

## Complete System Solution for the Determination of N-Nitrosamines in Drinking Water

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### Abstract

To provide a complete system solution for determining low parts per trillion levels (ng l<sup>-1</sup>) of EPA method 521<sup>1</sup> stated N-nitrosamines in drinking water.

### Introduction

The rapid and accurate analysis of drinking water is essential in protecting human health and well-being. The assurance of clean, safe drinking water has become more critical given the potential of accidental or intentional contamination, which has increased in recent years.

The detection of potentially carcinogenic N-nitrosamines in public water supplies has raised regulatory concerns and led to the requirement for surveillance of these unregulated contaminants. One source of concern is the probability of carcinogenicity of these compounds at low parts per trillion (ng l<sup>-1</sup>) concentrations.<sup>2</sup>

Water companies currently monitoring their supply have an opportunity to take the necessary steps to ensure the delivery of high quality water to their customers and prepare for the possibility of regulatory limits being applied. This will also help to demonstrate the supplier's commitment to public health.

N-nitrosamines are thought to occur in drinking water as a by-product of water treatment process operations.<sup>3</sup> They take the generic structure as shown in Figure 1 with variants arising from the substitution of the two R groups as shown in Table 1.

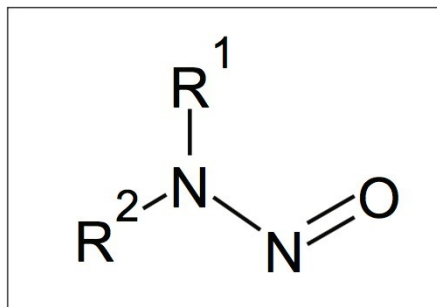


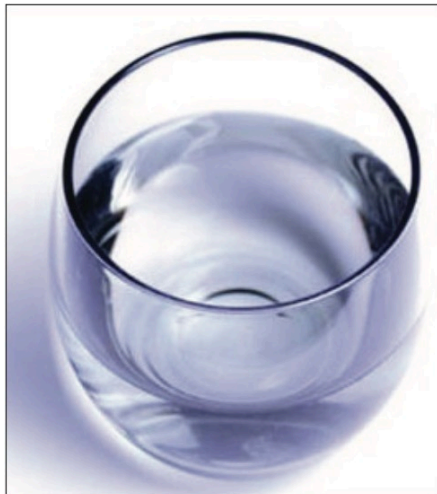
Figure 1. Generic N-nitrosamine structure.

Compound	R <sup>1</sup>	R <sup>2</sup>
N-Nitrosodimethylamine (NDMA)	CH <sub>3</sub> -	CH <sub>3</sub> -
N-Nitrosomethylethylamine (NMEA)	CH <sub>3</sub> -	CH <sub>3</sub> CH <sub>2</sub> -
N-Nitrosodiethylamine (NDEA)	CH <sub>3</sub> CH <sub>2</sub> -	CH <sub>3</sub> CH <sub>2</sub> -
N-Nitrosodi-n-propylamine (NDPA)	CH <sub>3</sub> CH <sub>2</sub> -CH <sub>2</sub> -	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> -
N-Nitrosodi-n-butylamine (NDBA)	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -
N-Nitrosopyrrolidine (NPYR)		
N-Nitrosopiperidine (NPIP)		

Table 1. N-nitrosamine variants arising from R<sup>1</sup> and R<sup>2</sup> substitution.

When relatively low molecular weight groups are present for R<sup>1</sup> and R<sup>2</sup>, N-nitrosamines are volatile and hydrophilic compounds. This adds difficulty to the analytical measurement in aqueous matrices. Addressing this challenge requires the use of an integrated approach of specialist chemistries and innovative instrumental technology.

The following method describes a complete system solution that allows the determination of N-nitrosamines in drinking water at low ng l<sup>-1</sup> concentrations.



## Experimental

### Sample extraction

Tap water samples were dechlorinated, fortified with varying levels of N-nitrosamines, spiked with 100  $\mu\text{l}$   $\text{d}_{14}$ -NPDA (10  $\text{ng ml}^{-1}$ ) and extracted using a Waters extraction manifold under vacuum in conjunction with Sep-Pak AC2 cartridges. Sep-Pak AC2 is a highly hydrophobic, low ash content, activated carbon used to remove or enrich highly polar organic molecules from water. Prior to extract elution, Sep-Pak AC2 cartridges were fitted directly to pre-conditioned Waters Sep-Pak Dry cartridges. Sep-Pak Dry is a high capacity desiccant used to remove residual water from water immiscible solvents. The procedure employed was a variation of EPA 521<sup>1</sup> methodology.

