METHODS OF ANALYSIS BY THE U.S. GEOLOGICAL SURVEY NATIONAL WATER QUALITY LABORATORY--EXTRACTION OF NITROAROMATIC COMPOUNDS FROM WATER BY POLYSTYRENE DIVINYLBENZENE CARTRIDGE AND DETERMINATION BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

By Chris E. Lindley, Mark R. Burkhardt, and Sabrina N. DeRusseau

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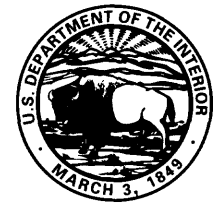

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CONVERSION FACTORS, ABBREVIATED WATER-QUALITY UNITS, AND OTHER ABBREVIATIONS

Multiply	By	To obtain
centimeter (cm)	3.94×10^{-1}	inch
gram (g)	3.53×10^{-2}	ounce
liter (L)	0.2642	U.S. gallon
milliliter (mL)	2.64×10^{-4}	U.S. gallon
milliliter per minute (mL/min)	3.38×10^{-2}	ounce per minute
micrometer (µm)	3.94×10^{-5}	inch
milligram (mg)	3.53×10^{-5}	ounce
millimeter (mm)	3.94×10^{-2}	inch

Degree Celsius (^{o}C) may be converted to degree Fahrenheit (^{o}F) by using the following equation:

$$^{o}F = 9/5(^{o}C) + 32.$$

The following terms and abbreviations also are used in this report:

HPLC	high-performance liquid chromatography
MDL	method detection limit
µg/L	microgram per liter
mg/L	milligram per liter
nm	nanometer
N-m	newton-meter
No.	number
NWQL	National Water Quality Laboratory
ODS	octadecylsilane
PSDVB	polystyrene divinylbenzene
SPE	solid-phase extraction
USATHAMA	U.S. Army Toxic and Hazardous Materials Agency
USEPA	U.S. Environmental Protection Agency
UV	ultraviolet

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ABSTRACT

Organic explosives are determined in samples of ground water and surface water with emphasis on identifying and quantifying trinitrotoluene (TNT) metabolites. Water samples are filtered to remove suspended particulate material and passed through a polystyrene divinylbenzene-packed cartridge by a vacuum-extraction system. The target analytes subsequently are eluted with acetonitrile. A high-performance liquid chromatograph (HPLC) equipped with a photodiode-array detector is used for sample analysis. Analytes are separated on an octadecylsilane column using a methanol, water, and acetonitrile gradient elution. The compounds 2,4- and 2,6-dinitrotoluene are separated through an independent, isocratic elution. Method detection limits, on the basis of a 1-liter sample size, range from 0.11 to 0.32 microgram per liter (µg/L). Recoveries averaged from 71 to 101 percent for 13 analytes in one set of HPLC-grade water fortified at about 1 µg/L. The method is limited to use by analysts experienced in handling explosive materials.

INTRODUCTION

Different techniques for the extraction of nitroaromatic compounds from water samples have been described, the most common being Porapak R extraction in Methods UW14 and UW32 (U.S. Army Toxic and Hazardous Materials Agency, written commun., 1990, 1991), a "salting-out" liquid-liquid extraction in U.S. Environmental Protection Agency (USEPA) Method 8330 (Miyares and Jenkins, 1991; U.S. Environmental Protection Agency, 1992), and extraction using Empore polystyrene divinylbenzene (PSDVB) discs (Le Brun and others, 1993; R.T. Medary, U.S. Army Corps of Engineers, written commun., 1992). The quantity of water sample extracted by these methods is often much less than 1 L, thereby reducing the total amount of analyte recovered. These methods also might require further concentration of the extract, risking loss of volatile analytes through Kuderna-Danish concentration (Miyares and Jenkins, 1991, p. 7) or other techniques. The method described in this report uses 0.25-g PSDVB-packed cartridges to extract 1 L of water and does not require a postextraction concentration step.

PSDVB has been used to extract nitroaromatic compounds by several researchers (Junk, Richard and others, 1974; Anspach and others, 1982; Maskarinec and others, 1984; Richard and Junk, 1986; M.G. Winslow, Environmental Science and Engineering, Inc., written commun., 1991). Recovery of the compounds has been described as "excellent" (Jenkins and others, 1992, p. 3), though interferences from contamination within the polymeric resin have been noted, both when the resin was not properly prepared, and when difficult sample matrices (for example, acidic samples or samples that had high ionic strength) caused contaminants from the PSDVB material to be released. This method relies on recently available cartridges containing un-derivatized PSDVB. The cartridges are commercially prepared and are intended for environmental analysis.

