

Sensitive determination of explosive compounds in water

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Keywords

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Goal

To develop an efficient high-performance liquid chromatography (HPLC) method for the sensitive determination of 14 explosives and related substances in tap water using on-line solid phase extraction (SPE) instead of the salting-out extraction specified in U.S. Environmental Protection Agency (EPA) Method 8330A

Introduction

There has been increased attention on the persistence of residual explosive materials and their degradation products, which are substances that are highly toxic to the environment. Gas chromatography (GC) is one of the methods used to determine these substances, but because some explosive materials and their byproducts are thermally unstable or nonvolatile, this can result in inexact determinations. HPLC with UV detection is ideally suited for low-level determination because it is not subject to these limitations.

EPA Method 8330A describes an HPLC method with UV detection to determine 14 priority explosives and related substances (structures shown in Figure 1).¹

This EPA method requires two columns for analysis. A C18 reversed-phase column is used as the primary column to separate the 14 compounds. However, not all compounds are resolved in this step, so a secondary cyano column is used as a complementary phase to resolve the peaks that are unresolved in the C18

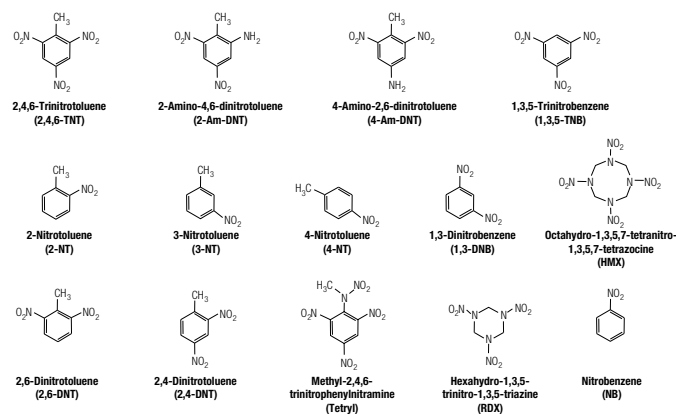


Figure 1. Structures of the 14 explosives and related compounds.

separation and to confirm the peaks found in the first separation. Therefore, the same sample is separated on two different columns. Additionally, because 2,4-DNT and 2,6-DNT elute at similar retention times (~0.2 min difference) on a C18 column, the EPA method requires the preparation of two groups of solutions containing 2,4-DNT and 2,6-DNT, respectively, when both are to be determined.

Previous methods have been used to complete the separation and confirmation simultaneously on the Thermo Scientific™ UltiMate™ 3000 Dual Gradient Rapid Separation (RS) LC system.² The sample preparation procedure for low concentrations of the target compounds in water, described in EPA Method 8830A, is a salting-out extraction. This approach is time consuming, requires large amounts of reagents, and is deficient in terms of process control. These limitations prompted development of an on-line method that eliminates the salting-out step for the determination of low-level concentrations of the target compounds.

Equipment

- An UltiMate 3000 Dual Gradient RSLC system, including:
 - Thermo Scientific™ UltiMate™ 3000 Series DGP-3600RS Dual Gradient Rapid Separation Pump (P/N 5040.0066) with SRD-3600 Integrated Solvent and Degasser Rack (P/N 5035.9230)
 - Thermo Scientific™ UltiMate™ 3000 WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020) with a 1000 µL sample loop (P/N 6820.2429) and a 1000 µL syringe (P/N 6822.0005)
 - Thermo Scientific™ UltiMate™ 3000 TCC-3000RS (P/N 5730.0000) or TCC-3000SD (P/N 5730.0010) Thermostatted Column Compartment equipped with one 2p–6p valve
 - Thermo Scientific™ UltiMate™ 3000 DAD-3000RS Diode Array Detector (P/N 5082.0020) with semi-micro flow cell, 2.5 µL, SST (P/N 6082.0300)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.1

Table 1. Preparation of calibration standards.

