for determination of the release of N-nitrosamines from rubber teats and soothers is also mentioned. Another China standard SN/T 4451-2016[3] demonstrates the determination of migration of N-nitrosamines in child feeding nipples by using the GC/MS system. Among those methods, NCD is welcomed by many labs because of its high selectivity and sensitivity. The 8255 NCD has been specifically designed with an integral nitrosamine mode. In the nitrosamine mode, the NCD response is proportional only to the presence of the thermally unstable nitroso moiety. The response to nitrogen compounds is equimolar based on NCD.

The purpose of this application note is to provide a detailed analytical method based on GC/NCD for the identification and determination of N-nitrosamines released from teats and soothers.

Experimental

Analyses were performed on an Agilent 8890 GC equipped with a multimode inlet (MMI) and an NCD. The instrumental conditions are listed in Table 1.

Chemicals and standards

A 12-component nitrosamine standard mixture and single standard of N-nitrosodiisopropylamine (NDiPA) were purchased from ANPEL Laboratory Technologies (Shanghai) Inc.

The standard stock solution was diluted appropriately to obtain calibration solutions of the following concentrations: 50, 100, 200, 500, and 1,000 µg/L, each prepared in hexane containing NDiPA as internal standard at a concentration of 200 µg/L.

The concentration of N-nitrosamine standard solutions may change during storage due to UV-light, evaporation or adsorption. Therefore, amber glassware or glassware protected from light by wrapping in aluminum foil should be used.

The N-nitrosamines listed in Table 2 have been identified in teats and tested for calibration standards.

Table 1. Conditions for N-nitrosamine analysis.

GC	Agilent 8890 Gas Chromatography System
Inlet	MMI, splitless 150 °C, then 100 °C/min to 250 °C (10 min)
Liner	Agilent Ultra Inert inlet liner, splitless, single taper, glass wool (p/n 5190-2293)
Column	Agilent J&W DB-WAX, 30 m × 0.53 mm, 1 µm (p/n 125-7032UI)
Carrier	Helium, 11 mL/min, constant flow
Oven	60 °C (0.2 min), then 15 °C/min to 82 °C then 1 °C/min to 88 °C then 15 °C/min to 140 °C (7 min) then 25 °C/min to 230 °C (15 min)
Detector (NCD)	Base Temperature: 250 °C Burner Temperature: 450 °C Chiller Temperature: ON Oxidizer Flow (O_2): 5 mL/min O_3 Generator Flow: ON O_3 Generator Power: ON Vacuum Pump: ON
Injection Size	2 µL

Table 2. Names, abbreviated names, and CAS numbers of N-nitrosamines.

No.	Compound Name	Abbreviated Name	CAS
1	N-nitrosodimethylamine	NDMA	62-75-9
2	N-nitrosodiethylamine	NDEA	55-18-5
3	N-nitrosodiisopropylamine (ISTD)	NDiPA	601-77-4
4	N-nitrosodipropylamine	NDPA	621-64-7
5	N-nitrosodiisobutylamine	NDiBA	997-95-5
6	N-nitrosodibutylamine	NDBA	924-16-3
7	N-nitrosopiperidine	NPIP	100-75-4
8	N-nitrosopyrrolidine	NPYR	930-55-2
9	N-nitrosomorpholine	NMOR	59-89-2
10	N-nitroso N-ethyl N-phenylamine	NEPhA	612-64-6
11	N-nitroso N-methyl N-phenylamine	NMPhA	614-00-6
12	N-nitroso-N, N-di(3,5,5-trimethylhexyl) amine	NDiNA	1207995-62-7
13	N-nitrosodibenzylamine	NDBzA	5336-53-8

Results and discussion

GC parameter optimization

The influence of inlet temperature, operating mode and oxygen flow on sensitivity was investigated. To investigate these parameters, several studies were carried out with a calibration solution at a concentration of 200 µg/L.

Inlet temperature: The temperature of the inlet plays an important role in sensitivity. Compounds with a relatively high boiling point will not be vaporized completely with low inlet temperature, while thermally unstable compounds will degrade with high inlet temperature. MMI was used in this application note and the temperature program was used.

Figure 1 shows the plot of the obtained peak area against the increasing inlet initial temperature. The influence of temperature is an analyte-specific function. The response increases with the increase of temperature for most compounds. However, for thermally unstable compounds (for example NEPhA, NMPhA, NDiNA, and NDBzA), these compounds begin to degrade with the increase of inlet initial temperature. Among the late eluting four compounds, NEPhA and NMPhA are most affected by temperature. Taking into account the sensitivity of each compound, 150 °C is used as the initial temperature for MMI.

