



# Quantitative Analysis of Explosives in Surface Water Comparing Off-Line Solid Phase Extraction and Direct Injection LC-MS/MS

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## Overview

Presented is an efficient method for measuring selected explosives in lake water at the sub-ng/L level applying either off-line Solid Phase Extraction (SPE) with LC-MS/MS detection and comparing it to direct injection LC-MS/MS.

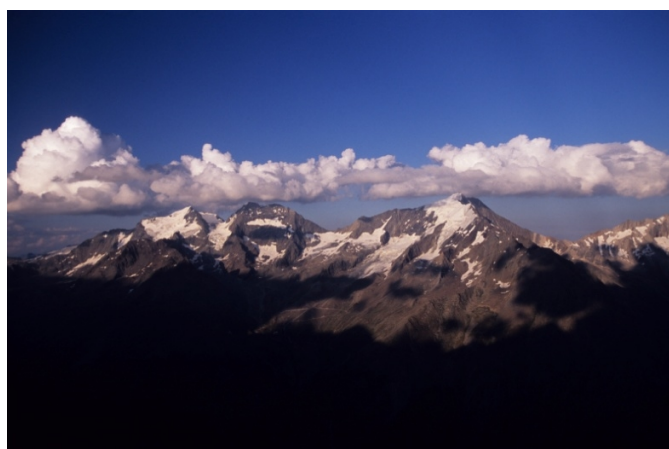
## Introduction

Between 1918 and 1967 some 8200 tons of ammunition, Trinitrotoluene (TNT) being the main explosive, was dumped to the lakes of Thun, Brienz and Lucerne in Switzerland.<sup>1</sup>

The amount of ecologically harmful compounds was considered to be negligible. In order for explosives to leak to the environment the casing must have rusted.<sup>2-3</sup> This corrosion process very much depends on environmental water conditions such as: temperature, oxygen content and pH value. Meanwhile a sediment layer of 20-30 cm covers the ammunition at the lakes' bottom and represents a natural barrier preventing the compounds to enter the aqueous phase.

Nevertheless water quality of the lakes should be monitored as lake water is frequently used as a source for drinking water.

Due to the very low concentrations of explosive residues expected in the lakes a powerful analytical set-up is important for a reliable detection and quantitation. LC-MS/MS analysis with Electrospray Ionization (ESI) is the method of choice to analyze polar and thermally labile compounds, such as explosives and their degradation products.



## Experimental

The following explosives and degradation products were investigated:

- 2,4,6-Trinitrotoluene (TNT)
- 2,4-Diamino-6-nitrotoluene (2,4-DA-6-NT)
- 2,6-Diamino-4-nitrotoluene (2,6-DA-4-NT)
- 2-Amino-4,6-dinitrotoluene (2-A-4,6-DNT)
- 4-Amino-2,6-dinitrotoluene (4-A-2,6-DNT)
- Hexogen (RDX)
- Nitroglycerin (NG)
- Octogen (HMX)
- Pentaerythritol tetranitrate (PETN)
- Tetryl

## Sample Preparation

50 mL of water samples were extracted on Phenomenex StrataX SPE cartridges. These extracts were analyzed by LC-MS/MS and compared to direct injections of filtered water samples.

Samples from different depths were analyzed within 48 hours after sampling. If water had to be stored for a longer period of time it was stabilized by acidifying to pH 3.5 with acetic acid and adding 2% of acetonitrile.

### Liquid Chromatography

- HPLC column: Xbridge Phenyl (2.1x150 mm), 3.5  $\mu$ m
- Eluent A: water + 2.5 mM ammonium acetate
- Eluent B: methanol + 2.5 mM ammonium acetate
- Gradient (A/B): 55/45 to 30/70 within 13 min and re-equilibration
- Flow: 200  $\mu$ L/min
- Injection volume: 100  $\mu$ L
- Oven temperature: 40°C

### Mass Spectrometry

- API 5000™ LC/MS/MS System
- Turbo V™ source with ESI probe
- Gas and source parameters: CUR: 20 psi, GS1: 40 psi, GS2: 40 psi, TEM: 350°C, CAD: 7, IonSpray voltage (IS): 5500 V (positive) and -4500 V (negative)
- Two periods with detection in positive and negative polarity using Multiple Reaction Monitoring (MRM) were programmed: 0 to 4.5 min (positive) and 4.5 to 15 min (negative). MRM transitions of detected explosives and MRM ratios are listed in Table 1.

