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Salting-Out Solvent Extraction Method for Determining Low Levels of Nitroaromatics and Nitramines in Water

Paul H. Miyares and Thomas F. Jenkins

August 1990

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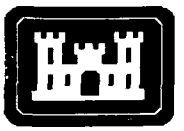
Special Report 90-30



U.S. Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

Salting-Out Solvent Extraction Method for Determining Low Levels of Nitroaromatics and Nitramines in Water

Paul H. Miyares and Thomas F. Jenkins

August 1990

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PREFACE

This report was prepared by Paul H. Miyares and Dr. Thomas F. Jenkins, Research Chemists, Geochemical Sciences Branch, U.S. Army Cold Regions Research and Engineering Laboratory. Funding for this research was provided by the U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, Maryland (R-90 Multi-Analytical Services), Martin H. Stutz, Project Monitor.

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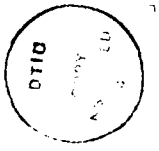

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INTRODUCTION

In 1984, Jenkins et al. developed a reversed-phase, high-performance liquid chromatographic (RP-HPLC) method for the simultaneous determination of 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and 2,4-dinitrotoluene (2,4-DNT) in munitions wastewater. TNT, RDX and HMX are explosives commonly used by the Army, and 2,4-DNT is a byproduct in the production of TNT. Although munitions wastewater is currently treated by carbon adsorption columns before discharge, these carbon columns have a finite sorption capacity and effluent must be tested to determine if it meets regulatory limits.

While developing a companion method for the determination of explosive residues in soil (Jenkins and Walsh 1987, Jenkins et al. 1988b, Bauer et al. 1989), an improved protocol was also developed for the direct determination of explosives in water (Jenkins et al. 1988a). This method allowed simultaneous determination of TNT, RDX, HMX and 2,4-DNT as well as 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB), methyl-2,4,6-trinitrophenylnitramine (tetryl), nitrobenzene (NB) and the ortho, meta and para isomers of nitrotoluene (o-NT, m-NT, p-NT). Certified Reporting Limits (USA THAMA 1987) for this method ranged from 4.0 µg/L for DNB up to 44 µg/L for tetryl. These limits satisfied the effluent discharge requirements, as well as drinking water limits, for TNT and RDX in effect at that time.

Recently the EPA issued two health advisories that specified substantial reductions in the acceptable levels of RDX and TNT in drinking water (USEPA 1988a, 1989). Lifetime Health Advisories (HA) limits of 2 µg/L are proposed for both RDX and TNT. The current RP-HPLC method, employing direct injection (Jenkins et al. 1988a), is not sufficiently sensitive for either analyte. Published solid-phase extraction methods for sample preconcentration (Bicking and Summer 1986, Valis et al. 1989) are also not sensitive enough to meet the EPA criterion for RDX (Table 1). The best published solvent extraction, gas chromatograph-electron capture detection (GC/ECD) method is capable of determining TNT at the 2-µg/L level but is not suitable for RDX due to poor extraction efficiency (Belkin et al. 1985).*

Table 1. Water quality criteria and measurement capability for RDX and TNT.

	Concentration (µg L)	
	RDX	*TNT*
Water quality criteria		
U.S. Navy (BUMED 1980)		50
U.S. AMBRDL (NRC 1982)	30	10
U.S. EPA (1988a, 1989)	2	2
Current measurement capability		
RP-HPLC		
Direct method (Jenkins et al. 1988a)	14.0	6.9
Solid-phase extraction (Bicking and Summer 1986	7.7	1.0
Solid-phase extraction (Valis et al. 1989)	7.5	1.3
GC-ECD		
Toluene extraction (Belkin et al. 1985)	*	1.0

* Method not recommended for RDX (Belkin et al. 1985)

The objective of this study is to develop a procedure for determining very low concentrations of munitions in ground water. The method must permit simultaneous determination of those solutes most often observed in munitions-contaminated ground water. This includes several explosives (HMX, RDX, TNT), production impurities (DNB, 2,4-DNT, 2,6-DNT) and degradation products such as TNB, 2-amino-4,6-dinitrotoluene (2-Am-DNT) and 4-amino-2,6-dinitrotoluene (4-Am-DNT). Certified reporting limits (CRLs) should be below the regulatory concentrations for RDX and TNT required by the EPA. Chlorinated and aromatic solvents should be avoided to minimize health risk to analysts and to reduce

* An unpublished method by R. Valis, U.S. Army Environmental Hygiene Agency, has successfully achieved a CRL for RDX of less than 1 µg/L using solvent extraction, GC-ECD (personal communication).

the introduction of environmentally incompatible chemicals from laboratory waste into the environment.

A method with some form of a preconcentration step is necessary to reduce CRLs below current regulatory limits. Three possible preconcentration procedures were considered:

- Conventional liquid–liquid extraction;
- Solid sorbent extraction; and
- Salting-out extraction.

Spanggord et al. (1982) described a conventional diethyl ether extraction of munitions wastewater prior to packed-column GC with flame ionization detection (FID). This method had the capability of determining 30 nitroaromatic compounds with at least 90% recovery and a precision of ±10% at the lower limit of the standard curve (100 µg/L). Phillips et al. (1983) employed a methylene chloride ($MeCl_2$) extraction of nitroaromatics from biosludge, and determination by gas chromatography thermal energy analyzer (GC/TEA). They reported a detection limit of 500 µg/L and a dynamic range covering four orders of magnitude. Neither of these methods satisfies current regulatory requirements. Also, extraction efficiency for HMX and RDX using $MeCl_2$ has been found to be poor (Miyares, unpublished). Belkin et al. (1985) described a conventional toluene extraction procedure for water prior to capillary GC/ECD. Excellent quantitative results were reported for TNT, 2,4-DNT, 2,6-DNT and tetryl, with detectable levels of approximately 1 µg/L. However, quantitation of RDX was impractical because of the low percent recovery with toluene.

Solid sorbents have also been used to extract explosives from water. Maskarinec et al. (1984) and Richard and Junk (1986) described methods that employ XAD series and Porapak series R and S resins. Analytes are recovered from the resins using polar solvents such as ethyl acetate or acetone. Maskarinec et al. employed HPLC-electrochemical detection (ED) for determination, while Richard and Junk employed GC/ECD. Maskarinec et al. indicated that breakthrough of the analytes occurred, resulting in low recovery. Their method of separation employs gradient elution RP-HPLC, which introduces an extended sample turnaround time. Richard and Junk reported that incomplete dissolution of compounds from the resins led to low recoveries. Despite the low percent recoveries, the use of solid sorbents is an option. The methods are easy to use and include the potential for extracting samples in the field as well as in the laboratory.

The concept that normally miscible solvents could be salted out of aqueous solutions has been known for many years. Salting-out has been used for extracting metals and organometallics from aqueous solutions by complexing the metals with organic ligands and extracting with a salted-out organic solvent (Bockris and Egan 1946, Matkovich and Christian 1973, Nagaosa 1980, Mueller and Lovett 1987). An inorganic salt such as sodium chloride (NaCl) or calcium chloride ($CaCl_2$) is added to an aqueous solution at concentrations near saturation, which then forces phase separation of otherwise miscible aqueous solutions from polar solvents such as acetone and acetonitrile (ACN). Any solutes present will partition between the two phases. While partition coefficients for organic solutes were unavailable in the literature, it seemed reasonable that favorable partitioning to the organic phase is likely.

Several theories have been proposed to explain the salting-out phenomenon. One postulates that an increase in the internal pressure of the solution created by the addition of a salt decreases the solubility of the solvent (Bockris and Egan 1946, Mukerjee 1965). Another suggestion is that preferential solvation of ionic salts by water reduces the availability of water to associate with solvent (Mukerjee 1965, Matkovich and Christian 1973). Other theories include a van der Waals hydration energy effect and electrostatic effects (Matkovich and Christian 1973). Despite the absence of a generally accepted, comprehensive theoretical description of this phenomenon, the technique can be used effectively. Matkovich and Christian (1973) discussed the many salts that are effective at salting-out organic solvents. Nagaosa (1980) and Mueller and Lovett (1987) demonstrated that acetonitrile can be salted out, which is of special interest to this study.

The idea of employing this technique for preconcentrating polar organic solutes arose out of an observation made during the development of a method to determine explosive residues in soil (Jenkins et al. 1988b, Bauer et al. 1989). In this method, aqueous $CaCl_2$ is routinely used to flocculate particulates in soil extracts. When the concentration of $CaCl_2$ was high, normally miscible water and acetonitrile were observed to form separate phases in the sample vial. Analysis of the two phases revealed that the nitroaromatics and nitramines were predominantly found in the acetonitrile layer. A review of the literature at that time did not indicate that salting-out solvent extraction had been used for extracting polar organic analytes from water. During the course of the work described below, Hertz et al. (1989) published an abstract in which they discussed the use of a salting-out extraction to recover polar organic solutes from an aqueous solution.*

EXPERIMENTAL METHODS

Instrumentation

All RP-HPLC determinations were performed on a modular system comprising the following components:

* We recently collaborated on a paper that introduces this concept to a larger audience (Leggett et al. 1990).

- A Spectra Physics Model SP8810 precision isocratic pump;
- A Dynatech Precision Autosampler, Model LC-241, equipped with a Rheodyne Model 7010A sample loop injector and a 100-µL loop;
- A Spectra Physics Model SP8490 variable-wavelength UV detector set at 254 nm;
- A Hewlett Packard Model HP3393A digital integrator equipped with a Hewlett Packard Model HP9114B disk drive; and
- A Linear Model 500 or Cole Palmer Model 8373-30 strip chart recorder.

An autosampler unit was used to introduce samples by flushing a 100-µL loop for 60 seconds at a rate of 0.5 mL/min. The analytes were separated on a 3.3-cm × 4.6-mm ID Supleco LC-8 (3-µm) reversed-phase column eluted with a ternary eluent of water, methanol (MeOH) and tetrahydrofuran (THF) (70.7/27.8/1.5, v/v/v) at a flow rate of 2.0 mL/min. The digital integrator was programmed to measure peak heights, which has shown better reproducibility than the automated peak area measurements for low-level samples.

Chemicals

Analytical standards for RDX, TNB, DNB, TNT, 2,4-DNT and 2,6-DNT were prepared from Standard Analytical Reference Materials (SARMs) obtained from the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA), Aberdeen Proving Ground, Maryland. Standards for 2-Am-DNT and 4-Am-DNT were obtained from Dr. David Kaplan, U.S. Army Natick Laboratories, Natick, Massachusetts, and their identity was confirmed by GC/MS analysis (Table B8). Standards were dried to constant weight in a vacuum desiccator over dry calcium chloride in the dark.

The methanol used in preparing the eluent was Baker HPLC-grade, the acetonitrile (ACN) used to extract samples and prepare standards was Mallinckrodt ChromAR HPLC-grade, and the THF used in the eluent was either Baker HPLC or Aldrich HPLC-grade. The water used for preparing eluent and spiked sample solutions was purified using a Milli-Q Type 1 Reagent-Grade Water System (Millipore Corp.). The mobile phase was prepared by combining the proper portions of each component and vacuum filtering through a Whatman CF-F microfiber filter to remove particulate matter and to degas the eluent. The ground water used to simulate field samples was acquired from deep ground water aquifers in either Hanover, New Hampshire, or Weathersfield, Vermont. Contaminated ground water samples were provided by the Missouri River Division Laboratory, Omaha, Nebraska, or the Waterways Experiment Station, Vicksburg, Mississippi. The NaCl was Baker reagent-grade crystals.

Preparation of individual stock standards

Individual stock standards of RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT were prepared by measuring about 100 mg of each dried standard material (weighed to the nearest 0.01 mg), transferring them to individual 250-mL volumetric flasks and diluting to volume with acetonitrile. Stoppered joints were wrapped with Parafilm to retard evaporation, and solutions were stored at 4°C in the dark. The concentrations of the analytes in these stock solutions were approximately 1000 mg/L.

Initial calibration

We prepared a combined analyte calibration stock standard by combining 2.00 mL of each of the RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT individual stock standards in a 100-mL volumetric flask and diluting to volume with ACN (STD A). The concentrations of the analytes in this standard were approximately 20 mg/L. From the combined analyte calibration standard, a series of calibration solutions were prepared as outlined in Table 2 (STDs B–M). Duplicate 2.00-mL aliquots of each calibration solution were each combined with 6.00 mL of Milli-Q water, shaken by hand and analyzed in random order. The acceptability of a linear model for each analyte was assessed by using the protocol specified in the USA THAMA QA Program (2nd ed., March 1987). Experience has shown that a linear model with a zero intercept is appropriate. Therefore, a response factor for each analyte can be taken as the slope of the best-fit regression line.

Daily calibration

Daily calibration was obtained using standard B (Table 2). A 3.00-mL aliquot of standard B was combined with

Table 2. Dilutions for initial calibration standards. All dilutions are in ACN.

Standard	Aliquot of standard (mL)	Size of flask (mL)	Concentrations (µg/L)
A*			20,000
B	25 of A	50	10,000
C	25 of A	100	5,000
D	10 of B	50	2,000
E	10 of B	100	1,000
F	10 of C	100	500
G	1 of A	100	200
H	1 of B	100	100
I	1 of C	100	50
J	1 of D	100	20
K	1 of E	100	10
L	1 of F	100	5
M	1 of G	100	2

*Solution A is the combined analyte calibration standard.

