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Rapid on-line chromatographic determination of trace-level munitions in aqueous samples

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Abstract

An on-line trace enrichment system is described that combines a divinylbenzene–vinylpyrrolidone co-polymer precolumn with a reversed-phase C_{18} HPLC analytical column. This arrangement allows quantitative preconcentration of munitions on the resin sorbent, followed by complete transfer of analyte to the analytical column for separation, followed by UV absorbance detection at 254 nm. Detection limits were approximately 0.10 ng/ml for TNT and RDX when 10 ml of sample was analyzed. The feasibility of increasing sample volume (up to 50 ml) to obtain detection limits down to 20 pg/ml was also demonstrated for TNT. Analyses of aquifer samples collected in the vicinity of a military installation are presented to show system utility.

Keywords: Water analysis; Environmental analysis; Explosives; Trinitrotoluene; Hexahydrotrinitrotriazine

1. Introduction

Decades of munitions manufacturing have resulted in numerous severely contaminated military installations. Often, munitions manufacturing and packing wastes were disposed of in trenches, cribs and settling lagoons. This practice has left areas of land heavily contaminated with military explosives and their environmental transformation products. Leaching of munitions from the soil into groundwater underlying the military production and packing plants has contaminated the aquifer water [1]. The principal explosives of concern are 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Aquifer pollution plumes laden with these munitions are spreading contamination beyond the boundaries of military reservations. Water for crop irrigation is often drawn from these contaminated aquifers. Trace-level munitions contamination observed in these agricultural wells is especially serious in light of studies demonstrating plant bioaccumulation of RDX [2]. Another, more immediate problem seems imminent as these pollution plumes encroach on potable municipal water supplies.

As munitions pollution plumes impact populated areas, regulatory agencies have adopted various exposure limits. One of these guidelines, the lifetime health advisory limit, has been set at 2 ng/ml in drinking water for both TNT and RDX [3]. Environmental transformation pathways for explosives have not been entirely delineated; however, it is clear that the toxicity of the intermediates is of concern. It is possible that one or more transformation products may eventually be regulated at the sub-ng/ml concentrations. Rapid analytical techniques are clearly needed that are capable of speciating munitions, their

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synthetic by-products and related environmental transformation products at sub-ng/ml concentrations. This methodology will be useful for monitoring aquifer drinking water, assessing suitability for agricultural irrigation, mapping pollution plume migration profiles and evaluating the effectiveness of remediation efforts.

A variety of approaches have been described for the trace analysis of munitions in water. Solvent extraction followed by capillary gas chromatographic analysis with electron-capture detection allowed early workers to achieve detection limits in the upper pg/ml range for TNT [4,5]. The current US Environmental Protection Agency method 8330 is also based on solvent extraction methods [6]. However, solvent extraction approaches, which are labor-intensive and produce large volumes of waste solvent, are rapidly being replaced by solid-phase extraction (SPE) procedures. Early workers found poor recoveries of munitions on octadecyl silica bonded phases and poly(styrene–divinylbenzene) polymer resins [7–9]. Maskarinec and co-workers [7] demonstrated that divinylbenzene–vinylpyrrolidone copolymer resin (commercially available as Porapak R) constituted a chromatographic matrix that allowed quantitative and apparently selective retention of nitro-containing explosives. Several investigators have noted possible limited utility of this resin for explosives analysis due to generation of resin-associated artifacts [3,7,10]. A significant recent development has been the commercial availability of exhaustively cleaned polymer that does not yield artifact peaks. Bouvier and Oehrle [10] have proposed a method based on off-line SPE of aqueous samples on this specially cleaned resin (Porapak RDX from Waters, Milford, MA, USA) followed by HPLC analysis of a portion of the acetonitrile eluate. This method requires a 500-ml sample and gives a detection limit of roughly 0.1 ng/ml for TNT and RDX. Off-line SPE with this specially cleaned resin was also evaluated by Jenkins and co-workers [11] and compared to extractions performed with a poly(styrene–divinylbenzene)-based membrane as well as a salting-out solvent extraction method. Other methods being developed include fiber optic immunosensors that, at present, do not offer the necessary sensitivity or specificity [12].

