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A platform for on-site environmental analysis of explosives using high performance liquid chromatography with UV absorbance and photo-assisted electrochemical detection

Ronita L. Marple, William R. LaCourse*

Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250, USA

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Abstract

High-performance liquid chromatography with photo-assisted electrochemical detection (HPLC–PAED) is used in conjunction with ultraviolet absorbance (UV) detection for determining explosives in environmental samples. The system utilizes an on-line solid-phase extraction technique for sample pretreatment (i.e., fractionation and concentration), thus reducing the required ground water sample size from 1 L to 2 mL and minimizing sample handling. Limits of detection for explosives using solid-phase extraction and PAED range from 0.0007 to 0.4 μg/L, well below those achieved with UV detection for several important explosives (e.g., RDX). The method has demonstrated good accuracy, precision, and recovery for all tested explosives, thus proving that the method is suitable for evaluation of explosives in ground water with competitive advantages over the U.S. Environmental Protection Agency (EPA) Method 8330. A system adaptable for the on-site environmental analysis of explosives has been developed and validated.
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1. Introduction

For many years, obsolete explosives were dumped in the sea, burned, or detonated in remote areas, constituting potentially serious and hazardous contamination problems [1]. These compounds leach into the soil and eventually get into ground water and, as a consequence, can travel distances from the contamination site. Many explosives are known toxins and carcinogens [2], so they pose a threat to living species as they find their way into the food chain.

Several detection techniques have been coupled to HPLC for the determination of explosives, including UV absorbance, refractive index (RI), mass spectrometry (MS), and dc amperometry [1–7]. Both RI and UV detectors suffer from a general lack of sensitivity and selectivity due to the presence of interfering compounds in environmental matrices. MS is complex and it is possible but difficult to use it for performing quantitative work [1,6], especially in the field.

Despite its inherent flaws, HPLC–UV is the accepted method used by the U.S. Environmental Protection Agency (EPA) for the determination of explosives in ground water and soil (EPA Method 8330). The method requires that samples be run on a C18 bonded-phase column and, subsequently, on a cyano bonded-phase column for confirmation of analyte identification. Furthermore, a ground water sample size of 1 L must be subjected to solvent extraction and be salted out and evaporated down to 5 mL prior to analysis. A more sensitive, selective method would aid in the analysis of explosive-containing samples.

To gain selectivity, reductive amperometry has been applied to the analysis of explosives. However, amperometric techniques in the reductive mode suffer from lack of sensitiv-

* Corresponding author. Tel.: +1 410 455 2969; fax: +1 410 455 2608.
E-mail address: lacourse@umbc.edu (W.R. LaCourse).

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ity and excessive noise, which is attributable to the reduction of dissolved oxygen present in the mobile phase and sample.

Post-column photochemistry in HPLC as a general derivatization approach for improved UV, fluorescence, and electrochemical detection has been used for a variety of analytes [8]. Photochemical derivatization provides the advantages of a reagent-free system, including the elimination of mobile phase restrictions, pulsations due post-column delivery, inadequate mixing of post-column reagents, and matrix effects due to chemical reagents [8]. Photochemical reactors are now commercially available with a variety of bulbs at different wavelengths and knitted reactor coils of various lengths and diameters. HPLC–photo-assisted electrochemical detection (HPLC–PAED), formerly referred to in the literature as HPLC-*hν*-EC, involves first the separation of the analytes of interest, followed by the photolytic generation of a new species that can then be detected electrochemically [8]. For nitro compounds, it has been reported that photolysis results in bond cleavage to generate inorganic nitrite (NO_2^-) which is then oxidized at a glassy carbon electrode (no other species have been identified at this time) [8–14]. Photo-assisted electrochemical detection (PAED), the detection scheme investigated here, operates in the oxidative mode and at applied potentials where dissolved oxygen is not a problem. Hence, PAED exhibits higher sensitivity than reductive amperometry. There have been numerous applications of HPLC–PAED for determining explosives in [3,6,8–14], revealing that nitro explosives are amenable to determination by HPLC–PAED with resulting detection limits in the range of 0.5–4 ng/20 uL injection, or about 25–200 μg/L. However, there are no applications of this technique to environmental samples.

This research takes advantage of the combination of HPLC–PAED, known for its inherent sensitivity and selectivity for organic nitro compounds [4,15], with on-line solid-phase extraction (SPE) for environmental samples in a field compatible platform [16,17]. SPE allows fractionation and preconcentration of the analytes of interest based on simple chromatographic principles. SPE eliminates sample handling and reduces dramatically (in combination with the increased sensitivity of PAED) the amount of sample required for analysis from 1 L (required by Method 8330) to 2 mL. It is this technique that makes the platform unique and compatible with the on-site analysis of explosives. PAED is compatible with EPA Method 8330 (HPLC–UV) in that only a buffer is added to the mobile phase, and the photochemical reactor and electrochemical cell are added in-line following the UV detector; therefore, only small adaptations are necessary for those systems currently running Method 8330.

The combination of photolysis and electrochemical detection is employed here in conjunction with EPA Method 8330 to develop an *enhanced* Method 8330 with on-line sample pretreatment, allowing a comprehensive analytical methodology for environmental field use superior in sensitivity and selectivity than current analytical techniques.

2. Experimental

2.1. Instrumentation and mobile phase

For simplicity, the system is described in two parts, the analytical system and the SPE unit.