NCD operating mode: NCD has two operating modes: standard mode and nitrosamine mode. The main differences between the two modes are different burner temperatures and lower hydrogen flow. The typical burner temperature of the standard mode is 900 °C, while 450 °C is recommended for nitrosamine mode. The typical lower hydrogen flow is 3 mL/min in standard mode, while hydrogen is not used in nitrosamine mode. The peak area comparison between the two modes is studied in this note. As shown in Figure 2, all compounds have a better response in nitrosamine mode.

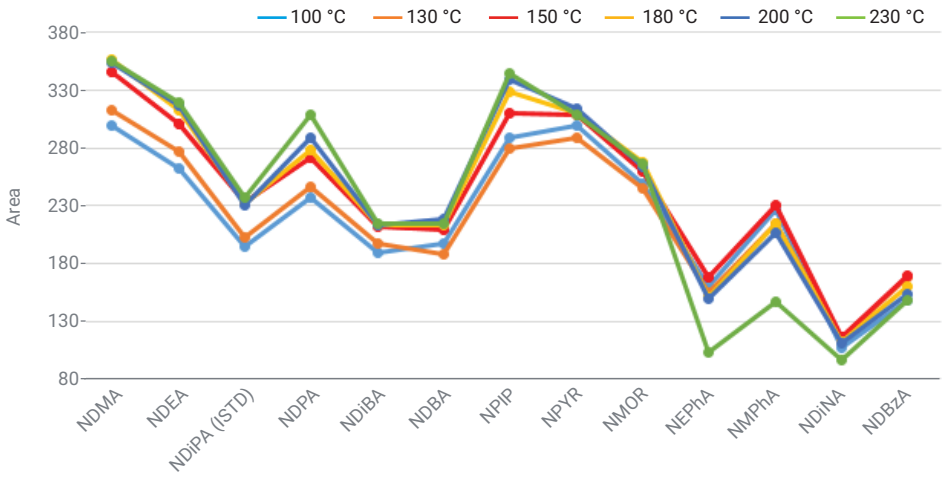

Figure 1. Inlet initial temperature versus peak area for nitrosamine compounds.

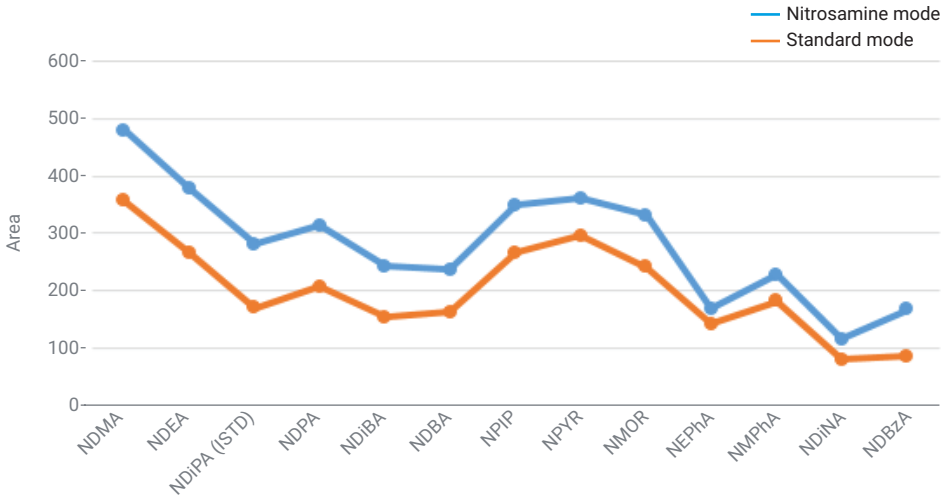

Figure 2. Analysis mode versus peak area for nitrosamine compounds.

Oxygen flow: The influence of oxygen flow on sensitivity was also investigated. One of the factors in the response is the conversion rate. Conversion rate depends on the stability of the compound itself, as well as the time that molecules spend in the burner. In nitrosamine mode, 5 to 10 mL/min of oxygen flow is recommended. As shown in Figure 3, a higher response was achieved by using a low oxygen flow.