The objective of our study was to construct an analytical system that would allow rapid on-line analysis of aqueous samples. This system will combine quantitative and selective preconcentration of munitions from aqueous samples on Porapak R sorbent followed by on-line transfer of the entire trace enrichment column contents to an analytical HPLC separation column. The explosives will then be separated and detected by UV absorbance.

2. Experimental

Acetonitrile and water were HPLC grade obtained from J.T. Baker (Phillipsburg, NJ, USA). Alternative suppliers of HPLC-grade water were Alltech (Deerfield, IL, USA) and Fisher (Pittsburgh, PA, USA). RDX and TNT were Standard Analytical Reference Material (SARM) provided by the US Army Toxic and Hazardous Material Agency (Aberdeen Proving Ground, MD, USA). Standards were prepared by dilution from aqueous 50 μg/ml concentrated stock solutions.

A trace enrichment chromatographic valving arrangement, which has been previously described [13], formed the core of our analytical system. The valve configuration, illustrated in Fig. 1, is composed of injection (Model 7120) and switching (Model 7000) valves manufactured by Rheodyne (Alltech). The injection valve was fitted with a standard Rheodyne 20-μl sample loop. To analyze aqueous samples for trace levels of munitions, solution was provided to the preconcentration column by the trace solution pump (Waters Model 660A), as shown in Fig. 1A. After sufficient explosive was enriched on the preconcentration column, analytes were analyzed by backflushing the cartridge column contents onto the analytical column with HPLC mobile phase as shown in Fig. 1C. Standards were conveniently analyzed with this system by filling the sample loop with aqueous standard while pumping pure water through the preconcentration column (Fig. 1A). Next, the standard was introduced to the preconcentration column by switching the valve to the configuration shown in Fig. 1B. Finally, standard material contained on the preconcentration column was analyzed by switching the valves to the trap analysis configuration shown in Fig. 1C.