### Calibration

Standards were prepared in MilliQ water and blank matrix water (matrix matched standards) over a range of 1-100 ng/L for off-line SPE and 0-1 ng/L for direct injection LC-MS/MS. Serial dilutions were obtained starting with a 10 ng/mL standard. All standards were prepared in water and kept at 4°C in the dark. Under these conditions standards were stable for at least three months – with the exception of TNT and Tetryl, which degrade rapidly and thus must be prepared freshly.

### Method validation data

- Recoveries (SPE): between 89% and 110% for all analytes
- Blank analysis: field blanks, travel blanks and laboratory blanks did not contain any traces of explosives (< 10% of lowest calibration standard)
- Linearity: 7 point equidistant calibration, statistical tests (Mandel, sensitivity plots and residual analysis) proved linearity of regression lines, residual analysis with normal distribution of the calibration points around the zero line
- Limit of Quantification (LOQ) with S/N=10 and Limit of Detection (LOD) S/N=3
- LOQ: 1 ng/L for DANT, NG and TNT, 0.03 ng/L for HMX, RDX, PETN and ADNT

**Table 1.** Retention times, MRM transitions of explosives with detected MRM ratio and tolerance intervals regarding the guideline 2002/657/EC<sup>5</sup>

Compound	$t_r$ (min)	MW	Precursor Ion	MRM Transition	MRM Ratio	Tolerance (%)	Tolerance Interval
2,4-DA-6-NT	3.7	167	168 [M+H] <sup>+</sup>	168/121 168/77	0.43	25	0.32-0.54
2,6-DA-4-NT	4.1	167	168 [M+H] <sup>+</sup>	168/121 168/77	0.37	25	0.28-0.46
HMX	5.0	296	355 [M+CH <sub>3</sub> COO] <sup>-</sup>	355/46 355/147	0.4	25	0.30-0.50
RDX	6.5	222	281 [M+CH <sub>3</sub> COO] <sup>-</sup>	281/46 281/93	0.04	50	0.02-0.06
NG	9.6	227	286 [M+CH <sub>3</sub> COO] <sup>-</sup>	286/62 286/46	0.83	20	0.67-1.00
4-A-2,6-DNT	9.9	197	196 [M-H] <sup>-</sup>	196/46 196/136	0.06	50	0.03-0.09
2-A-4,6-DNT	10.2	197	196 [M-H] <sup>-</sup>	196/46 196/136	0.26	25	0.20-0.33
Tetryl	11.9	287	286 [M-H] <sup>-</sup>	286/240 286/206	0.83	20	0.67/1.00
TNT	12.0	227	226 [M-H] <sup>-</sup>	226/46 226/196	0.49	25	0.37-0.61
PETN	13.1	316	375 [M+CH <sub>3</sub> COO] <sup>-</sup>	375/62 375/46	0.44	25	0.33-0.56

## Results and Discussion

Clearly, Electrospray Ionization turned out to be the method of choice for detecting traces of explosives in water samples.<sup>4</sup> Tests using either APCI or APPI were generally less sensitive (results not shown). As shown in Table 1 precursor ions of explosives were either detected as  $[M+H]^+$  or  $[M-H]^-$  for the DANT, ADNT, Tetryl and TNT, as  $[M+CH_3COO]^-$  for HMX, RDX, NG and PETN.

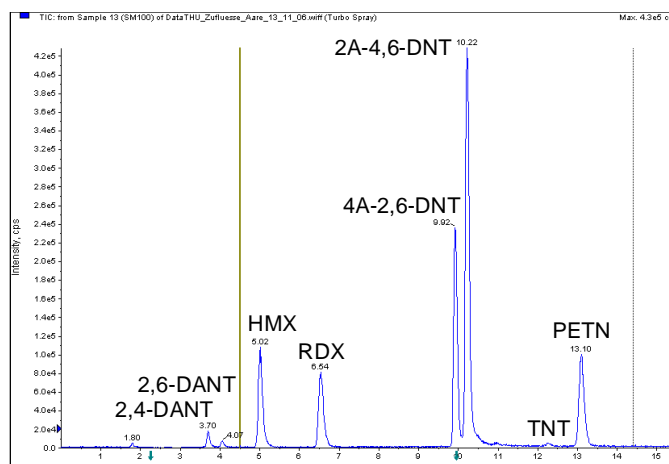
Selective detection was performed in MRM mode using two characteristic transitions for each compound. The ratio of both transitions was used to identify the presence of explosives in lake water regarding the guideline 2002/657/EC.<sup>5</sup>