Chromatogram

Figure 4 shows a chromatogram of target compounds at a concentration of 200 µg/L. The data were collected and analyzed using Agilent OpenLab chromatography data system 2.5 software. As shown in Figure 4, all target N-nitrosamine compounds can be baseline separated with excellent peak shapes except NEPhA and NMPhA.

Linearity and repeatability

Calibration curves were constructed from data obtained by 2 µL injections of standards. The correlation coefficients (R^2) for all 12 compounds were ≥0.9962 for the range of 50 to 1,000 µg/L. Table 3 shows detailed calibration information.

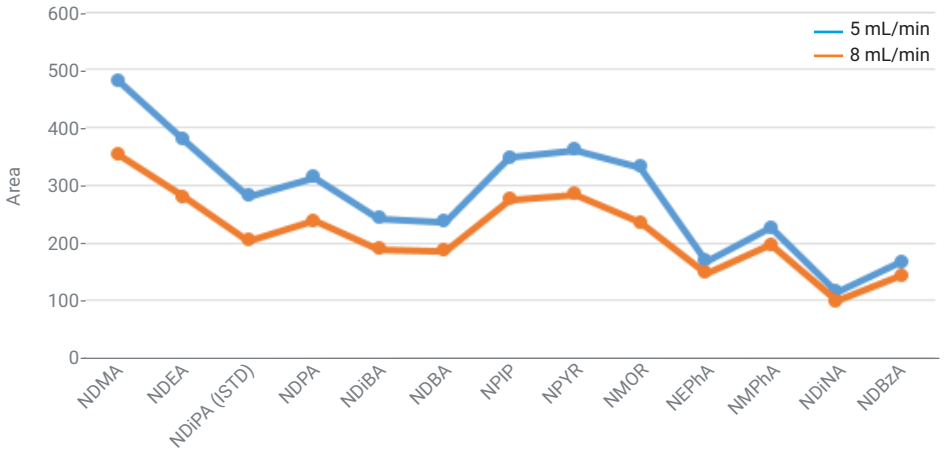

Figure 3. Oxygen flow versus peak area for nitrosamine compounds.

Repeatability was calculated by determining the percent relative standard deviation (RSD) from six replicate injections. After generating calibration curves for each compound, six replicate standards were analyzed at a concentration of 200 µg/L. Table 3 also summarizes the results of repeatability tests for retention time (RT) and area. For all compounds, the RT %RSD was ≤0.07%, while the area %RSD was ≤2.8%.

Table 3. Linearity and repeatability results for the analytes.

Name	RT	R^2	%RSD 200 µg/L (n = 6)	
			RT	Area
NDMA	3.81	0.9991	0.02	2.2
NDEA	5.287	0.9996	0.02	2.8
NDPA	8.976	0.9993	0.01	1.4
NDiBA	9.386	0.9995	0.02	2.5
NDBA	11.546	0.9994	0.01	1.7
NPIP	11.752	0.9995	0.01	1.7
NPYR	12.238	0.9995	0.01	1
NMOR	13.15	0.9993	0.02	2.8
NEPhA	17.272	0.9962	0.07	2.2
NMPhA	17.474	0.999	0.04	2.1
NDiNA	21.294	0.9992	0.01	2.6
NDBzA	26.341	0.9997	0.02	1.8