9.00 mL of Milli-Q water in a scintillation vial and shaken by hand. This daily calibration standard sample was analyzed in duplicate at the beginning of each day of analysis, singly at the midpoint and singly at the end of each day of analysis. The response factor for each analyte was obtained from the mean peak height and compared with the response factor obtained for the initial calibration. The mean response factor for the daily calibration must agree within ±25% of the response factor of the initial calibration for the first seven daily calibrations and within two standard deviations of the initial calibration for subsequent calibrations. If this criterion was not met, a new initial calibration was obtained.

Preparation of solutions for reporting limit tests

A combined analyte spiking stock solution was prepared by combining 2.00 mL each of RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-AM-DNT in a 250-mL volumetric flask and diluting to volume in ACN (STD N). The concentrations of the analytes in this solution are approximately 8000 µg/L. From the combined analytes spiking stock solution, a series of spiking solutions were prepared as outlined in Table 3 (STD P–Y).

Solutions used to estimate Certified Reporting Limits were prepared by pipetting a 400-mL volume of Milli-Q water into a 500-mL separating funnel and adding 1.00 mL of the appropriate combined analyte spiking solution (Table 4).

Sample extraction and analysis

One day prior to each day of extracting samples, all glassware used for sample preparation and extraction was washed with soap and water, rinsed with Milli-Q water, rinsed with acetone, and then rinsed again with Milli-Q water. The glassware was filled with Milli-Q water and allowed to stand overnight. Immediately prior to their use, the Kuderna–Danish concentrators were rinsed with ACN, and all other glassware (separating funnels, cylinders, etc) was given a final rinse with Milli-Q water.

A 400-mL aliquot of each ground water sample was measured with a graduated cylinder and added to a 500-mL separating funnel. A 130-g portion of NaCl was added to each sample, and the samples were shaken until *all* of the NaCl was dissolved. Then 100 mL of ACN was pipetted into each sample and the funnel shaken vigorously for 5 minutes (Nagaosa 1980). The phases were allowed to separate for 30 minutes prior to removal of the aqueous (lower) layer. The ACN (upper) layer (~23 mL) was collected in a 40-mL Teflon-capped vial. Each separatory funnel was rinsed with a 5-mL aliquot of fresh ACN, and the rinsate was added to the collected ACN sample. If the collected sample was turbid, it was centrifuged in the 40 mL vial at 4000 rpm's for 5 min, and the ACN (upper) layer was removed with a Pasteur pipette and transferred to a clean vial. The ACN volume was then reduced to less than 1 mL using a Kuderna–Danish microconcentrator, cooled and brought up to 1.00 mL with ACN, and the concentrate was combined with 3.00 mL of Milli-Q water in a scintillation vial. This diluted solution was poured back through the Kuderna–Danish to rinse the sides of the reflux column and flask, and then was transferred back into the vial. The entire procedure is outlined in Figure 1. Analytical control samples were prepared at a concentration of 2.0 µg/L each of RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT in reagent-grade water. This control sample was treated in an identical manner to the ground water samples described above.

Table 3. Dilutions series for spiking solutions for reporting limit tests. All dilutions are in ACN.

Standard	*Aliquot of standard (mL)*	*Size of of flask (mL)*	*Approx. analyte concentration (µg/L)*
N*			8000
P	25 of N	50	4000
Q	20 of N	100	1600
R	10 of N	100	800
S	5 of N	100	400
T	2 of N	100	160
U	1 of N	100	80
V	1 of P	100	40
W	1 of Q	100	16
X	1 of R	100	8
Y	1 of S	100	4

* Solution N is the combined analyte spiking stock standard.

Table 4. Dilution series for CRL study, spiking solution preparation.*

Spiking level	*mL of combined stock solution*
500X	50
200X	20
100X	10
50X	5
20X	2
10X	1
5X**	1 of 500X
2X**	1 of 200X
1X**	1 of 100X
0.5X**	1 of 50X

* Final concentrations are given in Table 2.
† All dilutions are to 100 mL in volumetric flasks.
** These spiking solutions were diluted from higher concentration dilutions as shown.

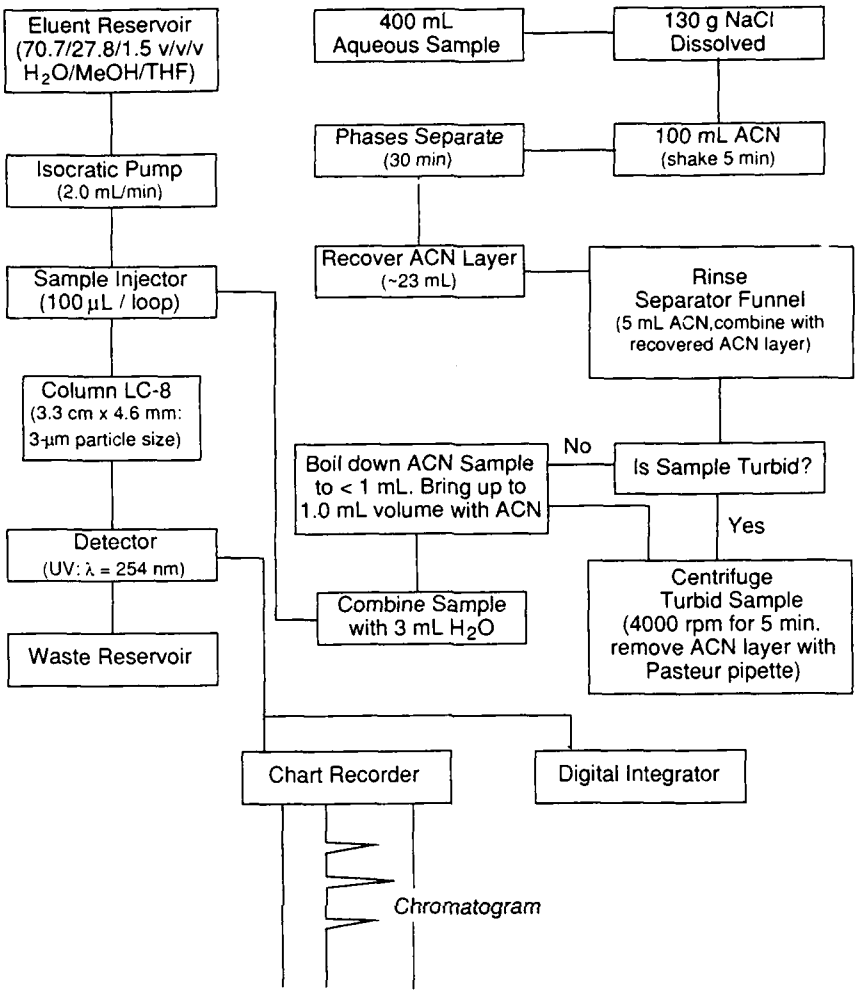


Figure 1. Salting-out extraction protocol.

RESULTS AND DISCUSSION

Sample extraction and preconcentration

A number of basic questions were addressed in the course of developing this method. The first of these was which salt–solvent combinations would be compatible with salting-out extraction. Matkovich and Christian (1973) tested many mono-, di- and tri-valent salts for their salting-out efficiency. They reported that aluminum chloride ($AlCl_3$), magnesium chloride ($MgCl_2$) and calcium chloride ($CaCl_2$) were the most efficient salting-out agents, but indicated that aluminum chloride would be a poor choice due to its vigorous reaction in water. Because Jenkins et al. (1988b) had reported phase separation of water and ACN using $CaCl_2$, and ACN is an excellent solvent for both nitroaromatics and nitramines, we decided to test this combination further. Also, since NaCl is available in high purity at a low cost, it was included in the study for comparison.

We first compared the ability of each compound to salt out ACN. Saturated aqueous solutions of each salt were prepared. The $CaCl_2$ solution required 298 g to saturate 400 mL of water, while the NaCl solution required only 130 g to saturate 400 mL of water. Upon saturation, the temperature of the $CaCl_2$ solution rose to 55°C; the NaCl solution temperature remained near ambient. Warming of the solution is undesirable because the compounds are thermally labile and because a significant length of time would be required to cool the samples before proceeding with extractions. When we added ACN to the saturated solutions, we observed phase separation for both the NaCl and $CaCl_2$ solutions when 100 mL of ACN was added, recovering ~23 mL and ~21 mL, respectively. Neither salt was observed to precipitate upon addition of ACN. The basic differences between using NaCl and $CaCl_2$, then, are the increase in sample temperature and the cost per sample. We chose NaCl because the sample temperature remained at ambient and because it was significantly less expensive.

The criteria for the choice of solvent included its ability

to be salted out, its extraction efficiency for the analytes, and its compatibility with reversed-phase HPLC-UV. Five solvents were considered: methanol, ACN, THF, isopropanol and acetone. The question of ability to be salted out was answered by measuring volumes recovered from 25 mL of Milli-Q water saturated with NaCl (8.8 g). The solvents were added in appropriate amounts and the volumes of the organic phases were measured (Table 5). The results indicate that methanol is not salted out of aqueous solution by NaCl. Acetone is salted out, but a substantial volume remains soluble in salt-saturated water. Consequently these solvents were excluded from further consideration.

The second criterion for solvent choice was extraction efficiency. The important points here are solubility and partitioning. Nitroaromatics and nitramines are very soluble in ACN and THF but much less so in isopropanol. Since extraction efficiency is generally correlated with solubility, isopropanol was expected to be less efficient than either ACN or THF, and therefore it was not considered further.

To aid in choosing between the two remaining solvents, THF and ACN, partition coefficients were compared for RDX and TNT. Aqueous solutions containing

Table 5. Volume recovery of solvents when added to 25 mL of water containing 8.8 g NaCl.

Solvent	*Volume added (mL)*	*Volume recovered (mL)*
AcN	10	2.3
Acetone	11	<1
IPA	10	8.9
MeOH	>10	0
THF	10	8.4

high levels of TNT and RDX were prepared. The solutions were saturated with NaCl, and sample aliquots were extracted with each of the solvents. Both solvent extracts and residual aqueous phases were analyzed in each case. The results indicate that THF is a significantly better extractant than ACN for these compounds (Table 6). THF also has a lower boiling point than ACN, so it is more easily concentrated by solvent evaporation. However, even HPLC-grade THF contains a stabilizer to prevent peroxide formation. When THF is concentrated, this stabilizer reaches detectable levels. When a sample of THF that had been concentrated by a factor of 33 was analyzed by RP-HPLC-UV, an interfering peak eluting at the same retention time as RDX was observed using our primary analytical column (LC-8). This impurity can be separated from RDX on an LC-CN column (Jenkins and Walsh 1987), but since RDX is of major interest, THF is undesirable for this application. Acetonitrile met all the criteria required: it can be salted out, the partition coefficients for the analytes of interest are acceptably large, and it is compatible with reversed-phase HPLC-UV.

Table 6. Partition coefficients for TNT and RDX between the salted-out organic solvent and NaCl-saturated water.

	Partition coefficients (K_p)	
Solvent	*RDX*	*TNT*
ACN	248	1920
THF	994	5308

The ratio of ACN to NaCl in solution required for maximum ACN recovery and optimum extraction efficiency was estimated by constructing a phase diagram. This experimental determination was patterned after a similar determination by Matkovich and Christian (1973) in their work on salting out of acetone with $CaCl_2$. They constructed a ternary-phase diagram by plotting the percent by weight of water, $CaCl_2$ and acetone and obtaining a curve that indicated the amount of $CaCl_2$ required for maximum acetone recovery.

We chose volumes of water (400 mL) and ACN (100 mL) so that the separation could be conducted in a 500-mL separatory funnel. Sodium chloride was added, initially in increments of 2 g starting at 120 g, and later in smaller increments as the concentration increased and the rate of dissolution decreased. The NaCl reached apparent saturation in the range of 130–132 g (5.56–5.64 molal) at room temperature (~23°C).

Our phase diagram was constructed by plotting the moles of ACN soluble in 400 mL of water vs the moles of NaCl soluble in 400 mL of water (Fig. 2, Table 7). Concentrations of NaCl ranging from 0.50 mol (29.2 g) to 2.23 mol (132 g) were dissolved in 400 mL of Milli-Q water. Upon dissolution of the NaCl, 100 mL of ACN was added. The samples were shaken vigorously for 5 minutes (Nagaosa 1980) and allowed to settle, and the volumes of recovered ACN were measured. The difference between the recovered volumes and the original 100 mL was taken as the amount soluble in the ternary system. The phase diagram was constructed by plotting the soluble amounts of ACN in the 400-mL solution against their respective amounts of NaCl (Fig. 2). In the diagram, the experimental curve (solid line) indicates a decrease in the soluble ACN in the solution as the NaCl concentration increases

Table 7. Data for phase diagram of moles NaCl and moles of ACN soluble in 400 mL of aqueous solution (Fig. 2).

NaCl (mol)	*ACN (mol)*
0.50	4.08
1.00	2.66
1.80	1.60
2.00	1.50
2.16	1.42
2.23*	—

* Precipitation of NaCl was observed upon addition of ACN at and above this amount of NaCl.