2.1.1. Analytical system

The chromatographic system (Fig. 1A) consists of an Advanced Gradient Pump (model GP-40) equipped with an Eluent Degas Module (Dionex Corporation, Sunnyvale, CA). The direct injection valve (model 9010; Rheodyne Inc., Rohnert Park, CA) is fitted with a 100 μL injection loop. All separations were performed on reversed-phase (C18; 4.6 mm × 250 mm, 5 μm) SelectaPore column (Vydac, Hesperia, CA) with guard column (C8; 4.6 mm × 3.0 mm, 5 μm; SecurityGuard; Phenomenex, Torrance, CA) using a mobile phase of 50% methanol in 20 mM acetate buffer (pH = 4.5). The flow rate was set at 1.0 mL/min. Absorbance detection was performed at 254 nm using a model AD-25 (Dionex) variable wavelength detector. The photochemical reactor (photochemical reactor for enhanced detection (PHRED), Aura Industries Inc., New York, NY) was placed in-line after the UV detector. The PHRED contains a "knitted" PTFE reactor coil that is 25 m in length and 0.25 mm in i.d. with a volume of 1.25 mL, and the bulb in the reactor is a low pressure 254 nmHg lamp. DC amperometry was performed on a Model ED-40 electrochemical detector (Dionex). The eluent from the photoreactor is passed through a thin-layer electrochemical cell which is fitted with a 1.0 mm diameter glassy carbon working electrode, a Ag/AgCl reference electrode, and a stainless steel body serving as the auxiliary electrode. The injection valve, chromatographic columns, and electrochemical cell were housed in a model LC-30 column oven (Dionex) which was set at 30 °C. The resultant data is represented by dual chromatograms using Dionex PeakNet software version 5.21 on a PC-compatible computer.

2.1.2. On-line SPE unit

The SPE unit depicted in Fig. 1B is used in place of the direct injection valve. It consists of two electronically operated valves, a prep injector (model RP750-102, version-01, Rheodyne) and a 6-port, 2-position valve (model RP750-100, version-01, Rheodyne). The injectors are operated by the GP40 pump through relay controls triggered under software control. The prep injector is fitted with a 2mL injection loop. The 2 mL sample from the prep injector is loaded onto a MetaSil^{TM} reversed-phase SPE column (C18; 4.6 mm × 75 mm, 5 μm particle size; Ansys Technologies Inc., Lake Forest, CA). The loading solvent of the SPE is 7.5% methanol in a solution of 0.5 mM sodium chloride and 20 mM sodium acetate trihydrate adjusted to pH = 4.5 with sodium hydroxide. It is delivered to the unit by an isocratic pump (model 510, Waters Corporation, Milford, MA) at a flow rate of 1.0 mL/min.

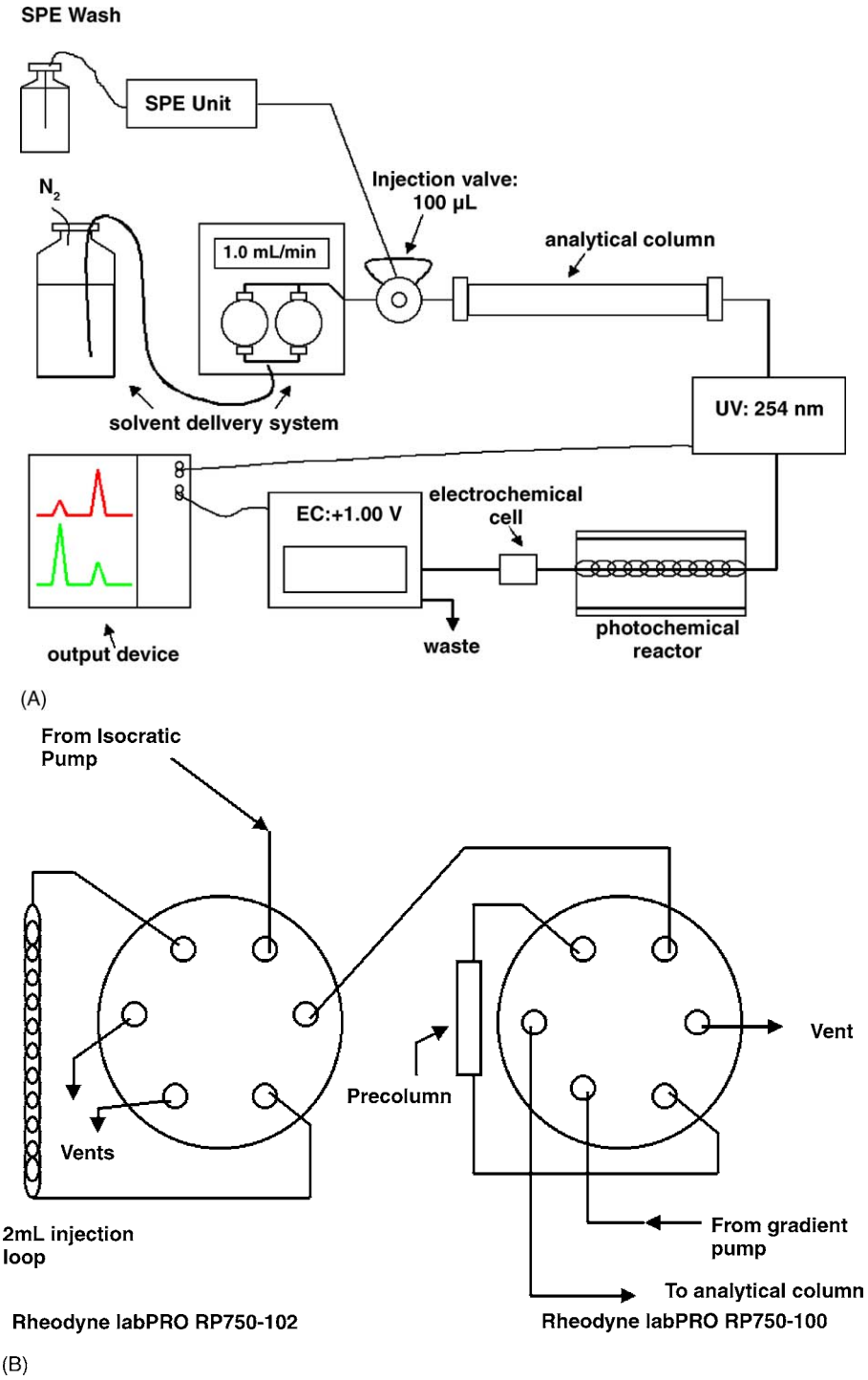

Fig. 1. Schematic of the (A) analytical system and (B) on-line SPE unit.