Stock Std of Explosives Cal. Mixture	Stock Std Volume of Explosives Cal. Mixture (mL)	Water Volume (mL)	Final Volume of Cal. Std (mL)	Final Conc of Cal. Std (µg/L)
Stock Standard Mix 2 0.1 µg/mL	0.2	19.8	20.0	1.0
	1.0	19.0		5.0
	2.0	18.0		10
Stock Standard Mix 1 10 µg/mL	0.1	19.9	20.0	50
	0.2	19.8		100
	1.0	19.0		500

Consumables

Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity
- Acetonitrile (CH₃CN), HPLC (Fisher Scientific P/N AC610010040)
- Methanol (CH₃OH), 99.9%, HPLC (Fisher Scientific P/N AC610090040)
- Method 8330-Explosives by HPLC, 1000 µg/mL in methanol:acetonitrile (1:1), consists of 14 explosives and related substances: 2-Am-DNT; 1,3-DNB; 2,4-DNT; HMX; NB; RDX; 1,3,5-TNB; 2,4,6-TNT; 4-Am-DNT; 2,6-DNT; 2-NT; 3-NT; 4-NT; and tetryl (AccuStandard P/N M-8330-R)

Preparation of Standards and Solutions

Stock Standard Mix 1

Mix 100 µL of the Method 8330-Explosives by HPLC standard (1000 µg/mL each) and 9.9 mL of H₂O/CH₃CN (1:1, v/v) in a 10 mL vial. The concentration of each explosive in the Stock Standard Mix 1 will be 10 µg/mL.

Stock Standard Mix 2

Mix 100 µL of Stock Standard Mix 1 (10 µg/mL each) and 9.9 mL of H₂O/CH₃CN (1:1, v/v) in a 10 mL vial. The concentration of each explosive in the Stock Standard Mix 2 will be 0.1 µg/mL.

Working Standard Solutions for Calibration

For calibration, prepare six working standard solutions with different concentrations by diluting the proper amount of either Stock Standard Mix 1 or 2 with water. The volumes of each solution needed to make the calibration standards are shown in Table 1.

Sample Preparation

The tap water samples were collected from local water supplies in Shanghai, China.

Filter water samples using the nylon syringe filters prior to injection. Add 100 µL of Stock Standard Mix 2 and 9.9 mL of each filtered water sample to a 10 mL vial. The concentration of each explosive in the spiked water samples will be 1 µg/L.

Note: Tetryl decomposes rapidly in CH₃OH/H₂O solutions, as well as with heat. Dilute all aqueous samples expected to contain tetryl with acetonitrile prior to filtration and acidify to pH <3 using acetic acid. Do not expose samples expected to contain tetryl to temperatures above room temperature (20–30 °C).

Conditions

On-line SPE	
Column:	Thermo Scientific™ Acclaim™ PolarAdvantage II (PA2), 3 µm Analytical, 3.0 × 33 mm (P/N 066276)
Mobile Phase:	A) H ₂ O; B) CH ₃ OH
Gradient:	0–12 min, 5% B; 20–25 min, 100% B; 27–40 min, 5% B
Flow Rate:	0.7 mL/min
Inj. Volume:	1000 µL on the on-line SPE cartridge
Separation	
Column:	Explosives E2, 4.6 × 250 mm (P/N 064309)
Mobile Phase:	H ₂ O/CH ₃ OH (52:48, v/v)
Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	UV absorbance at 254 nm
Valve Position	0 min, 1_2 4.0 min, 6_1 12 min, 1_2

Column:	Acclaim Explosives E2 (4.6 × 250 mm)
Mobile Phase:	48% Methanol, 52% Water
Flow Rate:	1.0 mL/min
Inj. Volume:	80 µL
Temperature:	30 °C
Detection:	UV absorbance at 254 nm