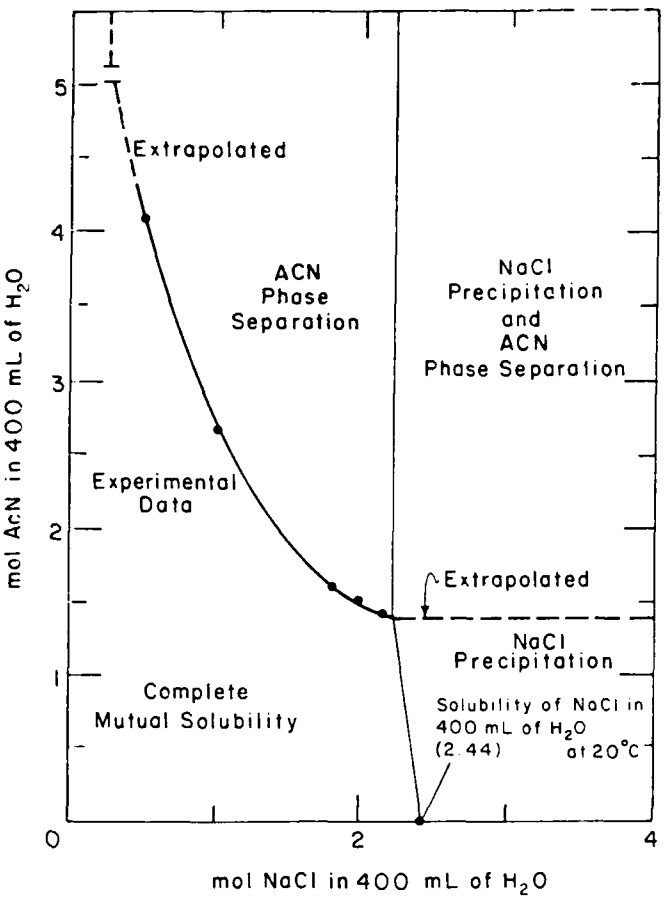


Figure 2. Phase diagram showing the mutual solubility of ACN and NaCl in 400 mL of H_2O at 22°C.

up to 2.23 mol of NaCl in 400 mL of water. Above this point the soluble amount of ACN remains constant at 1.40 mol in 400 mL as indicated by the horizontal dotted line, and NaCl precipitates upon the addition of additional NaCl to the system. The optimum ratio in the phase system was found to be 1.40 mol of ACN and 2.23 mol of NaCl in 400 mL of water. An excess of ACN, NaCl or both at this point will result in phase separation, precipitation or both.

The amount of salt used is critical since it has a direct effect on the partitioning of the analytes. If the concentration of salt is too high, the amount of salt associated with the organic solvent increases. This reduces the recovery of solvent and consequently the recovery of the analytes (Nagaosa 1980). To determine the point in the phase system that produced the greatest partitioning, the water samples used to construct the phase diagram had been previously spiked with the analytes of interest prior to phase separation. After measuring the volumes, the concentrations of the analytes were determined in each phase and the partition coefficients calculated (Table 8). The results show that the partition coefficients increase with the concentration of NaCl in solution up to 2.0 mol in 400 mL. Above this concentration the results indicate that there is no significant difference in the partition coefficients

Table 8. Partition coefficient (K_p) between ACN and water as a function of NaCl (g) in 400 mL of water.

	Mean K_p (moles NaCl/400 mL H_2O)*				
Analyte	*1.00*	*1.80*	*2.00*	*2.16*	*2.32*
RDX	170	201	239	248	246
TNB	396	477	565	582	605
DNB	261	316	380	406	403
TNT	1300	1610	1920	1920	1990
2,6-DNT	612	772	960	979	1010
2,4-DNT			831		
2-Am-DNT			641		

* Based on duplicate determinations.

(Duncan's multiple range test, $\alpha = 0.05$). These results indicate that the maximum partitioning occurs when the NaCl concentration in the sample is greater than 2.0 moles in 400 mL, near the optimum ratio of ACN to NaCl. It is concluded that the ratio of ACN to NaCl required for maximum recovery is the optimum ratio for the system.

Certified Reporting Limits (CRLs) for the direct RP-HPLC method range from 4.0 to 14 µg/L for the analytes frequently found in contaminated groundwater (Jenkins et al. 1988a). The desired levels for this method are

currently less than 0.1 µg/L for some analytes, such as 2,4- and 2,6-DNT. This would require a preconcentration factor of at least 140. For extracts to be analyzed by RP-HPLC, the solvent strength of the extract must be equal to or less than that of the eluent used, or band broadening and peak distortion will result. Thus, extracts must be diluted with water to decrease the solvent strength prior to introduction into the HPLC. As previously mentioned, the ACN sample was diluted 1:3 with water prior to analysis. This extra dilution of the sample can be compensated for by preconcentrating by a greater factor. A factor of 400 was chosen because adequate preconcentration is achieved and because a convenient volume of ACN (i.e. 100 mL) and a convenient size of separatory funnel are used for sample extraction. Therefore, a 400-mL water sample is extracted with 100 mL of ACN. Approximately 23 mL of ACN was recovered and was further preconcentrated to 1.00 mL with a Kudema–Danish microconcentrator. After dilution with 3.00 mL of water, the concentration factor achieved was 100.

One possible problem with this method is the evaporative preconcentration step using the Kudema–Danish apparatus. All of the compounds of interest are thermally labile to some extent. We thought that some analytes might be thermally degraded during this stage, but given the percent recovery data, this does not appear to be the case. Regardless, a thermal degradation experiment was conducted. In this test, spiked samples were prepared and analyzed before and after boiling down. The results showed that boiling had no significant effect for any of the analytes of interest. Clearly, thermal decomposition is not a problem under the conditions employed.

Separation and determination

Analyses of ground water samples from a number of explosives-contaminated areas have shown the presence of HMX, RDX, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT. The current direct-injection RP-HPLC method (Jenkins et al. 1988a) does not adequately separate the two most commonly observed DNT isomers or the amino-DNT isomers (Fig. 3) to allow accurate simultaneous quantitation of these compounds. A recent applications note from Supelco, Inc. (1987) describes an RP-HPLC separation of HMX, RDX, tetryl, TNT, 2,4-DNT and 2,6-DNT under isocratic conditions using a Supelcosil LC-8 column (3.3 cm × 4.6 mm, 3 µm packing) eluted with a mobile phase composed of 2% THF in MeOH:water (30:70) at a rate of 2 mL/min. We tested this separation with the analytes described above plus the addition of TNB and the two isomers of amino-DNT. The separation of 2,4-DNT and 2,6-DNT was adequate, but separations between 2,6-DNT and 2-Am-DNT, 2-Am-DNT and 4-Am-DNT, and RDX and TNB were not adequate under these conditions. A technique for

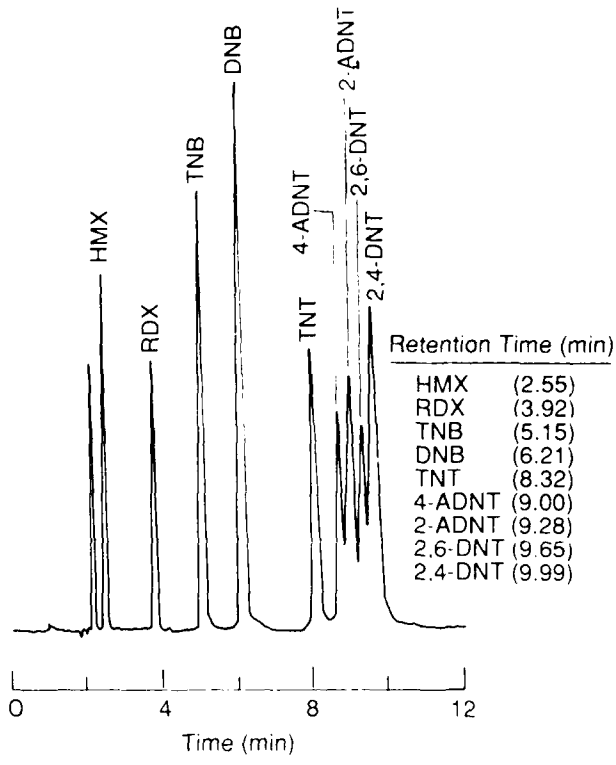


Figure 3. Separation for the direct injection RP-HPLC method (Jenkins et al. 1988a).
Column: LC-18 (25 cm × 4.6 mm, 5 µm).
Eluent: 50/50 (v/v) MeOH/H_2O.
Flow: 1.5 mL/min.
λ: 254 nm.

optimizing an isocratic separation (Meyer 1988) was employed in an attempt to better separate the analytes. A ternary combination of water:MeOH:THF (70.7/27.8/1.5) (v/v/v) eluted at a rate of 2.0 mL/min through the Supelco LC-8 column produced a separation of RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT in less than six minutes, with near separation for all analytes except the Am-DNT isomers (Fig. 4). Unfortunately we were unable to quantitate HMX due to substantial interference introduced by NaCl used to salt out the ACN. Fortunately the monitoring requirements for HMX are such that the direct injection method is adequate for HMX determination (US EPA 1988b). Table 9 contains the retention times and capacity factors for the primary analytes and potential interferences for this separation. We have not optimized the procedure to achieve complete separation of TAX, SEX and tetryl since our experience indicates that these analytes are not transported in ground water at detectable concentrations.

Because of the potential for interferences to co-elute with analytes and because of the very low concentrations of analytes for which this method is applicable, we found it necessary to use a confirmation analysis. Jenkins et al.

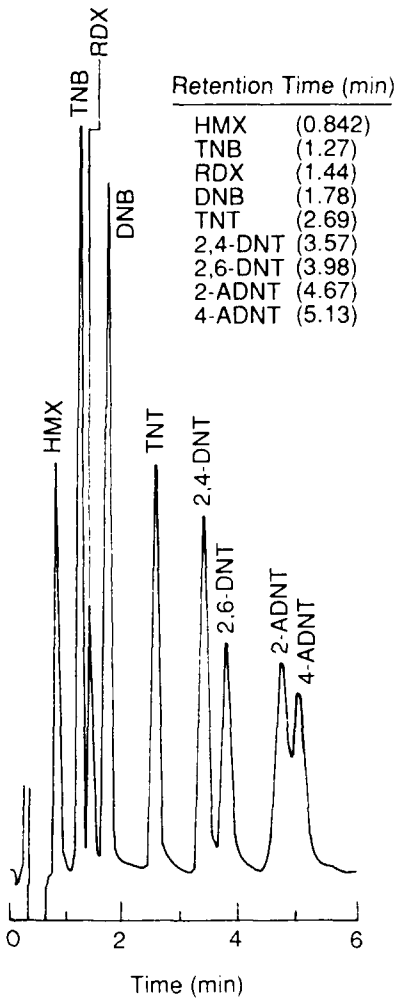


Figure 4. Primary separation for the salting-out procedure.

Column: LC-8 (3.3 cm × 4.6 mm, 3 µm)
Eluent: 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF
Flow: 2.0 mL/min
λ: 254 nm.

(1988b) demonstrated that the LC-CN reversed-phase column provided an adequate separation for confirmation of explosives in soil extracts. We found that by installing an LC-CN column (3.3 cm × 4.6 mm, 3 µm packing) in series with the LC-8 primary column and employing the same eluent at a flow rate of 1.5 mL/min, the separation was sufficiently different from the primary separation to be adequately used for confirmation. The retention times for this separation are presented in Table 9. Figure 5 is a chromatogram of this separation for the primary analytes.

Table 9. Retention times and capacity factors for LC-8 primary separation and LC-8/LC-CN confirmation separations. The columns were eluted with 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF eluent, LC-8 at 2.0 mL/min and LC-8/LC-CN at 1.5 mL/min.

Primary analytes	*Retention time (min)*		*Capacity factor* (K′)*	
	LC-8	*LC-8/LC-CN*	*LC-8*	*LC-8 LC-CN*
HMX	0.74	6.74	2.12	11.6
TNB	1.10	2.49	3.64	3.64
RDX	1.31	4.37	4.53	7.14
DNB	1.58	3.29	5.67	5.13
TNT	2.40	4.93	9.13	8.18
2,4-DNT	3.24	6.13	12.7	10.4
2,6-DNT	3.61	6.69	14.2	11.5
2-Am-DNT	4.56	9.01	18.2	15.8
4-Am-DNT	4.85	9.23	19.5	16.2
Potential interferences				
TAX	0.30	1.77	0.27	2.30
SEX	0.40	2.65	0.68	3.95
Benzene	2.24	3.71	8.45	5.91
Tetryl	3.50	9.19	13.8	16.1
Mono-NTs	4.1-4.5	7.0-7.6	17.1	12.6
Toluene	5.40	8.27	21.8	14.4

*Capacity factor is calculated on the unretained peak of KNO_3 at 0.238 min on LC-8 and 0.537 on LC-8/LC-CN.
$K' = (t_a - t_0)/t_0$.
K' = capacity factor.
t_a = retention time of analyte (min).
t_0 = retention time of KNO_3 (min).

Characteristics of salting-out extraction

Methylene chloride ($MeCl_2$) is often used for liquid–liquid extraction of water samples. While $MeCl_2$ is not miscible with water, it is relatively soluble (~1.6 g/100g H_2O at 20°C). The efficiency of a conventional $MeCl_2$ extraction for explosives was compared to the salting-out extraction with ACN (Table 10). Six identical 400-mL spiked water samples were prepared at ~2000 µg/L for five analytes. Three were extracted with 20 mL of $MeCl_2$ and three with 130 g of NaCl plus 100 mL of ACN. The aqueous layers were analyzed by RP-HPLC, and the percentage of each analyte remaining in the water sample was determined. The percent recovery was calculated by difference (100% remaining). Based on *t*-tests ($\alpha = 0.05$) the percent recovery for all of the analytes was significantly greater in ACN with the greatest improvement in recovery being RDX (59.6% with $MeCl_2$ vs 94.2% with ACN/NaCl).

