Adapting EPA Method 8330B for Analysis of Explosives in Water to SPE and LC/MS/MS

Application Note

Environmental

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Abstract

EPA Method 8330B for trace explosives in water by HPLC was transferred from UV detection to mass spectrometry detection using the Agilent 1290 Infinity LC System coupled to the Agilent 6460 Triple Quadrupole LC/MS System using APCI negative mode. Eleven explosive compounds were analyzed with this method at trace concentrations in water samples with limits of detection at the 5–50 ng/L concentration level. Solid phase extraction (SPE) was used and both a C18 and a polymeric cartridge were tested for recoveries. The polymeric cartridge gave the highest recoveries, from 80–101% using off-line SPE. The combination of UHPLC and the 6460 Triple Quadrupole LC/MS System is an effective method for measuring explosives in water.

Agilent Technologies

Introduction

EPA Method 8330B [1] was introduced in 2006 as an adaptation of the earlier EPA Method 8330, an HPLC method with UV detection for 17 explosives. The method measures trace levels of explosives in water at the sub-part-per-billion concentration level.

The detection of explosives in the environment is of concern because of both acute and chronic toxicity to humans, their introduction into the environment due to military operations, and the possibility of ecological damage. For example, acute toxicity has been shown for 2,4,6-TNT in the adult bullfrog [2]. Furthermore, explosive residues have leached from soils at a military site to underlying groundwater [3]. Therefore, the potential exists for environmental problems, and reliable and sensitive methods of detection are needed, such as those provided by mass spectrometry.

This application note describes the transfer of EPA Method 8330B to state-of-the-art mass spectrometry detection using both ultrahigh-pressure liquid chromatography (UHPLC) and triple quadrupole mass spectrometry. Using SPE, the method developed on the 1290 Infinity LC System coupled to the 6460 Triple Quadrupole LC/MS System provides limits of detection (LODs) for 11 explosives in the 5–50 ng/L concentration range and 80 to 101% recoveries.

Experimental

Reagents and Standards

All standards were purchased from AccuStandard (New Haven, CT, USA), M-8330-R set of 17 explosives. LC/MS-grade methanol, acetonitrile, and water were purchased from Burdick and Jackson (Morristown, NJ, USA). The SPE cartridges were polymeric RP and the C18 cartridges were from Agilent Technologies (p/n 12102052). All standards were 100 µg/mL in methanol and were serial diluted to appropriate levels.

Instruments

This method was developed on a 1290 Infinity LC System consisting of a binary pump, autosampler, thermostatted column compartment, and an UHPLC column. The LC system was coupled to a 6460 Triple Quadrupole LC/M System. The instrument run conditions are listed in Table 1.

Table 1. LC and MS Run Conditions

LC conditions			
Column	Agilent ZORBAX C18, 2.1 × 50 mm, 1.8 µm (p/n 95975-902)		
Column temperature	25 °C		
Injection volume	100 µL		
Mobile phase	A = acetonitrile B = 0.1% acetic acid in water		
Flow rate	0.4 mL/min		
Gradient	Time (min)	% A	% B
	0	20	80
	1.7	20	80
	10	100	0
	10.3	100	0
Post time	4 minutes		
Total run time	10.3 minutes		
MS conditions			
Ionization mode	APCI negative		
Gas temperature	350 °C		
Vaporizer temperature	275 °C		
Drying gas flow	4 L/min		
Nebulizer pressure	30 psig		
NCI Corona current	4 µA		
NCI capillary voltage	1,500 V		
MRM conditions	See Table 4		

Sample Preparation

Each 100 mL sample was concentrated using solid phase extraction (SPE) on polymeric RP cartridges or Bond Elut C18 with the Gilson 271-GX automated system (Gilson Inc., Madison, WI, USA). Cartridges were first conditioned with 5 mL of methanol followed by 5 mL of water. Sample was applied at 10 mL/min and eluted with 5 mL of methanol at 1 mL/min. The methanol was evaporated to 0.5 mL using a stream of nitrogen at a temperature of 45 °C in a water bath with the Turbovap concentration workstation (Caliper Life Sciences, Mountain View, CA) and injected into the UHPLC/MS/MS system.

