Application Note

Environmental Testing

Sensitive and Reproducible Determination of Nitrosamines by GC/MSD

Analysis of seven nitrosamines using the Agilent 5977 GC/MSD in positive chemical ionization mode with ammonia

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Abstract

A quantitative method has been developed for the determination of seven nitrosamines, including N-nitrosodimethylamine (NDMA), based on the analytical framework of US EPA Method 521. Regulatory bodies are establishing limits or guidance values for NDMA in drinking water, driving the need for sensitive and robust analytical approaches. This study demonstrates that the Agilent 5977 GC/MSD in positive chemical ionization mode with ammonia achieves parts-per-trillion instrument detection limits for the seven compounds (0.0063 to 0.0655 ppb). The method also provides good repeatability, with relative standard deviations of peak area < 4.8% for six measurements of 0.1 ppb concentration standard for NDMA, NMEA, NDEA, NDPA, NPIR, and NDBA, and 0.5 ppb concentration standard for NPYR.

Introduction

Nitrosamines are a well known class of carcinogenic compounds that have been the subject of extensive scientific investigation and regulatory assessment for several decades.[1] In recent years, various regulatory authorities have established drinking water guidelines for N-nitrosodimethylamine (NDMA), a disinfection byproduct of water treatment processes.

In 2004, the US Environmental Protection Agency (EPA) published Method 521, a GC/MS-based method for the analysis of seven nitrosamines including NDMA in drinking water, supporting monitoring and regulatory programs.[2] In 2010, Health Canada proposed a maximum acceptable concentration for NDMA in drinking water of 40 ng/L.[3] In the European Union (EU), several EU member states have introduced their own national limits for nitrosamines in drinking water. In Germany, for example, a concentration of 10 ng/L will trigger the initiation of remedial actions to reduce the concentration of NDMA.[4]

This application note demonstrates the ability of the Agilent 5977 Series GC/MSD to enable linear and reproducible detection of the seven nitrosamines included in EPA Method 521 using positive chemical ionization (PCI) with ammonia.

Chemical ionization (CI) is a soft ionization technique used in mass spectrometry that enhances molecular ion visibility through controlled ion–molecule reactions. In CI, a reagent gas is ionized first, and the resulting reagent ions subsequently transfer charge to the analyte, producing significantly less fragmentation than conventional electron ionization (EI).

PCI further improves sensitivity by generating predominantly protonated molecular ions $[M+H]^+$, concentrating the ion signal into a high-abundance species and delivering a stronger response and improved signal-to-noise ratios for trace-level compounds. Ammonia is an especially effective reagent gas for PCI due to its clean and efficient proton-transfer chemistry, low background spectral contribution, and ability to produce stable, high-intensity molecular ions.

These characteristics make ammonia PCI a highly suitable ionization mode for the sensitive and selective analysis of small polar contaminants such as nitrosamines, where low-level detection and confident molecular ion identification are critical.

Table 1. Seven nitrosamines included in EPA Method 521.

Compound Name	Acronym	CAS No.
N-Nitrosodimethylamine	NDMA	62-75-9
N-Nitrosomethylethylamine	NMEA	10595-95-6
N-Nitrosodiethylamine	NDEA	55-18-5
N-Nitrosopyrollidine	NPYR	930-55-2
N-Nitrosodi-n-propylamine	NDPA	621-64-7
N-Nitrosopiperidine	NPIR	100-75-4
N-Nitrosodi-n-butylamine	NDBA	924-16-3

Experimental

Reagents and standards

- Nitrosamines standards and dichloromethane were bought from Sigma-Aldrich.
- Calibration working solutions were prepared in dichloromethane.

Instrument parameters

The Agilent 5977 GC/MSD instrument conditions and analysis parameters are shown in Tables 2 and 3.

Table 2. Agilent 5977 GC/MSD parameters for nitrosamine analysis.