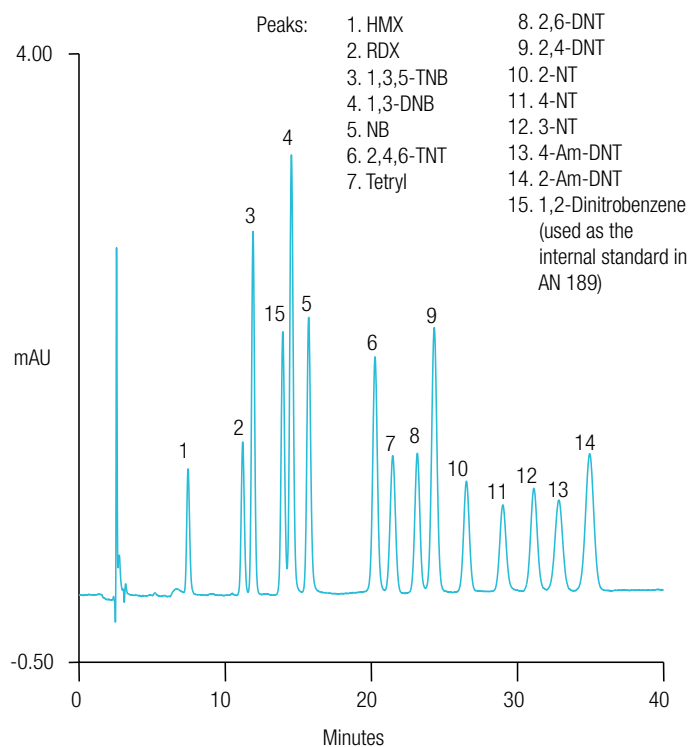


Figure 2. The 14 explosives and related compounds (160 µg/L each).

Results and Discussion

Separation of 14 Explosives and Related Substances on the Acclaim Explosives E2 Column

The 14 explosives and related substances must be divided into two groups for analysis because of the coelution of some compounds (i.e., 2,4-DNT and 2,6-DNT) when using the common columns C18 and CN recommended by EPA Method 8330A. However, because the Acclaim Explosives E2 column is specifically designed to separate these 14 substances, there is no need to prepare two groups of standards. Figure 2 shows that all 14 compounds are baseline resolved in one injection, which allows for significant time savings in both standard preparation and sample analysis. Peak 15, which eluted before Peak 4, is 1,2-dinitrobenzene, which was used as the internal standard in local water supplies in Shanghai, China.

Evaluation of On-Line SPE

Figure 3 shows a typical flow schematic of on-line SPE, which is directly coupled to the HPLC column using one 6-port (2p-6p) valve. The filtered sample is directly injected onto the system and delivered to the SPE column for enrichment (1_2 position) using the first pump; the analytical column is simultaneously equilibrated with the second pump of the dual-pump module. After the analytes are bound to the SPE column and impurities are washed out, the SPE column is switched into the analytical flow path to elute the bound analytes (6_1 position); the analytes are then separated on the analytical column and detected by the UV detector. This method is easily accomplished using the UltiMate 3000 Dual Gradient RS system to analyze local water supplies in Shanghai, China.

Optimization of Conditions for On-Line SPE

The polar-embedded Acclaim PA2 column is designed for enhanced hydrolytic stability within a wide range of pH values (pH 1.5–10) and for compatibility with 100% aqueous mobile phases.² It was selected as the SPE column for this application based on its excellent retention of the explosives and related compounds using 95% aqueous mobile phase (H₂O) and ease of elution using organic mobile phase (CH₃OH), which is compatible with the subsequent analytical separation on

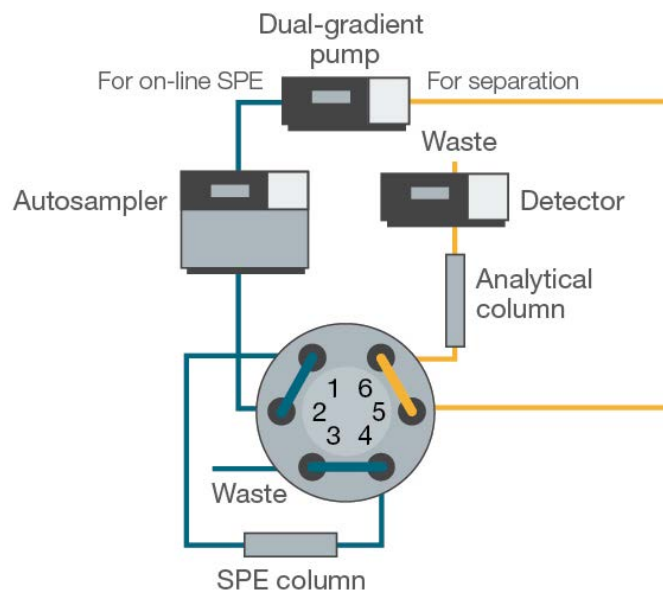


Figure 3. Flow schematic of on-line SPE.