Results and Discussion

Choosing the Ionization Mode

Table 2 lists the names of the 17 analytes of EPA Method 8330B, along with their abbreviations and CAS Numbers. The list includes new major explosives fabricated from nitramines, such as HMX, high melting explosive, and RDX, research department explosive. These 17 compounds were tested in both electrospray and atmospheric pressure chemical ionization (APCI) negative modes.

The APCI negative mode was much more successful at ionization than the electrospray negative source (Table 3), confirming a result published earlier [4]. Even in APCI negative mode there were several explosives that did not ionize, which was a result of their chemical structure. In particular, if the compounds did not contain at least two nitro groups then formation of the negative ion was not facilitated. This is noted by the lack of ionization for nitrobenzene, 2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene. Compounds with multiple nitro groups are capable of ionization in negative ion mode and form ions either by loss of a proton to form an even electron ion, or by capture of an electron in APCI negative mode to form a negative molecular ion, which is an odd electron ion.

Table 4 shows the chemical structures for the precursor ions that formed in APCI negative mode. Eleven of the 17 compounds were efficiently ionized. Note that the chemical structures of all of the compounds in Table 4 contain at least two or more nitro groups, which aid in the formation of negative ions in APCI. A method for the detection of these 11 explosive compounds was then developed using ultrahigh-pressure liquid chromatography (UHPLC) followed by triple quadrupole MS/MS analysis.

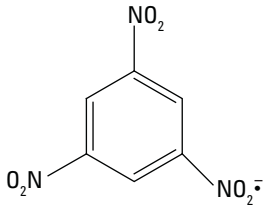
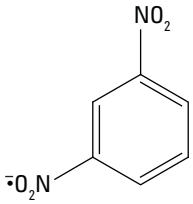
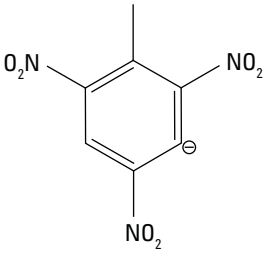
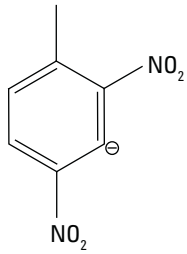
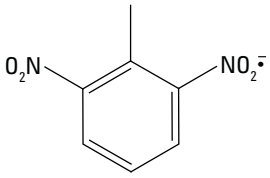
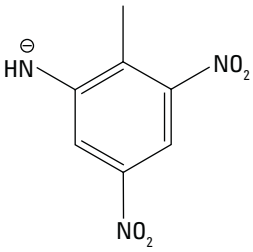
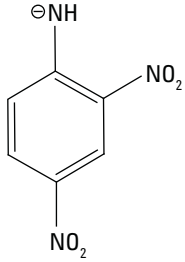
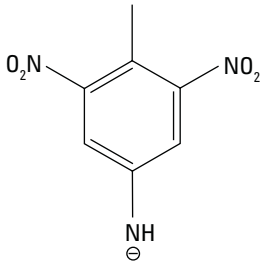
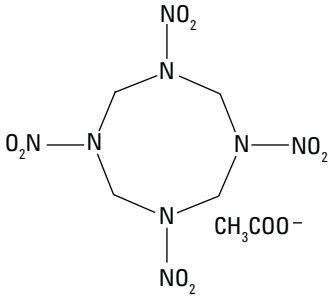
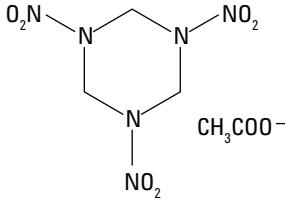
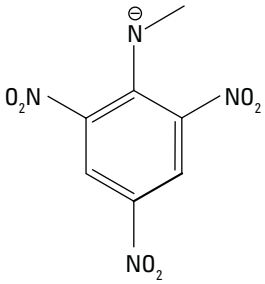
Table 2. Abbreviations, Names and CAS Numbers of Explosives Studied