Samples prepared in the laboratory using analytical-grade water are free of the background materials and compounds that can introduce matrix effects in normal liquid - liquid extraction. Field samples may contain varying amounts of natural organic matter or inorganic compounds, which could interfere with the analyte determination or affect the extraction efficiencies. When samples are extracted using the salting-out procedure, those matrix variations due to ionic strength differences should be largely reduced. This is because the addition of large amounts of salt will overwhelm the initial salt

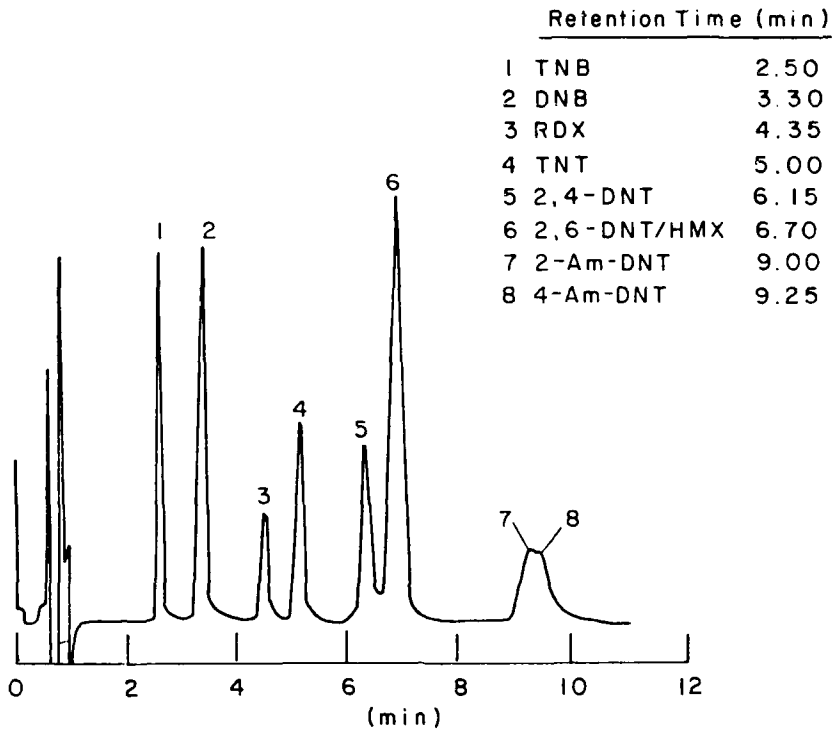


Figure 5. Confirmation separation for the salting-out procedure.
Column: LC-8/LC-CN series (both 3.3 cm × 4.6 mm, 3 μm)
Eluent: 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF
Flow: 1.5 mL/min
λ: 254 nm

Table 10. Comparison of mean percent recoveries for $MeCl_2$ and ACN/NaCl extraction.

	Percent recovery				*t value for*
	$MeCl_2$ extraction		*ACN/NaCl extraction*		*comparison of*
Analyte	*Mean**	*Std dev*	*Mean**	*Std dev*	*means†***
RDX	59.6	0.26	94.2	0.76	105
TNB	88.1	0.56	97.1	1.70	12.3
DNB	89.8	0.31	95.6	0.64	20.0
TNT	94.0	0.71	98.9	—	15.7
2,4-DNT	94.4	0.46	98.0	0.47	13.5

* Based on three replicates.
† Tabular *t* value at 95% confidence level is 2.78.
** Variances were pooled even though they were not homogeneous.

concentration, creating effectively equivalent ionic strengths for all samples.

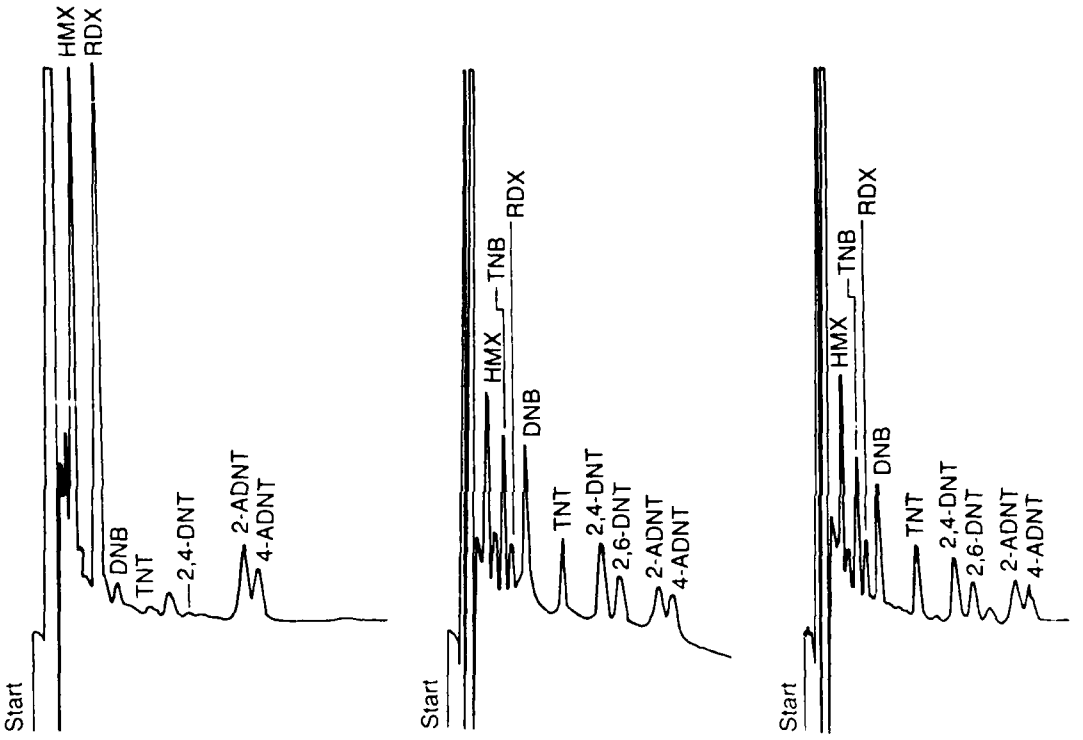
An experiment was run to compare the extraction efficiencies for explosives analytes in analytical-grade water to those in water from a local ground water well. The samples were treated identically: 400-mL aliquots of both reagent-grade water and well water were spiked with 1.00 mL of a common spiking solution. The resulting samples contained RDX, TNB, DNB, TNT and 2,4-DNT at about 2000 μg/L. Aliquots of both samples were extracted in triplicate using the salting-out procedure. The results are presented in Table 11. Statistical calculations indicate that the extraction efficiencies were not significantly different ($\alpha = 0.05$) for any of the analytes tested.

An experiment was conducted to determine if matrix effects from various water samples affected the performance of this method. Figure 6 shows three chromatograms for samples from three sources of water: reagent-

Table 11. Comparison of mean recoveries from Milli-Q and well water samples.

Analytes	Percent recovery: Milli-Q water samples Mean*	Milli-Q water samples Std dev	Well water samples Mean*	Well water samples Std dev	t value for comparison of means†
RDX	94.2	0.76	93.1	0.91	1.61
TNB	97.1	1.70	96.4	1.50	0.56
DNB	95.6	0.64	94.6	0.70	1.83
TNT	98.9	0.31	98.7	0.21	0.93
2,4-DNT	98.0	0.47	98.5	1.30	0.58

* Based on three replicates.
† Tabular t value at 95% confidence level is 2.78.



a. Contaminated field sample from Crane-Rockeye, Indiana. *b. Standard sample prepared in reagent-grade water.* *c. Standard sample prepared in local well water.*

Figure 6. Comparison of chromatograms for samples prepared in reagent-grade water, ground water and contaminated ground water. All are analyzed by the salting-out extraction method.

grade water and local well water spiked with the nine analytes of interest, and an actual contaminated field sample from Crane-Rockeye, Indiana. Chromatograms from all three water samples show similar peaks for the analytes, as well as a similar base line, solvent front and unretained peaks. Thus, the method appears to work equally well for all types of water tested.

Determination of CRLs and MDLs

The lower limits of detection and quantitation of a method can be estimated in a variety of ways. We chose two of the most frequently employed procedures for environmental methods. One is the Method Detection Limit (MDL) test outlined by the EPA (*Federal Register* 1984) and the other is the Certified Reporting Limit (CRL) test used by USATHAMA (1987). Grant et al. (1989) recently presented an extensive comparison of these two methods.

Both of these procedures were conducted for this salting-out extraction/RP-HPLC method. The MDL test consisted of ten replicates, which were processed and analyzed the same day (Table 12). The CRL test was

Table 12. Summary of CRL, MDL and percent recovery results for ACN/NaCl extraction and direct injection methods.

	ACN/NaCl extraction (µg/L)			*Direct injection method (µg/L)*††		
Analytes	*CRL**	*MDL†*	*% recovery***	*CRL**	*MDL†*	*% recovery***
RDX	0.836	0.407	101	14.0	12.0	99.4
TNB	0.258	0.125	137	7.3	5.0	95.3
DNB	0.108	0.144	99.0	4.0	2.4	97.7
TNT	0.113	0.251	88.8	6.9	2.6	99.8
2,4-DNT	0.0205	0.048	94.8	5.7	15.7	100.5
2,6-DNT	0.314	—	93.9	9.4	5.1	98.1
2-Am-DNT	0.0349	—	102	—	18.0	—
4-Am-DNT	0.0598	—	100	—	—	—

* USATHAMA (1987).

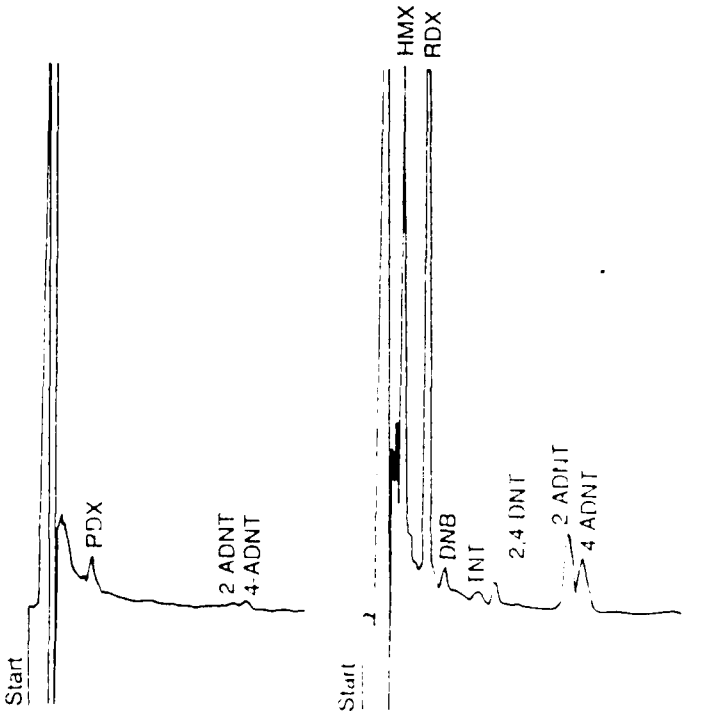
† *Federal Register* (1984).

** Calculated from slope of regression of target vs found concentration.

†† Jenkins et al. (1988a).

conducted using a series of spiked samples that were 0, 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 times a target reporting level for each analyte. Identical sets of samples were processed and analyzed on four consecutive days. A regression analysis of the data was conducted using statistical software provided by USATHAMA. Certified reporting limits and percent recoveries are shown in Table 12 along with similar results for the direct injection procedure.

Certified reporting limits for the eight analytes range from 0.0205 to 0.836 µg/L, an average of about a factor of 36 times lower than the CRLs for the direct injection method. The salting-out extraction step gives a preconcentration factor of 100, which is partially offset by the inherently lower analytical precision obtained when a more-complicated sample pretreatment protocol is required. The CRLs for RDX and TNT are well below the current 2-µg/L criterion established by the EPA (USEPA 1988a, 1989). The CRLs for 2,4- and 2,6-DNT do not satisfy current drinking water criteria for these two com-



a. Direct injection method. *b. Salting-out extraction method.*

Figure 7. Comparison of a water sample analyzed by both direct injection and salting-out methods.

pounds, and work continues to optimize this protocol to achieve sufficiently low CRLs for this application using RP-HPLC.

SUMMARY AND CONCLUSIONS

A method has been developed to determine sub-µg/L concentrations of nitroaromatics and nitramines in water (App. A). The compounds of interest were RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT. The method involves extraction of these compounds by salting out ACN with NaCl. The extract is further concentrated using a Kuderna–Danish microconcentrator. The sample is analyzed by RP-HPLC with UV detection (wavelength = 254 nm) using a Supelco LC-8 (3.3 cm × 4.6 mm) column eluted with a ternary mixture of H_2O/MeOH/THF, 70.7/27.8/1.5 (v/v/v), at 2.0 mL/min. The whole separation was completed in under seven minutes. The method has certified reporting limits ranging from 0.02 to 0.84 µg/L and is applicable up to concentrations of 10 µg/L. Above this concentration the direct injection method can be used.

This method is more labor intensive and more expensive than the current RP-HPLC direct injection method (Jenkins et al. 1988a). However, the detection capabilities are much greater and the separation is better for both the DNT and amino-DNT isomers (Fig.7).

LITERATURE CITED

Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USA Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.

