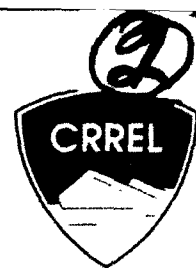


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SPECIAL REPORT

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Development of an Improved Confirmation Separation Suitable for Use With SW846 Method 8330

Thomas F. Jenkins and Susan M. Golden

June 1993

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Abstract

An improved RP-HPLC confirmation separation was developed that is suitable for use with EPA SW846 Method 8330. This separation provides adequate resolution of the analytes most commonly found in explosives-contaminated waters and soils. The separation is achieved on an LC-CN (cyanopropyl) column eluted with an eluent composed of water (65%), methanol (12%) and acetonitrile (23%) at 1.2 mL/min. Analysis of field-contaminated soil and groundwater samples indicate that this confirmation separation is an improvement over the confirmation separation currently recommended in SW846 Method 8330.

For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380-89a, *Standard Practice for Use of the International System of Units*, published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.

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Special Report 93-14



**US Army Corps
of Engineers**

Cold Regions Research &
Engineering Laboratory

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PREFACE

This report was prepared by Dr. Thomas F. Jenkins, Research Chemist, Geological Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory (CRREL) and Susan M. Golden, Chemist, Science and Technology Corporation. Funding was provided by the U.S. Army Environmental Center (formerly the U.S. Army Toxic and Hazardous Materials Agency), Aberdeen Proving Ground, Maryland, Martin H. Stutz, *Project Monitor*.

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Development of an Improved Confirmation Separation Suitable for Use With SW846 Method 8330

THOMAS F. JENKINS AND SUSAN M. GOLDEN

INTRODUCTION

Several years ago CRREL developed a method for the determination of residues of nitroaromatics and nitramines in explosives-contaminated soils, based on reversed-phase high performance liquid chromatography (RP-HPLC) (Jenkins et al. 1989). This method was collaboratively tested through the Association of Official Analytical Chemists (AOAC) (Bauer et al. 1990) and was subsequently adopted by the AOAC (1990), the American Society for Testing and Materials (1991) and the U.S. Environmental Protection Agency (1992) as the standard method for this analysis.

For this method air-dried soils are extracted with acetonitrile (ACN) in a temperature controlled ultrasonic (20°C) bath for 18 hours. The soil is allowed to settle and an aliquot of the ACN extract is mixed with an equal volume of an aqueous calcium chloride solution and filtered. The resulting extract is analyzed by RP-HPLC on a LC-18 column using an eluent composed of 1:1 methanol/water. If the chromatogram contains peaks corresponding to the target analytes (Table 1), the presence of those compounds is confirmed by RP-HPLC analysis on a second column. The confirmation column is an LC-CN (cyanopropyl) column eluted with 1:1 methanol/water at 1.5 mL/min. Retention times for the LC-CN separation and a summary of the abbreviations for the target analytes for Method 8330 are shown in Table 1. The selection of LC-CN for confir-

mation was based on the different mechanisms of separation found for the LC-18 and LC-CN (Jenkins 1989) and the ability to separate these compounds thought to occur most frequently in explosives-contaminated soil and water.

Over the past several years, this analytical method has been used extensively at CRREL, at other Corps of Engineers Division Laboratories, and at several contract laboratories working for the Army and the EPA. Recently an assessment of this method's ability to satisfy Army needs was conducted by examining the large data base of water and soil analyses accumulated at CRREL and the

Table 1. Retention times for target analytes in EPA SW846 Method 8330 on LC-CN column* (Jenkins et al. 1989).

Target analyte	Retention time (min)
nitrobenzene (NB)	3.81
1,3,5-trinitrobenzene (TNB)	4.05
1,3-dinitrobenzene (DNB)	4.18
o-nitrotoluene (2NT)	4.37
p-nitrotoluene (4NT)	4.41
m-nitrotoluene (3NT)	4.45
2,6-dinitrotoluene (2,6-DNT)	4.61
2,4-dinitrotoluene (2,4-DNT)	4.87
2,4,6-trinitrotoluene (TNT)	5.00
4-amino-2,6-dinitrotoluene (4-Am-DNT)	5.10
2-amino-4,6-dinitrotoluene (2-Am-DNT)	5.15
hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	6.15
methyl-2,4,6-trinitro-phenylnitramine (Tetryl)	7.36
octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	8.35

* Eluent 1:1 methanol/water at 1.5 mL/min
Column 25 cm x 4.6 mm (5 µm) (Supelco)

Missouri River Division Laboratory (Walsh et al., 1993). Two major conclusions of this study were 1) the target analyte list should be modified to include 3,5-dinitroaniline (DNA) and four current target compounds, nitrobenzene and the three isomers of nitrotoluene, should be dropped, and 2) an improved confirmation separation should be developed that provides a better separation for 2,4-dinitrotoluene (2,4-DNT) and 2,4,6-trinitrotoluene (TNT). A large TNT concentration, often several orders of magnitude higher than that of 2,4-DNT, interferes with confirmation of 2,4-DNT using the current confirmation separation.