2.2. Reagents and solutions

All solutions were made with purified water. Water was purified using a reverse osmosis system coupled to multi-tank/ultraviolet ultrafiltration stations (U.S. Filter/IONPURE, Lowell, MA). All solvents were HPLC grade. All solvents were filtered with a Fisher vacuum filtration apparatus utilizing a 0.2 μm PTFE membrane filter from Alltech Associates Inc. (Deerfield, IL). Sodium acetate trihydrate, sodium chloride, and methanol (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA).

The standard solutions (Restek Corporation, Bellefonte, PA) were purchased as mixtures denoted as either calibration mix 1 or calibration mix 2 at concentrations of 1000 μg/mL each in 1 mL acetonitrile. Table 1 lists the name, peak num-

Table 1
Peak legend for each chromatogram

Peak no.	Explosive	Abbreviation	EPA classification	Restek mix
1	Octagen	HMX	A	1
2	Hexagen	RDX	A	1
3	1,3,5-Trinitrobenzene	1,3,5-TNB	A	1
4	1,3-Dinitrobenzene	1,3-DNB	A	1
5	Nitrobenzene	NB	A	1
6	Methyl-2,4,6-trinitrophenylnitramine	Tetryl	B	2
7	2,4,6-Trinitrotoluene	2,4,6-TNT	A	1
8	4-Amino-2,6-dinitrotoluene	4-A-2,6-DNT	B	2
9	2,6-Dinitrotoluene	2,6-DNT	B	2
10	2-Amino-4,6-dinitrotoluene	2-A-4,6-DNT	A	2
11	2,4-Dinitrotoluene	2,4-DNT	A	1
12	2-Nitrotoluene	2-NT	B	2
13	4-Nitrotoluene	4-NT	B	2
14	3-Nitrotoluene	3-NT	B	2

ber, abbreviation, EPA classification (mix A or B), and Restek classification (1 or 2) of all the explosives of EPA Method 8330. Standard solutions were stored in a refrigerator at 4 °C. All stock solutions were prepared daily. Certified samples were obtained from Environmental Resource Associates (Arvada, CO) and stored at 4 °C until use. Ground water was obtained from Columbia Technologies (Baltimore, MD) and stored at 4 °C until use.

2.3. Procedures

The glassy carbon electrode must be cleaned daily prior to use. The electrochemical cell is disassembled, and the working electrode is detached for polishing. Pour a small amount of electrode polishing compound (Gamma Micropolish Alumina #3, 0.05u, Buehler, Lake Bluff, IL) onto a POLYPAD Gemstone Polishing Pad (Crystalite Corporation, Westerville, OH) and place the pad on a flat surface. Rub the electrode in a figure eight motion in the polishing compound for approximately 30 s. It is important to hold the electrode flat against the pad to avoid rounding of the block which would result in cell leakage. Rinse off all polishing compound from the working electrode with copious amounts of deionized water. Follow the water rinse with a rinse of methanol to remove oils deposited from the polishing compound. Follow up with a final rinse of deionized water. All traces of polishing compound must be removed because trace particulates on the electrode surface will alter electrode response. Reassemble the cell and place it back into the LC-30 oven.

2.4. Safety considerations

Nitro explosives are known toxins and carcinogens and should be handled with gloves in a fume hood. Skin and eye contact and ingestion should be avoided.

3. Results and discussion

3.1. Model compounds

The compounds studied in this research are the members of the U.S. Environmental Protection Agency (EPA) Method 8330 suite of 14 nitro explosives, with emphasis on RDX, TNT, and Tetryl, for they are the explosives most commonly found at contaminated sites. As stated earlier, their names and abbreviations are listed in Table 1. EPA Method 8330 utilizes UV detection at 254 nm exploiting the inherent optical activity of each aromatic explosive, while HMX and RDX, both nitramines, do not respond as well to this method. Additionally, each explosive contains at least one nitro ($-NO_2$) functionality, making them amenable to determination by PAED.

3.2. System optimization

The platform was configured and optimized first for direct injection of the analytes of interest and, subsequently, for online sample pretreatment.

3.2.1. Optimization of direct injection method

In order to optimize the PAED detection potential, the technique of hydrodynamic voltammetry (HDV) at a constant flow rate of 1.0 mL/min was performed at three different buffer pH values (3.5, 4.5, and 5.5; *only* pH = 4.5 is shown) over a range of 0.2–1.0 V. The resulting data was plotted as S/N (analytical signal-to-noise ratio) versus applied potential, as shown in Fig. 2A for HMX (—), RDX (· · ·), Tetryl (- - -), and TNT (·—·). The analytes show a maximum signal-to-noise ratio at 1.0 V. Beyond 1.0 V, however, the analyte response begins to decrease while the noise increases, thus decreasing the signal-to-noise ratio. All EPA Method 8330 explosives responded in a similar manner, which allows for a single set of detection parameters for all compounds. It should also be noted that in Fig. 2A HMX and RDX (nitramines) clearly show higher signal to noise ratios than Tetryl and TNT (nitro aromatic compounds). This is due to the fact the N–N bonds in nitramines are much weaker (average of 163 kJ/mol) than the C–N bonds of nitro aromatics (average of 293 kJ/mol), and the photolytic cleavage of N–N bonds is much more efficient. This leads generation of more nitrite from nitramines than from nitro aromatics (at isomolar concentrations) and thus affords higher S/N ratios for nitramines. Varying the pH showed little effect, so a pH of 4.5 and applied potential of 1.0 V were chosen as optimum values for PAED.

