Sensitive and Fast Screening for Explosives: Utilization of Online Preconcentration and High Resolution Mass Spectrometry

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Overview

Purpose: To develop a single liquid chromatography-mass spectrometry (LC-MS) method for screening various explosives and related compounds (Table 1) in water using large injection volumes, online extraction and ultrahigh resolution MS.

Methods: Automated large volume injection, preconcentration and high performance liquid chromatography (HPLC) separation was performed using the Thermo Scientific EQuan MAX system. A water sample spiked with a mixture of explosives was injected onto a loading column for analyte preconcentration. Analytes were then eluted and chromatographically separated on a reversed-phase HPLC column. Calibration curve samples were analyzed with 3 replicates and river water samples with 2 replicate injections, respectively. Detection was performed on an Thermo Scientific Exactive™ high performance benchtop mass spectrometer operated under atmospheric pressure chemical ionization (APCI) conditions. Ion source parameters were optimized using direct infusion of a mixture of 4-NT, NG and NB. Data were acquired in full scan mode at 50,000 resolution setting. Quantifier ions for analyzed compounds (Table 1) were chosen after data acquisition.

Results: Limits of detection (LODs) in range of <0.5 – 25 ppt and lower limits of quantitation (LLOQs) in range of ≤0.5 – 50 ppt were achieved for most of the investigated compounds in neat solvent (Table 1, Figure 2). Spiked in river water, most of the compounds were detected at concentration of 100 ppt, half of them at 10 ppt, and one third of them at 1 ppt (Figure 4, Table 2).

Introduction

Explosives are significant environmental pollutants and have been successfully analyzed using LC-MS, typically under APCI conditions. They tend to form miscellaneous molecule-related ions ($M^{\cdot-}$, $[M-H]^-$, adducts, decomposition-related ions).[1,2] Formation of those ions depends on various factors, especially ionization technique – APCI, electrospray ionization (ESI), atmospheric pressure photoionization (APPI) – and LC solvents and additives. Often multiple ions are simultaneously formed for a particular compound which can significantly complicate method development. For that reason, an ultrahigh resolution mass spectrometer operating in full-scan mode is a good choice since diagnostic ions can be determined after acquisition.[3] Another challenging aspect of analysis of environmental samples is sensitivity. To achieve desired sensitivity, solid phase extraction (SPE) can be used, but it is a material, labor and time demanding technique. Automated large-volume injection and online extraction systems, such as the EQuan MAX system (Figure 1), can be a good choice to improve sensitivity while reducing overall labor and analysis time. Here we present a sensitive and fast LC-MS screening method of 22 explosives and related compounds, including those from United States Environmental Protection Agency (USEPA) 8330 method.

Methods

Sample Preparation

A set of explosives and related compounds was diluted in a serial manner in 50% MeOH providing concentrated stock solutions. Calibration curve standards were prepared from a sample of water (LC-MS grade, 20 mL) spiked with a proper stock solution (100 µL). A river water sample was collected from the Delaware and Raritan Canal in South Bound Brook, NJ. The river water sample was spiked with stock solutions to target concentrations (1, 10, 100 and 1000 ppt) and filtered through a 0.45 µm PTFE filter.

Liquid Chromatography

EQuan MAX automated high throughput system

Analytical Column:	Thermo Scientific Hypersil GOLD PFP (2.1 x 100 mm, 1.9 µm)
Loading Column:	Hypersil GOLD™ aQ (2.1 x 20 mm, 12 µm)
Injection Volume:	4 mL
Loading Solvent:	H_2O
Sample Loading:	2.0 mL/min for 2.5 minutes
HPLC Mobile Phase:	**(A)** 0.1 mM NH_4Cl in H_2O; **(B)** 0.1 mM NH_4Cl in 99% MeOH
HPLC Flow Rate:	300 µL/min

HPLC Gradient:

Time	A%	B%
0.00	90	10
2.50	90	10
2.55	70	30
11.00	00	100

Time	A%	B%
11.50	90	100
11.55	90	90
12.00	90	90

Mass Spectrometry

Exactive™ high performance benchtop LC-MS powered by Thermo Scientific Orbitrap technology.

