



An ultra-high sensitivity analysis of nitrosamines in multiple water sources

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INTRODUCTION

Nitrosamines have been a cause for concern for years, with some known to be genotoxic [carcinogenic]. They are byproducts of industrial processes and can form during water treatment processes that use ozone or chlorine for disinfection. Current regulations for these chemicals in water vary widely across regions, however. For example, with no explicit limit set for nitrosamines in the EU Drinking Water Directive, some countries do not regulate these chemicals or they provide guidance instead of enforcing strict limits. Even when limits are set, they can differ significantly. While the World Health Organization (WHO) specifies a guideline value of 100 ng/L for NDMA,¹ the limit is 10 ng/L in Germany.² In the US, the Environmental Protection Agency (EPA) sets health reference levels of 30 ng/L for NMBA, 0.4 ng/L for NDEA, 0.6 ng/L for NDMA, 7 ng/L for NDPA, 3 ng/L for NMEA and 2 ng/L for NPYR.³ In the Netherlands, drinking water legislation sets a health-based limit of 12 ng/L for NDMA specifically, while other nitrosamines are covered by a more general signaling limit of 1 µg/L (1,000 ng/L) for anthropogenic substances. The method described here enables ultra-high sensitivity analysis of nitrosamines in different water sources to help ensure compliance with a wide range of regulatory requirements.

ANALYSIS DETAILS

Standard preparation: Standards with ISO 17034 certification were purchased from Supelco and used to prepare the calibration curve in ultra-pure water at concentrations of 1–250 ng/L.

Sample preparation: Water sample containers were cleaned with methylene chloride before sampling, and sodium thiosulfate was added for dichlorination for a final concentration of 100 mg/L. Isotopically labeled nitrosamine internal standards were added to blanks, calibration standards and water samples for a final concentration of 50 ng/L. The resulting solutions were filtered with a 0.45 µm regenerated cellulose filter before transfer to glass autosampler vials for injection.

Chromatography: Chromatographic separation was performed using a Shimadzu LC-40 system with a Waters ACQUITY Premier HSS T3 [2.1 x 100 mm, 1.8 µm] column, using 0.1% formic acid in ultra-pure water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. The column temperature was maintained at 45°C and the injection volume was 100 µL.

Time [min]	Mobile phase A [%]	Mobile phase B [%]	Parameter	Value
0.0	95	5	Ionization technique	APCI
2.0	50	50	Polarity	Positive
9.5	30	70	Ion source gas 1	40 psi
10.0	0	100	Curtain gas	50 psi
12.0	0	100	Temperature	275°C
12.1	95	5	Nebulizer current	1 µA
15	95	5	CAD gas	8

LOQ IN ULTRA-PURE WATER

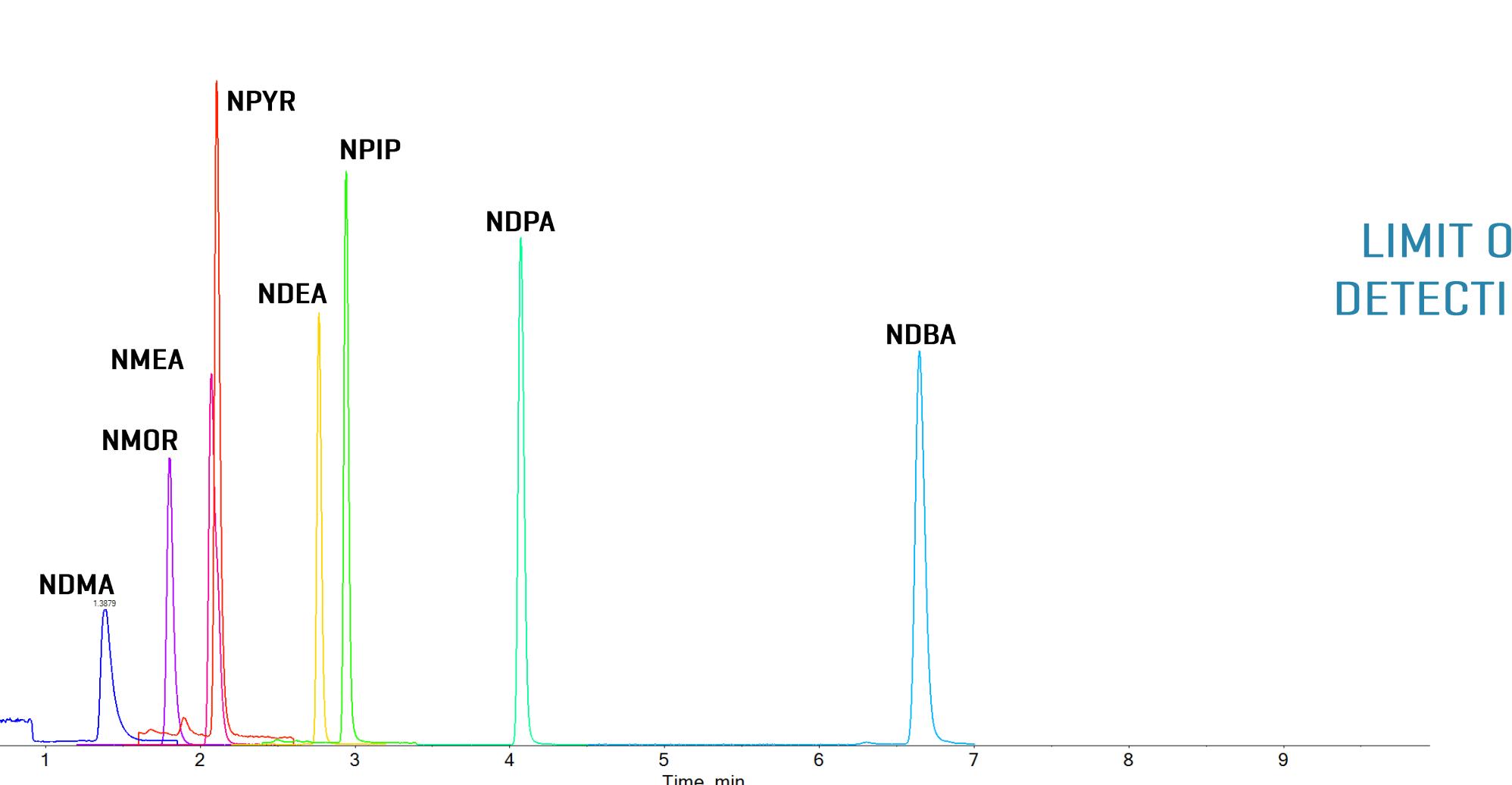
Component	LOQ [ng/L]
NDMA	3.0
NMOR	1.0
NMEA	1.0
NPYR	3.0
NDEA	1.0
NPIP	1.0
NDPA	1.0
NDBA	1.0