The optimal residence time in the photochemical reactor, necessary to achieve the maximum generation of photoproducts, is determined by holding the potential constant and varying the flow rate for each injection of a mixture of analytes. Here, the potential used was 1.0 V and the pH = 4.5, and the flow rate was varied between 0.4 and 1.4 mL/min. The resulting signal-to-noise ratio was plotted versus the flow rate, as depicted in Fig. 2B for HMX (—), RDX (· · ·), Tetryl (- - -),

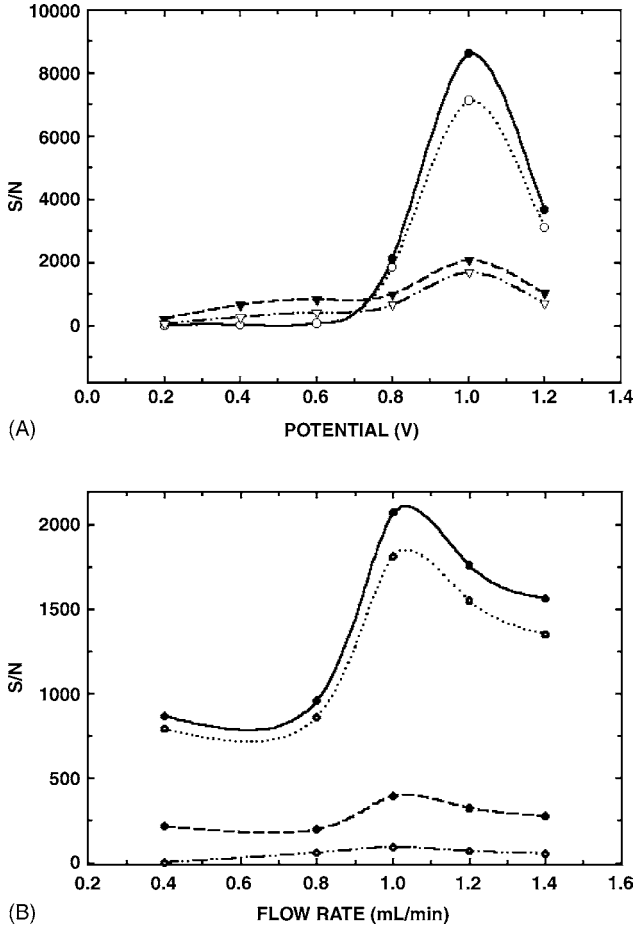

Fig. 2. (A) Hydrodynamic voltammograms for HMX (—), RDX (···), Tetryl (- - -), and TNT (·—·—·) at pH = 4.5. Mobile phase: 50% methanol in 20 mM acetate buffer, pH = 4.5; flow rate: 1.0 mL/min; guard column: Phenomenex SecurityGuard with 4 mm × 3.0 mm C8 cartridge; column: C18, 5 μm, 4.6 mm × 250 mm; column oven temperature: 30 °C; electrode: 1.0 mm glassy carbon; reference electrode: Ag/AgCl. (B) Plot of electrochemical S/N vs. flow rate for HMX (—), RDX (···), Tetryl (- - -), and TNT (·—·—·). Mobile phase: 50% methanol in 20 mM acetate buffer, pH = 4.5; guard column: Phenomenex SecurityGuard with 4 mm × 3.0 mm C8 cartridge; column: C18, 5 μm, 4.6 mm × 250 mm; column oven temperature: 30 °C; electrode: 1.0 mm glassy carbon; reference electrode: Ag/AgCl; applied potential: 1.0 V vs. Ag/AgCl.

and TNT (·—·—·). At flow rates below ca. 0.8 mL/min, the residence time in the photoreactor is too long and chemical degradation of electroactive species occurs [6]. The analytes show a maximum signal-to-noise ratio at 1.0 mL/min, and all faster flow rates do not allow enough time for the generation of the maximum amount of photoproducts. All nitro compounds responded similarly to those shown in Fig. 2B, and an optimum flow rate of 1.0 mL/min was chosen.

3.2.2. Optimization of on-line SPE

On-line SPE uses a short column that retains the analytes of interest under weak solvent conditions while allowing highly polar species to pass to waste. A stronger solvent is then passed through the miniature column and elutes the analytes of interest [18,19]. On-line SPE allows on-line sample preparation while eliminating the need for the "salting-out" extraction procedure required by EPA Method 8330. In addition, sample volume requirements are reduced, extensive sample handling is eliminated, and the system becomes field-compatible.

In order to perform on-line SPE, sample is loaded into the 2 mL injection loop, and the prep injector is electronically actuated, which allows the SPE solvent to flow through the loop and carry the sample to the SPE column. After an optimized "wash" time that preconcentrates the analyte and rinses off potential interferents, the prep injector is returned to the load position while the 6 port valve is turned to the inject position simultaneously. This procedure allows the HPLC mobile phase to backflush through the precolumn and elutes the analytes of interest onto the analytical column. This valve remains open throughout the entire chromatographic run. At the end of the run, a 5 min wash with 80% methanol cleans the SPE column, which is followed by a 5 min equilibration with "wash" solution prior to the next injection.

A wash solvent of 7.5% methanol in a solution of 20 mM sodium acetate trihydrate (pH = 4.5) and 0.5 M sodium chloride was determined to give the greatest sample cleanup. As in the "salting out" extraction process of EPA Method 8330, sodium chloride was added to increase the retention of the explosives from the matrix into the C18 phase of the SPE column. The methanol was needed to wet the C18 phase, and the sodium acetate buffer was added to matrix match the wash solvent and the HPLC mobile phase.

Optimization of the wash time was done by removing the analytical column, connecting the precolumn directly to the UV detector, injecting a standard, allowing it to be washed through the precolumn with the SPE wash solvent, and monitoring the detector for the time that the first explosive eluted. A wash time of 6.0 min at a flow rate of 1.0 mL/min was chosen.

