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Determination of Explosive Compounds in Drinking Water using Parallel-HPLC with UV Detection

INTRODUCTION

Determination of residual explosive materials and their degradation products in the environment has drawn increased attention due to their toxicity, persistence, and increased demand for forensic analysis concerning national security.

Gas chromatography has traditionally been used to detect and quantify explosive compounds; however, because some are thermally unstable or nonvolatile, using this method can result in inexact determinations. HPLC with UV detection, however, is ideally suited for low-level determination because it is not subject to these limitations. U.S. EPA Method 8330 describes an HPLC method with UV detection for determination of 14 priority explosives and related substances¹ (Figure 1). The method recommends the use of a C18 reversed-phase column as the primary column for separation, and a secondary column for confirmation.

Acclaim[®] Explosives E1 and E2 columns can provide baseline resolution for 14 compounds with complementary selectivity under identical chromatographic conditions (except mobile phase composition) while reducing analysis time.² These columns were used with EPA Method 8330 and the results are reported in this Application Note.

Analysis was performed by parallel LC using the UltiMate[®] x2 Dual HPLC system with UV detection. Seven additional related compounds were also determined using this method, two of which can be resolved from the 14 compounds in EPA Method 8330, along with an internal standard (1,2 dinitrobenzene).

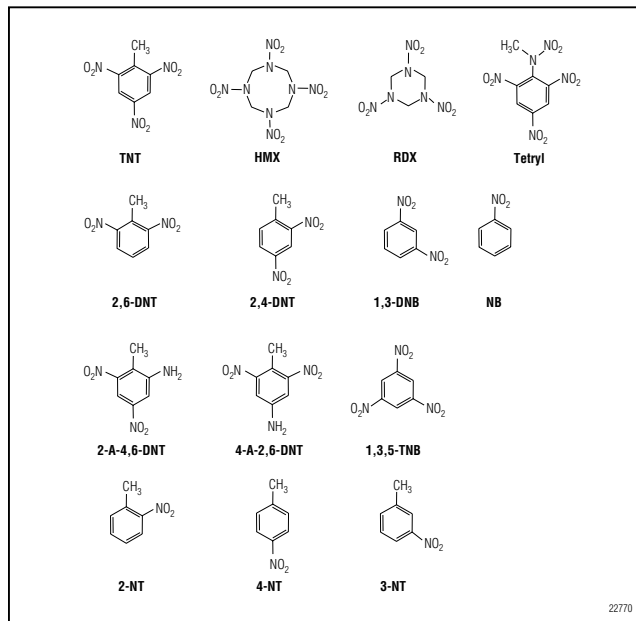


Figure 1. Structures of 14 explosive compounds and related substances.

EQUIPMENT

UltiMate-3000 HPLC system

DPG 3600A pump with SRD 3600 Air Solvent Racks

WPS-3000TSL autosampler

TCC-3200 thermostatted column compartment with one two-position six-port valve

VWD-3400 UV/Vis detectors

Chromeleon® 6.80 SP1 Chromatography Workstation

Note: Samples can be analyzed on an UltiMate system with a single pump and UV/Vis detector by first running the samples on the Acclaim Explosives E1 column then switching to the Acclaim Explosives E2 and reanalyzing the samples using the E2 program.

REAGENTS AND STANDARDS

Water: Milli-Q® Gradient A10

Methanol (CH₃OH), Fisher, HPLC grade

Acetonitrile (CH₃CN), Fisher, HPLC grade

Calcium chloride (CaCl₂), SCRC, ≥ 96.0%

Sodium chloride (NaCl), SCRC, ≥ 99.8%

Mixture A: consisting of 2-amino-4,6-dinitrotoluene, 1,3-dinitrobenzene, 2,4-dinitrotoluene, HMX, nitrobenzene, RDX, 1,3,5-trinitrobenzene, and TNT, 1.0 mg/mL in CH₃OH : CH₃CN (1:1) AccuStandard (M-8330A-R-10X).

Mixture B: consisting of 4-amino-2,6-dinitrotoluene, 2,6-dinitrotoluene, 2-nitrotoluene, 3-nitrotoluene, 4-nitrotoluene, and tetryl, 1.0 mg/mL in CH₃OH : CH₃CN (1:1) (AccuStandard (M-8330B-R-10X)

1,2-Dinitrobenzene, Fluka, ≥ 99.0%

2,4-Diamino-6-nitrotoluene, 0.1 mg/mL in CH₃CN, AccuStandard

2,6-Diamino-4-nitrotoluene, 0.1 mg/mL in CH₃CN, AccuStandard

Nitroglycerin, 10 mg/L in CH₃OH : H₂O (4:1), Dr. Ehrenstorfer GmbH

Nitropenta, 10 mg/L in CH₃OH : H₂O + 0.5% H₃PO₄ (2:1), Dr. Ehrenstorfer GmbH

Diphenylamine, 10 mg/L, Dr. Ehrenstorfer GmbH

Hexanitrodiphenylamine, 10 mg/L, Dr. Ehrenstorfer GmbH

2,4,6-Trinitrophenol, 10 mg/L, Dr. Ehrenstorfer GmbH

PREPARATION OF REAGENTS AND STANDARDS

Preparation of standards for calibration was based on conditions outlined EPA Method 8330.