A precolumn assembly manufactured by Brownlee

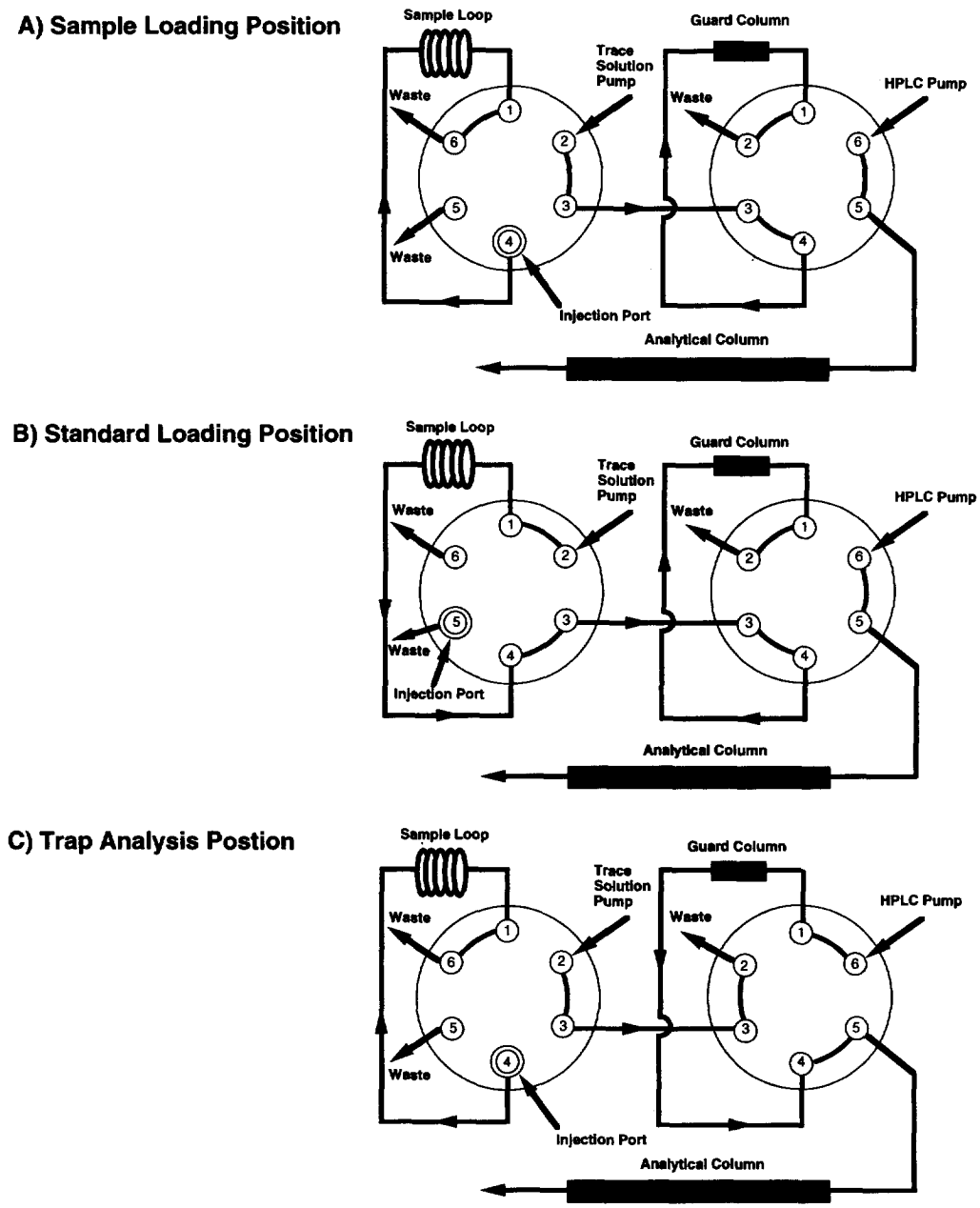

Fig. 1. On-line chromatographic valve configuration illustrating the (A) sample loading, (B) standard loading and (C) precolumn analysis positions.

(Applied Biosystems, Foster City, CA, USA) was used to house the 4.0×0.64 cm O.D. trace enrichment column. Divinylbenzene–vinylpyrrolidone copolymer resin (75–100 μm particle size) that had been exhaustively cleaned was obtained from Waters as Porapak RDX Sep-Pak cartridges and supplied to Higgins Analytical (Mountain View, CA, USA) with instructions to prepare columns that were compatible

with the Brownlee precolumn assembly. The custom trace enrichment columns contained a packed resin bed that was 3.7 cm×0.32 cm.

Analytical separations were performed on a Beckman (Irvine, CA, USA) Ultrasphere column (24× 0.46 cm I.D.) packed with 5-μm octadecyl silica. Components were eluted with isocratic acetonitrile–water mobile phase delivered by a Waters Model 590 pump at a flow-rate of 1.0 ml/min. An acetonitrile–water (50:50, v/v) mobile phase was used for analysis of samples that contained TNT or both TNT and RDX. A mobile phase of acetonitrile–water (40:60, v/v) was used to analyze samples containing only RDX. A Schoeffel (Westwood, NJ, USA) Model 770 UV detector, operated at 254 nm, was employed for detection of separated components. For several experiments a Waters Model 490E detector was substituted for the Schoeffel detector. Detector signal was recorded on a Hewlett–Packard (Avondale, PA, USA) Model 3390 recording integrator.

Gas chromatography (GC)–mass spectrometry (MS) studies utilized a Hewlett–Packard 5970A mass-selective detector interfaced to a Hewlett–Packard 5890 gas chromatograph. Aquifer samples were subjected to off-line SPE on Porapak RDX cartridges followed by elution of the sorbent with acetonitrile. The acetonitrile was then evaporated to dryness and the residue reconstituted with toluene. Analysis consisted of a 1-μl splitless injection onto a 30 m×250 μm I.D. DB-5 column that contained a 1.0-μm film of stationary phase (J&W, Folsom, CA, USA). Separation was effected with helium carrier by programming the column from 80 to 280°C at 6°C/min. Nominal resolution mass spectra were obtained by scanning the quadrupole mass analyzer from 40 to 600 amu at a rate of 200 amu/s.

3. Results and discussion

Injection volume was determined by filling the injection loop with mercury and injecting the loop contents into tared vial with a pentane mobile phase flush. The pentane was subsequently evaporated and the volume of mercury determined gravimetrically [14]. The volume determined in this manner was 24.3±0.2 μl (n=5).