Modifications to U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) Method UW14 for determination of explosives in water by high-performance liquid chromatography (HPLC) were used by the National Water Quality Laboratory (NWQL) from 1991-93 as a special method (LC 8202) for ground-water analysis (Schumacher and others, 1992). The NWQL method used C-18 solid-phase extraction (SPE) cartridges and recovered in excess of 80 percent of most analytes, but 1,3,5-trinitrobenzene (TNB) and 3,5-dinitroaniline (3,5-DNA) recoveries ranged from 40 to 65 percent, and nitramine recoveries generally were less than 10 percent (see table 1 for analyte abbreviations). The poorly recovered analytes had low octanol/water partition coefficients and would not be expected to be effectively retained on a C-18 cartridge (T.F. Jenkins, U.S. Army Corps of Engineers, oral commun., 1992). The USATHAMA Method UW14 does not account for possible interferences caused by the 2,4,6-trinitrotoluene (TNT) degradation products 2-amino-4,6-dinitrotoluene (2-Am) and 4-amino-2,6-dinitrotoluene (4-Am), which may interfere with the TNT peak, preventing successful quantitation.

Table 1. -- *List of compounds, abbreviations, and Chemical Abstracts Service registry numbers*
[CAS, Chemical Abstracts Service]

Compound	Abbrievation	CAS Number
Octahydro-1,3,5,7-tetranitro 1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro 1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	TNB	99-35-4
3,5-Dinitroaniline	3,5-DNA	618-87-1
m-Dinitrobenzene[1]	DNB	99-65-0
Nitrobenzene	NB	98-95-3
2-Amino-4,6-dinitrotoluene	2-Am	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am	1946-51-0
2,6-Dinitrotoluene	2,6-DNT	06-20-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,4,6-Trinitrotoluene	TNT	118-96-7
2-Nitrotoluene	2-NT	88-72-2
4-Nitrotoluene	4-NT	99-99-0
3-Nitrotoluene	3-NT	99-08-1

[1]The compound *m*-DNB coelutes with 3,5-DNA. See Introduction for more information.

The analytical procedure described in USATHAMA Method UW32 provides for the determination of 2-Am and 4-Am, and includes 3,4-dinitrotoluene (3,4-DNT) as a surrogate, but the common degradation product 3,5-DNA is not included. NWQL Method LC 8202 permitted separation of TNT, 2-Am, 4-Am, and 3,5-DNA by a gradient-elution profile. The gradient elution is particularly useful for the separation of unknown (degradation) products in complex samples. The disadvantage of the gradient profile is that 2,4-DNT and 2,6-DNT compounds coelute. Although the NWQL has used a single isocratic method to separate the DNT isomers and all other analytes, the method was not rugged enough for routine work. Separation of the DNT isomers is routinely achieved by the isocratic USATHAMA UW14 chromatographic conditions.

TNT can degrade into a wide range of products. For example, TNB is formed when TNT loses the methyl group through a photocatalytic degradation process. TNB may then transform through biological reduction to the compound 3,5-DNA, which is formed when one of the NO_2 groups on TNB is converted to the amine. TNT can degrade into the amino dinitrotoluenes (2-Am and 4-Am) through a hydroxylamine intermediate by a comparable process. Subsequent degradation of these amino DNTs can yield *diamino* nitrotoluene isomers. Several of these common TNT degradation products are identified and quantified in this method. Other classes of TNT degradation products also may be observed: While 2-nitrotoluene (2-NT) and 4-NT are the major isomers produced in the nitration of toluene (58 and 38 percent, respectively), about 4 percent of the nitrotoluenes appear as the 3-NT isomer. In TNT production, this *meta*-nitrotoluene isomer typically was not removed in the subsequent nitration processes (Davis, 1943, p. 143), leading to the production of TNT isomers (for example, 2,4,5-TNT). Mass spectrometry of samples from the abandoned Weldon Spring Ordnance Works, Missouri, have shown the presence of structural isomers of amino dinitrotoluenes that are believed to result from the degradation of TNT isomers. A similar situation may exist for the dinitrotoluenes.

The compound *m*-dinitrobenzene (DNB) coelutes with 3,5-DNA and is not as commonly observed as 3,5-DNA in samples received by the NWQL. Because of this difficulty, DNB was not included in the method detection limit (MDL) study or in work for the laboratory validation of this method. DNB may be distinguished from 3,5-DNA in a chromatogram by comparing the observed spectrum to a library of standard spectra (for an example of a library match, see fig. 2 later in this report).

This report describes a method for determining nitroaromatic compounds developed for use by the U.S. Geological Survey in the Survey's National Water Quality Laboratory. The method incorporates PSDVB-packed cartridges for removal of organic explosives from water samples and an HPLC equipped with a photodiode-array detector for identification and quantification of the analytes. The method supplements other methods of the U.S. Geological Survey for determination of organic substances in water that are described by Wershaw and others (1987). The method was implemented in the National Water Quality Laboratory in January 1994.

This report provides a detailed description of all aspects of the method from sampling protocol through calculation and reporting of results. Accuracy and precision data, and method detection limits for nine explosive compounds and four TNT metabolites are presented.

ANALYTICAL METHOD

Parameter and Code: Nitroaromatic compounds, dissolved, high-performance liquid chromatography, O-1124-94

1. Scope and application

This method is intended for the determination of explosive ingredients and selected degradation products in environmental water samples. The method has been used successfully for ground-water, surface-water, and lysimeter samples, and is expected to be applicable to most environmental water matrices [soil concentrations of explosive materials also have been measured using the soil extraction procedure described in USEPA Method 8330 (U.S. Environmental Protection Agency, 1992) in conjunction with the HPLC analytical conditions of this method]. The MDLs for individual analytes (table 1) were determined by multiplying the standard deviation from the results of seven replicates by the Student's *t* -value for a 99 percent confidence level, as given in the USEPA procedure for determination of the method detection limit (U.S. Environmental Protection Agency, 1990).