Stock Standard Solution 1

Pipet 200 µL Mixtures A and B, respectively, into a 1.8-mL vial, add 400 µL methanol. The concentration of each analyte in stock standard solution 1 is 250 µg/mL.

Stock Standard Solution 2

Pipet 300 µL Stock Standard Solution 1 into a 1.8-mL vial, add 1200 µL methanol. The concentration of each analyte in stock standard solution 2 is 50 µg/mL.

Stock Standard Solution 3

Pipet 100 µL Stock Standard Solution 2 into a 1.8-mL vial, add 900 µL methanol. The concentration of each analyte in stock standard solution 3 is 5 µg/mL.

Intermediate standard solutions

Pipet volumes of 200 and 400 µL Stock Standard Solution 3 into two separate 25 mL volumetric flasks, respectively, then bring to volume with methanol. The concentration of each solution is 40 and 80 µg/L.

Pipet volumes of 100, 160, 250, 400, and 500 µL Stock Standard Solution 2 into five 25 mL volumetric flasks respectively, then bring each to volume with methanol. The concentration of each solution is 200, 320, 500, 800 and 1000 µg/L.

Stock Internal Standard (I.S.) solution

1,2-Dinitrobenzene was used as an internal standard. Dilute 12.5 mg 1,2-dinitrobenzene with a 1:1 mixture solution of methanol and acetonitrile to 25 mL, for a final concentration of 500 µg/mL.

Intermediate I.S. solution

Pipet 500 µL stock I.S. solution and dilute with methanol to 25 mL, for a final concentration of 10 µg/mL.

Calcium chloride solution

Dissolve 0.5 g CaCl₂ in 100 mL water and filter through a 0.45-µm filter, for a final concentration of 5 g/L. This solution is used for diluting intermediate standard solutions following the instructions in EPA Method 8330.

Working standard solutions

Pipet 800 μL of each intermediate standard solution into six 1.8-mL vials. Add 760 μL CaCl_2 (5 g/L) solution and 40 μL intermediate I.S. solution to each vial, to yield working standard solutions of the 14 analytes with concentrations of 20, 40, 100, 160, 250 and 400 $\mu\text{g/L}$, respectively, and an internal standard concentration of 250 $\mu\text{g/L}$.

SAMPLES AND SPIKED SAMPLES PREPARATION

Tap water and soil samples were collected at the Dionex Shanghai Applications Lab located in the Pudong District of Shanghai, China.

According to the instructions in EPA Method 8330, samples and spiked samples should be diluted 1:1 with CaCl_2 solution (5 g/L) prior to analysis.

Tap water samples

Tap water samples were prepared as high- and low-level samples according to the sample preparation method specified in EPA method 8330.

To prepare the high-level sample: Mix 5 mL tap water and 5 mL methanol, filter the solution through a 0.45- μm filter.

To prepare the high-level (200 $\mu\text{g/L}$) spike: Add 5 mL of the 800 $\mu\text{g/L}$ intermediate standard solution to 5 mL tap water, filter through a 0.45- μm filter, discarding the first 3 mL of filtrate. Pipet 500 μL of filtrate, 25 μL of the 10 $\mu\text{g/mL}$ internal standard solution, and 475 μL of the 5 g/L CaCl_2 solution into a 1.5-mL vial and mix.

To prepare the low-level sample: See Section 7.1.1.1 of U.S. EPA Method 8330, entitled “Low-level Method (salting out extraction).”

To prepare the low-level (5.2- $\mu\text{g/L}$) spike: Add 4 mL of the 1000 $\mu\text{g/L}$ intermediate standard solution and 500 μL of the 10 $\mu\text{g/mL}$ I.S. solution to 770 mL NaCl solution (251.3g NaCl in 770 mL tap water). Following the steps specified in Section 7.1.1.1 in U.S. EPA Method 8330, obtain an extraction solution of approximately 5 mL and bring to 10 mL volume with methanol.