Gas Chromatography Conditions	
GC Column	Agilent HP-5 ms UI, 30 m × 0.25 mm, 0.25 µm (p/n 19091S-433 UI)
Column Pneumatics	Constant flow
Carrier Gas	Helium
Injection Volume	1.0 µL
Injector Mode	Pulsed splitless
Purge Flow to Split Vent	50 mL/min at 2.5 min
Inlet Temperature	60 °C for 0.1 min, then to 280 °C at 900 °C/min
Injector Liner	Agilent Ultra Inert fritted splitless liner (p/n 5190-5112)
Flow Rate	1.5 mL/min
Oven Temperature Program	40 °C for 2 min 10 °C/min to 150 °C 15 °C/min to 200 °C, hold 0 min 20 °C/min to 270 °C, hold 0 min
Mass Spectrometer Conditions	
Acquisition Mode	Selected ion monitoring (SIM)
Gain	1
Ion Source Temperature	300 °C
Quad Temperature	150 °C
Transfer Line Temperature	280 °C
CI Gas Flow	Ammonia, 20%

Table 3. Agilent 5977 GC/MSD selected ion monitoring (SIM) parameters for nitrosamine analysis.

SIM Group	Start Time (min)	Compound	Retention Time (min)	Target Ion	Qualifier Ion
1	1.5	NDMA-d6	2.288	98	81
	–	NDMA	2.324	92	75
2	3	NMEA	4.096	89	106
3	5	NDEA	5.584	103	120
4	7	NDPA-d14	8.586	145	162
	–	NPYR	8.646	101	118
	–	NDPA	8.711	131	148
5	9	NPIR	9.316	115	132
6	11	NDBA	11.641	159	176

Results and discussion

Calibration curves

A ten-level calibration curve for the seven nitrosamines was evaluated over the concentration range of 0.1 to 20 ppb (0.1, 0.2, 0.25, 0.5, 1, 2, 5, 8, 10, and 20 ppb). The results are presented in Table 4 and Figure 1. Excellent linearity was obtained for NDMA, NMEA, NDEA, NDPA, NPIR, and NDBA, with correlation coefficients (R^2) of 0.9989 or better across the full calibration range from 0.1 to 20 ppb. NPYR also demonstrated good linearity, exhibiting an R^2 value of 0.9987 over the concentration range of 0.5 to 20 ppb.

Table 4. Calibration results of nitrosamines.

Name	Linearity Range (ppb)	R^2
NDMA	0.1–20	0.9993
NMEA	0.1–20	0.9998
NDEA	0.1–20	0.9993
NPYR	0.5–20	0.9987
NDPA	0.1–20	0.9992
NPIR	0.1–20	0.9989
NDBA	0.1–20	0.9992