The maximum concentration of constituents in samples reliably analyzed by this method has not been determined. Sample concentrations that exceed the retention capacity of the SPE cartridge would effectively attenuate the method dynamic range. This method, however, is intended for low-level (<1,000 µg/L) concentrations of nitroaromatic compounds.

Filtration of the sample takes approximately 15 minutes, but high particulate levels in the sample can increase filtration times. Conditioning of the SPE cartridge takes approximately 5 minutes. An extraction rate of less than 40 mL/min is recommended for optimal analyte recovery. Sample volumes of 1 L are extracted in about 30 minutes. Several cartridges may be extracted at the same time. The duration of the HPLC analysis is 33 minutes.

The recommended time for extraction of submitted samples is within 7 days from the time of collection. No chemical preservative is used, but the sample must be chilled from the time of collection to the time of extraction. Sample extracts need to be analyzed within 21 days. Any remaining sample is kept for 60 days from the date of sample receipt.

2. Summary of method

2.1 A sample volume of 1 L is recommended for low-level (< 1,000 µg/L) determinations. Smaller volumes can be used, with a consequent increase in the detection limit. All samples need to be filtered to remove suspended particulate material. Glass-fiber filters with a nominal 0.7-µm pore diameter are required. Samples are submitted in 1-L amber-glass pesticide bottles sealed with a Teflon-lined cap.

2.2 Sample extracts are prepared by conditioning an SPE cartridge with successive rinses of two cartridge volumes of acetonitrile and one of reagent-grade water. The sample is poured through the reservoir onto the cartridge. After the sample has passed through, any remaining sample water is expelled from the cartridge. The nitroaromatic components are desorbed from the cartridge with 2.0 mL of acetonitrile immediately after sample extraction to reduce loss of the more volatile nitroaromatic compounds (nitrotoluenes, nitrobenzene) from the cartridge through evaporation.

2.3 The extract is brought to 4.0 mL with reagent-grade water. Approximately 2 mL of the extract is placed into an HPLC vial, capped, and saved for analysis. The remaining extract is not used.

2.4 Extracts are analyzed using an HPLC equipped for reverse-phase chromatography. Analytes are detected by ultraviolet (UV) photodiode array.

3. Interferences

3.1 Polystyrene divinylbenzene material has been shown to contain uncrosslinked prepolymer that can interfere with analyte integration (U.S. Army Toxic and Hazardous Materials Agency, written commun., 1991, p. 5; Jenkins and others, 1992, p. 2). Thorough cleaning of polystyrene divinylbenzene is essential in USATHAMA Method UW32 and in other methods that use the bulk material.

3.2 Compounds that demonstrate an affinity for the solid adsorbent of the cartridge also may cause interferences with the analytes of interest. These may include organic compounds having nonpolar, hydrophobic character that are introduced from cartridge conditioning or eluting solvents, or any naturally occurring or introduced organic compounds already present in the water sample.

4. Apparatus and equipment

4.1 The apparatus and equipment required for this method are listed as follows; specific sources and models used during the development of the method also are listed, where applicable.

4.1.1 Volumetric flasks -- 25- and 50-mL volumetric flasks.

4.1.2 Cartridge reservoirs -- 75-mL or larger cartridge reservoirs; J.T. Baker, Inc., or equivalent.

4.1.3 Cartridge adapters -- Varian, or equivalent.

4.1.4 Vacuum manifold -- Varian VacElut system, or equivalent.

4.1.5 Micropipettes -- 50-µL fixed volume and 20- to 100-µL variable volume; Eppendorf, Brinkmann Instruments, Inc., or equivalent.

4.1.6 Analytical column -- Ultrasphere octadecylsilane (ODS), 5 µm (4.6 mm x 25 cm); Beckman Instruments, Inc., or equivalent.

4.1.7 Data system -- Hewlett-Packard (Pascal) Chemstation, or equivalent.

4.1.8 Liquid chromatograph -- A Hewlett-Packard Model 1090 HPLC equipped with a 250-µL injection loop and photodiode-array detector with a 6-mm fixed-pathlength UV flow cell, or equivalent.

4.1.8.1 HPLC conditions: The method uses a linear gradient elution that begins with acetonitrile:water:methanol (15:80:05) and changes to a (47:48:05) ratio over 28 minutes. The flow rate is 1.0 mL/min. The injection volume is 100 µL. Maintain the column temperature at 40°C. Retention time of TNT is about 25.4 minutes. A typical chromatogram of an extracted sample is shown in figure 1. A separate, isocratic elution of 15:45:40 acetonitrile:water:methanol is used to separate the DNT isomers. Both methods use detection of components by photodiode array, and quantitation is based on the chromatogram generated by the detector signal at 250 nm.