CHROMATOGRAPHIC CONDITIONS

Column:	Acclaim Explosives E1 5 μ , 4.6 \times 250 mm (primary column) Acclaim Explosives E2 5 μ , 4.6 \times 250 mm (secondary column)
Column Temp.:	30 $^{\circ}\text{C}$
Mobile Phase:	(E1 column) 57% water: 43% methanol (E2 column) 52% water: 48% methanol
Flow Rate:	1.0 mL/min
Injection Volume:	80 μL

Parallel System Set-up

A parallel system requires a column compartment which includes one two-position six-port valve and a capillary kit for installation of a parallel application. For instructions on hardware setup, see the “Quick Installation Guides for UltiMate 3000 \times 2 System Capillary Kits” included with each kit. Additionally, two new timebases are added in the Chromeleon Server Configuration, one for each pump. The WPS-3000TSL autosampler and TCC-3200 thermostatted column compartment are shared components. Each system sequence runs the analyses independently, and can be controlled using its own Chromeleon control panel.

RESULTS AND DISCUSSION

Separation of 14 explosive compounds and related substances on Acclaim E1 and E2 columns based on EPA Method 8330

Analytes are traditionally divided into two groups because some compounds coelute (e.g. 2,4-DNT and 2,6-DNT) when using the C18 and CN columns recommended by EPA Method 8330. Similarly, if 2,4-DNT and 2,6-DNT are present in a sample, they may coelute on the same column.¹ The Acclaim Explosives E1 and E2 columns were developed to separate explosive compounds and related substances without having to prepare two groups of standards. All analytes are baseline resolved in a single injection, with significant savings in standard preparation and sample analysis times.²

Figure 2: Panels A and B show baseline separation of the 14 explosive formulations and related substances on the E1 and E2 columns respectively, at concentrations specified in EPA Method 8330. For this Application Note, 80 μL sample volume was injected instead of 100 μL as outlined in the EPA Method.

Each chromatogram also contains 1, 2-dinitrobenzene as an internal standard (I.S.). This is not specified in EPA Method 8330, but may be useful for studies of explosives and related compounds that may deviate from the procedure in that method.

The E2 column has a different selectivity than the E1 column, as shown in Figure 2; For example, the elution order of tetryl peak (# 7) and 2,4,6-trinitrotoluene peak (# 8) is different, and the retention times of 4-amino-2,6-dinitrotoluene peak (# 9) and 2-amino-4,6-dinitrotoluene peak (#10) are significantly different.

Parallel HPLC system

While an HPLC system with a single pump and detector can be used to analyze samples according to EPA Method 8330, a parallel LC system is ideal for this analysis. The system used for the analyses presented here performs as two independent HPLC systems (pump, column, and detector) with the autosampler shared between both. Injection onto the E2 column occurs just after injection onto the E1 column (i.e. E1 separation is in progress during injection onto E2). The two sequences (E1 and E2 analyses) run simultaneously. Each sample is quantified with independent calibration (i.e. E1 or E2 calibration). The flow schematic for UltiMate 3000 parallel LC mode is shown in Figure 3.

Reproducibility, linearity and detection limits

Reproducibility was determined by making six replicate injections of the 250 µg/L calibration standard. Table 1 summarizes retention time and peak area precision data.

Calibration linearity was obtained by making replicate injections of a mixed standard of analytes prepared at six different concentrations. The external standard calibration method is used in EPA Method 8330, therefore calibration curves with the six concentrations of each standard were prepared and used for sample analysis. Table 2 shows a summary of the data. The calibration curve was also calculated using the internal standard, with similar results, as shown in Table 3. The higher relative standard deviation (RSD) and correlation coefficient of calibration for HMX on the E1 column can be attributed to a small impurity peak with a retention time that changed from 4.61 in the third injection to 5.15 min in the last injection during calibration. (Throughout the analysis the retention time of HMX was 5.00 min). Experiments demonstrated that the impurity originated in the purchased standard solution, and not in the reagent water or CaCl₂ solution (data not shown).