Belkin, F., R.W. Bishop and M.V. Sheely (1985) Analysis of explosives in water by capillary gas chromatography. *Journal of Chromatographic Science*, **24**: 532–534.

Bicking, M.K.L. and S.J. Summer (1986) Evaluation of solid sorbent and detector technology for determination of explosives in water. Contract No. DAAL03-86-D-0001, Battelle Laboratories, Columbus Division.

Bockris, J.O.M. and H. Egan (1946) The salting-out effect and dielectric constant. *Transactions of the Faraday Society*, **44**: 151–159.

BUMED (1980) Letter to the Committee on Toxicology, National Research Council. Washington, D.C., February 11, p. 5. Bureau of Medicine and Surgery, Department of the Navy, Washington, D.C. Cited in NRC (1982).

Federal Register (1984) Definition and procedure for the determination of the method detection limit. Code of Federal Regulations, Part 136, Appendix B, October 26.

Grant, C.L., A.D. Hewitt and T.F. Jenkins (1989) Comparison of low concentration measurement capability estimates in trace analysis: Method Detection Limits and Certified Reporting Limits. USA Cold Regions Research and Engineering Laboratory, Special Report 89-20.

Hertz, C.D., J.G. Schnable and I.H. Suffet (1989) Isolation of polar organic compounds from water using salting-out liquid–liquid extraction systems. Preprint from American Chemical Society Conference, Dallas, Texas, 1989.

Jenkins, T.F., C.F. Bauer, D.C. Leggett and C.L. Grant (1984) Reversed-phase HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater. USA Cold Regions Research and Engineering Laboratory, CRREL Report 84-29.

Jenkins, T.F. and M.E. Walsh (1987) Development of an analytical method for explosive residues in soil. USA Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.

Jenkins, T.F., P.H. Miyares and M.E. Walsh (1988a) An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 88-23.

Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1988b) Development of an analytical method for the determination of explosive residues in soil. Part II: Further development and ruggedness testing. USA Cold Regions Research and Engineering Laboratory, CRREL Report 88-8.

Leggett, D.C., T.F. Jenkins and P.H. Miyares (1990) Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water. *Analytical Chemistry*, **62**: 1355–1356.

Maskarinec, M.P., D.L. Manning, R.W. Harvey, W.H. Griest and B.A. Tomkins (1984) Determinations of munitions components in water by resin adsorption and high-performance liquid chromatography-electrochemical detection. *Journal of Chromatography*, **304**: 51–63.

Matkovich, C.E. and G.D. Christian (1973) Salting-out of acetone from water: Basis of a new solvent extraction system. *Analytical Chemistry*, **45**(11): 1915– 1921.

Meyer, V.R. (1988) *Practical High Performance Liquid Chromatography*. New York: John Wiley and Sons.

Mueller, B.J. and R.J. Lovett (1987) Salt-induced phase separation for the determination of metals as their diethyldithiocarbamate complexes by high-performance liquid chromatography. *Analytical Chemistry*, **59**: 1405– 1409.

Murkerjee, P. (1965) Salt effects on nonionic association colloids. *The Journal of Physical Chemistry*, **69** (11), 4038–4040.

Nagaosa, Y. (1980) Salting-out of polar solvents from aqueous solution and its application to ion-pair extractions. *Analytica Chimica Acta*, **120:** 279–287.

NRC (1982) Evaluation of the health risks of ordnance disposal waste in drinking water. National Research Council, Committee on Toxicology. Washington, D.C.: National Academy Press.

Phillips, J.H., R.J. Coraor and S.R. Prescott (1983) Determination of nitroaromatics in biosludges with gas chromatography/thermal energy analyzer. *Analytical Chemistry*, **55:** 889–892.

Richard, J.J. and G.A. Junk (1986) Determination of munitions in water using macroreticular resins. *Analytical Chemistry*, **58**: 725–727.

Spanggord, R.J., B.W. Gibson, R.G. Keck, D.W. Thomas and J.J. Barkley (1982) Effluent analysis of wastewater generated in the manufacture of 2,4,6-trinitrotoluene. 1. Characterization study. *Environmental Science and Technology*, **16**: 229–232.

Supelco, Inc. (1987) Supelco chromatographic supplies. Supelco Park, Belfonte, Pennsylvania., p. 150.

USATHAMA (1987) U.S. Army Toxic and Hazardous Materials Agency installation restoration program. Quality Assurance Program. Aberdeen Proving Ground, Maryland.

USEPA (1988a) Health advisory for hexahydro-1,3,5-trinitro-1,3,5-triazine. U.S. Environmental Protection Agency, Office of Drinking Water, Washington, D.C.

USEPA (1988b) Health advisory for octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazozine. U.S. Environmental Protection Agency, Office of Drinking Water, Washington, D.C.

USEPA (1989) Health advisory for 2,4,6-trinitrotoluene. U.S. Environmental Protection Agency, Office of Drinking Water, Washington, D.C.

Valis, R.J., T.A. Bishop and K.E. Williams (1989) Validation of a sorbent tube/high-performance liquid chromotographic procedure for the determination of eight explosives in water. U.S. Army Environmental Hygiene Agency (AEHA), Aberdeen Proving Ground, Maryland.

APPENDIX A: DOCUMENTATION OF PRECERTIFICATION CALIBRATION IN USATHAMA (1987) FORMAT

I. SUMMARY

A. Analytes: The following analytes can be determined using this analytical method: RDX, 135TNB, 13DNB, 246TNT, 24DNT, 26DNT, 2ADNT and 4ADNT.

B. Matrix: This method is suitable for the determination of nitroaromatics and nitramines in ground and surface water samples.

C. General Method: This method involves the extraction of a 400-mL water sample by the addition of 130 g of NaCl and 100 mL of acetonitrile (ACN). Upon phase separation, the ACN phase is collected, reduced to 1.0 mL by means of a Kuderna–Danish microconcentrator, then combined with 3.0 mL of water. Determination is by reversed-phase HPLC on an LC-8 (3.3 cm × 4.6 mm, 3 µm) column eluted with a ternary eluent of 70.7/27.8/1.5 (v/v/v) water–methanol–tetrahydrofuran at 2.0 mL/min. Detection is by UV at 254 nm.

II. APPLICATION

A. Calibration Range: The linear range of each analyte concentration for this method is shown in Table A1.

B. Tested Concentration Range: The range of each analyte concentration over which this method was tested is shown in Table A2.

C. Sensitivity: The response of the UV detector at λ = 254 nm for each analyte is presented in Table A3.

D. Interferences: While baseline separation is not achieved for 2ADNT and 4ADNT, resolution is sufficient so that the two can be determined simultaneously if present at similar concentrations. It is not possible to quantitate HMX due to substantial interferences introduced by the salt. EPA monitoring requirements for HMX are 1.8 mg/L (McLellan et al. 1988) and the CRL for HMX using the direct injection method is 13 µg/L (Jenkins et al. 1988). The monitoring requirements for HMX then are such that the direct injection method is adequate for HMX determination. The retention times for the certified analytes as well as potential interferences for the LC-8 and LC-8/LC-CN separations are presented in Table A4. The LC-8/LC-CN series of columns was shown to be satisfactory for confirmation of the primary analytes.

All of the glassware must be meticulously cleaned because of the large preconcentration factor used. NaCl must be reagent grade or better and must have been stored in glass bottles only (not plastic). Plastic containers introduce unknown interfering compounds.

E. Safety Information: The normal safety precautions appropriate to use of flammable organic solvents should be employed.

Table A1. Concentration ranges for calibration of analyte standards.

	Concentration of standards (µg/L)	
Analyte	*Low*	*High*
RDX	2.01	10,037
135TNB	2.01	10,026
23DNB	2.00	10,013
246TNT	2.03	6,079
24DNT	2.00	10,014
26DNT	2.05	10,228
2ADNT	2.11	10,528
4ADNT	2.30	11,486

Table A2. Tested concentration ranges of the analytes.

	Concentration of samples (µg/L)	
Analyte	*Low*	*High*
RDX	0.010	10.0
135TNB	0.010	10.0
13DNB	0.010	10.0
246TNT	0.010	10.1
24DNT	0.010	10.0
26DNT	0.010	10.2
2ADNT	0.010	10.0
4ADNT	0.012	12.4

Table A3. Sensitivity of UV detector for analytes at λ = 254 nm.

Analytes	*Sensitivity (Abs/µg/L)*	*Absorbance[a] at CRL*
RDX	3.97×10^{-6}	1.33×10^{-3}
TNB	1.17×10^{-5}	1.20×10^{-3}
DNB	1.14×10^{-5}	4.92×10^{-4}
TNT	5.75×10^{-6}	3.05×10^{-4}
24DNT	5.81×10^{-6}	4.76×10^{-5}
26DNT	3.65×10^{-6}	4.58×10^{-4}
2ADNT	2.56×10^{-6}	3.57×10^{-5}
4ADNT	1.68×10^{-6}	4.02×10^{-5}

[a] Calculated based on a concentration factor of 400.

Table A4. Retention times and capacity factors for LC-8 primary separation and LC-8/LC-CN confirmation separations. Columns eluted with 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF eluent, LC-8 at 2.0 mL/min and LC-8/LC-CN at 1.5 mL/min.

	Retention time (min.)		*Capacity factor* * *(K')*	
Analyte	*LC-8*	*LC-8/LC-CN*	*LC-8*	*LC-8/LC-CN*
SEX	0.4	2.65	0.68	3.95
TAX	0.3	1.77	0.27	2.30
HMX	0.74	6.74	2.12	11.6
RDX	1.31	4.37	4.53	7.14
TNB	1.10	2.49	3.64	3.64
DNB	1.58	3.29	5.67	5.13
TNT	2.40	4.03	9.13	8.18
Tetryl	3.50	9.19	13.8	16.1
Benzene	2.24	3.71	8.45	5.91
24DNT	3.24	6.13	12.7	10.4
26DNT	3.61	6.69	14.2	11.5
2ADNT	4.56	9.01	18.2	15.8
4ADNT	4.85	9.23	19.5	16.2
Toluene	5.4	8.27	21.8	14.4
Mono-NTs	4.1–4.5	6.97–7.55	17.1	12.6
KNO_3	0.238	0.537		

* Capacity factor is calculated on the unretained peak of KNO_3.

$K' = \left(\bar{t}_r - \bar{t}_{no_3}/\bar{t}_{no_3}\right)$.

$\bar{t}_r$ = mean retention time of the analyte (min).

$\bar{t}_{no_3}$ = mean retention time of unretained KNO_3(min).

III. APPARATUS AND CHEMICALS

A. Instrumentation

1. HPLC System: HPLC Spectra Physics SP8810 pump (or equivalent), an injector equipped with a 100-µL injection loop and a Spectra Physics SP8490 UV detector set to 254 nm (or equivalent variable wavelength or fixed 254 nm detector). The RP-HPLC column is eluted with water, methanol and tetrahydrofuran (70.7/27.8/1.5) (v/v/v) at 2.0 mL/min.

2. Strip chart recorder (Linear 500 or equivalent).

3. Digital Integrator (HP3393A or equivalent) equipped with an external disc drive (HP9114B or equivalent).

4. Autosampler (optional) (Dynatech LC-241 or equivalent).

5. LC-8 (Supelco) RP-HPLC column, 3.3 cm × 4.6 mm (3 µm).

B. Analytes

1. RDX (hexahydro-1,3,5-trinitro- 1,3,5-triazine)
   BP: decomposes
   MP: 203.5°C
   Solubility in water at 25°C: 60 mg/L
   Octanol/water partition coefficient: 7.5
   CAS #121-82-4
2. 135TNB (1,3,5-trinitrobenzene)
   BP: decomposes
   MP: 122°C
   Octanol/water partition coefficient: 15
   CAS #99-35-4
3. 13DNB (1,3-dinitrobenzene)
   BP: 302°C
   MP: 122°C
   Octanol/water partition coefficient: 31
   CAS #99-65-0
4. 246TNT (2,4,6-trinitrotoluene)
   MP: 80.1°C
   Solubility in water: 130 mg/L
   Octanol/water partition coefficient: 68
   CAS #118-96-7
5. 24DNT (2,4-dinitrotoluene)
   BP: 300°C (decomposes)
   MP: 70°C
   Solubility in water: 300 mg/L
   Octanol/water partition coefficient: 95
   CAS #121-14-2
6. 26DNT (2,6-dinitrotoluene)
   MP: 66°C
   Solubility in water (25°C): 206 mg/L
   Octanol/water partition coefficient: 97
   CAS #606-20-2
7. 2ADNT (2-amino-4,6-dinitrotoluene)
   Octanol/water partition coefficient: 88.2*
   CAS #35572-78-2
8. 4ADNT (4-amino-2,6-dinitrotoluene)
   Octanol/water partition coefficient: 81.5*
   CAS #1946-51-0

C. Reagents and SARM

1. RDX (SARM quality)
2. 135TNB (SARM quality)
3. 13DNB (SARM quality)
4. 246TNT (SARM quality)
5. 24DNT (SARM quality)
6. 26DNT (SARM quality)
7. 2ADNT (reagent grade)
8. 4ADNT (reagent grade)
9. Methanol (HPLC grade)
10. Water (reagent grade)
11. Acetonitrile (HPLC grade)
12. Tetrahydrofuran (HPLC grade)

IV. PRECERTIFICATION CALIBRATION

1. Preparation of Standards

Solid material (SARM or reagent grade) for each analyte are dried to constant weight in a vacuum des-

* Estimated (Jenkins 1989).

sicator in the dark. Approximately 0.1 g (100 mg) of each dried SARM or dried reagent is weighed out to the nearest 0.1 mg and transferred to individual 100-mL volumetric flasks and diluted to volume with HPLC-grade ACN. Stock standards are stored in a refrigerator at 4°C in the dark. Stock standards are usable for a period up to 1 year after the date of preparation. The concentration of each stock standard is presented in Table A5.