For On-Line SPE

Column: Acclaim PA2, 3 μ m Analytical, 3.0 \times 33 mm
Mobile Phase: A. H₂O; B. CH₃OH
Gradient: 0–12 min, 5% B; 20–25 min, 100% B; 27–40 min, 5% B
Flow Rate: 0.7 mL/min
Inj. Volume: 1000 μ L onto the on-line SPE cartridge

For Separation

Column: Acclaim Explosives E2, 4.6 \times 250 mm
Mobile Phase: H₂O/CH₃OH (52:48, v/v)
Flow Rate: 1.0 mL/min
Temperature: 30 $^{\circ}$ C
Detection: UV absorbance at 254 nm
Valve Position: 0 min, 1_2
4.0 min, 6_1
12 min, 1_2

Sample: 14 explosives and related compounds (100 μ g/L each)

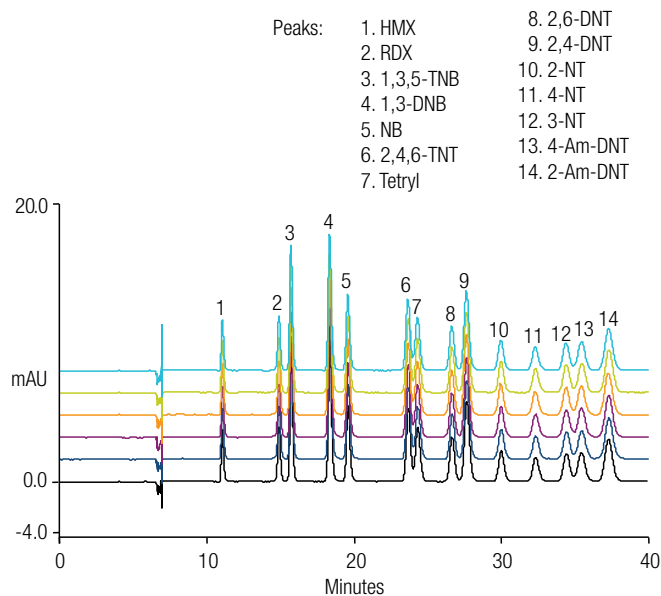


Figure 4. Six consecutive injections of the 14 explosives and related compounds standard mixture following on-line SPE.

the Acclaim Explosives E2 column. Figure 4 illustrates good separation of the 14 explosives and related compounds following on-line SPE under the specified chromatographic conditions.

Reproducibility, Linearity, and Detection Limits

Method precision was estimated using UV detection by making six consecutive 1000 µL injections of a calibration standard with a concentration of 100 µg/L of each analyte (Figure 4). The retention time and peak area reproducibilities (RSD) show good precision for the on-line SPE HPLC method (Table 2).

Calibration linearity for UV detection of the 14 explosives and related compounds was investigated by making three consecutive 1000 µL injections of a mixed standard prepared at six different concentrations (i.e., 18 total injections). An external standard method was used to establish the calibration curve and quantify the analytes in the tap water sample. Linearity was observed from 1.0–500 µg/L when plotting the concentration versus the peak area with all coefficients of determination ≥ 0.9904 (Table 3).

Method detection limits (MDLs) of each compound using UV detection were calculated with the single-sided Student's *t* test method (at the 99% confidence limit). Six consecutive injections of a tap water sample mixed with 1 µg/L of the standard mixture were used to determine the standard deviation value for calculating MDLs. The results were MDLs ≤ 0.35 µg/L for each analyte.

Tap Water Analysis

Figure 5 shows chromatograms of a tap water sample. No target analytes were found. An unknown peak with retention time (*t* = 14.513 min) close to RDX (Peak 2, *t* = 14.840 min) was found in the reagent water (blank) and tap water samples, which might interfere with the determination of RDX (with 120% recovery at 1 µg/L spiked concentration). Method recovery was investigated by determining a spiked tap water sample (1 µg/L for each explosive); the recovery range was from 83 to 120%, demonstrating that this on-line SPE HPLC method provides good selectivity and suitability for the analysis of explosives and related compounds in water samples.

Table 2. Reproducibility of retention time and peak areas for the 14 target compounds.

Analyte	Retention Time RSD	Peak Area RSD
HMX	0.05	1.49
RDX	0.04	1.47
1,3,5-TNB	0.04	1.53
1,3-DNB	0.04	1.31
NB	0.04	1.33
2,4,6-TNT	0.05	1.36
Tetryl	0.04	1.32
2,6-DNT	0.05	1.38
2,4-DNT	0.05	1.25
2-NT	0.04	1.27
4-NT	0.05	1.76
3-NT	0.06	1.14
4-Am-DNT	0.03	1.27
2-Am-DNT	0.05	1.35

Note: Six consecutive injections of the 100 µg/L mixed standard

Table 3. Calibration data.