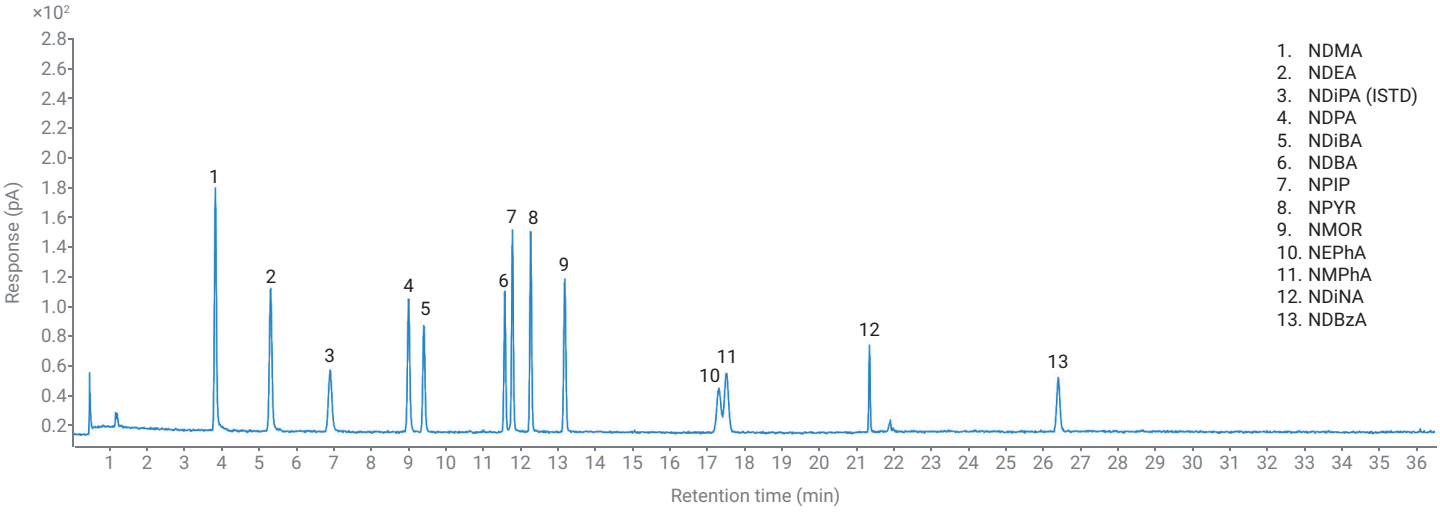

Figure 4. GC/NCD chromatogram of a calibration solution at a concentration of 200 µg/L.

Limit of detection (LOD) evaluation

To determine a practical LOD for the NCD, the 12 analytes, along with NDiPA as the internal standard, were diluted. The chromatograms in Figure 5 show the NCD response to the combined mix at both 25 µg/L (Figure 5A) and 10 µg/L (Figure 5B). At 25 µg/L, all analytes show excellent peak shape and resolution, although NEPhA and NMPhA are not completely resolved and show some broadening. At 10 µg/L, most analytes can be easily detected from the baseline noise except NDiNA and NDBzA. Signal-to-noise ratio (S/N) (ASTM)[4] was used for the LOD evaluation. The S/N values for all analytes at the concentration of both 25 µg/L and 10 µg/L are listed in Table 4. Table 4 indicates a practical LOD of 10 µg/L for the early eluting eight compounds (except ISTD) under the instrumental conditions listed in Table 1. For the late four compounds (from peak 10 to 13), the LOD is around 25 µg/L.

Table 4. S/N values for all analytes.

No.	Name	S/N (ASTM)	
		25 µg/L	10 µg/L
1	NDMA	26.7	8.2
2	NDEA	16.6	5.1
4	NDPA	14.7	6.6
5	NDiBA	10.9	5.8
6	NDBA	16.4	7
7	NPIP	21.3	8.8
8	NPYR	22.4	7.4
9	NMOR	19	8.4
10	NEPhA	5.6	2.6
11	NMPhA	7.8	3.7
12	NDiNA	9.8	4
13	NDBzA	7	3.1