5. Reagents and consumable materials

5.1 SPE cartridges -- 6 mL, 0.25 g ENVI-Chrom P (styrene divinylbenzene); Supelco, or equivalent.

5.2 Glass-fiber filters (142-, 47-, or 25-mm diameter), nominal 0.7-µm pore diameter; Baxter Scientific Products, or equivalent.

5.3 Solvents -- Methanol, water, and acetonitrile; B&J Brand, HPLC grade, or equivalent.

5.4 Disposable plastic pipet tips, 100 and 1,000 µL; Eppendorf.

5.5 RP-18 guard column; Applied Biosystems, or equivalent.

5.6 HPLC vials -- 2-mL sample vials; Hewlett-Packard, or equivalent.

5.7 Vial caps -- Aluminum Teflon-lined crimp-top vial caps; Hewlett-Packard, or equivalent.

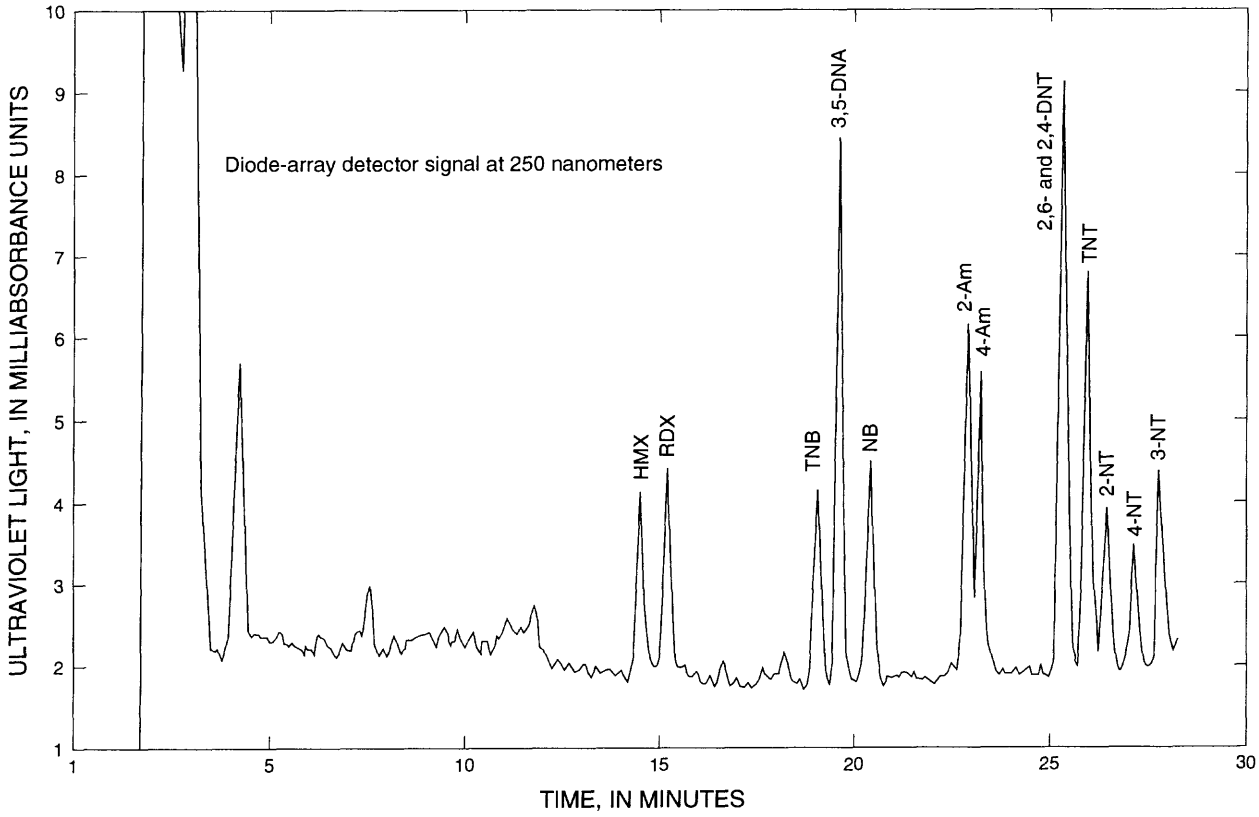

Figure 1.--High-performance liquid chromatogram of extracted sample fortified at 1 microgram per liter. (See table 1 for compound abbreviations.)

5.8 Solvent filters -- Nylon membrane 47-mm solvent filters, 0.2-µm pore size; Gelman Sciences, or equivalent.

5.9 Sample containers -- 1-L, amber-glass bottles fitted with Teflon-lined screw caps.

5.10 Stock standard solutions: Prepare stock standard solutions by weighing 10 mg of the analyte to the nearest 0.01 mg. Place in a 10-mL volumetric flask, and dilute to volume with acetonitrile.

NOTE 1. HMX, RDX, TNB, TNT, 2-Am, and 4-Am standard materials were acquired for the initial custom analysis as an interagency cooperative effort with the Indian Head Naval Weapons Center, Maryland. All other standard materials were purchased from commercial vendors.

5.11 Working standard solutions: Obtain a 1:500 dilution of the stock standard solutions by combining a 50-µL aliquot from each stock standard into a 25-mL volumetric flask and add 12.5 mL of water at 20°C. Bring the flask *close* to the final volume with acetonitrile.