Optimization of the compound dependent parameters was obtained by automatic Quantitative Optimization in Analyst® Software. The ion source temperature was a crucial parameter during source optimization. TNT, Nitroglycerine and above all Tetryl, known as being very labile, could only be detected using a rather low temperature of 350°C. As Nitroglycerine and Tetryl are not expected to persist for a longer time in the environment they were not included in the final target method.

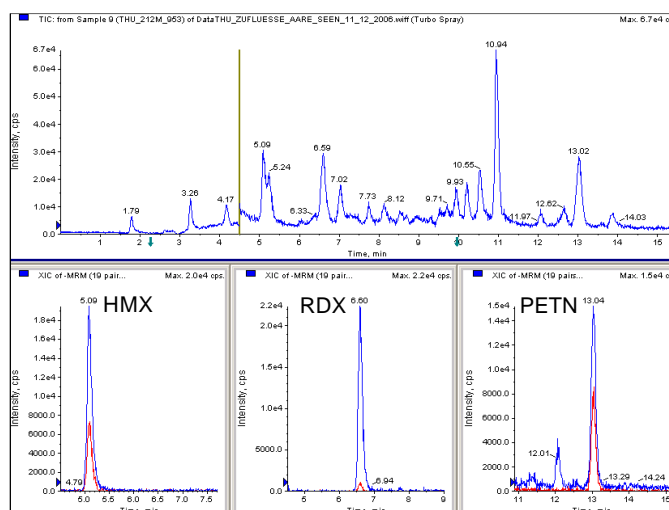
The separation of the different isomers of the diaminitrotoluenes and aminodinitrotoluenes became difficult on traditional C<sub>18</sub> stationary phases. Figure 1 presents a total ion chromatogram (TIC) with baseline separated analytes on the selected phenyl type phase.

Concentrations of explosive residues in lake water were assumed to be very low if present at all. Therefore, in a first attempt an off-line SPE enrichment procedure of the water samples was performed using an enrichment factor of 100. Using this procedure a typical TIC as shown in Figure 2 was obtained. Quantitation of the compounds revealed concentrations between 0.1-0.4 ng/L. Concentrations at different depths were very similar assuming a homogeneous distribution of the explosives in the water body.

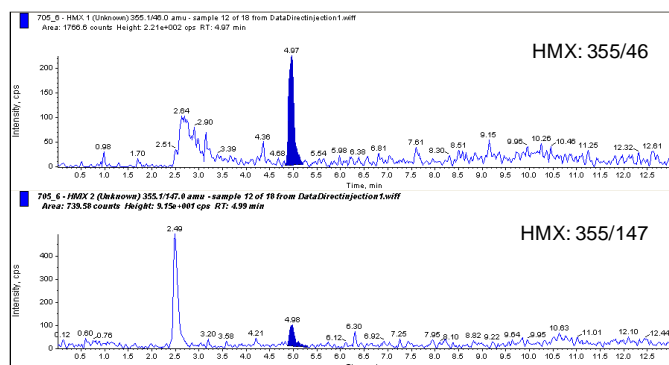
In a second step, direct injection of 100 µL of water samples was performed. A representative chromatogram of HMX is shown in Figure 3. The calibration curve (working range 0-1 ng/L) is presented in Figure 4. Quantitation of the sample resulted in a concentration of 0.21 ng/L. The calculated MRM ratio of 0.42 was well within the limits of the ratio obtained from the calibration line (0.40). Note the excellent agreement between the intensity (cps) of HMX in the concentrated sample ( $2.0 \times 10^4$  cps; enrichment factor 100) and the directly injected sample (200 cps).