3.3. Direct injection of explosives

The UV and PAED chromatograms under optimized separation and detection conditions are shown in Fig. 3 (A: UV; B: PAED). The chromatograms are scaled so that peaks 12, 13, and 14 are at approximately the same S/N ratio. The use of two detectors offers three distinct modes of selectivity. First, inherent to all chromatographic methods, is the selectivity afforded by the chromatography and the comparison of retention times of standards versus analytes in a sample. Because nitro compounds cannot be oxidized directly, there is no detectable analytical signal for the compounds of interest when the lamp in the photochemical reactor is turned off. Hence, the second mode of selectivity is that the compounds must be photoreactive and produce oxidizable products to be detected. Finally, the use of two detectors allows for the determination of response ratios for standards and those for analytes in a sample. The ratios are found by dividing the EC signal by the UV signal for a particular analyte. Using a certified sample purchased from Environmental Resource

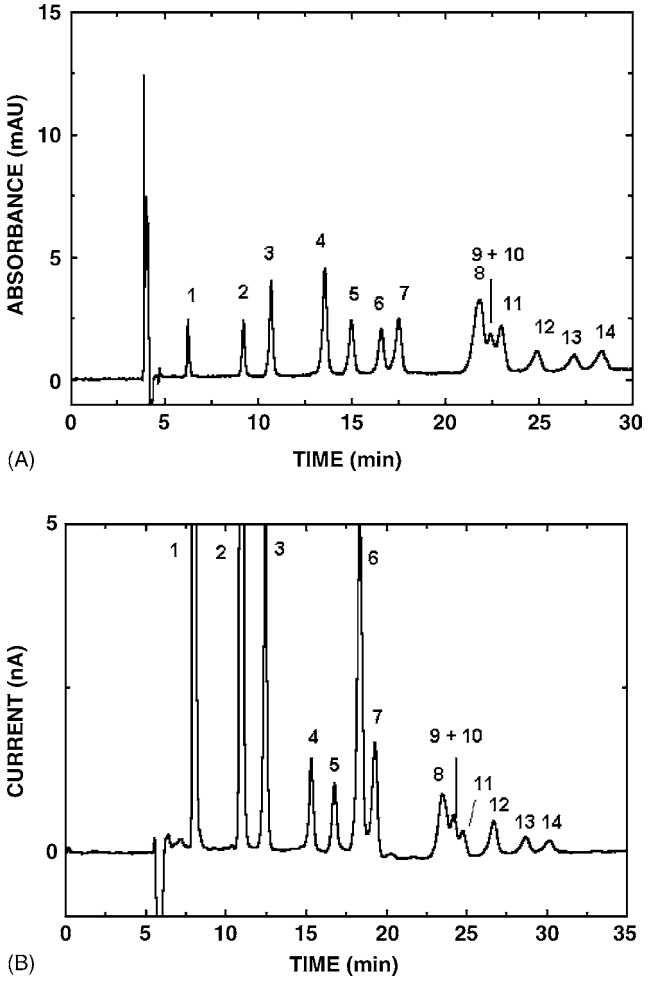

Fig. 3. Optimized separation of explosives: (A) UV at 254 nm chromatogram; (B) PAED chromatogram. Mobile phase: 50% methanol in 20 mM acetate buffer, pH = 4.5; flow rate: 1.0 mL/min; guard column: Phenomenex SecurityGuard with 4 mm × 3.0 mm C8 cartridge; column: C18, 5 μm, 4.6 mm × 250 mm; column oven temperature: 30 °C; electrode: 1.0 mm glassy carbon; reference electrode: Ag/AgCl; applied potential: 1.0 V vs. Ag/AgCl.

Associates, Arvada, CO, the response ratios for selected explosives are given in Table 2. Statistically, there is no significant difference between the ratios for the standards and the analytes in the samples at the 95% confidence level.

Upon examination of the two chromatograms (Fig. 3A and B), the expected increased sensitivity achieved by PAED for the nitramines (HMX and RDX) and other compounds such as Tetryl and TNT is easily seen. The figures of merit for the model compounds are presented in Table 3A. These

Table 2
Response ratios for listed explosives

Explosive	Response ratio for standard (n = 3) (EC/UV)	Response ratio for analyte (n = 6) (EC/UV)
RDX	104.2 ± 4.8	104.3 ± 2.5
Tetryl	30.6 ± 0.4	30.6 ± 0.6
TNT	8.3 ± 0.2	8.2 ± 0.3

were determined using the direct injection method with the 100 μL injection loop, and all solutions were made in deionized water. The figures of merit for 2,6-DNT and 2-A-4,6-DNT were tabulated by running calibration curves for each of these compounds separately. When comparing limits of detection, PAED (LODs 0.007–3 μg/L) is more sensitive than UV detection (LODs 0.9–5 μg/L) for almost all of the model compounds. For some of the more common explosives, there is an approximate 1000- and 100-fold increase in sensitivity for HMX and RDX, respectively, and an approximate 100- and 10-fold increase in sensitivity for Tetryl and TNT, respectively, over UV detection. Furthermore, the PAED limits of detection are much lower here than those previously described for $h\nu$-EC in [3,6,8–14].

The slope of the line (m) and the y-intercept (b), were included to show the calibration sensitivity of the detection methods and that there is no system bias in the method, respectively. Also, R^2 values determined by linear regression analysis show that UV and PAED are of comparable linearity over the concentration range tested, which is at least four orders of magnitude in both cases. The percent relative standard deviation (%R.S.D.), determined by seven injections at the approximate limit of quantitation for each explosive, ranged from 0.80 to 3.41% for UV detection and 0.46 to 6.70% for PAED, all under 15% as required by the Resource Conservation and Recovery Act (RCRA) described in Section 3.5.