Sonicate the flask for 20 minutes to remove any dissolved gases, and bring to final volume with acetonitrile (solvent ratio is 50:50 acetonitrile:water). The concentration of the working standard solution is 2,000 µg/L. Store working standard solutions in a dark freezer at -15°C and keep for up to 60 days after the date of preparation.

5.12 Perform serial dilutions for concentrations of 50, 200, and 1,000 µg/L in 50:50 acetonitrile:water.

6. Sampling

Collect water samples that represent the character of the surface or ground water at a given time and location. Specific sample collection procedures are described in U.S. Geological Survey publications by Edwards and Glysson (1988) and Hardy and others (1989). Use sample-collection equipment that is free of plastic parts and oils that may contaminate water samples or sorb the nitroaromatic components from the water. Wash all sample-collection equipment with an appropriate detergent, and rinse the equipment with water to remove all traces of detergent; finally, rinse with methanol (contained in a glass or Teflon bottle). Clean all equipment before each sample is taken.

7. Instrument performance evaluation

7.1 HPLC performance is typically indicated by peak shape, peak retention time, peak resolution, and deviations of analyte response observed in the graphical representation of continuing calibration standard data. If the chromatography deteriorates, the HPLC solvents, guard column, analytical column, pump system, or injection apparatus might be at fault. An aging detector lamp (or other factors) might cause sudden changes in analyte response factors or chromatography.

7.2 Check instrument performance by injecting a midlevel (for example, 200 µg/L) working standard. If any analyte deviates from the reported concentration by more than ±15 percent, the standard curve must be regenerated.

8. Calibration and quality control

8.1 Obtain initial calibration data by using a new analytical column and freshly prepared calibration solutions. Use these data to compare subsequent HPLC performance.

8.2 Calibrate the instrument by injecting working standard solutions of 2,000, 1,000, 200, and 50 µg/L. Analyze a midlevel (200 µg/L) continuing calibration standard solution at the beginning of a sample set to evaluate the calibration. If any analyte differs from the reported concentration by more than ±15 percent, regenerate the standard curve.

8.3 Analyze quality-control samples as part of every sample batch to demonstrate that the method is performing as intended. The quality-control samples will constitute a minimum of 10 percent of the sample batch for each of the following types of checks.

8.3.1 Laboratory blanks are prepared by extracting 1 L of water and analyzing in accordance with method procedures. The purpose of the blank sample is to determine whether any artifacts are being introduced into the sample from materials or reagents used in sample preparation that would interfere with the determination of the analytes. Eliminate any interferences that are detected before samples are analyzed.

8.3.2 Reagent blanks consist of 50:50 water:acetonitrile. Analyze after a continuing calibration standard solution to ensure that interferences caused by instrumental aberrations are not present and there is no carryover from the standard injection.

8.3.3 Spiked samples are a known amount of the working standard solution that is added to 1 L of water. The spiked sample is intended to demonstrate the precision and accuracy of the entire sample preparation procedure. Prepare and analyze the spiked sample exactly as the environmental sample. Recovery of the spiked sample needs to be within method warning limits as determined by data from the 45 most recently analyzed spiked samples.

8.3.4 Duplicate samples indicate the reproducibility of the method. Analyze at least 10 percent of samples in duplicate (when duplicate samples are available). Prepare and analyze the duplicate sample as described in Section 9. Results from a duplicate sample analysis must agree within ±15 percent of the original results. If duplicate results fall outside of this acceptance criteria, determine the cause and re-extract the sample, if the sample volume is sufficient.

8.3.5 Continuing calibration standards will be analyzed once every 10 samples in the analytical batch. They serve to demonstrate the consistency of the instrument for the duration of the analytical run. If the concentration of any analyte in the check standard falls outside of ±15 percent of the actual value for that analyte, determine the cause for the deviation and reanalyze.

8.3.6 Matrix spikes are replicate environmental samples fortified with a known amount of analyte. They are prepared by the regular sample preparation procedure. The matrix spike determines how well the analyte is recovered from the sample matrix. Fortification amounts should range from 25 to 100 percent of the mean concentration of the analytes found in the original sample, if possible. Results from the matrix spike should be within ±30 percent of the original concentrations and added material.

9. Procedure

9.1 Weigh the bottle containing the sample to the nearest gram. Attach the solid-phase cartridge(s) to the vacuum manifold. Pour two successive cartridge volumes of acetonitrile through each cartridge. (Note: *The cartridge must not be permitted to become dry once the conditioning process has started.*) Allow the acetonitrile meniscus to drain to the (upper) cartridge frit by gravity. Pour one cartridge volume (about 6 mL) of water through the cartridge. After the water has reached the frit, pour the sample water into the cartridge until the cartridge is three-fourths full. Attach a clean 75-mL reservoir to the top of the cartridge using the cartridge adaptor. Pour the sample water into the reservoir until full and adjust the vacuum to 15 cm Hg. Use low manifold vacuums (10-20 cm Hg) to ensure low-flow rates (<25 mL/min) and high analyte recovery. Continue refilling the reservoir with the sample water until all of the water has passed through the cartridge. Turn off the vacuum. Reweigh the bottle that contained the sample to determine the sample volume extracted. Clean the reservoir and adaptor with ample amounts of water and rinse with methanol.