Ionization Mode:	Negative ion APCI
Corona Needle Current:	80 µA
Vaporizer:	200 °C
Ion Transfer Tube:	125 °C
Scan Mode:	Full MS 100-500 amu
Resolution:	50,000; External calibration

Data Analysis

Thermo Scientific LCQUAN software was used for data acquisition and processing. Recorded data were processed using a 5 ppm mass tolerance filter.

FIGURE 1. EQuan MAX LC-MS system equipped with an Exactive benchtop mass spectrometer.

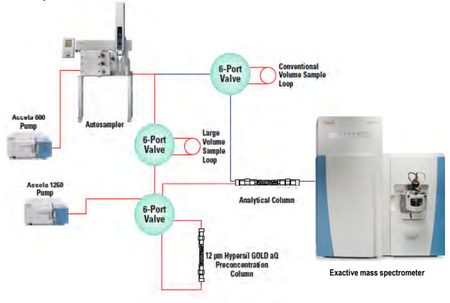


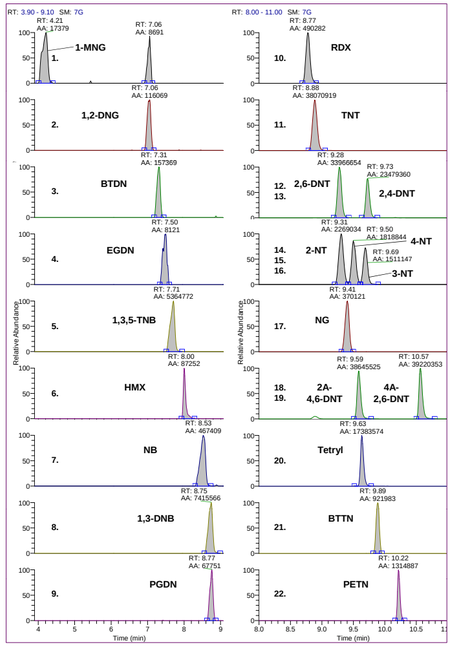
Table 1. Explosives and related compounds used in the LC-MS assay. Included are the types of ions which were used as diagnostic ions for particular compounds.

	Chemical name	Abbreviation	Quantifier Ions					
			$[M-H]^-$	$[M]^-$	$[M+^{35}Cl]^-$	$[M+^{37}Cl]^-$	$[M-NO_2]^-$	$[M+MeO]^-$
1	1-Mononitroglycerin	**1-MNG**			172.0018	173.9989		
2	1,2-Dinitroglycerin	**1,2-DNG**			216.9869	218.9840		
3	1,2,4-Butanetriol 1,4-dinitrate	**BTDN**			231.0026	232.9996		
4	1,2-Ethanediol dinitrate	**EGDN**			186.9763			
5	1,3,5-Trinitrobenzene	**1,3,5-TNB**		213.0027				
6	Cyclotetramethylene tetranitramine	**HMX**			331.0159	333.0130		
7	Nitrobenzene	**NB**		123.0326				
8	1,3-Dinitrobenzene	**1,3-DNB**		168.0177				199.0360
9	1,2-Propanediol dinitrate	**PGDN**			200.9920	202.9890		
10	Cyclotrimethylene trinitramine	**RDX**			257.0043	259.0013		
11	2,4,6-Trinitrotoluene	**TNT**	226.0106	227.0184				
12	2,6-Dinitrotoluene	**2,6-DNT**		182.0333				
13	2,4-Dinitrotoluene	**2,4-DNT**	181.0255	182.0333				
14	2-Nitrotoluene	**2-NT**		137.0482				
15	4-Nitrotoluene	**4-NT**		137.0482				
16	3-Nitrotoluene	**3-NT**		137.0482				
17	Nitroglycerin	**NG**			261.9720	263.9690		
18	2-Amino-4,6-Dinitrotoluene	**2A-4,6-DNT**	196.0364	197.0442	232.0131	234.0101		
19	4-Amino-2,6-Dinitrotoluene	**4A-2,6-DNT**	196.0364	197.0442	232.0131	234.0101		
20	2,4,6-Trinitrophenyl-N-methylnitramine	**Tetryl**					241.0215	
21	1,2,4-Butanetriol trinitrate	**BTTN**			275.9876	277.9847		
22	Pentaerythritol tetranitrate	**PETN**			350.9833	352.9803		