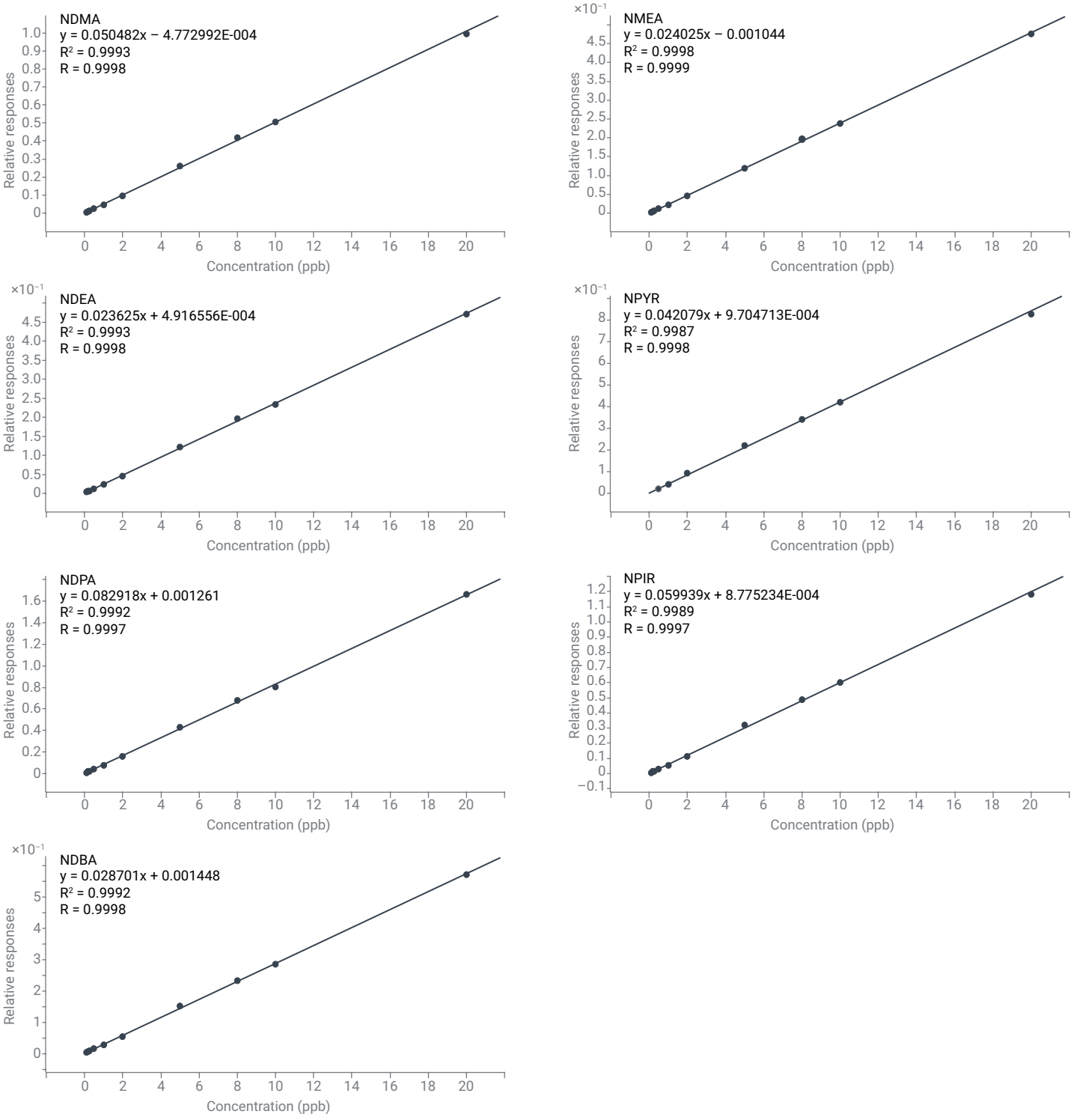

Figure 1. Calibration curves for nitrosamine standards in dichloromethane.

Repeatability

Repeatability was evaluated at the lowest calibration level of 0.1 ppb for NDMA, NMEA, NDEA, NDPA, NPIR, and NDBA, and at 0.5 ppb for NPYR, using six replicate injections for each analyte. The results are summarized in Table 5, Figure 2A, and Figure 2B. Excellent repeatability was demonstrated, with peak area RSD values < 4.8% and calculated concentration RSD values < 3.7%, indicating highly consistent instrument response and stable quantitation performance at the low-level calibration points.

Table 5. %RSD results for nitrosamines standard at the lowest calibration level.

Name	RT (min)	Average Concentration (ppb, n = 6)	Std. Dev. (ppb)	Conc. %RSD	Area %RSD
NDMA	2.324	0.1134	0.0019	1.7	4.4
NMEA	4.096	0.104	0.0037	3.6	2.7
NDEA	5.584	0.1024	0.0028	2.7	4.8
NPYR	8.646	0.5514	0.0195	3.7	2.3
NDPA	8.711	0.0845	0.0033	3.9	4.7
NPIR	9.316	0.0946	0.0028	2.9	3.4
NDBA	11.641	0.0896	0.0034	3.7	3.6