Table A5. Concentrations of stock standards for initial calibration.

Analyte	Concentration (mg/L)
RDX	1003.72
TNB	1002.60
DNB	1001.32
TNT	1013.24
24DNT	1001.40
26DNT	1022.80
2ADNT	701.87
4ADNT	765.70

Two identical combined analyte stock solutions labeled A and AA are prepared by combining 2.00 mL with 6.00 mL of water in individual scintillation vials and manually shaken at least 10 times. The precertification calibration standards are analyzed singly in random order. The acceptability of a linear model for each analyte is assessed using the protocol specified in the USATHAMA QA Program (2nd edition, March 1987). Experience has shown that a linear model with a zero intercept is appropriate. Therefore, the response factor for each analyte is taken to be the slope of the best-fit regression line.

V. PROCEDURE

1. Procedure

A. Separations: The separation of analytes is achieved by means of RP-HPLC employing an LC-8 (3.3 cm × 4.6 mm) (3 µm) column eluted with a ternary eluent composed of 70.7/27.8/1.5 (v/v/v) of water/

Table A6. Concentrations of the analytes in the stock, combined and calibration standards (µg/L).

Label		Level	RDX	TNB	DNB	TNT	24DNT	26DNT	2ADNT	4ADNT
		Stocks	1,004,000	1,003,000	1,001,000	1,013,000	1,001,000	1,023,000	701,900	765,700
A	AA	Combined	20,100	20,100	20,000	20,300	20,000	20,500	21,100	23,000
B	BB	10,000	10,100	10,100	10,000	10,200	10,000	10,300	10,600	11,500
C	CC	6,000	6,030	6,030	6,000	6,090	6,000	6,150	6,330	6,900
D	DD	2,000	2,010	2,010	2,000	2,030	2,000	2,050	2,110	2,300
E	EE	1,000	1,010	1,010	1,000	1,020	1,000	1,030	1,030	1,150
F	FF	600	603	603	600	609	600	615	633	690
G	GG	200	201	201	200	203	200	205	211	230
H	HH	100	101	101	100	102	100	103	106	115
I	II	60	60.3	60.3	60.0	60.9	60.0	61.5	63.3	69.0
J	JJ	20	20.1	20.1	20.0	20.3	20.0	20.5	21.1	23.0
K	KK	10	10.0	10.0	10.0	10.2	10.0	10.3	10.5	11.5
L	LL	6	6.03	6.03	6.00	6.09	6.00	6.15	6.33	6.90
M	MM	2	2.01	2.01	2.00	2.03	2.00	2.05	2.11	2.30

each of this RDX, 135TNB, 13DNB, 246TNT, 24DNT and 26DNT analyte stock standards, and 3.00 mL each of this 2ADNT and 4ADNT analyte stock standards in 100-mL volumetric flasks and diluting with ACN. The analyte concentration of solution A and AA are ~20,000 µg/L for all analytes (Table A6). Two identical series of precertification calibration standards are prepared following the dilutions presented in Table A7. These standard solutions are labeled B through M and BB through MM, respectively.

Precertification calibration standards are stored in a refrigerator at 4°C in the dark and are usable for up to 28 days.

2. Instrument Calibration

A 2.00-mL aliquot of each precertification calibration standard B through M and BB through MM is combined

Table A7. Dilutions for calibration standards.

	Dilution pattern				Approximate concentrations
	Series 1		Series 2		
Std	Aliquot (mL)	Std	Aliquot (mL)	Flask (mL)	of analytes (µg/L)
B	25 of A	BB	25 of AA	50	10,000
C	15 of A	CC	15 of AA	50	6,000
D	10 of B	DD	10 of BB	50	2,000
E	10 of B	EE	10 of BB	100	1,000
F	10 of C	FF	10 of CC	100	600
G	1 of A	GG	1 of AA	100	200
H	1 of B	HH	1 of BB	100	100
I	1 of C	II	1 of CC	100	60
J	1 of D	JJ	1 of DD	100	20
K	1 of E	KK	1 of EE	100	10
L	1 of F	LL	1 of FF	100	6
M	1 of G	MM	1 of GG	100	2

*See Table A6 for exact concentrations for each analyte.

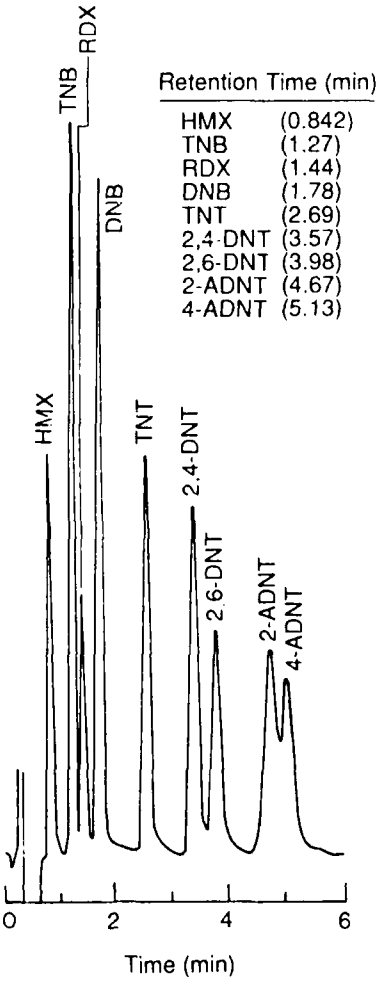


Figure A1. Primary separation for the salting-out procedure.
Column: LC-8 (3.3 cm × 4.6 mm, 3 µm)
Eluent: 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF
Flow: 2.0 mL/min
λ: 254 nm

methanol/THF at 2.0 mL/min. Retention times and capacity factors for the separation are found in Table A4. A chromatogram of the separation is shown in Figure A1.

B. Instrumental Analysis: Samples are introduced onto the column by means of an injection valve equipped with a 100-µL sample loop. This loop is flushed with 500 µL of sample. Detection is by UV at λ = 254 nm. Peak height determination is by manual measurement of an analog (strip chart) trace or by digital integration.

VI. CALCULATION

To obtain a precertification calibration curve for each analyte, the protocol for precertification outlined in USATHAMA QA Program (2nd ed. March 1987) is employed.

VII. REFERENCES

1. **Hubaux, A. and G. Vos** (1970) Decisions and detection limits for linear calibration curves. *Analytical Chemistry*, **42**: 849–855.

2. **Jenkins, T.F.** (1989) Development of an analytical method for the determination of extractable nitroaromatics and nitramines in soil. Ph.D. Thesis, University of New Hampshire.

3. **Jenkins, T.F., P.H. Miyares and M.E. Walsh** (1988) An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 88-23.

4. **McLellan, W., W.R. Hartley and M. Brower** (1988) Health advisory for octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, D.C., August.

5. **Spanggord, R.J., B.W. Gibson, R.G. Keck and G.W. Newell** (1978) Mammalian toxicological evaluation of TNT wastewaters. SRI International, Menlo Park, California, vol. 1.

6. **USATHAMA** (1987) U.S. Army Toxic and Hazardous Materials Agency installation restoration program, quality assurance program. Aberdeen Proving Ground, Maryland.

APPENDIX B: DOCUMENTATION OF CERTIFICATION IN USATHAMA (1987) FORMAT

I. SUMMARY

A. Analytes: The following analytes can be determined using this analytical method: RDX, 135TNB, 13DNB, 246TNT, 24DNT, 26DNT, 2ADNT and 4ADNT.

B. Matrix: This method is suitable for the determination of nitroaromatics and nitramines in ground and surface water.

C. General Method: This method involves the extraction of a 400-mL water sample by adding 130 g of NaCl and 100 mL of acetonitrile (ACN). Upon phase separation, the ACN phase is collected, reduced to 1 mL by means of a Kuderna–Danish microconcentrator, then combined with 3 mL of water. Determination is by reversed-phase HPLC on an LC-8 (3.3 cm × 4.6 mm, 3 μm) column eluted with a ternary eluent of 70.7/27.8/1.5 (v/v/v) water–methanol–tetrahydrofuran at 2.0 mL/min. Detection is by UV at 254 nm.

II. APPLICATION

A. Tested Concentration Range: The ranges of analyte concentration over which this method was tested are shown in Table A2.

B. Sensitivity: The response of the UV detector at λ = 254 nm for each analyte is presented in Table A3.

C. Reporting Limits: Certified Reporting Limits (CRL) for the following analytes were determined over a four-day period using the method of Hubaux and Vos as described in the USATHAMA Installation Restoration Program Quality Assurance Program (1987). CRL values for the analytes are presented in Table B1.

Table B1. Certified Reporting Limits (CRL) for the salting-out extraction method.

Analytes	CRL (μg/L)
RDX	0.836
135TNB	0.258
13DNB	0.108
246TNT	0.113
24DNT	0.0205
26DNT	0.314
2ADNT	0.0349
4ADNT	0.0598

D. Interferences: While baseline separation is not achieved for 2ADNT and 4ADNT, resolution is sufficient so that the two can be determined simultaneously. It is not possible to quantitate HMX due to substantial interference introduced by the salt. EPA monitoring requirements for HMX are 1.8 mg/L (McLellan et al. 1988) and the CRL for HMX using the direct injection method is 13 mg/L (Jenkins et al. 1988). Thus, current monitoring requirements for HMX can be met with the direct injection method. The retention times for the certified analytes as well as potential interferences for the LC-8 and LC-8/LC-CN separations are presented in Table A4. The LC-8/LC-CN series of columns was shown to be satisfactory for confirmation of the primary analytes.

All of the glassware must be meticulously cleaned because of the preconcentration of the sample. NaCl must be reagent grade or better and must have been stored in glass bottles only (not plastic). Plastic containers can introduce unknown interfering compounds.

E. Analysis Rate: In an 8-hour work day, 12 samples can be processed and analyzed along with appropriate calibration standards.

F. Safety Information: The normal safety precautions appropriate to the use of flammable organic solvents, hot plates and preconcentrators should be employed.

III. APPARATUS AND CHEMICALS

A. Glassware/hardware

1. 500-mL separatory funnels equipped with Teflon stopcock (1/sample)
2. Volumetric pipettes: 200 mL (1), 100 mL (2), 50 mL (1), 20 mL (1), 10 mL (2), 7 mL (1), 5 mL (1), 4 mL (7), 3 mL (1), 2 mL (1), 1 mL (15), glass
3. Volumetric flasks: 250 mL (10), 100 mL (10)
4. Teflon-capped vial: 40 mL, glass (1/clear sample; 2/turbid sample)
5. Scintillation vials: 20 mL, glass (2/sample)
6. Autosampler vials: glass, Teflon-faced septa (1/sample)
7. Disposable syringes: Plastipak, 5 mL (1/sample)
8. Filters: 0.5-μm Millex SR, disposable (1/sample)
9. Powder funnel: glass (1)
10. Balance: accuracy (±0.01 g), capacity (> 150 g)
11. Kuderna–Danish microconcentrators: 2.0-mL

receiving vessel, 40-mL flask, 10-cm distillation column (1/sample)

B. Instrumentation

1. HPLC System: HPLC Spectra Physics SP8810 pump (or equivalent), an injector equipped with a 100-µL injection loop and a Spectra Physics SP8490 UV detector set to 254 nm (or equivalent variable wavelength or fixed 254-nm detector). The RP-HPLC column is eluted with an eluent comprised of water, methanol, tetrahydrofuran (70.7/27.8/1.5) (v/v/v) at 2.0 mL/min.

2. Strip chart recorder (Linear 500 or equivalent)

3. Digital Integrator (HP3393A or equivalent) equipped with an external disc drive (HP9114B or equivalent)

4. Autosampler (optional) (Dynatech LC-241 or equivalent)

5. LC-8 (Supelco) RP-HPLC column, 3.3 cm×4.6 cm (3 µm)

C. Analytes

1. RDX (hexahydro-1,3,5-trinitro- 1,3,5-triazine)
   BP: decomposes
   MP: 203.5°C
   Solubility in water at 25°C: 60 mg/L
   Octanol/water partition coefficient: 7.5
   CAS #121-82-4
2. 135TNB (1,3,5-trinitrobenzene)
   BP: decomposes
   MP: 122°C
   Octanol/water partition coefficient: 15
   CAS #99-35-4
3. 13DNB (1,3-dinitrobenzene)
   BP: 302°C
   MP: 122°C
   Octanol/water partition coefficient: 31
   CAS #99-65-0
4. 246TNT (2,4,6-trinitrotoluene)
   BP: 280°C (explodes)
   MP: 80.1°C
   Solubility in water: 130 mg/L
   Octanol/water partition coefficient: 68
   CAS #118-96-7
5. 24DNT (2,4-dinitrotoluene)
   BP: 300°C (decomposes)
   MP: 70°C
   Solubility in water: 300 mg/L
   Octanol/water partition coefficient: 95
   CAS #121-14-2
6. 26DNT (2,6-dinitrotoluene)
   MP: 66°C
   Solubility in water (25°C): 206 mg/L
   Octanol/water partition coefficient: 97
   CAS #606-20-2
7. 2ADNT (2-amino-4,6-dinitrotoluene)
   Octanol/water partition coefficient: 88.2*
   CAS #35572-78-2
8. 4ADNT (4-amino-2,6-dinitrotoluene)
   Octanol/water partition coefficient: 81.5*
   CAS #1946-51-0

D. Reagents and SARMs

1. RDX (SARM quality)
2. 135TNB (SARM quality)
3. 13DNB (SARM quality)
4. 246TNT (SARM quality)
5. 24DNT (SARM quality)
6. 26DNT (SARM quality)
7. 2ADNT (reagent grade)
8. 4ADNT (reagent grade)
9. NaCl (reagent grade)
10. ACN (HPLC grade)
11. Methanol (HPLC grade)
12. Water (reagent grade)
13. THF (HPLC grade)

IV. CALIBRATION

A. Initial Calibration

1. Preparation of Standards: Analyte material (SARM or reagent grade) was dried to constant weight in a vacuum dessicator in the dark. Approximately 0.1 gm (100 mg) of each dried SARM or dried reagent was weighed out to the nearest 0.1 mg and transferred to individual 100-mL volumetric flasks and diluted with HPLC-grade ACN. Stock standards are stored in a refrig-

Table B2. Dilutions for calibration standards. All dilutions are in ACN.