### SPE conditions

Cartridge:	Sep-Pak AC2, (Waters part no. JJAN20229)
Condition:	6 ml dichloromethane 6 ml methanol 10 ml $\text{H}_2\text{O}$ (analyte free)
Load:	500 ml sample ( $<10 \text{ ml min}^{-1}$ )

Dry:	Under vacuum (35 mins)
Condition:	Sep-Pak Dry cartridges (Waters part no. WAT054265) 4 ml dichloromethane
Elute:	Connect Sep-Pak AC2 Cartridges directly to conditioned Sep-Pak Dry cartridges Elute 3 x 2 ml dichloromethane
Concentrate:	To 500 µl under N <sub>2</sub> at 20 °C
GC conditions	
Instrument:	Quattro micro GC

## Chromatographic conditions

GC column:	BPX-VOL 30 m x 250 µm x 1.4 µm <i>df</i>
Injector :	PTV operated in solvent vent mode
Liner:	Tenax TA packed
Injector temp:	20 °C (0.65 min), 200 °C min <sup>-1</sup> to 300 °C (3 min), 300 °C min <sup>-1</sup> to 325 °C (5 min)
Vent pressure:	0 KPa
Vent flow :	70 ml min <sup>-1</sup>
Vent end :	0.65 min
Purge time:	4.05 min

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Injection: 20  $\mu\text{l}$  @ 0.5  $\mu\text{l s}^{-1}$

Oven temp: 35  $^{\circ}\text{C}$  (4 min), 25  $^{\circ}\text{C min}^{-1}$  to 280  $^{\circ}\text{C}$ , 30  $^{\circ}\text{C min}^{-1}$  to 300  $^{\circ}\text{C}$  (4 min)

Column flow: 1.3  $\text{ml min}^{-1}$  constant flow

## MS conditions

Ion mode: EI+

Electron energy: 70 eV

Source temp: 180  $^{\circ}\text{C}$

Acquisition: Multiple Reaction Monitoring (MRM)

Collision gas: Argon ( $3.0 \times 10^{-3}$  mBar)

<b>COMPOUND</b>	<b>MRM</b>	<b>Collision Energy (eV)</b>
NDMA	74 > 44	5
	74 > 74	5
NMEA	88 > 71	5
	88 > 73	5
NDEA	102 > 56	10
	102 > 85	5
d <sub>14</sub> NDPA	144 > 78	15
	144 > 126	5
NDPA	130 > 88	5
	130 > 113	5
NPYR	100 > 55	7
	100 > 70	7
NPIP	114 > 84	7
	114 > 97	5
NDBA	116 > 99	5
	158 > 99	7

Table 2. Multiple Reaction Monitoring MS parameters.

### Data acquisition and processing

Data was acquired using Waters MassLynx v. 4.1 Software. Data processing was carried out using Waters TargetLynx Application Manager.

### Results and Discussion

The volatile nature of these N-nitrosamines favors measurement by gas chromatography with a sensitive detector such as MS. However, the low molecular weights of these compounds make them difficult to detect

against background in real samples when using single stage nominal mass spectrometry. Quattro micro GC allows a greater opportunity to detect low levels in real samples without suffering from matrix saturation problems associated with other MS/MS instruments. This can also be achieved without the need for source reagent gas, which has the added advantage of further improving longer term measurement stability.

N-nitrosamine analysis requires pre-concentration and large volume injection to achieve the required ppt detection levels. Large volume injection presents its own problems due to the relative vapor pressures of the lower molecular weights species when compared to common GC injection solvents. Use of Tenax TA packed GC inlet liners aids retention of the N-nitrosamines during solvent venting and allows large injection volumes to achieve low detection levels. Figure 2 shows Quattro micro GC MRM chromatogram of N-nitrosamines at  $1 \text{ ng ml}^{-1}$  using  $20 \mu\text{l}$  solvent vent injection.