Initial studies were conducted with 10-ml samples. This sample volume provided sufficient analyte for analysis within a reasonable sampling time. An accurate volume of sample was determined gravimetrically by collecting and weighing fluid emerging from the trace enrichment column (Model 7000 valve, port 2). After the sample was loaded, a pure water chase of 7.0 ml was delivered at 1.0 ml/min to ensure complete transfer of sample to the polymer sorbent bed. At a signal-to-noise ratio of 3, the limits of detection for RDX and TNT were approximately 0.2 ng/ml when the Schoeffel detector was employed. To give a more realistic appraisal of detection limits that could be achieved with modern analytical instrumentation, these experiments were repeated with the Waters Model 490E UV detector. Fig. 2 (bottom) presents a representative chromatogram that resulted from preconcentration of 10-ml water that contained 0.2 ng/ml TNT. The

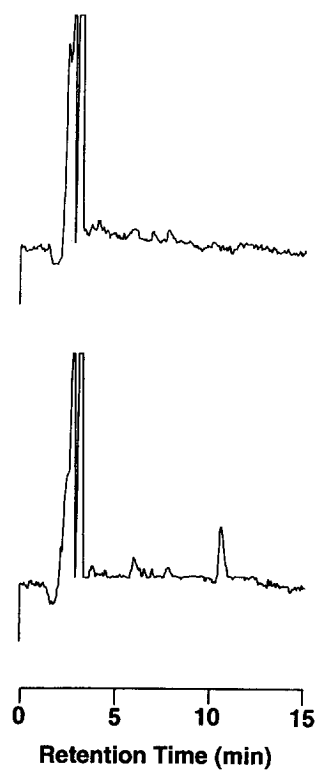

Fig. 2. Chromatograms of a 0.200 ng/ml TNT standard (bottom) and a corresponding water blank (top). Both chromatograms resulted from preconcentrating 10.0 ml of sample. TNT elutes with a retention time of 10.51 min in the bottom chromatogram. Detector sensitivity was 0.00085 absorbance units full scale.

peak due to TNT is clearly present at roughly 6 times background noise. The chromatogram in the top of Fig. 2 is an analysis blank resulting from trace enrichment of 10 ml of HPLC-grade water. The water blank chromatogram is included to emphasize that the on-line system does not suffer from the resin-associated artifact peaks described by previous investigators [3,7].

Percent recoveries for TNT were assessed at two different concentrations by comparing TNT peak areas resulting from loop injections to results obtained from analysis of 10-ml aliquots of trace-level standards. Sample loop injections were followed by a 7-min water chase to quantitatively transfer the loop contents to the resin precolumn before transfer to the analytical column. Recoveries at higher concentration compared a 5.0 μg/ml standard loop injection to the trace enrichment of 10 ml of a 10 ng/ml solution (introduction of ~100 ng). The lower concentration recovery compared a 1.0-μg/ml loop injection to trace enrichment of 10 ml of a 2.0 ng/ml solution (introduction of ~20 ng). Recoveries of TNT at the 10 and 2.0 ng/ml levels, as described above, were 100.0±2.7% (n=6) and 95±3.4% (n=3), respectively. These quantitative recoveries indicate that breakthrough did not occur during preconcentration. Importantly, these studies also confirm complete elution of analyte from the polymer sorbent with mobile phase compositions compatible with chromatography on the octadecyl silica analytical column. The high precision of the analyses reflects elimination of variation typically introduced by manual sample manipulation. Next, a standard curve for TNT was constructed. This curve was linear in the range of standards tested (r=0.997 for injections ranging from 0.0243 to 1.22 μg).

Similar studies were conducted for RDX. Again it was found that the acetonitrile–water (40:60, v/v) mobile phase caused complete elution of the explosive from the resin polymer. Percent recovery from 10 ml at the 2.0 ng/ml level was 103±11% (n=4). These values again indicate quantitative trace enrichment of explosive from the sample. The higher relative standard deviation reflects the smaller molar absorptivity and hence lower signal generated at 254 nm for RDX compared to TNT. The standard curve for RDX was linear in the range from 0.0243 to 1.22 μg (r=0.983).