The objective of this study is to develop an improved confirmation separation that provides adequate resolution of the target analytes of Method 8330, even when TNT or RDX is present in large concentrations, a common occurrence for explosives-contaminated soils.

EXPERIMENTAL

Chemicals

Stock standards for the target analytes for Method 8330 (Table 2) were prepared from Standard Analytical Reference Materials (SARM) obtained from the U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland. In addition, a stock standard for 3,5-dinitroaniline (DNA) was prepared from material obtained from Aldrich, and its identity was confirmed by GC/MS. Water used in the preparation of the aqueous calcium chloride (CaCl_2) solution and in the preparation of HPLC eluent was reagent grade water from a Milli-Q Type 1 Reagent Grade Water System (Millipore Corp.). Methanol used in the preparation of eluent was Alltech HPLC grade. Acetonitrile used for soil extraction and for preparation of eluent was Baker Analyzed HPLC grade. Eluent was prepared daily by combining the appropriate volumes of water, acetonitrile and methanol and vacuum filtering through a nylon-66 membrane (0.45 μm) to degas and remove particulate matter.

Instrumentation

RP-HPLC retention time data were obtained on a modular system composed of the following:

1. Spectra Physics Model 8800 ternary HPLC pump.
2. Spectra Physics Spectra 100 variable wavelength UV detector set at 254 nm with a cell path length of 0.6 cm.

3. Hewlett-Packard model HP 3393A digital integrator equipped with a Hewlett-Packard Model HP911B disk drive.

4. Linear Model 500 strip chart recorder.

Separations

All separations were obtained on a Supelco LC-CN column (25 cm $\times$ 4.6 mm, 5 μm) using either binary or ternary eluents composed of water, methanol and acetonitrile.

Field-contaminated soil and groundwater samples

Air-dried soil from Department of Defense installations including Hawthorne Army Ammunition Plant (Hawthorne, Nevada), Nebraska Ordnance Works (Mead, Nebraska) and Hastings East Industrial Area (Hastings, Nebraska), and contaminated groundwater from Hastings and the Rockeye Site, Naval Surface Warfare Center, Crane, Indiana were used to test various eluents. Soil extracts were obtained as described by Jenkins et al. (1989).

Preparation of stock standards

All individual analyte stock standards were prepared by weighing out approximately 100 mg of dried standard material to the nearest 0.1 mg, transferring it to individual 100-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. Combined analyte test solutions were prepared by combining appropriate volumes of these stock standards and diluting to volume with acetonitrile.

RESULTS AND DISCUSSION

Initial experiments were conducted using the LC-CN column with different eluents than used in the standard method (50/50 V/V water/methanol). The first eluent tested was composed of 65/35 (V/V) water/methanol (MeOH) at a flow rate of 1.2 mL/min; this eluent is used at the Corps of Engineers Missouri River Division Laboratory.* Retention times of the 15 analytes of interest using this separation are shown in Table 2. This separation is inadequate, however, in several respects.

* P.S. Schnitker, Missouri River Division Laboratory, personal communication, 1992.

Table 2. Retention times of target nitroaromatic and nitramine analytes on LC-CN column with various eluents (1.2 mL/min).

	% Water/% Methanol/% Acetonitrile (V/V/V)							
	65/35/0	65/30/5	65/25/10	65/20/15	65/15/20	15/10/25	65/5/30	65/0/35
Nitrate ion	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1
NB	6.2	—	—	6.1	—	—	—	—
TNB	6.4	6.8	6.8	7.1	7.3	7.4	7.6	8.0
DNB	6.8	6.9	6.8	6.8	6.8	6.6	6.7	6.8
2-NT	8.3	8.1	7.8	7.8	7.5	7.3	7.4	7.5
4-NT	8.4	8.1	7.7	7.7	7.6	7.3	7.4	7.5
3-NT	8.6	8.3	7.9	7.9	7.7	7.5	7.5	7.1
TNT	9.1	9.6	9.7	10.1	10.3	10.2	10.7	11.1
2,4-DNT	9.3	9.2	8.9	8.9	8.7	8.3	8.4	8.5
2,6-DNT	9.3	9.2	9.1	9.1	8.8	8.5	8.6	8.7
DNA	9.9	—	—	8.9	8.3	7.7	7.6	7.5
4-Am-DNT	12.3	11.9	11.0	10.6	9.9	9.0	8.8	8.7
RDX	12.7	11.6	10.2	9.4	8.5	7.7	7.4	7.2
2-Am-DNT	12.9	12.1	11.1	10.6	9.9	9.0	8.8	8.6
Tetryl	19.5	18.8	17.2	16.5	15.7	14.8	14.5	14.3
HMX	22.0	19.1	16.3	14.4	12.7	10.9	10.2	9.5

The resolution between TNT and 2,4-DNT is only 0.2 min. When TNT is present in much higher concentration, as is often the case, the ability to confirm the presence of a much smaller concentration of 2,4-DNT is poor. Inadequate separation of 2,4-DNT and TNT was the major problem with the standard confirmatory separation. An additional problem is the very late elution of HMX, which reduces the sensitivity for that analyte and substantially increases analytical run times. Nevertheless, this separation proved to be a useful starting point for this evaluation.