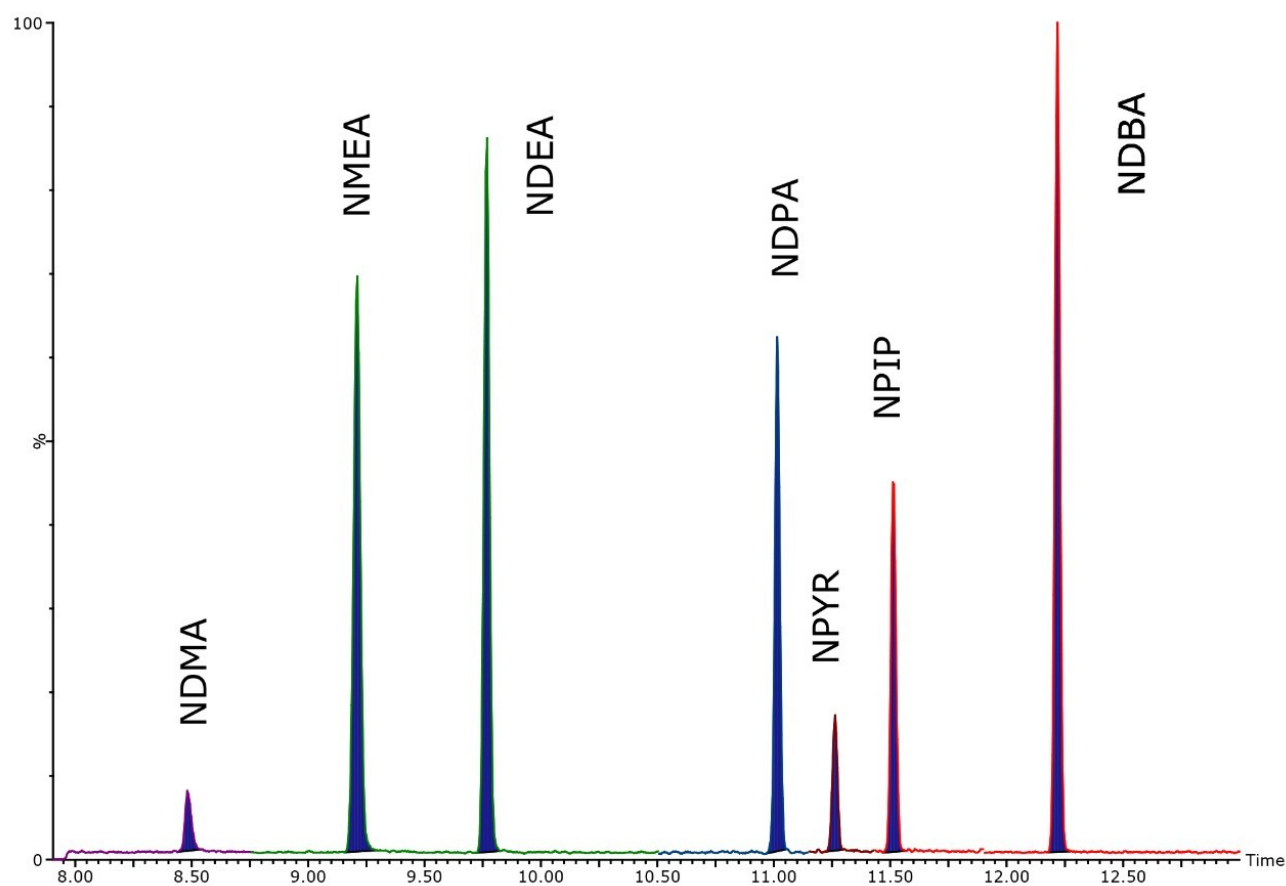


Figure 2. Extracted MRM chromatogram of N-nitrosamines at  $1 \text{ ng ml}^{-1}$  using  $20 \mu\text{l}$  solvent vent injection ( $1 \text{ ng l}^{-1}$  equivalent).

Sample pre-concentration is achievable using solid phase extraction (SPE) but due to the hydrophilicity of N-nitrosamines, it is critical to select the correct chemistry for extraction. When large volume injection is used along

with Sep-Pak chemistries and MRM on the Quattro micro GC, it is possible to achieve highly reproducible results at low concentrations with high recoveries. Figure 3 shows NDMA and NDPA in spiked drinking water at 1 ng l<sup>-1</sup>. Table 3 shows reproducibility and recovery data of N-nitrosamines spiked at 1 ng l<sup>-1</sup> in tap water.