Sample dilutions were next run to determine the minimal detectable concentration of TNT that would produce a signal of 3 times the noise after preconcentration. Several studies were conducted where sample volumes of up to 50 ml were enriched. The chromatograms shown in Fig. 3 result from enrichment of 50 ml of a 0.040 ng/ml sample (bottom) and the corresponding water blank (top). The TNT signal in this case was approximately six times background noise. Comparison to a loop injection allowed calculation of a 92% recovery for this analysis. This high recovery indicates that near quantitative recoveries are maintained even when 50-ml sample volumes are preconcentrated. For analysis at low pg/ml levels (Fig. 3) it is evident that preconcentration of trace contaminants from the high-purity HPLC water becomes a significant factor and would likely inhibit dramatic reductions in detection limits. Water ob-

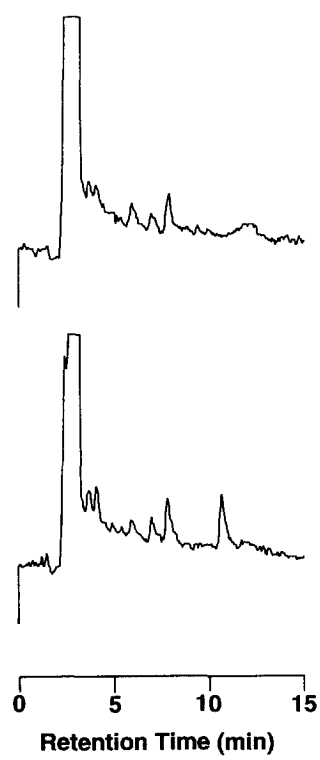

Fig. 3. Chromatograms of a 40 pg/ml TNT solution (bottom) and a corresponding water blank (top). Both chromatograms resulted from preconcentrating 50.0 ml of sample. TNT elutes with a retention time of 10.66 min in the bottom chromatogram. Detector sensitivity was 0.00085 absorbance units full scale.

tained from J.T. Baker produced the cleanest chromatographic blanks for this analysis of the three high-purity HPLC-grade water suppliers tested (including Fisher and Alltech).

The heterogeneous dual-column on-line system described here consists of a chromatographically compatible resin preconcentration stage combined with a silica-based reversed-phase separation stage. This approach allows several advantages over off-line sample preparation strategies. The principal advantages include lower detection limits achieved in a shorter analysis time. These features arise from the ability to quantitatively transfer all the sample to the analytical column for analysis, thereby permitting efficient sample use. In contrast, off-line methods are inherently wasteful of sample. The method of Bouvier and Oehrle, which is typical of off-line methods, processes 500 ml of sample into 5.0 ml of final extract [10]. An equivalent of only 40 μl of the 5.0-ml extract (0.8% of the sample) is used for analysis. On-line transfer of analyte permits either lower detection limits to be achieved or smaller sample volumes to be analyzed. For example, the on-line strategy described here allows roughly equivalent detection limits as the Bouvier and Oehrle method with a factor of 50 less sample. This approach allows collection of higher integrity data in shorter time periods with a corresponding increase in sample throughput. The procedure described by Bouvier and Oehrle takes approximately 100 min; 70 min is needed for sample preparation and an additional 30 min for chromatographic analysis [10]. The on-line procedure described here requires less than 30 min per sample. Additional advantages include higher recoveries and lower standard deviations that can be traced to the elimination of manual manipulation steps. The fact that over 300 standards and/or samples have been analyzed on the on-line system without noticeable change in the chromatographic performance or preconcentration efficiency testifies to the ruggedness of the analytical system. Finally, sample preparation and analysis are all conducted in a closed system that excludes light, a feature that is useful when dealing with low concentrations of photolabile explosives such as TNT.

The on-line system presented here was developed under isocratic conditions with an analytical run time of 15 min, thus limiting the range of separation. Several authors have described longer isocratic runs using conditions designed to separate a wide variety of explosives and munitions-related compounds [3,9]. These systems often utilize either binary or ternary mobile phases in combination with reversed-phase separation on alkyl or cyanopropyl silica columns [3,9]. It is possible that these separation systems may also be amenable to the on-line analysis approach described here. Future developments will explore these possibilities as well as implementation of gradient elution column development in combination with the on-line analysis scheme to extend the range of applicable analytes.