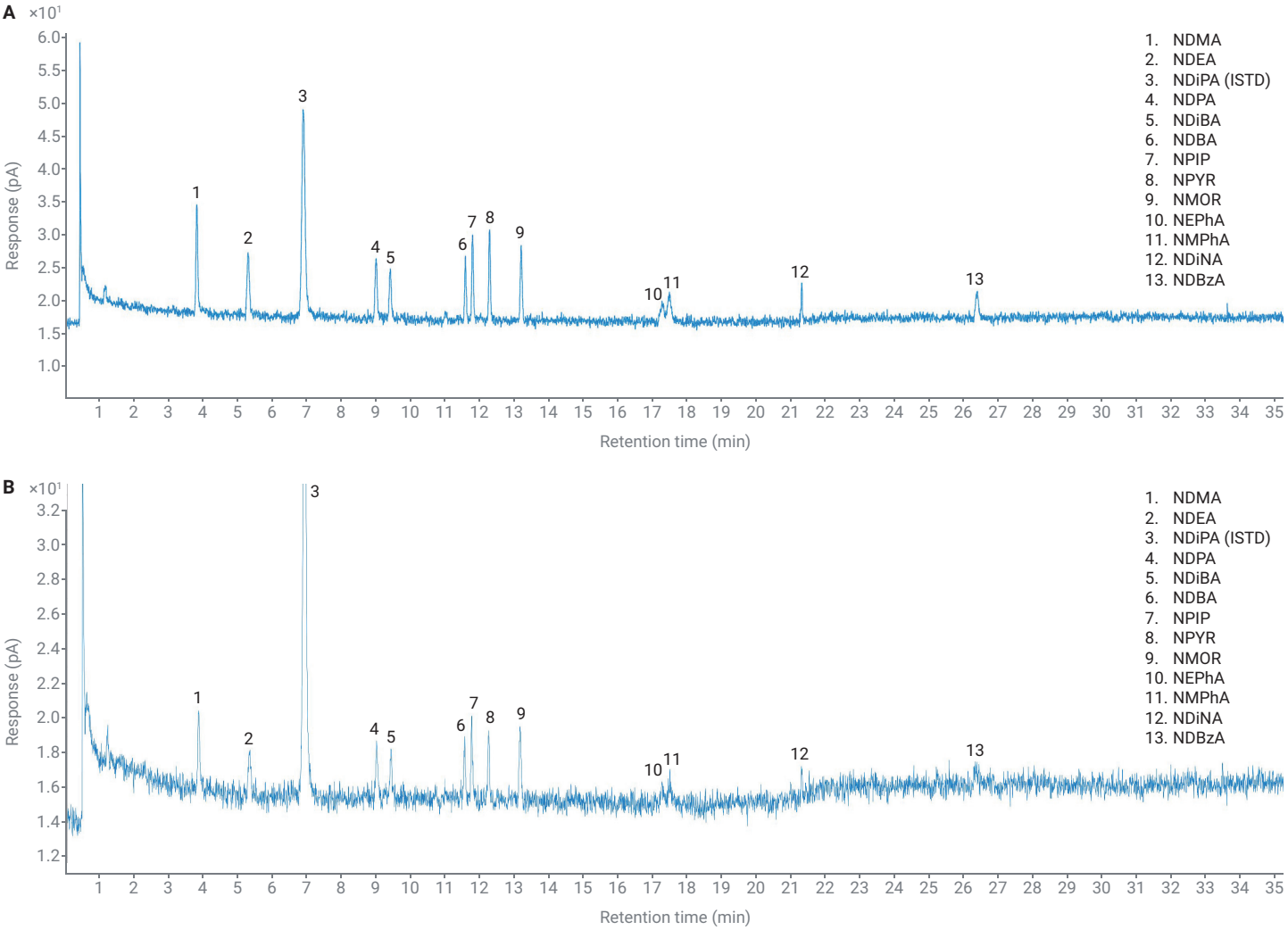

Figure 5. (A) GC chromatogram of a calibration solution at a concentration of 25 µg/L. (B) GC chromatogram of a calibration solution at a concentration of 10 µg/L.

Real sample analysis

The real sample was from an Agilent customer. Samples were weighed, cut into pieces, and transferred to a beaker with boiling water. After preboiling, N-nitrosamines were migrated into a nitrite-containing artificial saliva salt solution under specified conditions. Dichloromethane was used for extraction and a rotary evaporator was used for concentration following cleanup. Details can be found in BS EN 12868:2017. The sample was analyzed both by NCD and MSD. Figure 6A shows the chromatogram obtained on NCD. No target peaks were observed except for the internal standard. Figure 6B shows the chromatogram on the Agilent 5977B GC/MSD. Many more peaks were observed on MSD. MSD is a universal detector. Even using the selected ion monitoring mode, the MSD selectivity on N-nitrosamine compounds is not as good as NCD. This is why more interference peaks from the sample matrix can be observed in the MSD total ion chromatogram (TIC) single ion monitoring (SIM) chromatogram. For the analytical lab, if mass spectrometry is the preferred identification technique, it is recommended to couple the more selective triple quadrupole mass spectrometry with GC for accurate N-nitrosamine compound identification. NCD is another good choice for accurate identification due to its high selectivity on N-nitrosamine-containing compounds as shown in our real sample chromatogram.