3.4. On-line SPE analyses

When a real ground water sample was injected on the existing system, a large background was present on the electrochemical detector that completely overwhelmed any signal from the explosives present, as shown in Fig. 4A. This large background is due to electroactive species present in the groundwater (e.g., salts, urea, etc.). This effect is a limitation to PAED for directly analyzing ground water samples without any sample pretreatment, and on-line SPE was chosen for reasons stated earlier. When the same ground water sample was run on the system using optimized on-line SPE, the high background was virtually eliminated, as seen in Fig. 4B.

Table 3B displays the figures of merit for the nitro explosives in deionized water using the SPE system. On average, preconcentration lowered the limits of detection by an order of magnitude for both detection methods while retaining a good linear response and low R.S.D.s. Preconcentrated limits of detection ranged from 0.0007 to 0.4 μg/L for PAED and from 0.04 to 0.4 μg/L for UV detection. It should be noted that on-line SPE could be used with Method 8330 alone to increase sensitivity without incorporating PAED, but PAED should be used for Method 8330 enhancement. This concentration factor will eliminate the need for 1 L of ground water required by Method 8330, and these limits of detection are achieved using only 2 mL of water. Linearity is retained when using SPE, with an average R^2 value of 0.99962 (PAED and UV). The %R.S.D.s ranged from 1.17 to 5.38%, com-

Table 3
(A) Analytical figures of merit using the direct injection method and (B) analytical figures of merit by on-line SPE

Explosive	Detection	LOD[a] (μg/L)	*m*	*b*	R^2	LOQ[b] (μg/L)	%R.S.D. (*n* = 7)
(A) Direct injection method							
1	UV	2	90673	68	0.99949	4	0.80
	PAED	0.007	19602576	10417	0.99935	0.02	4.08
2	UV	2	142265	272	0.99915	4	1.44
	PAED	0.02	20120735	13029	0.99950	0.08	2.26
3	UV	1	306047	266	0.99908	3	1.75
	PAED	0.06	5791715	6931	0.99955	0.2	2.43
4	UV	1	467947	376	0.99980	2	0.85
	PAED	0.5	1978307	325	0.99996	2	3.65
5	UV	0.9	325421	−164	0.99997	3	2.88
	PAED	0.3	2032216	−4097	0.99974	1	5.00
6	UV	2	235262	228	0.99905	5	1.47
	PAED	0.03	9040367	3099	0.99986	0.3	0.91
7	UV	2	328575	328	0.99975	4	1.88
	PAED	0.1	3141376	250	0.99989	1	2.83
8	UV	1.8	238989	−24	0.99991	6	0.95
	PAED	2	964574	1460	0.99990	6.8	1.76
9	UV	2	226891	−19	0.99990	6.7	1.52
	PAED	2	1259294	1986	0.99994	6.7	1.46
10	UV	1	356023	−289	0.99994	3.2	0.50
	PAED	3.5	504355	−839	0.99992	11.5	0.46
11	UV	1	463620	66	0.99993	3.5	0.80
	PAED	2	1458181	−593	0.99990	6.4	1.63
12	UV	3	191530	−461	0.99952	7	3.41
	PAED	0.3	2011157	−5032	0.99997	3	5.35
13	UV	4	166670	−591	0.99930	12	2.01
	PAED	1	697940	−3275	0.99915	3	6.70
14	UV	5	210125	−1010	0.99927	15	2.77
	PAED	3	440613	−3193	0.99910	11	5.52
(B) On-line SPE							
1	UV	0.04	2375046	3611	0.99931	0.6	1.53
	PAED	0.0007	530804816	32445	0.99987	0.01	5.38
2	UV	0.1	3688869	1922	0.99919	2	3.55
	PAED	0.002	511876487	74400	0.99922	0.03	3.16
3	UV	0.07	7587023	58	0.99917	1	2.23
	PAED	0.008	120733258	−11449	0.99948	0.1	3.04
4	UV	0.06	10817046	1632	0.99955	1	2.11
	PAED	0.03	33333127	11235	0.99974	0.5	1.72
5	UV	0.05	6572014	569	0.99971	2	1.72
	PAED	0.04	35730659	−15748	0.99957	0.9	1.69
6	UV	0.2	5181264	804	0.99982	3	1.61
	PAED	0.007	146103947	66998	0.99950	0.1	1.88
7	UV	0.09	7408222	2870	0.99917	2	2.18
	PAED	0.02	63700692	2833	0.99973	0.3	2.64
8	UV	0.08	5147257	1078	0.99990	0.3	3.70
	PAED	0.1	16781705	−118	0.99992	0.4	3.80
9	UV	0.05	5145178	3289	0.99995	0.17	2.23
	PAED	0.05	25886203	10101	0.99991	0.17	3.79
10	UV	0.04	8107759	4710	0.99994	0.15	2.36
	PAED	0.2	12842597	3849	0.99990	0.5	3.50
11	UV	0.04	10441174	1917	0.99998	0.14	1.17
	PAED	0.09	27704511	7444	0.99996	0.3	2.76
12	UV	0.3	3987859	−940	0.99974	5	4.02
	PAED	0.1	26831251	−7335	0.99941	2	3.91
13	UV	0.4	3566579	−1080	0.99987	7	4.97
	PAED	0.2	13729809	−8603	0.99919	3	4.09
14	UV	0.4	4526009	−1550	0.99987	5	4.25
	PAED	0.4	8779970	−1160	0.99874	6	4.31

Linear range nA (mAU for UV) = *m*(ppm) + *b*.
[a] LODs are calculated at S/N = 3.
[b] LOQs are calculated at S/N = 10.