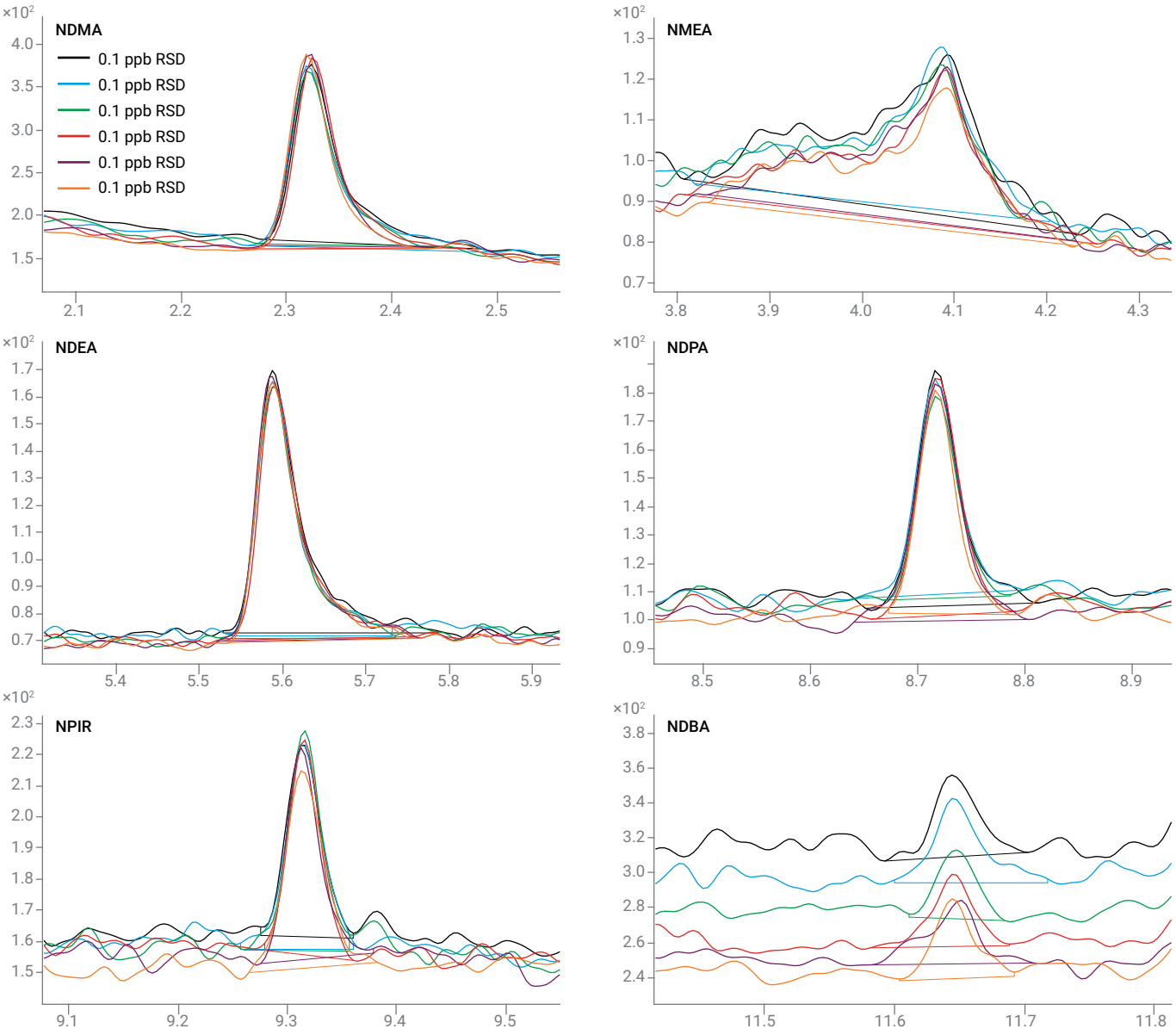

Figure 2A. Overlaid chromatograms of NDMA, NMEA, NDEA, NDPA, NPIR, and NDBA at 0.1 ppb.