Std	*Aliquot (mL)*	*Size of flask (mL)*	*Concentrations* (µg/L)*
N	25 of M	50	10,000
P	15 of M	50	6,000
Q	10 of N	50	2,000
R	10 of N	100	1,000
S	10 of P	100	600
T	1 of M	100	200
U	1 of N	100	100
V	1 of P	100	60
W	1 of Q	100	20
X	1 of R	100	10
Y	1 of S	100	6
Z	1 of T	100	2

*See Table B3 for exact concentrations for each analyte.

* Estimated (Jenkins 1989).

Table B3. Concentrations of the analytes in the stock, combined and calibration standards (µg/L).

Label		*Level*	*RDX*	*TNB*	*DNB*	*TNT*	*24DNT*	*26DNT*	*2ADNT*	*4ADNT*
		Stocks	1,004,000	1,003,000	1,001,000	1,013,000	1,001,000	1,023,000	701,900	765,700
A	AA	Combined	20,100	20,100	20,000	20,300	20,000	20,500	21,100	23,000
B	BB	10,000	10,100	10,100	10,000	10,200	10,000	10,300	10,600	11,500
C	CC	6,000	6,030	6,030	6,000	6,090	6,000	6,150	6,330	6,900
D	DD	2,000	2,010	2,010	2,000	2,030	2,000	2,050	2,110	2,300
E	EE	1,000	1,010	1,010	1,000	1,020	1,000	1,030	1,030	1,150
F	FF	600	603	603	600	609	600	615	633	690
G	GG	200	201	201	200	203	200	205	211	230
H	HH	100	101	101	100	102	100	103	106	115
I	II	60	60.3	60.3	60.0	60.9	60.0	61.5	63.3	69.0
J	JJ	20	20.1	20.1	20.0	20.3	20.0	20.5	21.1	23.0
K	KK	10	10.0	10.0	10.0	10.2	10.0	10.3	10.5	11.5
L	LL	6	6.03	6.03	6.00	6.09	6.00	6.15	6.33	6.90
M	MM	2	2.01	2.01	2.00	2.03	2.00	2.05	2.11	2.30

erator at 4°C in the dark. Stock standards are usable for up to 1 year after the date of preparation. The concentration each stock standard is presented in Table A5.

A combined analyte stock solution labeled M is prepared by combining 2.00 mL each of the RDX, 135TNB, 13DNB, 246TNT, 24DNT and 26DNT analyte stock standards, and 3.00 mL each of the 2ADNT and 4ADNT analyte stock standards in a 100-mL volumetric flask and diluting with ACN. The analyte concentration of solution A is ~20,000 µg/L for all analytes (Table B3). A series of calibration standards are prepared following the dilutions presented in Table B2 and labeled N through Z, respectively.

Calibration standards are stored in a refrigerator at 4°C in the dark. Calibration standards are usable for up to 28 days.

2. Instrument Calibration: A 2.00-mL aliquot of each calibration standard N through Z is combined with 6.00 mL of water in individual scintillation vials and shaken well (by hand). The calibration standards are analyzed in duplicate in random order. The acceptability of a linear model for each analyte is assessed using the protocol specified in the USATHAMA QA Program (2nd edition, March 1987). Experience has shown that a linear model with a zero intercept is appropriate. Therefore, the response factor for each analyte is taken to be the slope of the best-fit regression line.

B. Daily Calibration

1. Preparation of Standards: Stock standards for each analyte are prepared in an identical manner to that described for the initial calibration above. The concentrations of the stock standards are presented in Table B4. A combined analyte standard solution labeled MM is prepared by combining 4.00 mL of 4ADNT stock standard and 2.00 mL each of RDX, 135TNB, 13DNB, 246TNT, 24DNT, 26DNT and 2ADNT stock standards in a 250-mL volumetric flask and diluting to volume with ACN. The concentrations of the analytes in solution MM are listed in Table B4. The daily calibration standard (labeled NN) is prepared by pipetting 25 mL of solution MM into a 50-mL volumetric flask and diluting to volume with ACN. The analyte concentrations in solution NN are listed in Table B4. The daily calibration standard solution is stored in a refrigerator at 4°C in the dark and is usable for 28 days.

2. Instrumental Analysis: For analysis, a 3.00-mL aliquot of solution NN is combined with 9.00 mL of water in a scintillation vial and shaken (by hand). This dilution is required to match to mobile-phase polarity. This standard solution is analyzed in duplicate at the beginning of each day of analysis and singly at the midpoint and at the end of each day of analysis. Response factors for each analyte are obtained from the mean peak height and compared with the response factors obtained for the initial calibration.

The mean response factor for the daily calibration must agree within ±25% of the response factor of the

Table B4. Concentrations of combined analyte calibration solution (MM) and daily calibration solution (NN).

Analyte	*Stock (mg/L)*	*MM (µg/L)*	*NN (µg/L)*
RDX	1004	8030	4015
135TNB	1023	8021	4010
213DNB	1001	8011	4005
246TNT	1013	8016	4053
24DNT	1001	8011	4006
26DNT	1023	8182	4091
2ADNT	999.6	7997	3998
4ADNT	620.0	9920	4960

initial calibration for the first seven daily calibrations and within two standard deviations of the initial calibration for subsequent calibrations.

If the criteria are not met, a new initial calibration must be obtained.

V. CERTIFICATION TESTING

A. Preparation of Spiking Standards

Individual analyte certification stock solutions are prepared in the identical manner to that described for the calibration stock standards above. A combined analyte certification solution labeled PP is prepared by combining 2.00 mL of RDX, 135TNB, 13DNB, 246TNT, 24DNT, 26DNT and 2ADNT certification stock solutions and 4.00 mL of 4ADNT certification stock solution in a 250-mL volumetric flask and diluting with ACN. A series of spiking certification solutions is prepared in the manner outlined in Table B5. Concentrations of analytes in the spiking solutions are presented in Table B6. Certification stocks and combined and spiking solutions are stored in a refrigerator at 4°C in the dark. Stock solutions are usable up to 1 year after the date of preparation. Combined solutions and spiking solutions are usable for up to 28 days.

Table B5. Dilution outline of certification spiking solutions.* All dilutions are in acetonitrile.

Solution	*Level*	*Aliquot (mL)*	*Size of flask (mL)*
QQ	500X	25 of PP	50
RR	200X	20 of PP	100
SS	100X	10 of PP	100
TT	50X	5 of PP	100
UU	20X	2 of PP	100
VV	10X	1 of PP	100
WW	5X	1 of QQ	100
XX	2X	1 of RR	100
YY	X	1 of SS	100
ZZ	0.5X	1 of TT	100

*Concentrations of analytes in the certification spiking solutions are listed in Table B6.

B. Preparation of Certification Samples

Eleven 400-mL volumes of Milli-Q water are pipetted into 500-mL separatory funnels (10 spiked samples and one blank). A 1.00-mL aliquot of the appropriate spiking solution (PP through ZZ) is added to each sample. The samples are shaken vigorously by hand. A 130-g sample of NaCl is added to each sample, and the samples are shaken until the NaCl has completely dissolved. Once dissolved, a 100-mL volume of ACN is pipetted into each sample, and the sample is shaken vigorously (by hand) for 5 minutes, venting as needed. The sample is allowed to stand for 30 minutes to allow the phases to separate.

The aqueous layers (lower) are drawn off and discarded. The ACN layers (upper) are collected in a 40-mL vial. A 5.00-mL aliquot of fresh ACN is pipetted into each separatory funnel to rinse the walls of the funnel and is then combined with the collected ACN extract. The collected ACN extract is examined for large water drops. If any are present, they are removed with a Pasteur pipette.

Each ACN sample is transferred to a Kuderna–Danish microconcentrator. The volume is reduced to less than 1 mL. The volume is then brought up to 1.0 mL with fresh ACN. The sample is then combined with 3.0 mL of Milli-Q water in a scintillation vial. The ACN–water sample is poured down through the Kuderna–Danish system to rinse the sides of the glass, then returned to the vial. The sample is then filtered through a Millex SR 0.5-µm filter into a clean scintillation vial.

VI. SAMPLE HANDLING AND STORAGE

A. Sampling Procedure: In collecting the sample, the sample container is rinsed three times with sample water.

Table B6. Concentrations of certification stock combined and spiking solutions.

	Level	*RDX*	*135TNB*	*13DNB*	*246TNT*	*24DNT*	*26DNT*	*2ADNT*	*4ADNT*
Stock		1,004,000	1,002,000	1,001,000	1,013,000	1,001,000	1,023,000	999,600	620,000
PP		8,032	8,020	8,010	8,110	8,010	8,180	8,000	9,920
QQ	500X	4,020	4,010	4,010	4,060	4,010	4,090	4,000	4,960
RR	200X	1,610	1,600	1,600	1,620	1,600	1,640	1,600	1,980
SS	100X	803	802	801	811	801	818	800	992
TT	50X	402	401	401	406	401	409	400	496
UU	20X	161	160	160	162	160	164	160	198
VV	10X	80.3	80.2	80.1	81.1	80.1	81.8	80.0	99.2
WW	5X	40.2	40.1	40.1	40.6	40.1	40.9	40.0	49.6
XX	2X	16.1	16.0	16.0	16.2	16.0	16.4	16.0	19.8
YY	X	8.03	8.02	8.01	8.11	8.01	8.18	8.00	9.92
ZZ	0.5X	4.02	4.01	4.01	4.06	4.01	4.09	4.00	4.96

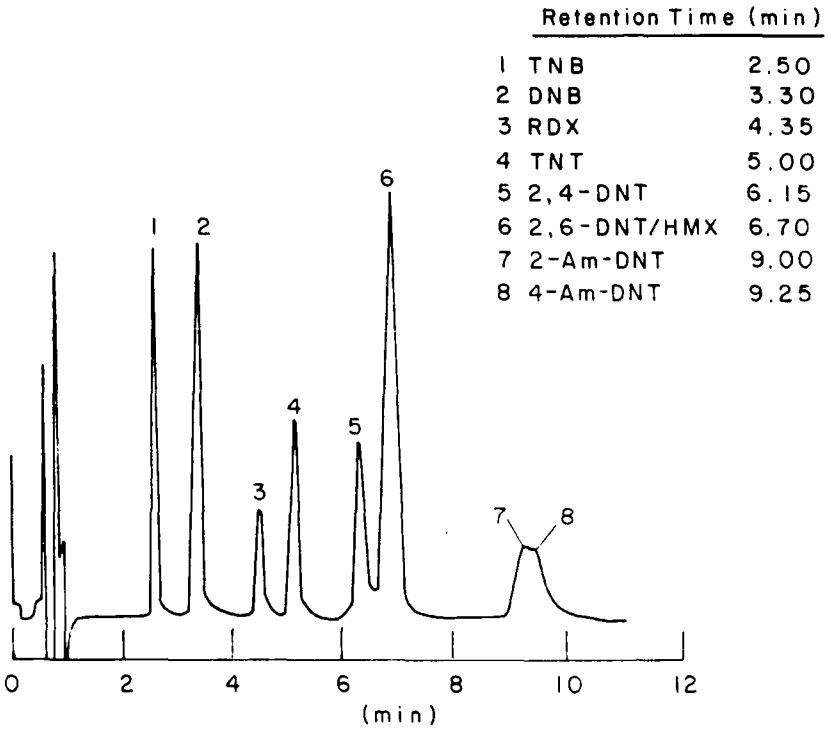


Figure B1. Confirmation separation for the salting-out procedure.
Column: LC-8/LC-CN series (both 3.3 cm×4.6 mm, 3 µm)
Eluent: 70.7/27.8/1.5 (v/v/v) H_2O/MeOH/THF
Flow: 1.5 mL/min
λ: 254 nm

then filled to the very top of the container and capped.

B. Containers: The sample container is an amber glass bottle equipped with a Teflon-lined screw cap.

C. Storage Conditions: Samples in the field are stored in coolers in the dark that are kept cool with ice or cold packs. Samples in the laboratory are stored in a refrigerator at 4°C in the dark.