4 Determination of Explosive Compounds in Drinking Water using Parallel-HPLC with UV Detection

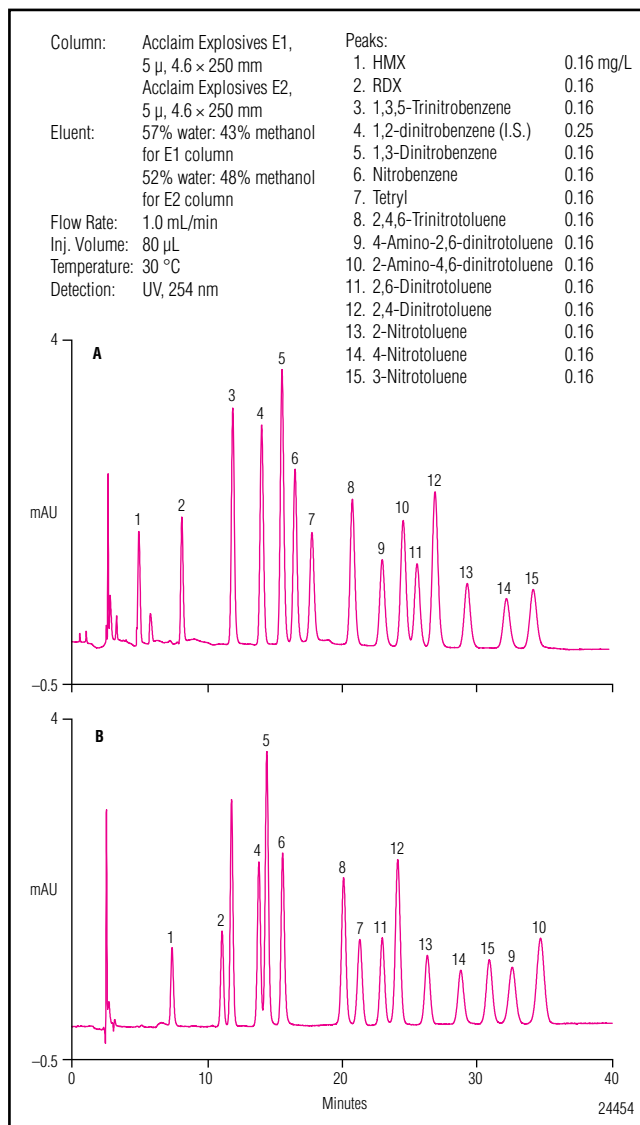


Figure 2. Chromatogram of a standard mixture of 14 explosives (160 µg/L) and 1,2-dinitrobenzene (used as I.S.) on A) E1 column and B) E2 column.

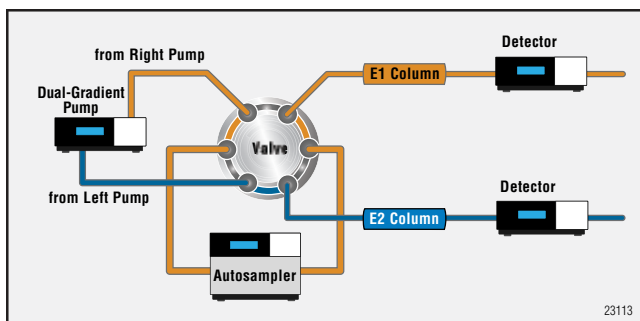


Figure 3. Configuration of the parallel HPLC system.

Table 1. Reproducibility of Retention Time and Peak Areas for 14 Explosive Compounds

Analyte	E1 Column		E2 Column	
	RT RSD (%)	Peak Area RSD (%)	RT RSD (%)	Peak Area RSD (%)
HMX	0.038	1.026	0.097	0.713
RDX	0.033	0.832	0.077	0.537
1,3,5-Trinitrobenzene	0.023	0.472	0.051	0.660
1,3-Dinitrobenzene	0.022	0.667	0.056	0.727
Nitrobenzene	0.021	0.472	0.063	0.213
Tetryl	0.038	1.136	0.090	0.801
2,4,6-Trinitrotoluene	0.028	0.776	0.075	0.625
4-Amino-2,6-dinitrotoluene	0.041	0.808	0.093	0.498
2-Amino-4,6-dinitrotoluene	0.041	0.595	0.081	0.985
2,6-Dinitrotoluene	0.037	0.989	0.083	0.742
2,4-Dinitrotoluene	0.034	0.836	0.100	0.835
2-Nitrotoluene	0.047	0.621	0.101	0.922
4-Nitrotoluene	0.035	0.989	0.089	0.587
3-Nitrotoluene	0.038	1.026	0.082	0.406

Note: Six injections of the 250 µg/L mixed standard were made on each column.

Table 2. Method Linearity Data¹ and Method Detection Limits (MDL)² on the Acclaim E1 and E2 Columns

Analyte	E1 Column			E2 Column		
	r	Area RSD (%)	MDL (µg/L)	r	RSD (%)	MDL (µg/L)
HMX	0.9981	3.45	4.5	0.9990	2.55	4.1
RDX	0.9996	1.56	0.6	0.9998	1.22	4.6
1,3,5-Trinitrobenzene	0.9999	1.00	1.6	0.9999	0.88	2.4
1,3-Dinitrobenzene	0.9999	0.83	2.5	0.9999	0.83	1.8
Nitrobenzene	0.9998	1.11	3.2	0.9999	1.13	3.3
Tetryl	0.9994	2.02	3.2	0.9998	0.76	3.4
2,4,6-Trinitrotoluene	0.9999	0.96	2.3	0.9998	1.06	3.0
4-Amino-2,6-dinitrotoluene	0.9998	1.29	2.7	0.9999	1.09	3.4
2-Amino-4,6-dinitrotoluene	0.9998	0.97	1.7	0.9997	0.88	2.4
2,6-Dinitrotoluene	0.9998	1.11	3.3	0.9996	1.37	4.5
2,4-Dinitrotoluene	0.9998	1.12	2.6	0.9997	1.64	2.7
2-Nitrotoluene	0.9997	1.11	3.9	0.9998	1.37	4.8
4-Nitrotoluene	0.9998	1.18	4.5	0.9998	1.35	4.3
3-Nitrotoluene	0.9996	1.57	4.0	0.9999	1.22	4.6