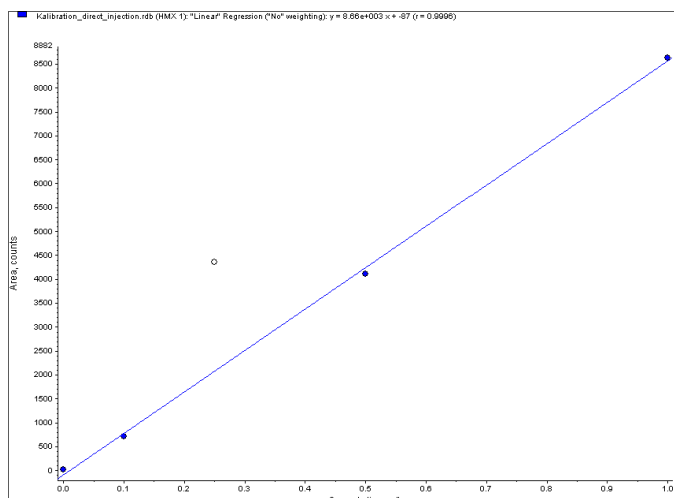
**Figure 1.** Total ion chromatogram of a 100 ng/L standard: 0 to 4.5 min in positive polarity 4.5 to 15 min in negative polarity



**Figure 2.** TIC of a lake water sample taken at a depth of 212 m showing the presence of HMX, RDX and PETN using off-line SPE

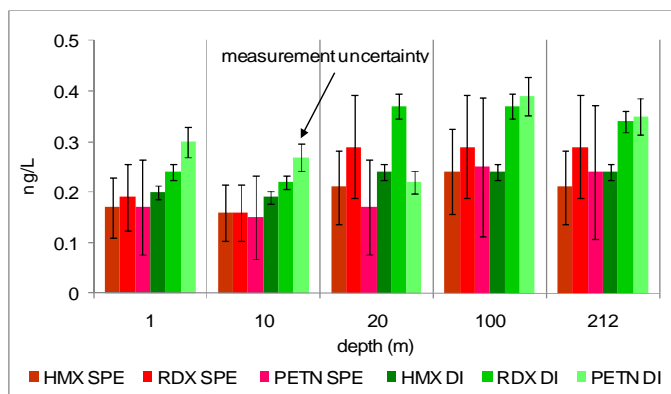


**Figure 3.** Direct injection of a lake water sample taken at a depth of 212 m showing the two transitions of HMX: 355/46 (upper trace), 355/147 (lower trace)



**Figure 4.** Calibration curve of HMX with a working range of 0-1 ng/L ( $r = 0.9996$ ) used for direct injection analysis

A comparison of the concentrations of direct injection and SPE enriched samples from different depths of the lake for HMX, RDX and PETN is shown in Figure 5. Concentrations of direct injection do not significantly deviate from the SPE samples. The lower concentrations detected after SPE can be explained by a recovery less than 100% and/or stronger ion suppression due to increased matrix concentration after extraction. However, uncertainty of measurement can drastically be reduced using direct injection LC-MS/MS.



**Figure 5.** Comparison of concentrations between direct injection and off-line SPE for HMX, RDX and PETN with error bars for uncertainty of measurement

## Summary

A highly sensitive LC-MS/MS method for the analysis of sub-ng/L levels of selected explosives such as TNT and the corresponding monoamino and diamino metabolites, HMX, RDX, and PETN has been presented. Specificity was obtained using Multiple Reaction Monitoring with identification based on ion ratio calculation using two transitions for each analyte. Sensitivity turned out to be optimal using Electrospray Ionization (ESI) with positive or negative polarity on an API 5000™ LC/MS/MS System equipped with a Turbo V™ source. Using direct injection analysis of water samples comparable results were obtained as from SPE enriched samples for the three main explosives HMX, RDX and PETN. In addition reproducibility was found to be much better using direct injection LC-MS/MS analysis.

## Acknowledgements

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## References

- 1 Van Stuijvenberg et al: Gefährdungsabschätzung zu militärischen Munitionsversenkungen in Schweizer Seen, Generalsekretariat VBS, September 2005
- 2 J. Sjöstrom et al: Environmental Risk Assessment of Dumped Ammunition in Natural Waters of Sweden, User Report, September 2004
- 3 H. Stucki: Chimia 58, 409-413, 2004
- 4 X. Xu et al: J Forensic Sci, 49, 6, 1-10, 2004
- 5 J.D. Berset et al: Chimia 61, 532, 2007
- 6 Chr. Borton et al: Application Note AB SCIEX #1282110-01

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