Analyte abbreviation	Analyte name	CAS number
1,3,5-TNB	1,3,5-Trinitrobenzene	99-35-4
1,3-DNB	1,3-Dinitrobenzene	99-65-0
2,4,6-TNT	2,4,6-Trinitrotoluene	118-96-7
2,4-DNT	2,4-Dinitrotoluene	121-14-2
2,6-DNT	2,6-Dinitrotoluene	606-20-2
2-Am-DNT	2-Amino-4,6-Dinitrotoluene	35572-78-2
2-NT	2-Nitrotoluene	88-72-2
3,5-DNA	3,5-Dinitroaniline	618-87-1
3-NT	3-Nitrotoluene	99-08-1
4-Am-DNT	4-Amino-2,6-Dinitrotoluene	19406-51-0
4-NT	4-Nitrotoluene	99-99-0
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	2691-41-0
NB	Nitrobenzene	98-95-3
NG	Nitroglycerin	55-63-0
PETN	Pentaerythritol tetranitrate	78-11-5
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine	121-82-4
Tetryl	Methyl-2,4,6-trinitrophenylnitramine	479-45-8

Table 3. Ionization Source for Explosives Studied (X = No Response; ✓ = Response)

Analyte abbreviation	ESI -	APCI -
1,3,5-TNB	✓	✓
1,3-DNB	X	✓
2,4,6-TNT	✓	✓
2,4-DNT	✓	✓
2,6-DNT	X	✓
2-Am-DNT	✓	✓
2-NT	X	X
3,5-DNA	✓	✓
3-NT	X	X
4-Am-DNT	✓	✓
4-NT	X	X
HMX	✓	✓
NB	X	X
NG	X	X
PETN	X	X
RDX	✓	✓
Tetryl	X	✓

Table 4. Chemical Structures for Precursor Ions of the Explosives Using APCI in Negative Ion Mode

Analyte abbreviation	Chemical structure	Precursor ion
1,3,5-TNB		$[M]^{-\bullet}$
1,3,-DNB		$[MH]^-$
2,4,6-TNT		$[MH]^-$
2,4-DNT		$[M]^{-\bullet}$
2,6-DNT		$[M]^{-\bullet}$
2-Am-DNT		$[MH]^-$
3,5-DNA		$[MH]^-$
4-Am-DNT		$[MH]^-$
HMX		$[M+CH_3COO^-]^-$
RDX		$[M+CH_3COO^-]^-$
Tetryl		$[M+NO_2]^-$

Optimizing the Method Parameters

The fragmentor voltage for each of these 11 compounds was then optimized to find the value that would enable the largest production of each precursor ion. In all but three cases, the precursor ion was either the deprotonated molecule or the molecular ion. Both HMX and RDX (the newer nitramines) formed an acetate adduct to the molecule to make a negative ion. Tetryl, on the other hand, lost a nitro group (presumably in the ion source) before the collision cell, to form its precursor ion.

Table 5 shows the precursor ion, fragmentor voltage, and collision energy required to form two product ions for each of the 11 explosives. The quadrupole resolution included a wide setting on MS1 and a unit setting on MS2 in order to capture the most ion signal and yet retain low baseline background noise from spurious ions in the matrix and solvents. Common product ions included the *m/z* 46 ion, a nitrate group (NO_2-), which is not surprising given that nitro groups are an important leaving group in each of the explosives. A dwell time of 5 ms was used for all analytes. The MRM transitions for all 11 analytes and their product ions are also shown in Table 5. These MRM transitions were used to build a solid and reliable triple quadrupole mass spectrometry method.