9.2 Blow (ambient) air through the cartridge to remove excess sample water from the solid-phase sorbent (the excess water dilutes the eluting solution, resulting in lower recovery). Immediately desorb the nitroaromatic components from the SPE cartridge after the sample extraction to ensure optimum recovery of the more volatile nitroaromatic compounds (nitrotoluenes, nitrobenzene). Fix the cartridge above a graduated tube, and place 2.0 mL acetonitrile onto the cartridge. The acetonitrile elution might have to be started by placing a rubber pipet bulb over the opening of the cartridge and applying air pressure. After elution is complete, use the bulb to blow any remaining liquid from the cartridge. Bring the extract to 4.0 mL volume with water. Place approximately 2 mL of the extract into the HPLC vial, cap, and save for analysis.

9.3 The analyte concentrations in some samples may be great enough (greater than 100 µg/L) to allow determination by direct injection: Add a 3-mL aliquot of the water sample to an equal volume of acetonitrile. Mix the solutions and filter through a 0.45-µm syringe filter. Place the sample in a vial, cap, and save for analysis. If the direct injection technique is used, however, the MDL achieved through the solid-phase extraction will not be applicable.

10. Calculation and reporting of results

10.1 Prior to HPLC analysis of environmental samples, ensure that the performance criteria specified in paragraph 7.2 have been met.

10.2 For positive confirmation of an analyte, the retention time of the analyte peak must be within 1 percent of the relative retention time of the target compound. In addition, comparison of a peak's spectrum to a spectral library assists in identification: An analyte can be considered to be positively identified when its retention time and UV spectrum match those of the standard material. An example of a match between a library spectrum and the spectrum of a target compound is shown in figure 2.

Confirmation of tentatively identified analytes having concentrations five times the MDL or greater requires a library match in excess of 990 (on the Hewlett-Packard Chemstation). Confirmation of those analytes that have concentrations less than five times the MDL and that have a poor library match are conservatively tagged as "not spectrally confirmed," though the experience of the analyst should weigh heavily in the interpretation.

10.3 Base quantitation on the chromatogram generated by the detector signal at 250 nm. Integrate using optimized integration parameters loaded into an automated postacquisition routine (such as that on the Hewlett-Packard Chemstation). Check computer-generated results for applicability.

10.4 Compound concentrations are calculated as follows:

$$\text{Compound concentration} = \frac{M}{(Vs \,/\, Ve)}$$

where M = compound concentration in extract, in micrograms per liter;
Vs = sample volume, in milliliters; and
Ve = extract volume, in milliliters.

Example Calculation: If a 1,024-mL sample was extracted to a final volume of 4.0 mL, and the concentration of the extract was 92.3 µg/L, the compound concentration reported would be 0.36 µg/L.

10.5 Report concentrations of nitroaromatic compounds, dissolved, as follows: Less than 1.0 µg/L, two significant figures (for example, 0.321 will round to 0.32); 1.0 µg/L and greater, three significant figures. Indicate values that are not spectrally confirmed and that are greater than the MDL.