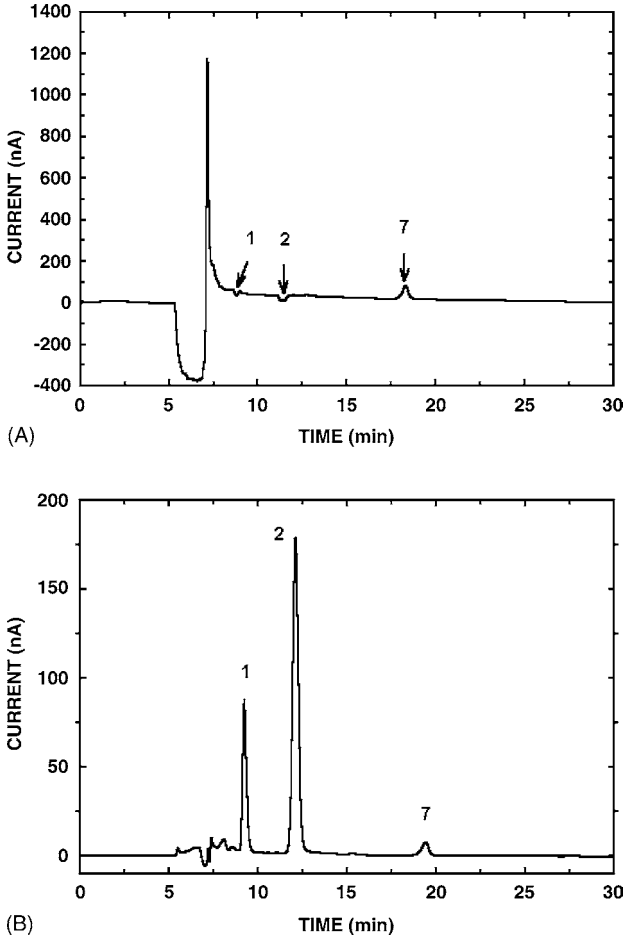

Fig. 4. PAED chromatograms of real ground water sample contaminated with explosives: (A) without SPE; (B) with SPE. SPE wash solvent: 7.5% methanol in 20 mM sodium acetate and 0.5 M sodium chloride; flow rate: 1.0 mL/min; column: C18, 5 μm, 4.6 mm × 75 mm; column temperature: ambient; HPLC–UV–PAED conditions are the same as listed in Fig. 3.

parable to those by direct injection and still within RCRA guidelines.

3.5. Validation

Because this system will be used for environmental fieldwork, it has been validated using the procedures set forth by the Resource Conservation and Recovery Act (RCRA), enacted by Congress in 1976. RCRA requires an evaluation of the method with respect to accuracy (80–120%), precision (<20%), repeatability (<15%), and quantitation and detection limits in the matrix of interest. Analytical figures of merit for explosives spiked into ground water are listed in Table 4. Limits of detection ranged from 0.004 to 0.9 μg/L for PAED and from 0.07 to 2 μg/L for UV detection. Both detectors retain good linearity, very similar to that achieved with SPE on standards in deionized water, and both precision and accuracy fall within RCRA guidelines (%R.S.D. 1.06 to 14.97%). A high %R.S.D. is seen for HMX on both detectors (UV = 14.97 and PAED = 13.00). This is probably due to the fact that this compound elutes early in the chromatographic run, and it will be the most affected by the on-line clean-up process. It is important to note, however, that high R.S.D.s are observed using *both* detection methods, but they are still both within RCRA guidelines.

For the final phase of validation for ground water samples, a certified sample was purchased from Environmental Resource Associates, Arvada, CO. The sample was analyzed by both the direct injection method and by on-line SPE, and the results are listed in Table 5. The table reports the amount of explosive contained in the sample (target value), the acceptable range of quantitation, the results found by direct injection and SPE (by PAED and UV detection), the standard deviation based on six injections, and the percent recovery. All recoveries of explosives, from 93.2 to 104.9%, fell within ranges accepted by RCRA (80–120%). An important note is that values obtained from the UV and PAED detectors agree with each other, and that the direct injection method agrees with the on-line SPE method. Hence, the validation of the EPA Method 8330 performed as expected, and the PAED results are equivalent to the UV detection as used in Method 8330.

3.5.1. Chemometric approach

The above validation was performed using standards and samples, using retention times and response ratios for analyte confirmation. However, 2,6-DNT and 2-A-4,6-DNT co-elute (also reported by the EPA), and it is impossible to accurately report the amount of each present in the sample. Instead of reporting a mixture of both compounds, as suggested by the EPA, one would rather achieve a more efficient separation in order to report the presence of these compounds in a sample with complete confidence.

A way to resolve the overlapping peaks is through chemometrics. One is able to do this because of the use of two detectors, each with a characteristic response for the explosives in question. The response equation for a particular peak by either detector is as follows:

$$Y_{\text{unknownUV}} = m_{\text{UVA}}C_{\text{A}} + m_{\text{UVB}}C_{\text{B}} \quad (1)$$

$$Y_{\text{unknownEC}} = m_{\text{ECA}}C_{\text{A}} + m_{\text{ECB}}C_{\text{B}} \quad (2)$$

where Y_{unknown} is the signal due to the overlapping peaks, m_{A} the slope of the standard curve for one of the two peaks, and C_{A} is the concentration of the same overlapped peak in the unknown. Those terms with a "B" subscript have similar meanings but apply to the second overlapped peak. This equation also applies to the signals and slopes obtained from the electrochemical detector as denoted by Eq. (2). This approach will always work because the sensitivities of the two detection methods are similar for these particular compounds; if the compounds are at a level detectable by PAED, they will also be detectable by UV. Because the responses of the two compounds are additive and using their response factors obtained from calibration curves during the analysis, one can find an algebraic solution for C_{A} and C_{B} in the unknown sample dur-

Table 4
Analytical figures of merit for explosives spiked into blank ground water