REFERENCES

1. World Health Organization (WHO). Guidelines for drinking-water quality: fourth edition incorporating the first and second addenda.
2. German Federal Environment Agency (UBA). Hygiene-related assessment of "non-relevant" metabolites (as defined in chemicals law) of active substances of plant protection products in drinking water.
3. US Environmental Protection Agency (EPA). Six-Year Review 3 Technical Support Document for Nitrosamines.

XIC SEPERATION



LIMIT OF DETECTION

LOW LEVEL MATRIX SPIKE RECOVERY

MID LEVEL MATRIX SPIKE RECOVERY

QUANTITATIVE RESULTS

Compound name	Spike level [ng/L]	Calculated LOD Drinking water [ng/L]	Calculated LOD Ground water [ng/L]	Calculated LOD Surface water [ng/L]
NDMA	5	0.9	1.0	0.9
NMOR	1	0.4	0.3	0.5
NMEA	1	0.5	0.8	0.7
NPYR	5	1.1	1.4	1.3
NDEA	1	0.7	0.5	0.4
NPIP	1	0.4	0.4	0.5
NDPA	1	0.1	0.2	0.2
NDBA	1	0.7	0.5	0.7

Spike level and detection limit in 3 water matrices tested. This table shows the calculated detection limit in each of the 3 sample types based on 9 replicate injections over 8 separate days. The LOD is defined as 3 times the standard deviation across all injections for each sample type. The spike level [either 1 ng/L or 5 ng/L] was determined based on the LOQ data

Nitrosamine	%CV for 5 ng/L spike			Accuracy for 5 ng/L spike		
	Drinking water	Ground water	Surface water	Drinking water	Ground water	Surface water
NDMA	6.3%	6.1%	6.0%	97.3%	104%	102%
NMOR	6.9%	7.2%	7.4%	101%	102%	105%
NMEA	7.9%	8.6%	9.9%	94.3%	103%	103%
NPYR	7.3%	8.9%	8.3%	102%	102%	105%
NDEA	9.1%	9.7%	12%	94.0%	99.1%	98.7%
NPIP	5.9%	6.5%	9.3%	96.9%	100%	100%
NDPA	4.6%	7.9%	9.2%	98.9%	103%	104%
NDBA	8.5%	5.3%	10.2%	99.4%	99.2%	96.2%