¹ A standard mix was prepared at the following concentrations: 20, 40, 100, 160, 250 and 400 µg/L, and was used for calibration; each concentration was injected six times. Calculated values are those reported by Chromeleon. The internal standard was not used to obtain these calibration curves. ² MDL was calculated using S/N = 3, where S = signal, N = noise.

Sample analysis

Figure 4 shows chromatograms of tap water and spiked tap water prepared as high-level samples. Results are summarized in Table 4. No explosive or related compounds were detected, and excellent spike recovery was demonstrated for all 14 analytes.

Figure 5 shows chromatograms of tap water and spiked tap water prepared as low-level samples. Results are summarized in Table 5. The analyte spike levels of the 770 mL tap water samples were 5.2 µg/L for each compound; after extraction, their desired concentrations in 10 mL acetonitrile were 400 µg/L each. Prior to injection, the spiked samples were diluted 1:1 by CaCl₂ solution.

Nitrobenzene was tentatively identified in the tap water sample at low concentration (near the lowest calibration concentration). Spike recoveries for nitrobenzene were low (73 and 67% for the E1 and E2 columns, respectively).

Separation of related compounds

Seven additional explosive related compounds were evaluated using the conditions specified in EPA Method 8330. Their retention times are shown in Table 6. Of these seven, only nitropenta and 2,4,6-trinitrophenol can be separated together with the 14 explosive compounds (Figure 6). Diphenylamine and hexanitrodiphenylamine are strongly retained using the conditions outlined in this method.

PRECAUTIONS

Solvents, reagents, glassware and other sample processing hardware may contain impurities which may yield unknown peaks, elevated baselines, or misinterpretation of chromatograms. All materials must be demonstrated to be free from interference by running appropriate controls (e.g., blank samples, injection blanks, etc.)

Tetryl decomposes rapidly in MeOH/H₂O solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