Analyses of aquifer samples were conducted to evaluate the on-line system for quantification of TNT and RDX in real sample matrices. Grab samples were collected in amber glass jars fitted with Teflon-lined caps from wells surrounding the Cornhusker Army Ammunition Plant near Grand Island, NE, USA. Samples were shipped on ice and analyzed immediately upon arrival. Fig. 4 presents chromatographic profiles of water obtained from different wells. All chromatograms represent preconcentration of 10-ml samples. Due to large quantities of material in sample C, this sample was diluted by a factor of 10 before trace enrichment. Previous analysis of water from these aquifer wells had indicated TNT and RDX contamination; therefore, a mobile phase that would elute both explosives (acetonitrile–water, 50:50, v/v) was used. The retention times of RDX and TNT under these conditions were 5.41 and 10.28 min, respectively. The on-line system allows introduction of standards (through the sample loop) to occur simultaneously with sample preconcentration. Chromatographic co-elution experiments can conveniently be performed in this manner. Identification of RDX and TNT in the aquifer samples was verified by co-injection experiments. Chromatographic co-elution was particularly useful in determining which of several closely eluting peaks corresponded to RDX in samples C and D in Fig. 4. The concentration of RDX ranged from below the detection limit (samples A and B) to 5.8 ng/ml in sample C. The RDX content of sample D was determined to be 1.7 ng/ml and was therefore below the 2.0 ng/ml EPA threshold limit. TNT was well above the threshold limit in all samples and ranged in concentration from 3.0 to 18.7 ng/ml in samples B and C, respectively.