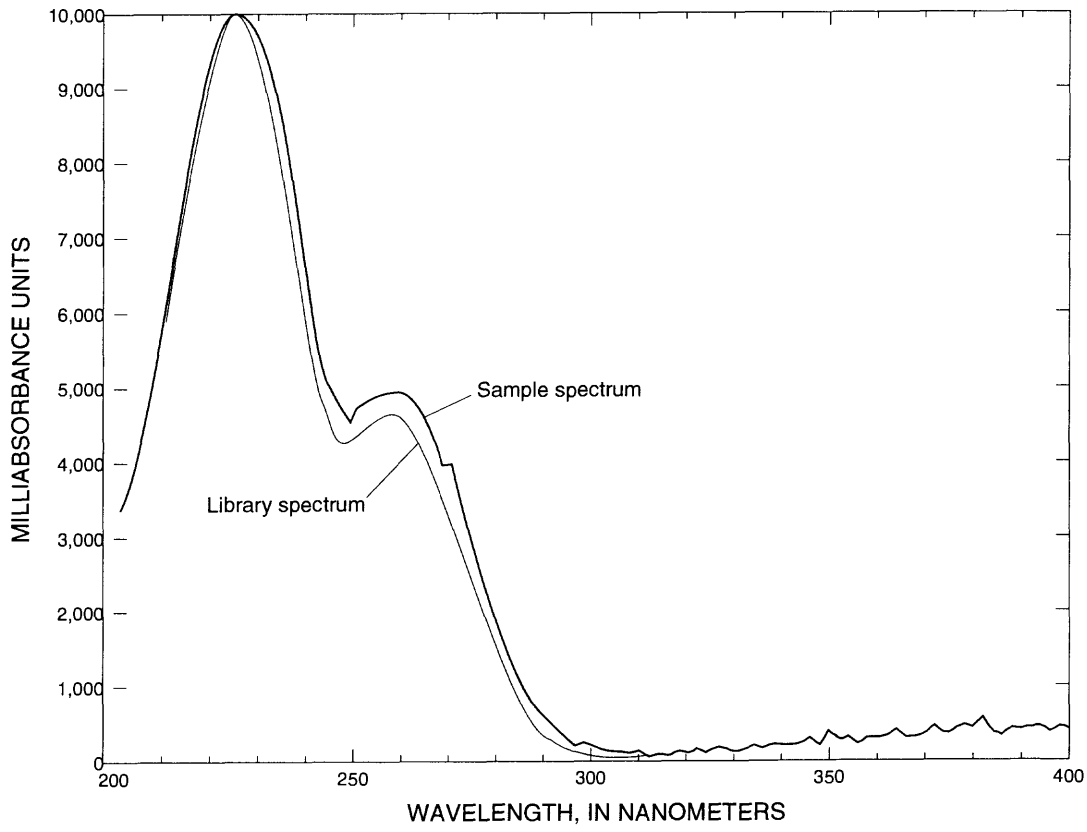

Figure 2.--Example of a spectral match of 3,5-dinitroaniline library ultraviolet spectrum.

11. Safety issues

11.1 The methanol and acetonitrile used for the HPLC mobile phase are both flammable and toxic. Care must be taken to avoid inhalation and contact with these solvents. The DNT isomers are considered to be extremely carcinogenic. In addition to the hazards involved when working with an HPLC (for example, mobile phase toxicity/flammability and analyte toxicity), there are special safety concerns when handling explosive materials.

11.2 While most explosive materials of the type used in this method are relatively safe in solution, extra caution is required when manipulating the solid standard material. In climates with low relative humidity, electrostatic discharge is a potential hazard; persons should take care to ground themselves before handling these materials. Metal spatulas should not be used in weighing (dry wooden tongue depressors are recommended). When working with an explosive, keep other explosive materials out of the vicinity. The materials should not be dropped, tumbled, or slid along surfaces. TNT has an impact sensitivity of 15 N-m, but still can be detonated if confined between moving metal surfaces. TNB has an intermediate impact sensitivity of 7.4 N-m, nearly the same as RDX. **CAUTION:** *The method is limited to use by analysts experienced in handling explosive materials.*

METHOD PERFORMANCE

Sets of reagent-water samples, surface-water samples collected from Clear Creek at Prospect Park in Wheat Ridge, Colorado, and ground-water samples collected in Jefferson County, Colorado (Arvada Well No. 14), were used to test method performance. Each of the three water matrices was split into two subsamples. At least seven aliquots of approximately 1 L from each subsample were individually fortified at about 1 µg/L, and the other subsample at about 100 µg/L. The samples were analyzed by two analysts in one laboratory (USGS National Water Quality Laboratory), using one HPLC instrument. Accuracy and precision data from the analyses are presented in tables 2 through 7.

The MDL was calculated for each set of aliquots for each analyte using the USEPA procedure for the determination of method detection limits (U.S. Environmental Protection Agency, 1990). The MDLs calculated for the reagent-water samples fortified at about 1 µg/L range from 0.11 to 0.32 µg/L and average 0.19 µg/L (table 2). The MDLs for surface-water samples fortified at about 1 µg/L range from 0.08 to 0.70 µg/L and average 0.24 µg/L (table 4). The MDLs for ground-water samples fortified at about 1 µg/L range from 0.16 to 0.51 µg/L and average 0.30 µg/L.

Table 2.--Accuracy and precision data from seven determinations of the method analytes at about 1 microgram per liter in reagent water

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)	Method detection limit (µg/L)
HMX	1.02	1.04	0.041	4	101	0.12
RDX	.99	.93	.043	5	94	.14
TNB	.97	.88	.055	6	91	.19
3,5-DNA	.71	.65	.067	10	91	.32
NB	1.00	.90	.052	6	90	.18
2-Am	.96	.97	.041	4	101	.13
4-Am	.97	.69	.056	8	71	.26
2,6-DNT	1.35	1.02	.069	7	75	.21
2,4-DNT	.99	.76	.043	6	77	.18
TNT	1.27	1.19	.042	4	94	.11
2-NT	1.15	.93	.070	8	81	.24
4-NT	1.05	.88	.081	9	84	.29
3-NT	1.45	1.22	.051	4	84	.13
Mean		.93	.055	6	87	.19

Table 3.--Accuracy and precision data from seven determinations of the method analytes at about 100 micrograms per liter in reagent water

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)
HMX	102	92.5	3.90	4	91
RDX	98.9	89.6	3.60	4	91
TNB	97.0	88.0	3.40	4	91
3,5-DNA	71.1	66.3	5.70	9	93
NB	100	91.4	4.10	5	91
2-Am	96.3	89.3	6.20	7	93
4-Am	97.4	85.5	16.70	20	88
2,6-DNT	135	135	7.20	5	100
2,4-DNT	98.9	79.6	4.60	6	80
TNT	127	118	5.40	5	93
2-NT	115	101	5.30	5	88
4-NT	105	93.9	4.80	5	89
3-NT	145	128	6.70	5	88
Mean		96.8	5.97	6	90