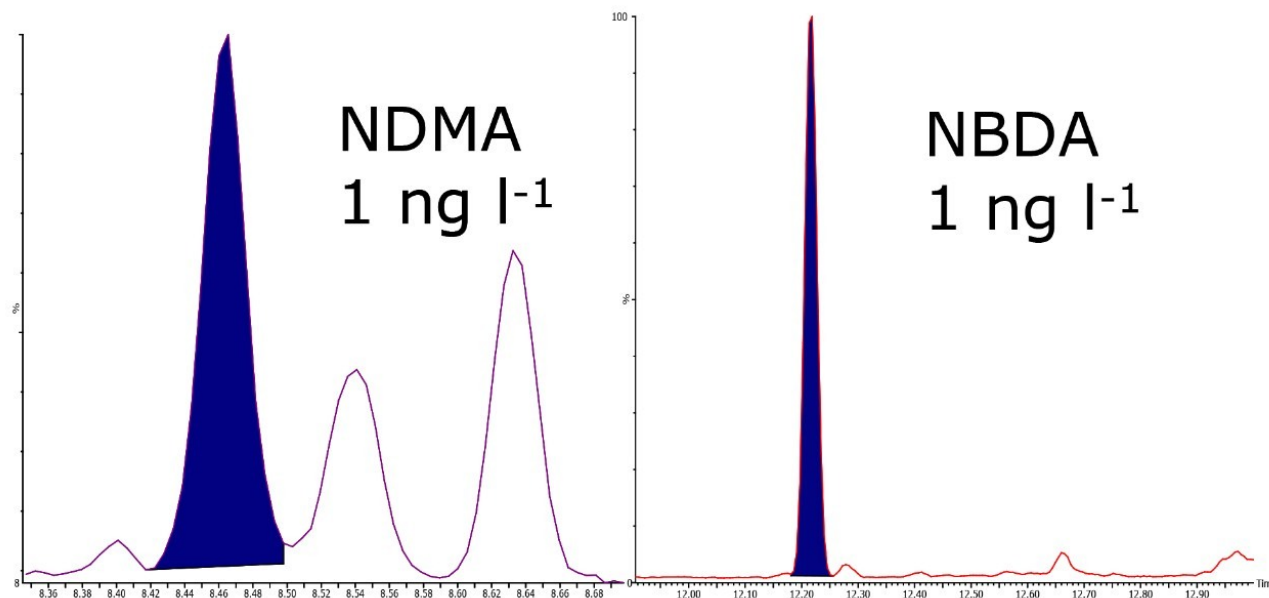


Figure 3. Tap water spike NDMA and NDPA 1 ng l<sup>-1</sup>.

<b>Compound</b>	<b>EPA MRL<sup>a</sup> ng l<sup>-1</sup></b>	<b>Concentration<sup>b,c</sup> ng l<sup>-1</sup></b>	<b>Recovery<sup>c</sup> %</b>	<b>RSD<sup>d</sup> %</b>
NDMA	2.0	1.0	100.5	6.6
NMEA	3.0	1.0	104.0	5.4
NDEA	5.0	1.0	100.0	4.2
NDPA	7.0	1.1	110.1	5.8
NPYR	2.0	1.1	107.5	5.4
NPIP	-	1.1	106.0	3.2
NDPA	4.0	1.1	106.7	2.8

Table 3. Reproducibility and recovery data of N-nitrosamines spiked at 1 ng l<sup>-1</sup> in tap water. <sup>a</sup>EPA minimum reporting level<sup>4</sup>, <sup>b</sup>calculated concentration, <sup>c</sup>mean (n=6), <sup>d</sup>percent relative standard deviation (n=6).



To assess method linearity, calibration curves (0.2 - 10 ng l<sup>-1</sup>) were prepared by extracting spiked tap water. High recoveries and reproducibility allowed excellent linearity for each N-nitrosamine. Table 4 gives calibration curve coefficient of determination values for each N-nitrosamine spiked. Figure 4 shows calibration curve and residual plot for NMEA (0.2 - 10 ng l<sup>-1</sup>). Calibration curves were produced using the TargetLynx Application Manager. Figure 5 shows the TargetLynx browser window.

<b>Compound</b>	<b>Coeff. Of Det (R<sup>2</sup>)</b>
NDMA	0.9987
NMEA	0.9997
NDEA	0.9969
NDPA	0.9994
NPYR	0.9989
NPIP	0.9994
NDBA	0.9995

Table 4. Coefficient of determination (R<sup>2</sup>) for N-nitrosamine spiked tap water (0.2 - 10 ng l<sup>-1</sup>), extracted with the full Sep-Pak AC2 and Sep-Pak Dry SPE procedure.