Results

- Chromatographic separation and signal response of 22 explosives and related compounds in neat water are shown in Figure 2. Quantifier ion(s) for every individual compound were chosen after data acquisition (Table 1).
- Calibration curves for the tested compounds were fit to either linear or quadratic regression. Quantitation dynamic ranges were defined from LLOQ – 1000 ppt (Table 2 A). The LLOQs (lowest calibration concentrations) were defined as concentrations with relative CVs <25% for 3 replicate injections.
- For several compounds (e.g. TNT) signal response at the lowest tested level (0.5 ppt) indicated possible further improvements of their detection limits.
- Most of the compounds were detected in spiked river water at concentration of 100 ppt, half of them at 10 ppt, and one third of them at 1 ppt (Table 2B, Figure 4). Calculated concentrations are generally lower than the nominal ones which can be attributed to a matrix effect.

FIGURE 2. Extracted ion chromatograms (XICs) of the investigated compounds in neat solvent at concentrations of 500 ppt (5000 ppt for EGDN and PGDN). Diagnostic ions used for each particular compound are listed in Table 1. All chromatograms are reconstructed with 5 ppm mass tolerance.



- The overall sensitivity of each compound obviously depended on two factors: mass spectrometric sensitivity and trapping efficiency on the preconcentration column. It can be expected that compounds with higher affinity to the stationary phase exhibit more efficient preconcentration than earlier eluting ones. (Note that relative affinities to the trap column don't necessarily follow the ones to the analytical column due to different stationary phases.) For example, using regular HPLC injection and setup, HMX, RDX, and 1,3-DNB showed a similar response.[3] However, because of their different trapping efficiency, 1,3-DNB exhibits significantly better sensitivity than HMX and RDX (Table 2).
- Sensitivity for EGDN and PGDN was significantly lower comparing to other compounds. This observation is consistent with data published elsewhere.[2,3]
- Data obtained for the river water sample generally showed little to no chemical noise in chromatograms due to the ultrahigh resolution and narrow mass window of ± 5 ppm. The importance of these two factors is demonstrated in Figure 3 showing chromatograms for 1,3-DNB with various mass tolerances applied. Obviously, mass tolerance of ≥10 ppm prohibits any quantitation at concentration of 10 ppt.

FIGURE 3. XIC of 1,3-DNB spiked in river water at level of 10 ppt. Chromatograms are reconstructed using different mass tolerances

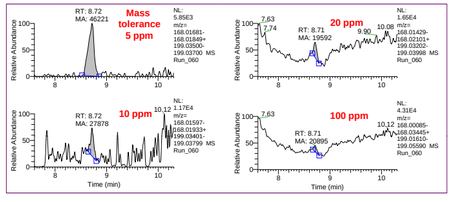
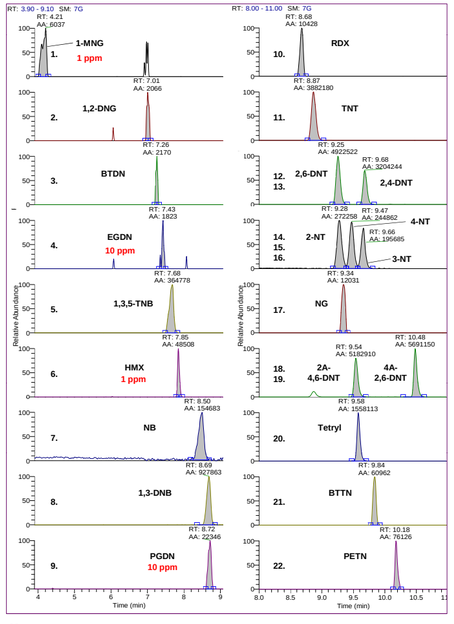


Table 2. A) Lower detection limit, quantitation dynamic range, and statistic data for the investigated compounds measured in neat water; B) Calculated concentrations of the compounds spiked in river water sample. Obtained values are based on averages of two replicate measurements.