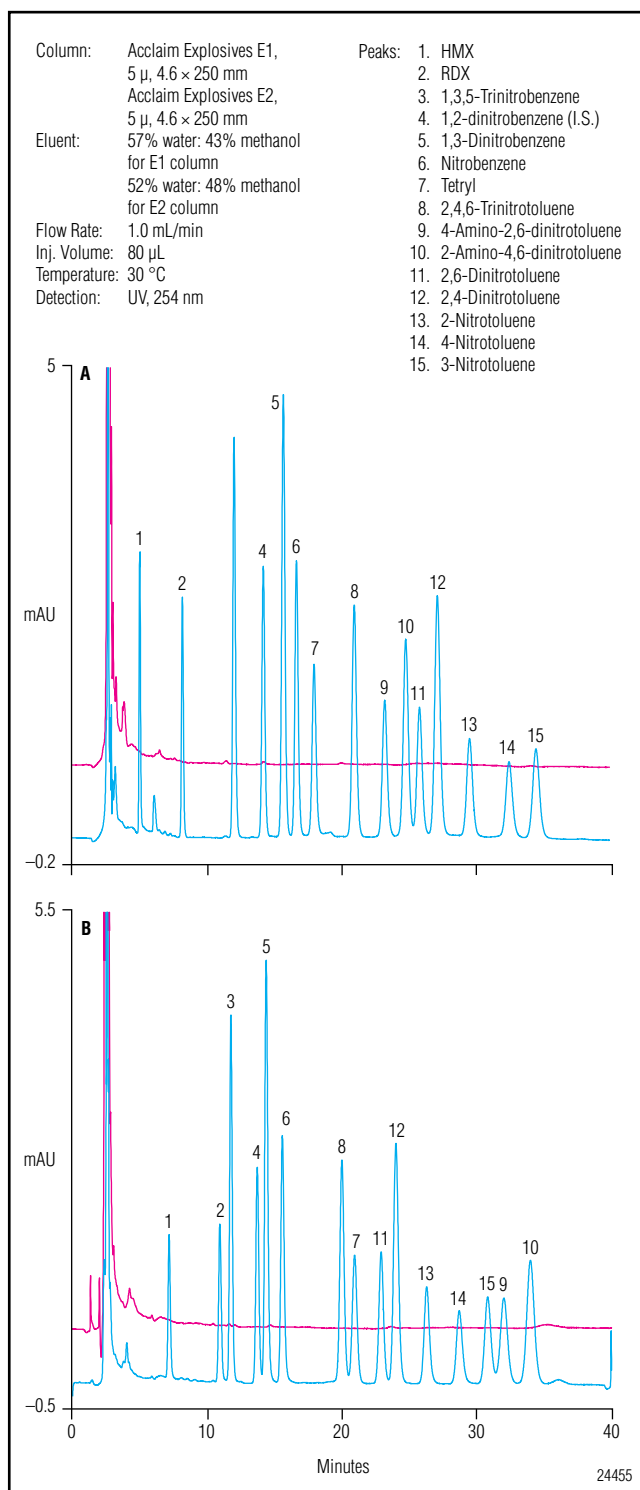


Figure 4. Overlay chromatograms of (a) tap water and (b) spiked tap water analyzed using E1 and E2 columns.

Table 3. Method Linearity Data for the Acclaim E1 and E2 Columns

Analyte	E1 Column		E2 Column	
	r	RSD (%)	r	RSD (%)
HMX	0.9967	4.49	0.9979	3.60
RDX	0.9985	3.07	0.9994	2.01
1,3,5-Trinitrobenzene	0.9993	2.25	0.9994	1.97
1,3-Dinitrobenzene	0.9994	2.07	0.9996	1.64
Nitrobenzene	0.9995	1.86	0.9996	1.65
Tetryl	0.9984	3.38	0.9995	1.83
2,4,6-Trinitrotoluene	0.9989	2.89	0.9994	2.06
4-Amino-2,6-dinitrotoluene	0.9989	2.90	0.9991	2.50
2-Amino-4,6-dinitrotoluene	0.9990	2.63	0.9974	4.29
2,6-Dinitrotoluene	0.9991	2.46	0.9996	1.62
2,4-Dinitrotoluene	0.9992	2.40	0.9996	1.64
2-Nitrotoluene	0.9993	2.23	0.9996	1.75
4-Nitrotoluene	0.9992	2.35	0.9993	2.27
3-Nitrotoluene	0.9993	2.28	0.9995	1.93

Table 4. Analysis Results of Tap Water Prepared as High-Level Sample

Analyte	E1 Column				E2 Column			
	Detected (µg/L)	Added (µg/L)	Found ¹ (µg/L)	Recovery ² (%)	Detected (µg/L)	Added (µg/L)	Found ¹ (µg/L)	Recovery ² (%)
HMX	NA	200	224	112	NA	200	199	100
RDX	NA	200	204	102	NA	200	208	104
1,3,5-Trinitrobenzene	NA	200	206	103	NA	200	207	104
1,3-Dinitrobenzene	NA	200	206	103	NA	200	205	103
Nitrobenzene	NA	200	203	102	NA	200	203	102
Tetryl	NA	200	200	100	NA	200	205	103
2,4,6-Trinitrotoluene	NA	200	208	104	NA	200	208	104
4-Amino-2,6-dinitrotoluene	NA	200	208	104	NA	200	204	102
2-Amino-4,6-dinitrotoluene	NA	200	208	104	NA	200	203	102
2,6-Dinitrotoluene	NA	200	208	104	NA	200	206	103
2,4-Dinitrotoluene	NA	200	204	102	NA	200	209	105
2-Nitrotoluene	NA	200	204	102	NA	200	207	104
4-Nitrotoluene	NA	200	204	102	NA	200	205	103
3-Nitrotoluene	NA	200	202	102	NA	200	205	103

Note: 1. Two tap water samples were prepared, with two injections made for each preparation. 2. Four spiked samples were prepared, with five injections made for each preparation.

Table 5. Analysis Results of Tap Water Prepared as a Low-Level Sample

Analyte	E1 Column				E2 Column			
	Detected (µg/L)	Added (µg/L)	Found ¹ (µg/L)	Recovery ² (%)	Detected (µg/L)	Added (µg/L)	Found ¹ (µg/L)	Recovery ² (%)
HMX	NA	200	168	83	NA	200	210	105
RDX	NA	200	177	89	NA	200	153	77
1,3,5-Trinitrobenzene	NA	200	214	107	NA	200	258	129
1,3-Dinitrobenzene	NA	200	172	86	NA	200	230	115
Nitrobenzene	NA	200	159	73	26	200	158	67
Tetryl	NA	200	178	89	NA	200	200	100
2,4,6-Trinitrotoluene	NA	200	188	94	NA	200	190	95
4-Amino-2,6-dinitrotoluene	NA	200	190	95	NA	200	191	96
2-Amino-4,6-dinitrotoluene	NA	200	181	91	NA	200	176	88
2,6-Dinitrotoluene	NA	200	184	92	NA	200	205	102
2,4-Dinitrotoluene	NA	200	183	92	NA	200	184	92
2-Nitrotoluene	NA	200	196	98	NA	200	195	98
4-Nitrotoluene	NA	200	193	97	NA	200	179	90
3-Nitrotoluene	NA	200	169	85	NA	200	183	91