Analyte	Regression Equation	r^2	Range of Standards (µg/L)
HMX	$A = 0.0069c + 0.0797$	0.9932	1.0–500
RDX	$A = 0.0086c + 0.1165$	0.9924	
1,3,5-TNB	$A = 0.0196c + 0.2307$	0.9925	
1,3-DNB	$A = 0.0257c + 0.3024$	0.9925	
NB	$A = 0.0157c + 0.1722$	0.9938	
2,4,6-TNT	$A = 0.0183c + 0.2199$	0.9919	
Tetryl	$A = 0.0149c + 0.1803$	0.9923	
2,6-DNT	$A = 0.0125c + 0.1532$	0.9920	
2,4-DNT	$A = 2.8730c + 0.2777$	0.9925	
2-NT	$A = 0.0239c + 0.0966$	0.9953	
4-NT	$A = 0.0099c + 0.0877$	0.9939	
3-NT	$A = 0.0087c + 0.1069$	0.9945	
4-Am-DNT	$A = 0.0105c + 0.1361$	0.9904	
2-Am-DNT	$A = 0.0192c + 0.2103$	0.9904	

Conclusion

This work describes an on-line SPE-HPLC with UV absorbance detection method for determining the 14 explosives and related compounds specified in EPA Method 8330A in water samples. This analysis was performed on the UltiMate 3000 Dual gradient RSLC system controlled by Chromeleon CDS software. Use of on-line SPE with UV detection eliminates the labor associated with off-line extraction and provides a convenient method that achieves reduced MDLs ($<0.35 \mu\text{g/mL}$) when determining these compounds in water.

References

1. Method 8330A: Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC); Revision 1; U.S. Environmental Protection Agency: Cincinnati, OH, 2007.
2. Additional HPLC applications can be found at the Thermo Fisher Scientific Appslab Library.

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For Separation

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Mobile Phase: $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ (52:48, v/v)
Flow Rate: 1.0 mL/min
Temperature: 30 $^\circ\text{C}$
Detection: UV absorbance at 254 nm
Valve Position: 0 min, 1_2
4.0 min, 6_1
12 min, 1_2

Samples: a. Blank (reagent water)
b. Tap water sample
c. Sample b spiked with a mixture of 14 explosives and related compounds (1 $\mu\text{g/L}$ each)

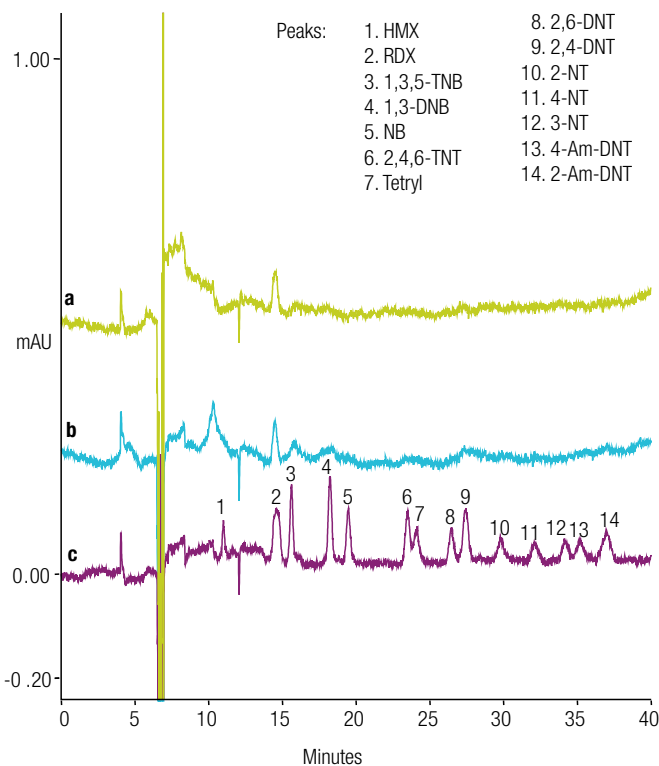


Figure 5. A tap water sample following on-line SPE.

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