Table 5. MRM Parameters for the Detection of Explosives in APCI in Negative Ion Mode

Compound name	Precursor ion	MS1 res	Product ion	MS2 res	Dwell	Fragmentor	Collision energy	Cell accelerator voltage
1,3,5-TNB	213	Wide	183	Unit	5	70	5	3
1,3,5-TNB	213	Wide	95	Unit	5	70	20	3
1,3-DNB	168	Wide	138	Unit	5	50	5	3
1,3-DNB	168	Wide	46	Unit	5	50	5	3
2,4,6-TNT	226	Wide	196	Unit	5	90	5	3
2,4,6-TNT	226	Wide	46	Unit	5	90	20	3
2,4-DNT	181	Wide	135	Unit	5	90	20	3
2,4-DNT	181	Wide	46	Unit	5	90	20	3
2,6-DNT	182	Wide	152	Unit	5	50	20	3
2,6-DNT	182	Wide	46	Unit	5	50	10	3
2-Am-DNT	196	Wide	136	Unit	5	90	20	3
2-Am-DNT	196	Wide	46	Unit	5	90	20	3
3,5-DNA	182	Wide	152	Unit	5	90	10	3
3,5-DNA	182	Wide	46	Unit	5	90	20	3
4-Am-DNT	196	Wide	119	Unit	5	90	10	3
4-Am-DNT	196	Wide	46	Unit	5	90	20	3
HMX	355	Wide	147	Unit	5	50	5	3
HMX	355	Wide	46	Unit	5	50	10	3
RDX	281	Wide	59	Unit	5	50	10	3
RDX	281	Wide	46	Unit	5	50	20	3
Tetryl	241	Wide	213	Unit	5	70	0	3
Tetryl	241	Wide	196	Unit	5	70	10	3

The experimental conditions were also optimized for sensitivity, vaporizer temperature, drying gas temperature, flow rate, corona current, and capillary voltage. A large increase in signal was observed by lowering the gas flow rate from 6 L/min to 4 L/min, an observation made in a previous application note using APCI and LC/TOF-MS [4]. Because some of the compounds are thermally labile, the vaporizer temperature was lowered to 275 °C for optimal performance.

Detection and Quantitation

Figure 1 shows the UHPLC chromatographic separation of the 11 analytes. Many of the explosives were baseline separated. However, the two isomers, 2-amino-DNT and 4-amino-DNT, were partially separated. Because each has a different product ion due to fragmentation differences arising from the location of the nitro and methyl groups, it is possible to distinguish these two isomers by mass spectrometry using their multiple reaction monitoring (MRM) transitions.

Good linearity was found for all 11 explosives when standard curves were developed for each compound. Figure 2 shows the standard curve for 2,4-DNT, which has a calibration coefficient (R^2) of 0.999. The concentrations used for the external standard curve were 1, 10, 25, 50, and 100 µg/L, or parts per billion (ppb), which was the typical sensitivity for most of the explosives in APCI negative mode after concentration by solid phase extraction.

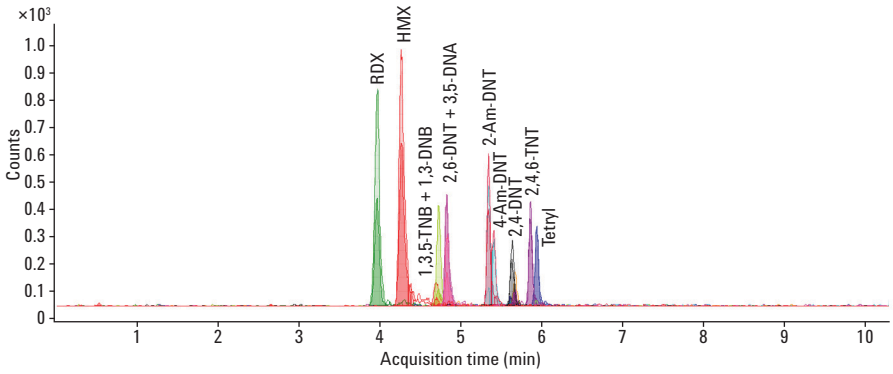


Figure 1. Chromatogram showing the separation of the explosives using a C18 column.