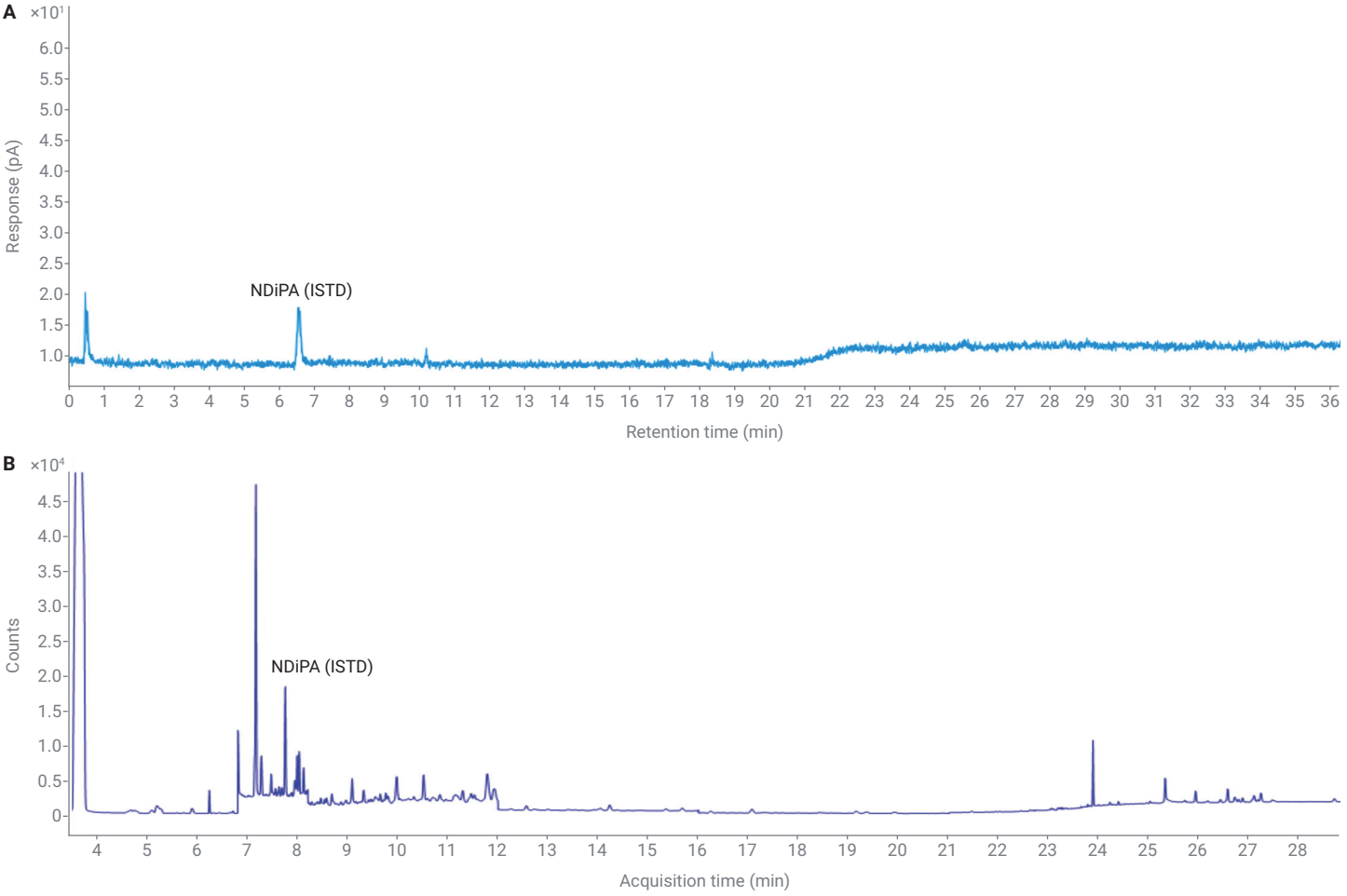

Figure 6. (A) Chromatogram of the real sample based on NCD. (B) Chromatogram of the same real sample based on Agilent 5977B MSD SIM mode on an Agilent DB-WAX 30 m × 0.25 mm, 0.25 µm column. The GC parameters of inlet and injection size are the same as the NCD method, which is shown in Table 1.

Conclusion

This application note demonstrates a highly sensitive GC/NCD method for N-nitrosamine compound analysis from rubber teats and soothers. Good linearity and repeatability were achieved. Correlation coefficients were found to be 0.9962 or greater. The area RSD was 1 to 2.8%. A practical LOD was also evaluated for all analytes. The real sample analysis shows great applicability and selectivity of the NCD method.

References

1. BS EN 12868:2017, Child Use and Care Articles-Method for Determining the Release of N-Nitrosamines and N-Nitrosatable Substances from Elastomer or Rubber Teats and Soothers.
2. China National Standard GB 28482-2012, Safety Requirements of Soothers for Babies and Young Children.
3. China National Standard SN/T 4451-2016, Determination of Migration of N-Nitrosamines in Child Feeding Nipples – GC/MS.
4. Agilent OpenLab CDS Data Analysis Reference Guide.

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