Explosive	Detection	LOD[a] (μg/L)	*m*	*b*	R^2	LOQ[b] (μg/L)	%R.S.D. ($n = 7$)
1	UV	0.2	3727396	1736	0.99939	0.7	14.97
	PAED	0.004	335572515	93978	0.99982	0.01	13.00
2	UV	0.6	3934298	−1824	0.99998	2	9.00
	PAED	0.006	402950611	−153648	0.99821	0.02	8.00
3	UV	0.3	7039598	−4032	0.99932	1	7.38
	PAED	0.05	100549387	−48786	0.99860	0.1	7.19
4	UV	0.1	9598515	25124	0.99831	0.2	6.41
	PAED	0.07	38545526	38057	0.99831	0.2	6.53
5	UV	0.2	6673138	−153	0.99778	0.6	5.70
	PAED	0.02	30686076	−1952	0.99925	0.2	5.65
6	UV	0.1	10357198	5106	0.99606	0.3	6.72
	PAED	0.02	160833997	−58796	0.99879	0.07	6.44
7	UV	0.2	7546564	521	0.99908	0.6	4.63
	PAED	0.06	61342625	−22311	0.99865	0.1	5.00
8	UV	0.2	2740651	−1256	0.99999	0.7	1.13
	PAED	0.5	8931537	−871	1.00000	1.7	1.10
9	UV	0.1	2450297	2714	0.99999	0.4	1.33
	PAED	0.3	11801884	−6877	0.99995	1.2	1.06
10	UV	0.07	8054742	5699	0.99997	0.2	1.77
	PAED	0.9	3688344	−181	0.99994	4	2.06
11	UV	0.08	4921502	6218	0.99998	0.3	1.62
	PAED	0.4	12491873	−8692	0.99993	1.2	1.09
12	UV	1	4268597	−2284	0.99816	3	2.03
	PAED	0.08	21849583	2454	0.99874	0.3	2.18
13	UV	2	3716345	−2830	0.99732	4	1.66
	PAED	0.2	12709611	1086	0.99919	0.8	1.29
14	UV	2	4584982	−3187	0.99969	7	2.89
	PAED	0.2	10152957	−11917	0.99191	5	2.10

Linear range nA (mAU for UV) = m(ppm) + b.
[a] LODs are calculated at S/N = 3.
[b] LOQs are calculated at S/N = 10.

Table 5
Certified sample results by direct injection and SPE

Explosive	Target value (μg/L)	Accepted range	Direct injection		% Recovery		SPE		% Recovery	
			UV	PAED	*UV*	PAED	UV	PAED	*UV*	*PAED*
1	15.20	8.44–19.8	15.21 ± 0.31	15.28 ± 0.06	100.1	100.5	15.08 ± 0.45	15.14 ± 0.19	99.2	99.6
2	9.20	6.71–11.40	8.84 ± 0.17	8.93 ± 0.26	96.0	97.0	8.79 ± 0.09	8.76 ± 0.05	95.5	95.3
3	7.00	4.51–7.42	7.30 ± 0.05	7.29 ± 0.25	104.3	104.1	7.35 ± 0.26	7.28 ± 0.04	104.9	104.1
4	10.40	8.17–11.50	10.08 ± 0.07	9.98 ± 0.11	96.9	95.9	10.11 ± 0.05	10.11 ± 0.06	97.2	97.2
5	10.00	6.29–13.80	9.39 ± 0.10	9.40 ± 0.17	93.9	94.0	10.09 ± 0.07	10.05 ± 0.23	100.9	100.5
6	15.70	9.45–19.80	15.18 ± 0.13	15.24 ± 0.34	96.7	97.1	15.22 ± 0.14	15.15 ± 0.06	96.9	96.5
7	9.82	7.04–12.70	9.43 ± 0.16	9.43 ± 0.12	96.0	96.1	9.45 ± 0.05	9.45 ± 0.07	96.3	96.2
8	8.20	5.22–9.68	8.30 ± 0.09	8.31 ± 0.11	101.2	101.3	8.41 ± 0.02	8.44 ± 0.11	102.6	103.0
9[a]	9.42	6.62–10.70	7.25 ± 0.36		77.0		7.26 ± 0.06		77.1	
10[a]	6.21	4.99–7.08	6.28 ± 0.38		101.1		6.20 ± 0.02		99.8	
11	5.62	4.69–6.13	5.58 ± 0.21	5.68 ± 0.12	99.2	101.1	5.63 ± 0.06	5.77 ± 0.23	100.2	102.6
12	16.80	12.70–17.80	17.09 ± 0.44	17.05 ± 0.40	101.7	101.5	17.26 ± 0.33	17.20 ± 0.34	102.7	102.4
13	6.21	4.26–6.52	6.54 ± 0.34	6.51 ± 0.50	105.2	104.8	6.25 ± 0.03	6.27 ± 0.04	100.7	100.9
14	7.40	6.13–7.62	6.90 ± 0.33	7.01 ± 0.34	93.2	94.7	7.01 ± 0.08	6.86 ± 0.30	94.7	92.7

[a] Determined chemometrically (see Section 3.5).

ing post-data analysis. The results obtained by this method for 2,6-DNT and 2-A-4,6-DNT are listed in Table 5. The % recovery for 2,6-DNT and 2-A-4,6-DNT ranged from 77.0 and 101.1%, respectively. Importantly, direct analysis and the SPE approach were in excellent agreement. This shows that the chemometric approach is a feasible one for determining the amount of these explosives at a contaminated site.

4. Conclusions

The instrumentation for determining explosives in ground water has been developed, optimized, and validated. It provides the advantages of increased sensitivity and selectivity over EPA Method 8330. On-line SPE allows for on-site analysis compatibility, reducing the required sample amount

from 1 L (EPA Method 8330) to 2 mL, and performing sample preparation on-line, thus minimizing sample handling and allowing for "real-time" analyses. The developed platform is a unique analytical tool with increased sensitivity and selectivity, enabling faster, more accurate site assessment.

Although daily polishing of the electrode is required, as with all dc amperometric methods, acceptable reproducibility and ruggedness has been observed throughout 4 years of running this assay. Addition of PAED to Method 8330 instrumentation will allow for a slightly modified Method 8330, so those currently using this method can switch to HPLC–UV–PAED with only minimal modifications and achieve enhanced sensitivity and selectivity.

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