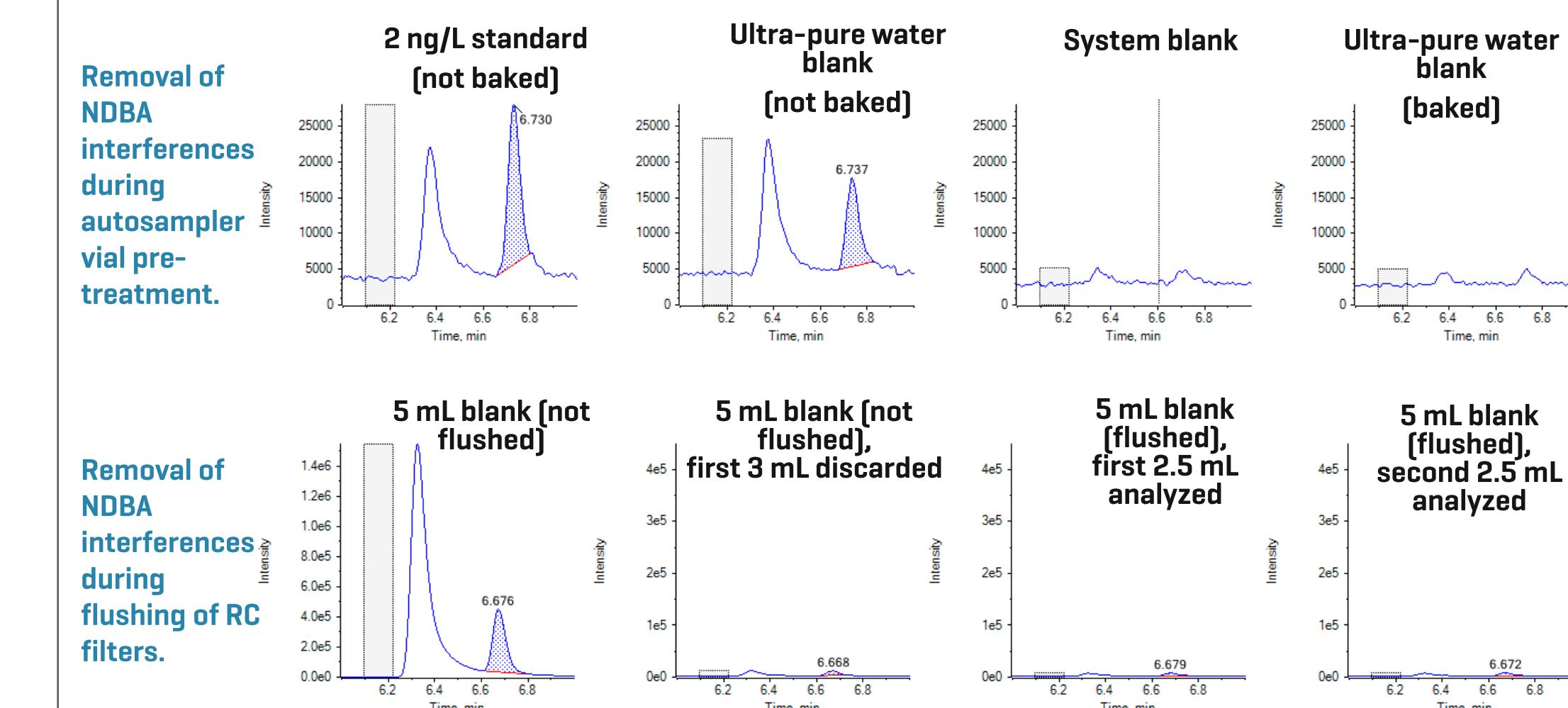
Quantitative performance for nitrosamine analysis in water samples. Inter-day reproducibility and accuracy results were determined from 5 ng/L spiked water samples across 9 replicate injections performed over 8 days.

Nitrosamine	%CV for 50 ng/L spike			Accuracy for 50 ng/L spike		
	Drinking water	Ground water	Surface water	Drinking water	Ground water	Surface water
NDMA	6.0%	7.9%	11%	98.9%	103%	106%
NMOR	6.4%	7.6%	9.7%	99.5%	102%	104%
NMEA	4.6%	6.7%	8.5%	98.4%	103%	104%
NPYR	5.1%	7.2%	8.0%	100%	103%	103%
NDEA	6.1%	7.2%	8.9%	99.8%	104%	103%
NPIP	5.0%	6.5%	8.7%	97.3%	102%	98.9%
NDPA	3.6%	6.1%	8.6%	99.8%	103%	104%
NDBA	3.5%	6.5%	7.8%	98.9%	102%	103%

Quantitative performance for nitrosamine analysis in water samples. Inter-day reproducibility and accuracy results were determined from 50 ng/L spiked water samples across 9 replicate injections performed over 8 days.

CONTAMINATION MITIGATION

- Ultra-pure water was treated with a UV module to break down trace amounts of nitrosamines.
- Dedicated glassware was used for nitrosamine sample preparation and analysis, and a autosampler vials were baked for several hours at 550°C, which removed blank interference for NDPA and a significant ghost peak for NDPA that previously prevented low-level quantitation.
- RC filters were flushed before use with 10 mL of ultra-pure water, which removed significant interferences for NDPA.
- Vials were not reused for re-injections due to the potential for nitrosamines to leach from the septum.



CONCLUSIONS

- High levels of sensitivity for nitrosamines in drinking, ground and surface water were achieved using the SCIEX 7500 system with LOQ levels of 1 ng/L and linearity across ≥ 3 orders of magnitude.
- Calculated detection limits as low as 0.1 ng/L were achieved in the 3 water sources.
- The method demonstrated accurate (94–105%) and highly reproducible (%CV≤12%) quantitative performance for 5 ng/L and 50 ng/L spikes in the 3 water sources.
- Solvent selection and pre-treatment of equipment are vital to the successful low-level quantitation of nitrosamines in water.

TRADEMARKS/LICENSING

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