Note: 1. One tap water sample was prepared, with four injections made. 2. Two spiked samples were prepared, with eight injections made for each preparation. 3. The detected concentrations of nitrobenzene shown were calculated from the tap water prepared as low-level samples (concentrated). Results showed 14 µg/L, using the E1 column, and 26 µg/L using the E2 column.

CONCLUSION

A parallel HPLC system equipped with the Acclaim Explosives E1 and E2 columns is an efficient way to determine 14 explosive compounds and related byproducts as specified in U.S. EPA Method 8330. This method saves time by running the primary and secondary (confirmatory) methods simultaneously, and achieves detection limits equivalent to those demonstrated in the EPA method. This method is part of the LCi concept of x2 dual applications from Dionex, designed to offer convenient, accurate results with minimal sample handling and fast turnaround. The E1 and E2 columns provide baseline resolution of all compounds, allowing a 14-component standard to be analyzed rather than running two lower component number standards separately as required for other columns designed to meet the requirements of EPA Method 8330.

REFERENCES

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2. X.Liu, Abordunov, M. Tracy, C. Pohl, New Columns for Baseline Separation of 14 Explosives-Related Compounds in EPA Method 8330, Dionex HPLC 2006 Presentation.
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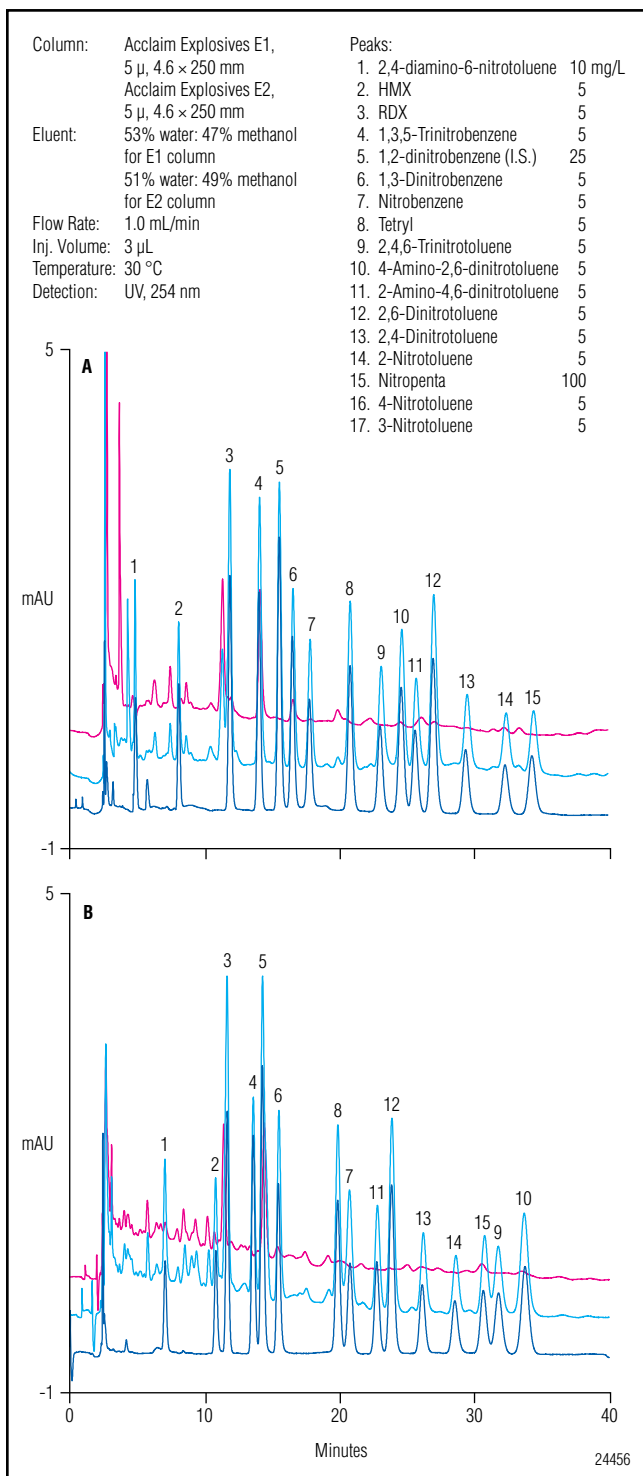


Figure 5. Overlay of chromatograms of (a) tap water and (b) spiked tap water prepared as low-level samples, and (c) mixture of standard solution (160 μ g/L) on (A) E1 and (B) E2 columns.