D. Storage Limits: Samples must be extracted within 7 days of collection and extracts analyzed within 30 days.

VII. PROCEDURE

A. Preparation of Samples: A 400-mL volume of a water sample is measured by graduated cylinder into a 500-mL separatory funnel. A 130-g sample of NaCl is added to the sample, and the sample is shaken until the NaCl has completely dissolved. Once dissolved, a 100-mL volume of acetonitrile is pipetted into the sample. The sample is shaken vigorously (by hand) for 5 minutes, venting as needed. The sample is allowed to stand for 30 minutes to allow the phases to separate.

The aqueous layer (lower) is drawn off and discarded. The acetonitrile layer (upper) is collected in a 40-mL vial. A 5.00-mL aliquot of fresh acetonitrile is pipetted into the separatory funnel to rinse the walls of the funnel and is then combined with the collected extract. If the collected extract is turbid, it is centrifuged at 4000 rpm for 5 minutes. The acetonitrile extract is drawn off the centrifuged sample by Pasteur pipette and transferred to a clean vial. The acetonitrile extract is then examined for large water drops. If any are present, they are removed with a Pasteur pipette.

The extract is transferred to a Kuderna–Danish micro-concentrator. The volume is reduced to less than 1 mL, then brought up to 1.0 mL with fresh acetonitrile. The sample is then combined with 3.00 mL of Milli-Q water in a scintillation vial. The sample is poured down through the Kuderna–Danish system to rinse the sides of the glass, then returned to the vial. The sample is then filtered through a Millex SR 0.5-µm filter into a clean scintillation vial.

B. Determination: Determination of the analyte concentration in the sample solution is obtained by RP-HPLC-UV at λ = 254. A 100-µL sample is injected onto an LC-8 column eluted with 2.0 mL/min of 70.7/27.8/1.5 (v/v/v) water/methanol/THF. Retention times and capacity factors for the analytes of interest and a number of potential interferences are presented in Table A4 for both LC-8, the primary column, and LC-8/LC-CN series, the confirmation column. Chromatograms obtained for the primary analytes are shown in Figures A1 and B1.

VIII. CALCULATION

A. Response Factors: Since a linear calibration curve with a zero intercept is to be expected, calculations of results on a daily basis are obtained using response factors calculated for each analyte. The mean response ($\bar{R}$) for each analyte from repeated determinations of

Table B7. Initial control limits for percent recoveries from duplicates certification samples (10X) and certification samples (2X) using three-point moving averages.

	TNB	*RDX*	*DNB*	*TNT*	*24DNT*	*26DNT*	*2ADNT*	*4ADNT*
a. Duplicates certification samples (10X).								
For means								
UWL	180.8	104.6	128.3	109.3	115.3	114.4	116.9	116.7
UCL	198.0	106.4	146.6	119.8	129.1	127.8	127.9	129.0
LWL	112.7	97.3	55.4	67.6	60.6	61.1	73.3	68.1
LCL	95.5	95.5	37.0	57.1	46.8	47.7	62.3	55.9
For range								
UWL	68.4	7.3	73.2	41.9	55.0	53.5	43.8	48.8
UCL	89.0	9.5	95.2	54.6	71.5	69.6	57.0	63.5
LCL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LWL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
b. 2X certification samples using three-point moving averages.								
For means								
UWL	176.9	170.9	132.7	122.4	119.7	111.2	125.0	120.0
UCL	178.5	190.0	142.1	131.6	125.1	116.9	139.4	128.2
LWL	170.4	94.4	94.9	85.7	98.4	88.6	67.3	87.2
LCL	168.8	75.3	85.5	76.5	93.1	82.9	52.9	79.0
For range								
UWL	9.8	115.0	56.7	55.2	32.1	34.0	86.7	49.3
UCL	12.2	144.4	71.3	69.3	40.3	42.7	108.9	61.9
LCL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
LWL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

solution P is obtained in peak height units. The response factor (RF) for each analyte is obtained by dividing the mean response by the known concentration (C) in units of µg/L:

$$RF = \frac{R}{C}.$$

B. Analyte Concentration: The concentrations (µg/L) of each analyte (C_a) are obtained by dividing the response for each analyte (R_a) by the appropriate response factor (RF_a), then dividing by the preconcentration factor of 400:

$$C_a = \frac{R_a}{RF_a} \div 400.$$

IX. DAILY QUALITY CONTROL

A. Control Spikes: Spiked water samples are prepared as described for Class I method in the USATHAMA QA Program (2nd Edition, March 1987). This requires the use of a method blank, a single spike at two times the certified reporting limit and duplicate spikes at ten times the certified reporting limit for each analytical lot. Control spikes are prepared using the appropriate spiking solution in an identical manner as described in Section V.

B. Control Charts: The control charts required are described for Class I methods in USATHAMA QA Program (2nd Edition, March 1987). This will require use of standard Shewhart $\bar{X}$ and R charts for the duplicate high spike (Table B7a) and moving average $\bar{X}$ and R charts for the single low spike (Table B7b). Details on the charting procedures required are specified in USA THAMA QA Program (2nd Edition, March 1987).

X. CERTIFICATION OF NON-SARMS

The 2ADNT and 4ADNT were obtained from Dr. David Kaplan, U.S. Army Natick Laboratories (Natick, Massachusetts). These materials were not SARMs but

Table B8. Top ten fragments of mass spectrum for 2ADNT and 4ADNT.

2ADNT		*4ADNT*	
m/e	*Relative abundance*	*m/e*	*Relative abundance*
180	100	180	100
197	78	197	60
78	64	104	51
104	41	78	33
77	34	105	30
52	32	52	24
51	26	77	24
105	23	51	22
134	19	93	17
133	17	94	15

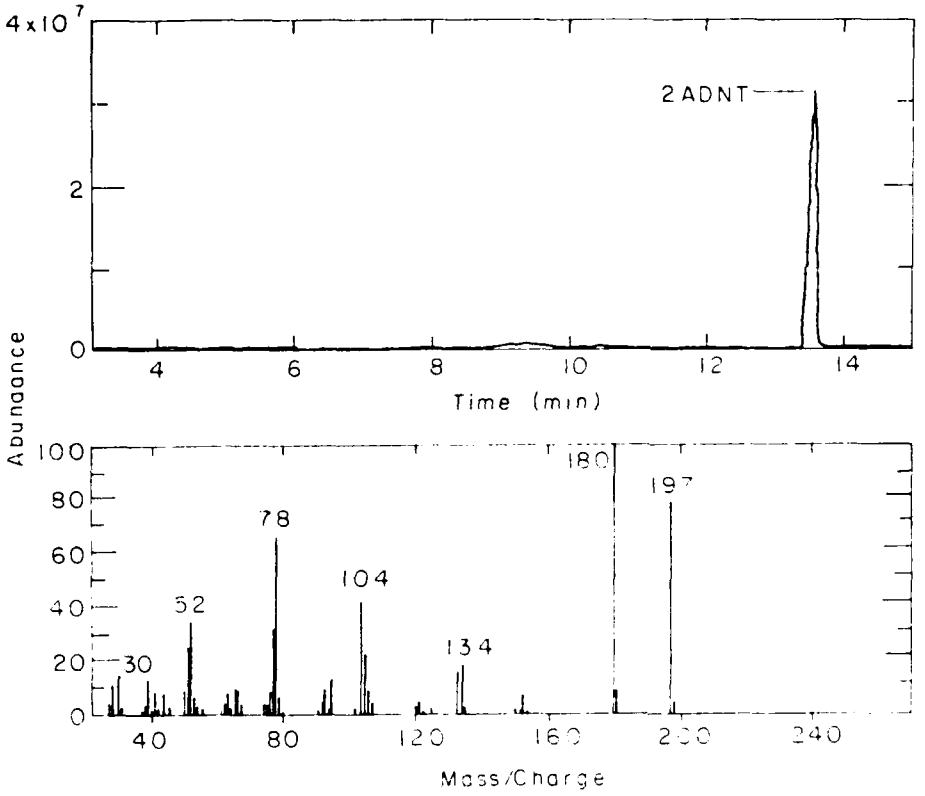


Figure B2. Chromatogram and mass spectrum of 2ADNT. Top: Total ion chromatogram using an HP-1 fused silica capillary column, a temperature program of 100 to 240°C at 20°C/min, and a scan of 30 to 300 m/e. Bottom. Mass spectrum of the 2ADNT peak in the chromatogram.

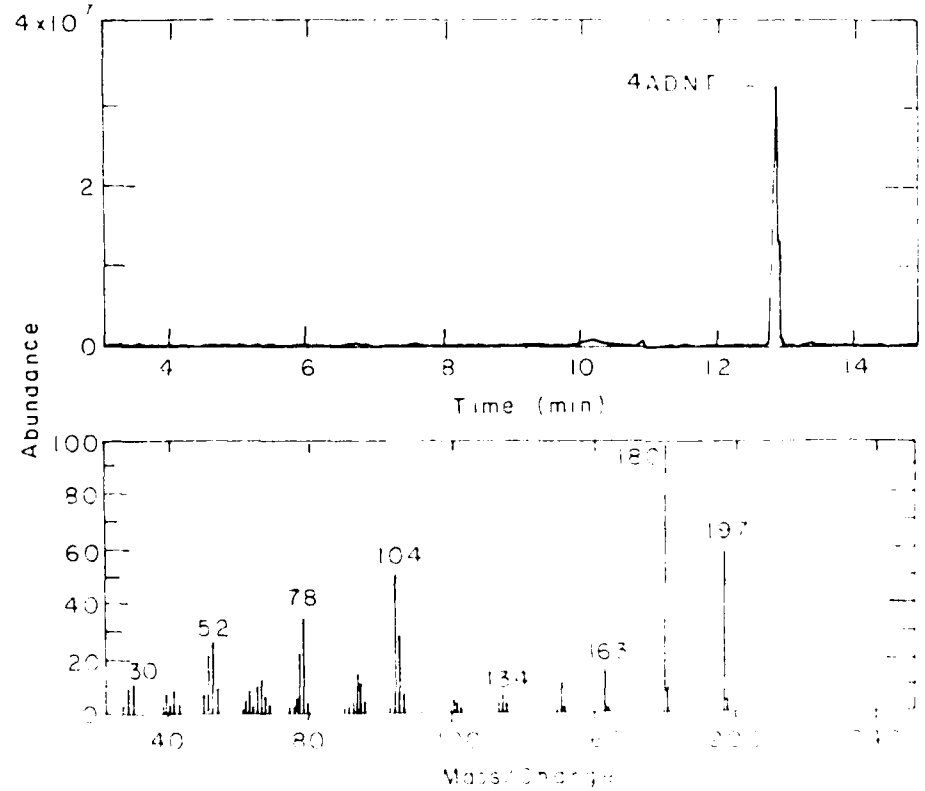


Figure B3. Chromatogram and mass spectrum of 4ADNT. Top: Total ion chromatogram using an HP-1 fused silica capillary column, a temperature program of 100 to 240°C at 20°C/min, and a scan of 30 to 300 m/e. Bottom: Mass spectrum of the 4ADNT peak in the chromatogram.

their purity was verified by GC/MS. The 2ADNT mass spectrum (Fig. B2) was verified against the Hewlett Packard mass spectrum library entry number #16458: Benzenamine, 2-methyl-3,5 dinitro-. The top ten fragments are presented in Table B8. Although a standard mass spectrum (Fig. B3) for 4ADNT was unavailable, the mass spectrum obtained was consistent with the structure of the compound. The top ten fragments are presented in Table B8.

XI. REFERENCES

1. **Hubaux, A. and G. Vos** (1970) Decisions and detection limits for linear calibration curves. *Analytical Chemistry*, **42**:8 49–855.

2. **Jenkins, T.F.** (1989) Development of an analytical method for the determination of extractable nitroaromatics and nitramines in soil. Ph.D. Thesis, University of New Hampshire.

3. **Jenkins, T.F., P.H. Miyares and M.E. Walsh.** (1988) An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USA Cold Regions Research and Engineering Laboratory, Special Report 88-23.

4. **McLellan, W., W.R. Hartley and M. Brower** (1988) Health advisory for octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine. Office of Drinking Water, U.S. Environmental Protection Agency, Washington, D.C., August.

5. **Spanggord, R.J., B.W. Gibson, R.G. Keck and G.W. Newell** (1978) Mammalian toxicological evaluation of TNT wastewaters. SRI International, Menlo Park, California, vol. 1.

6. **USATHAMA** (1985) U.S. Army Toxic and Hazardous Materials Agency installation restoration program, quality assurance program. Aberdeen Proving Ground, Maryland.

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13. ABSTRACT (*Maximum 200 words*)

A protocol was developed for determining low levels of nitroaromatics and nitramines in ground water. Sample preparation employs salting-out extraction with acetonitrile and NaCl, further preconcentration of extract by solvent evaporation on a Kuderna–Danish concentrator, dilution of the concentrate with water, and filtration through a 0.5-μm Millex-SR filter. Separation is achieved using reversed-phase high-performance liquid chromatography with an LC-8 (3.3 cm) column using a 70.7/27.8/1.5 (v/v/v) water–methanol–THF eluent, and determination is obtained on a UV detector at 254 nm. This procedure provides far lower detection limits than the earlier protocol, which involved direct injection onto an LC-18 (25 cm) column eluted with 50/50 (v/v) methanol–water. The new method is capable of simultaneously determining RDX, TNB, DNB, TNT, 2,4-DNT, 2,6-DNT, 2-Am-DNT and 4-Am-DNT in less than five minutes, with reporting limits ranging from 0.02 to 0.84 μg/L. Analytical recovery averaged greater than 95%.

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