	Compound	A) Calibration curve in neat solvent			B) Calculated concentrations in spiked river water (ppt)			
		LOD (ppt)	Dynamic Range (ppt)	CV(%) at LLOQ	*1 ppt spiked*	*10 ppt spiked*	*100 ppt spiked*	*1000 ppt spiked*
1	1-MNG	100	100-1000	5.5 %	N/D	N/D	51	218
2	1,2-DNG	25	25-1000	8.4 %	N/D	N/D	26	315
3	BTDN	25	25-1000	22.5 %	N/D	N/D	26	240
4	EGDN	2500	2500-10000	19.5 %	N/D	N/D	N/D	1870 *
5	1,3,5-TNB	1	1-1000	7.4 %	0.77	3.6	39	525
6	HMX	25	50-1000	18.7 %	N/D	N/D	7.8	252
7	NB	10	25-1000	4.9 %	0.77	28.0	176	2415
8	1,3-DNB	1	1-1000	7.1 %	N/D	3.9	66	905
9	PGDN	500	1000-10000	5.4 %	N/D	N/D	N/D	1930 *
10	RDX	10	25-1000	6.9 %	N/D	N/D	23	174
11	TNT	<0.5	≤0.5-1000	5.5 %	0.49	3.9	53	679
12	2,6-DNT	<0.5	≤0.5-1000	3.6 %	0.43	5.6	76	888
13	2,4-DNT	<0.5	≤0.5-1000	9.7 %	0.72	4.4	71	937
14	2-NT	2.5	2.5-1000	7.6 %	N/D	6.1	65	1022
15	4-NT	2.5	2.5-1000	12.8 %	N/D	7.2	71	1027
16	3-NT	2.5	5-1000	7.0 %	N/D	6.4	67	973
17	NG	10	25-1000	5.2 %	N/D	N/D	31	373
18	2A-4,6-DNT	<0.5	≤0.5-1000	6.0 %	0.57	4.8	70	911
19	4A-2,6-DNT	<0.5	≤0.5-1000	8.0 %	0.90	5.5	72	845
20	Tetryl	<0.5	≤0.5-1000	9.4 %	0.46	3.9	51	517
21	BTTN	5	10-1000	21.9 %	N/D	N/D	45	540
22	PETN	5	10-1000	5.1 %	N/D	N/D	40	479

** Spiked at concentration level of 10000 ppt.*

FIGURE 4. Extracted ion chromatograms of the compounds spiked in river water to final concentrations of 100 ppt (except for 1-MNG, EGDN, PGDN, HMX).



Conclusion

- An automated LC-MS method using large injection volumes, online extraction, and ultrahigh resolution MS was developed for screening of 22 explosives and related compounds.
- Using a 4 mL injection volume, most of the tested compounds showed low- or sub-ppt level detection and quantitation limits in neat water.
- Spiked in river water, most of the compounds were detected at a concentration of 100 ppt, half of them at 10 ppt and one third of them at 1 ppt.
- Further improvement of the LC-MS method can include improving trapping efficiency to increase sensitivity for some of the compounds. Also, the method robustness can be tested using other matrix samples, such as wastewater and soil.

References

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2. Zhao, K.; Yinon, J. J. *Chromatogr. A* **2002**, *977*, 59-68.
3. Ruzicka, J.; McHale, K. J.; Sanders, M. *Proceedings of the 57th ASMS Conference on Mass Spectrometry and Allied Topics.* Philadelphia, PA. **2001**.