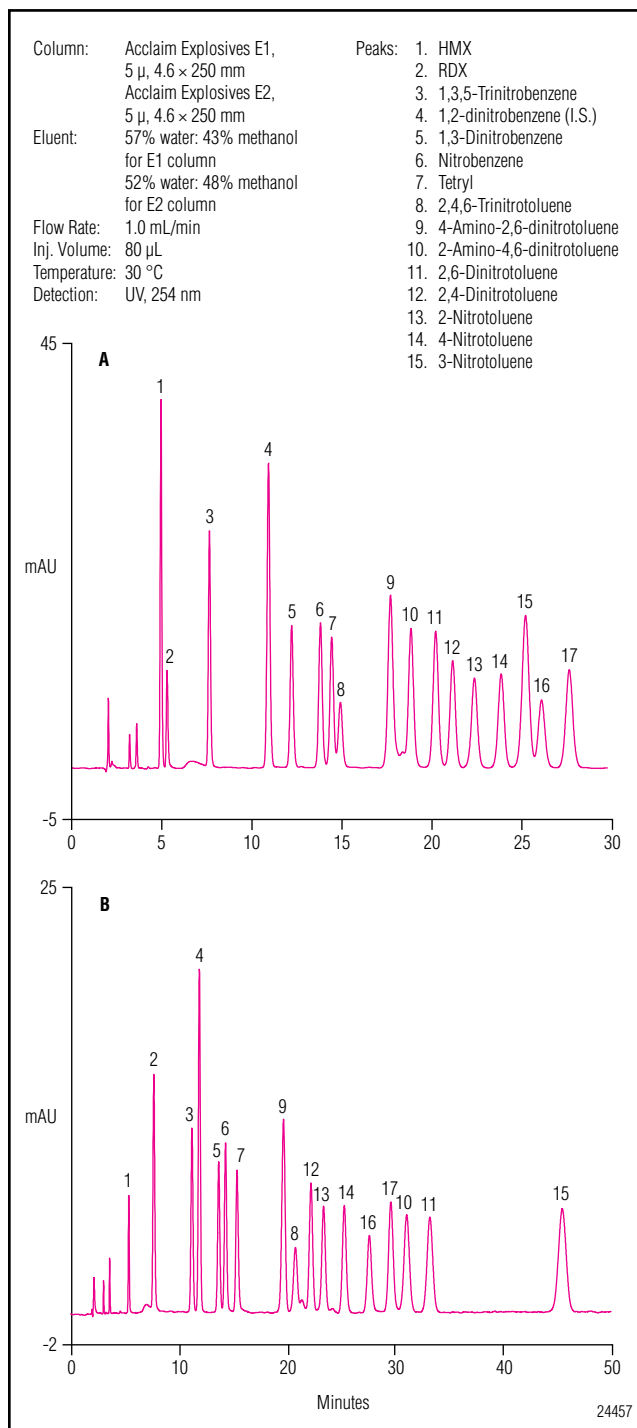


Figure 6. Chromatogram of a standard mixture of 17 explosives (5 mg/L) on A) E1 and B) E2 columns.

Table 6. Retention Times of the 14 Analytes Specified in EPA Method 8330, Plus Seven Added Compounds

		Retention Time (min)	
		E1 Column	E2 Column
Added Compounds	2,4,6-trinitrophenol	2.22	undetermined
	2,6-diamino-4-nitrotoluene	5.053	4.993
	2,4-diamino-6-nitrotoluene	5.417	5.157
	Nitroglycerin	16.44	21.55
	Nitropenta	31.70	46.92
	Diphenylamine	99.51	67.04
	Hexanitrodiphenylamine	No absorbance at 205, 210, and 254 nm*	
Compounds Specified in EPA Method 8330	HMX	5.000	7.196
	RDX	8.200	10.95
	1,3,5-Trinitrobenzene	12.05	11.78
	1,3-Dinitrobenzene	15.71	14.38
	Nitrobenzene	16.68	15.57
	Tetryl	17.98	20.91
	2,4,6-Trinitrotoluene	20.99	19.98
	4-Amino-2,6-dinitrotoluene	23.24	31.85
	2-Amino-4,6-dinitrotoluene	24.81	33.88
	2,6-Dinitrotoluene	25.84	22.89
	2,4-Dinitrotoluene	27.17	23.98
	2-Nitrotoluene	29.57	26.24
	4-Nitrotoluene	32.51	28.66
	3-Nitrotoluene	34.50	30.77

*Hexanitrophenylamine is not eluted with the standard conditions for the E1 and E2 columns.

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