Table 4.--Accuracy and precision data from seven determinations of the method analytes at about 1 microgram per liter in surface water (Clear Creek)

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)	Method detection limit (µg/L)
HMX	1.03	0.96	0.214	22	94	0.70
RDX	.99	.94	.085	9	95	.29
TNB	.97	.90	.125	14	92	.44
3,5-DNA	.71	.69	.039	6	98	.18
NB	1.00	.92	.055	6	92	.19
2-Am	.96	.91	.046	5	95	.16
4-Am	.97	.96	.030	3	98	.10
2,6-DNT	1.35	1.56	.041	3	115	.08
2,4-DNT	.98	.86	.044	5	87	.16
TNT	1.27	1.17	.072	6	92	.19
2-NT	1.15	.77	.054	7	67	.22
4-NT	1.05	1.02	.056	5	97	.17
3-NT	1.45	1.40	.086	6	97	.19
Mean		1.00	.073	7	94	.24

Table 5.--Accuracy and precision data from seven determinations of the method analytes at about 100 micrograms per liter in surface water (Clear Creek)

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)
HMX	103	92.0	1.18	1	90
RDX	98.9	90.8	1.36	1	92
TNB	97.0	71.1	11.73	16	73
3,5-DNA	71.1	77.3	13.64	18	109
NB	100	76.1	12.76	17	76
2-Am	96.3	87.6	4.42	5	91
4-Am	97.4	90.0	.65	1	92
2,6-DNT	135	144	4.33	3	107
2,4-DNT	99.2	79.6	4.60	6	80
TNT	127	97.1	7.40	8	77
2-NT	115	98.9	4.78	5	86
4-NT	105	91.6	2.21	2	87
3-NT	145	123	3.40	3	84
Mean		93.7	5.36	6	88

Table 6.--Accuracy and precision data from seven determinations of the method analytes at about 1 microgram per liter in ground water (Arvada Well 14)

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)	Method detection limit (µg/L)
HMX	1.03	1.04	0.061	6	101	0.18
RDX	.99	.90	.093	10	91	.32
TNB	1.20	1.07	.070	7	89	.21
3,5-DNA	.71	.67	.048	7	95	.23
NB	1.00	.90	.081	9	91	.28
2-Am	1.03	.93	.130	14	91	.44
4-Am	1.14	.95	.118	12	83	.39
2,6-DNT	.99	.95	.077	8	96	.25
2,4-DNT	.93	.87	.104	12	93	.38
TNT	1.17	1.10	.070	6	95	.20
2-NT	1.08	.73	.119	16	69	.51
4-NT	.99	.90	.082	9	91	.29
1.535	1.54	1.34	.070	5	88	.16
Mean		.95	.086	9	90	.30

Table 7.--Accuracy and precision data from seven determinations of the method analytes at about 100 micrograms per liter in ground water (Arvada Well 14)

[µg/L, micrograms per liter. Abbreviated compounds in column 1 are defined in table1]

Compound	Fortified concentration (µg/L)	Mean observed concentration (µg/L)	Standard deviation (µg/L)	Relative standard deviation (percent)	Mean accuracy (percent of true concentration)
HMX	103	86.6	3.36	4	84
RDX	101	84.2	3.29	4	84
TNB	120	107	8.12	8	89
3,5-DNA	71.1	64.0	5.95	9	90
NB	100	87.7	7.31	8	88
2-Am	103	93.5	6.50	7	91
4-Am	114	107	8.44	8	94
2,6-DNT	99.2	82.9	6.45	8	84
2,4-DNT	93.4	84.5	7.77	9	90
TNT	117	106	9.01	8	91
2-NT	108	89.0	8.42	9	83
4-NT	98.9	82.6	7.94	10	84
3-NT	154	125	12.00	10	82
Mean		92.4	7.27	8	87

The low percent recovery (mean accuracy) for 4-Am in samples of reagent water was probably a result of some unknown chemical activity of the reagent-grade water: The concentration of 4-Am in spiked reagent-grade water would decrease with time.

The MDL was calculated using the following formula:

$$\text{MDL} = S \times t_{(n-1,1-\alpha=0.99)}$$

where S = standard deviation of the replicate analyses,
n = number of replicate analyses, and
$t_{(n-1,1-\alpha=0.99)}$ = Student's t -value appropriate for a 99 percent confidence level and a standard deviation estimate with n-1 degrees of freedom.

CONCLUSIONS

Extraction of explosive residues from water samples, including TNT and its degradation products, is reliably achieved through use of polystyrene divinylbenzene SPE cartridges. Recoveries for nine explosive compounds and four metabolites ranged from 67 to 115 percent for seven determinations each of HPLC-grade water and two natural-water samples fortified at approximately 1 and 100 µg/L. The MDLs, on the basis of a 1-L sample size, ranged from 0.11 to 0.32 µg/L in HPLC-grade water fortified at about 1 µg/L, and averaged 0.19 µg/L. Advantages of the method include use of commercially prepared solid-phase extraction cartridges, which reduce the need for much of the labor, time, and materials used by alternative procedures.

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