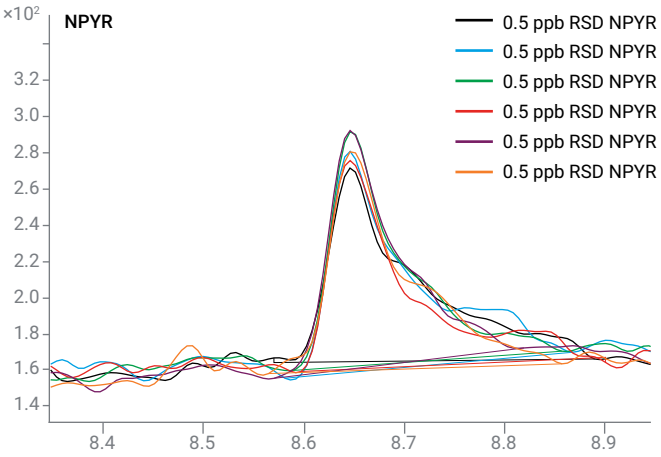

Figure 2B. Overlaid chromatograms of NPYR at 0.5 ppb.

Instrument detection limits (IDLs)

Instrument detection limits (IDLs) were calculated with 99% statistical confidence that the measured analyte signal represents a true concentration greater than zero. For this study, the IDLs were derived from the analysis of six replicate injections of the lowest calibrationlevel standard.

The IDL was calculated using the following equation:

$IDL = (t_{\alpha, n-1}) \times (\%RSD) \times (\text{amount of standard})/100$

Where

$t_{\alpha, n-1}$ is the Student's tvalue corresponding to the selected confidence level and degrees of freedom (n = 6, therefore n – 1 = 5),

%RSD is the relative standard deviation of the six replicate injections, and

"amount of standard" is the concentration of the lowest calibration standard used for IDL determination.

At the 99% confidence level and with five degrees of freedom, the corresponding $t_{\alpha, 5}$ value is 3.365.

Applying this calculation, the IDLs ranged from 0.0063 to 0.0655 ppb, as summarized in Table 6.

Table 6. Agilent 5977 GC/MSD IDLs for nitrosamines.

Name	RT (min)	Concentration (ppb, n = 6)	IDL (ppb)
NDMA	2.324	0.1	0.0063
NMEA	4.096	0.1	0.0125
NDEA	5.584	0.1	0.0095
NPYR	8.646	0.5	0.0655
NDPA	8.711	0.1	0.0111
NPIR	9.316	0.1	0.0093
NDBA	11.641	0.1	0.0113

Conclusion

The study has shown that the Agilent 5977 GC/MSD, operated in PCI mode with ammonia, delivers the sensitivity, accuracy, and reproducibility needed for trace-level nitrosamine analysis.

The method demonstrated excellent linearity, with regression coefficients (R^2) of 0.9989 or better across the 0.1 to 20.0 ppb range for six nitrosamines, and 0.9987 across the 0.5 to 20.0 ppb range for NPYR. Analytical repeatability was robust, with %RSD values < 4.8% at the lowest calibration level, indicating a stable and consistent instrument response. Instrument Detection Limits ranged from 0.0063 to 0.0655 ppb, demonstrating the system's ability to reliably distinguish analyte signals from instrumental noise.

Overall, these results confirm the suitability of the 5977 GC/MSD as a sensitive and dependable platform for nitrosamine analysis in applications that require stringent trace-level detection.

References

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4. Maria, J. F.; *et al.* Determination of 15 N-Nitrosodimethylamine Precursors in Different Water Matrices by Automated On-Line Solid-Phase Extraction Ultra-High-Performance-Liquid Chromatography Tandem Mass Spectrometry. *J. Chromatogr. A* **2016**, *1458*, 99–111.

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DE-012901

Printed in the USA, March 25, 2026
5994-9063EN

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