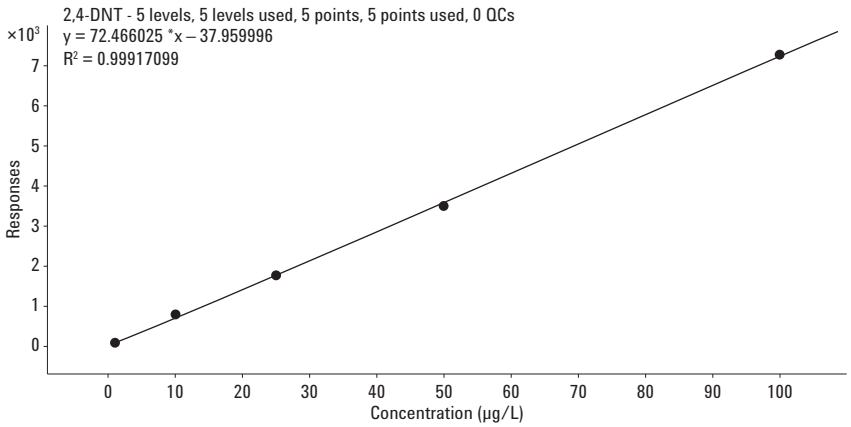


Figure 2. An example of the external calibration curve for 2,4-DNT showing good linearity (R^2 =0.999).

Sample Preparation

In order to develop methods at the ng/L level in water, it is necessary to use solid phase extraction (2–3). Thus, the recovery of all 11 explosives was measured on both C18 and a polymer phase, as shown in Table 6. Both SPE materials gave similar recoveries, which varied from 72 to 101%, with the exception of HMX and RDX that had lower recoveries on C18 due to their polarity.

The combination of off-line SPE using the polymeric cartridge and UHPLC chromatography with MRM enables a sensitive method for 11 explosive analytes. Table 7 shows the method limits of detection (LODs) for the 11 explosives after preconcentration of 100 mL surface-water samples spiked with the explosives mixture. The LODs varied from 5 to 50 ng/L, depending on the sensitivity of the compound in APCI negative ion mode.

Conclusion

A sensitive method for 11 explosives from EPA Method 8330B was developed using solid-phase extraction followed by UHPLC and LC/MS/MS with the 1290 Infinity LC System coupled to the 6460 Triple Quadrupole LC/MS System. This robust method uses two MRM transitions for quantitation and confirmation of the explosives in surface and groundwater sample with limits of detection at the 5–50 ng/L concentration level. Recoveries from 80–101% were obtained using off-line SPE polymeric cartridges.

*Table 6. Percent Recoveries**

Analyte abbreviation	C18 Bond Elut cartridges (%)	Polymer Phase cartridges (%)
1,3,5-TNB	72	80
1,3-DNB	82	101
2,4,6-TNT	101	95
2,4-DNT	91	86
2,6-DNT	95	83
2-Am-DNT	91	97
3,5-DNA	95	83
4-Am-DNT	91	88
HMX	9.2	84
RDX	19	88
Tetryl	88	82

*Solid phase extraction of 100 mL of surface water samples spiked at 100 ng/L.

*Table 7. Method Limits of Detection**

Analyte abbreviation	LOD (ng/L)
1,3,5-TNB	25
1,3-DNB	50
2,4,6-TNT	5
2,4-DNT	25
2,6-DNT	10
2-Am-DNT	5
3,5-DNA	5
4-Am-DNT	10
HMX	10
RDX	25
Tetryl	20

*Solid phase extraction of 100 mL of surface water samples spiked at 100 ng/L.

References

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2. N.E Paden, E. E.Smith, R. J. Kendal. "Acute toxicity of 2,4,6-trinitrotoluene, 2, 4-dinitrotoluene, and 2,6-dinitrotoluene in the adult bullfrog (Lithobates catesbiana)." *Bulletin of Environmental Contamination and Toxicology*. **80**, 487-491(2008)
3. K. Spiegel, J.V. Headley, K.M. Peru, N. Haidar, N.P. Gurprasard. "Residues of explosives in groundwater leached from soils at a military site in Eastern Germany", *Communications in Soil Science and Plant Analysis* **36**, 133-153 (2005).
4. R. Kinghorn, C.Milner, J. .Zweigenbaum. Analysis of Trace Residues of Explosive Materials by Time-of-Flight LC/MS, Agilent Application Note 5989-2449EN.

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