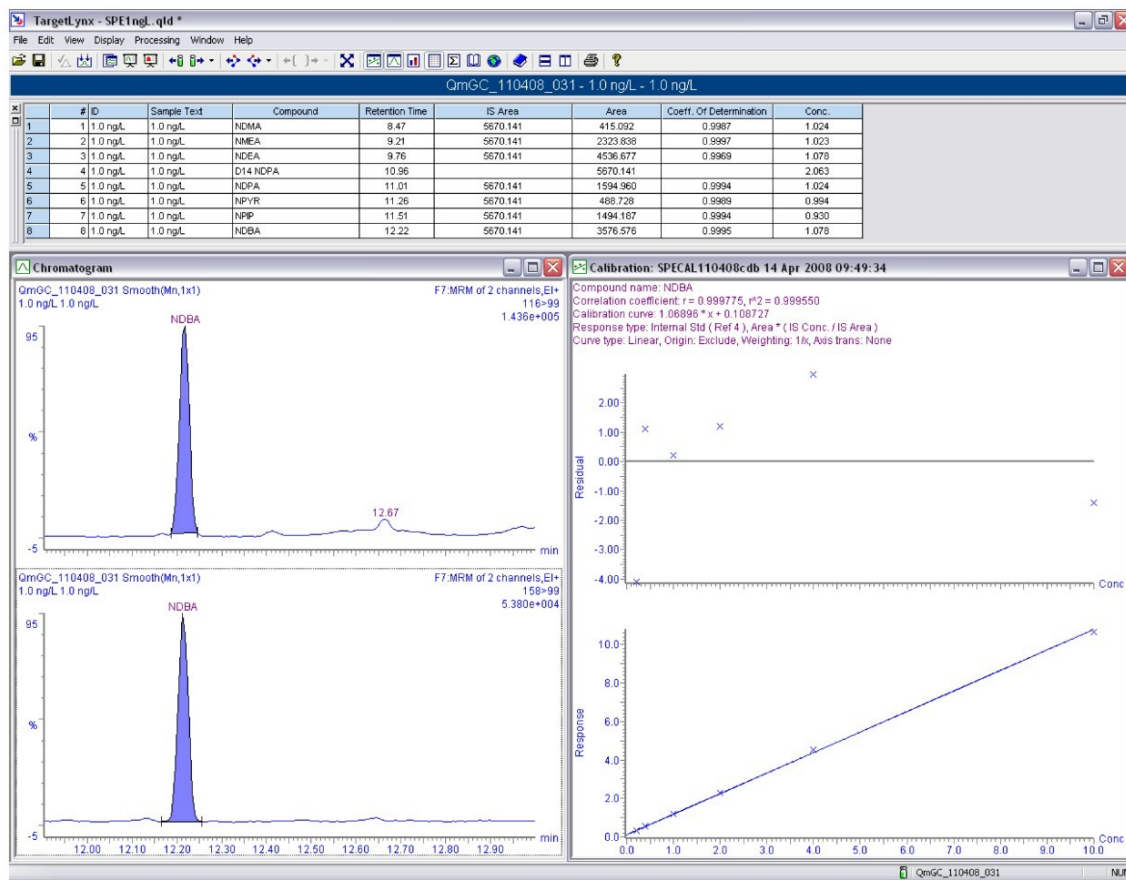


Figure 4. N-nitrosomethylethylamine calibration curve and residual plot for spiked tap water ( $0.2 - 10 \text{ ng l}^{-1}$ ) processed through the full SPE procedure.

Compound name: NMEA  
 Correlation coefficient:  $r = 0.999841$ ,  $r^2 = 0.999681$   
 Calibration curve:  $0.787081 * x + 0.0148846$   
 Response type: Internal Std ( Ref 4 ), Area \* ( IS Conc. / IS Area )  
 Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