All aquifer samples contained a large peak that

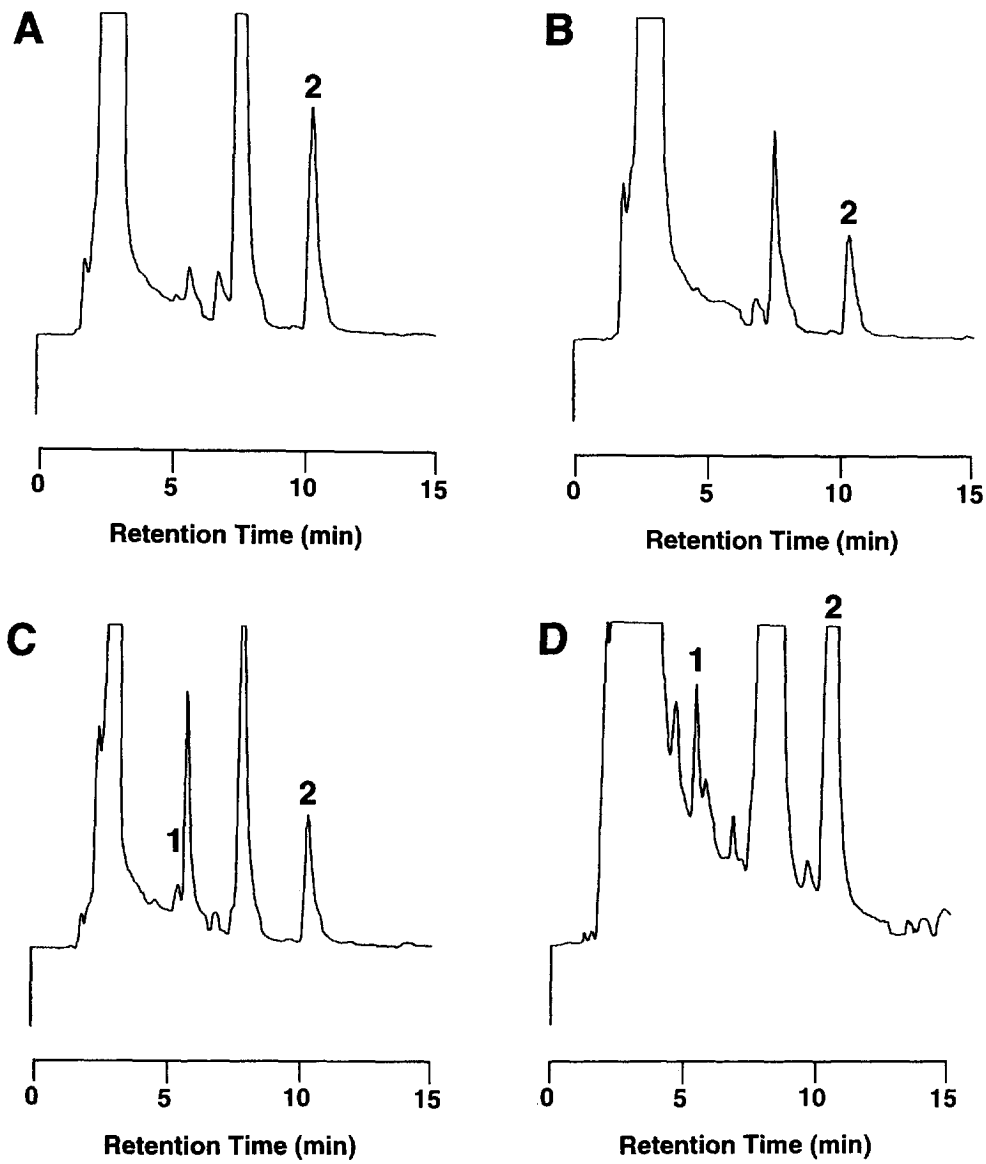

Fig. 4. Chromatograms of aquifer water samples collected in the vicinity of Cornhusker Army Ammunition Plant. Peaks labeled 1 and 2 are due to RDX and TNT, respectively. All chromatograms represent 10.0 ml of sample. Due to unusually high concentrations of munitions, sample C was diluted a factor of 10 before preconcentration. Detector sensitivity for these chromatographic runs was 0.0069 absorbance units full scale.

eluted between RDX and TNT (retention time of 7.79 min). Initial chromatographic co-injection studies indicated a retention time for the unknown that was coincident with 4-amino-2,6-dinitrotoluene, a known environmental transformation product of TNT [15,16]. It is known from previous studies that 2-amino-4,6-dinitrotoluene and 4-amino-2,6-dinitrotoluene co-elute under the HPLC conditions utilized. Identification was further pursued by off-line SPE extraction with subsequent GC–MS analysis. These studies indicated that the aminodinitrotoluene isomers were present as the principal sample constituents with 2-amino-4,6-dinitrotoluene accounting for 62.5% of the combined isomer concentration.

Additional HPLC studies demonstrated a total aminodinitrotoluene concentration of approximately 52 ng/ml for the water profiled in Fig. 4C.

4. Conclusions

An on-line trace enrichment system is described that allows rapid analysis of nitro munitions in the ng/ml to low pg/ml concentration range. The system features preconcentration on a trace enrichment column packed with divinylbenzene–vinylpyrrolidone co-polymer resin followed by complete transfer of analyte to a reversed-phase chromatographic column. Detection of the components was performed by UV absorption. The chromatographic compatibility of the resin with the separation stage was demonstrated by high recoveries of RDX and TNT. Detection limits determined for TNT were roughly 0.10 ng/ml for 10 ml of sample. A conservative detection limit of 20 pg/ml was demonstrated for TNT by preconcentrating 50 ml of sample. To our knowledge this is the lowest detection limit that has been demonstrated for the analysis of TNT by HPLC. Further drastic reductions in detection limit are unlikely with UV detection due to the increasing prominence of matrix interferences with larger sample volumes. Further reductions in detection limits could, however, proceed by implementing selective detection methods such as mass spectrometry.

The on-line system was applied to the analysis of contaminated aquifer water from the Cornhusker Army Ammunition Plant in Nebraska. Analysis confirmed the presence of trace quantities of TNT and, in some samples, of RDX. Additionally, a major peak in the HPLC profiles was identified by GC–MS studies as a combination of the aminodinitrotoluene isomers.

The on-line system offers several advantages over off-line methods. Efficient sample use allows lower detection limits or the analysis of smaller volumes of sample. Generally, analysis is faster and, because sample is not lost through manual manipulations, recoveries are higher and more precise. Analysis of a 10-ml sample can be completed in less than 30 min. Further reductions in analysis time may be possible by loading sample at higher flow rates or by substituting short analytical columns packed with 3-μm particles. Success of the latter approach depends on either providing a sufficiently small injection volume to the analytical column or choosing chromatographic conditions such that preconcentration of components occurs on the head of the analytical column. Future studies will focus on automation of the on-line system and implementation of gradient elution techniques.

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