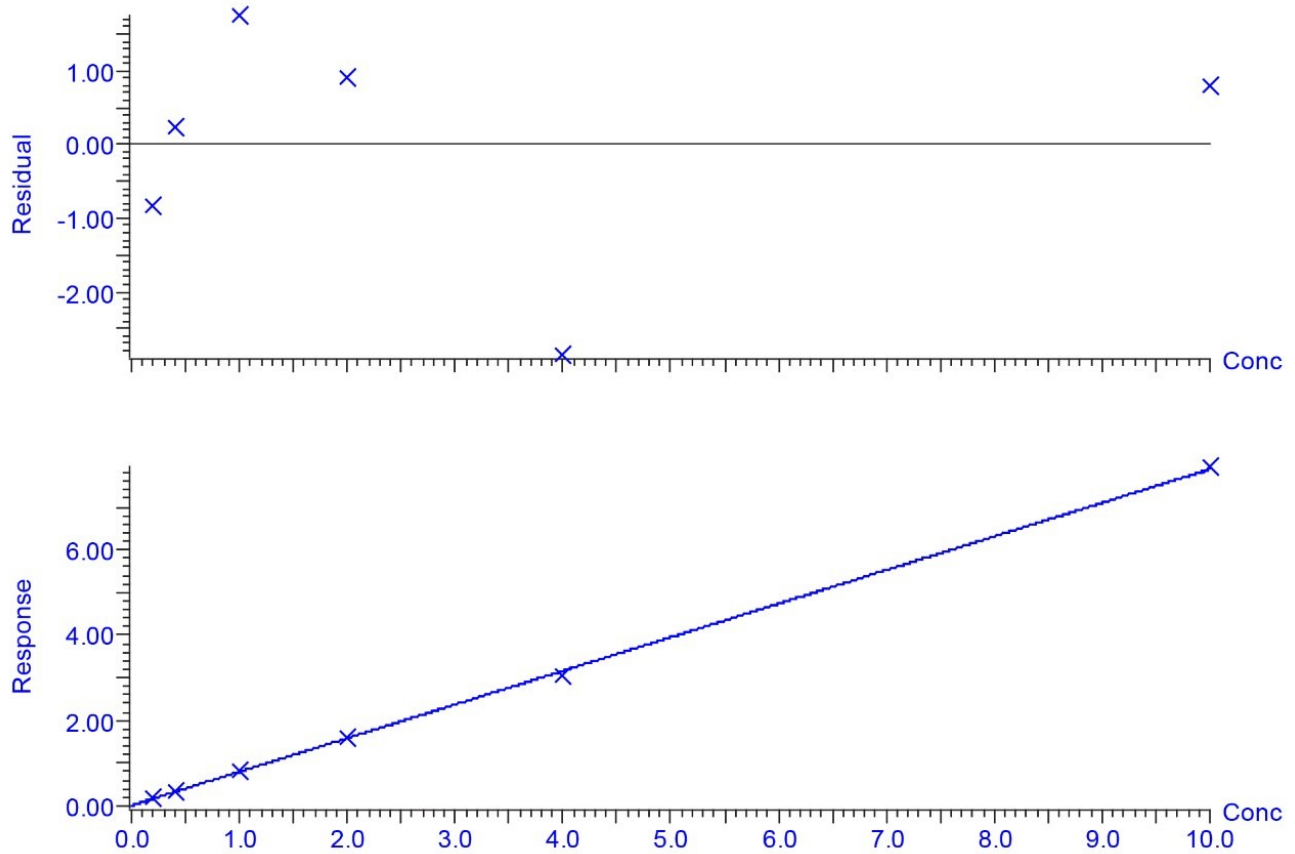


Figure 5. TargetLynx browser, customizable windows for efficient data processing.

## Conclusion

- There is a clear need for innovative solutions for the analytical challenges faced by the water industry with respect to emerging contaminants such as N-nitrosamines.
- Surveillance monitoring requirements, along with regulatory requirements to improve the quality of water supplies to populations, adds pressure to laboratories charged with delivering quality results every time.
- In the case of N-nitrosamines, specialist chemistry products namely Sep-Pak AC2 and Sep-Pak Dry have provided high quality and highly reproducible sample enrichments.
- Quattro micro GC provides accurate, reproducible trace-level quantitative analysis.

- It also offers greater selectivity to reduce matrix interferences, high sensitivity to reach the low detection levels required for monitoring, and quantitative and confirmatory data in a single injection.
- When specialist SPE chemistries and GC-MS/MS technologies are combined, the result is a powerful complete system solution that addresses the needs of water suppliers in difficult analytical areas.

## References

1. Website: [http://www.epa.gov/nerlcwww/m\\_521.pdf](http://www.epa.gov/nerlcwww/m_521.pdf)
2. Website: <http://www.epa.gov/ogwdw/ucmr/ucmr2/index.html>
3. W A Mitch and D L Sedlak. Water Supply. Vol 2 No 3; 191-198, 2001.
4. Website: <http://www.epa.gov/ogwdw/ucmr/ucmr2/methods.html>

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## Featured Products

- [MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)
- [TargetLynx <https://www.waters.com/513791>](https://www.waters.com/513791)

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