Since ACN has been found to have a special affinity for HMX and RDX (Jenkins 1989), we felt that incorporating ACN in the eluent would reduce the retention time for HMX. In addition, changing from MeOH to ACN on some columns reverses the retention order of TNT and 2,4-DNT, and hence incorporation of ACN in the eluent could have a positive effect on the TNT/2,4-DNT separation as well.

To pursue this possibility, a series of experiments were conducted in which the aqueous portion of the eluent was maintained constant at 65% (by volume), but the 35% organic portion was varied between pure MeOH and pure ACN in 5% increments. Retention times of the 15 analytes of interest were obtained at each composition (Table 2). The retention times for nitrate (an unretained species) was also obtained to facilitate calculation of capacity factors (Table 2).

The effect of ACN incorporation was dramatic for HMX and RDX. Retention times for these two analytes were reduced from 22.0 and 12.7 minutes in water/methanol to 9.5 and 7.2 minutes, respectively, in water/ACN. Retention times for tetryl, 4-Am-DNT, and 2-Am-DNT and DNA, were also reduced significantly and smaller decreases were observed for the three isomers of NT and the two isomers of DNT. Retention times for TNT and TNB, interestingly, increased as the percentage of ACN was increased.

Recently, Walsh et al. (1993), summarized the results obtained from analysis of hundreds of soil and water samples from Army ammunition plants, arsenals and depots. The eight nitroaromatic and nitramine explosives, impurities and degradation products most commonly found in soils and waters from these sites are listed in Table 3, along with their frequency of occurrence. A plot of the retention times for these eight compounds, as a function of the percentage of acetonitrile in the eluent, is shown in Figure 1. It should be emphasized, however, that the total organic content of this eluent is maintained at 35% for this plot, the difference between 35% and the percentage of ACN being MeOH.

Clearly, the separation using the binary eluents (water/MeOH or water/ACN) is inadequate. For water/MeOH, the resolution between TNT and 2,4-DNT is poor, as is that for RDX and 2-Am-DNT. For water/ACN, the separation between 2,4-DNT

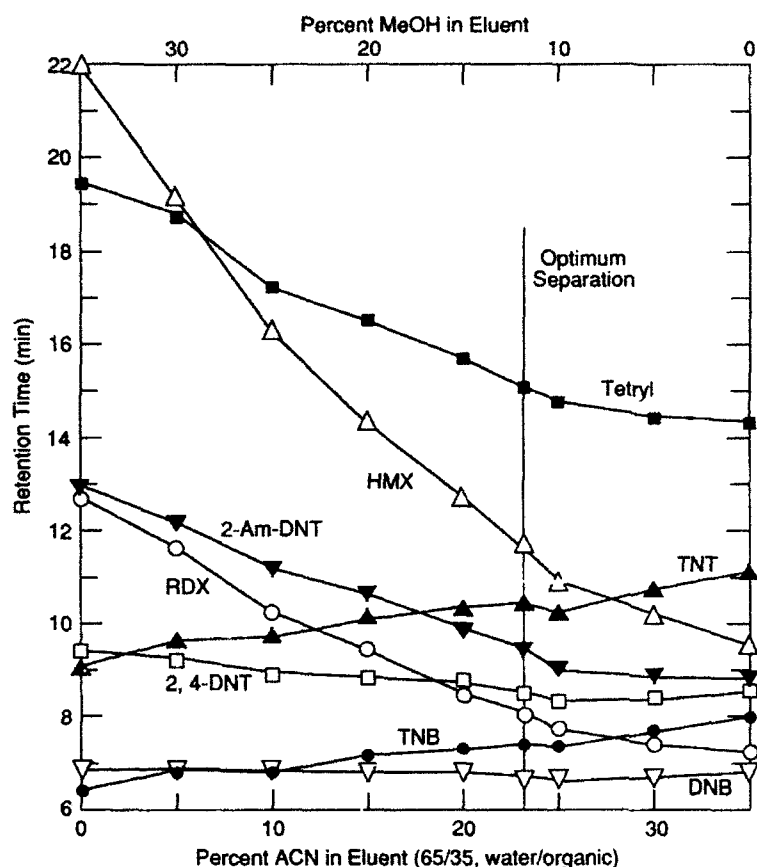


Figure 1. Retention times of target analytes on LC-CN column as a function of the percentage of acetonitrile and methanol in the eluent.

and 2-Am-DNT is inadequate. At several ternary eluents, however, adequate separation for all eight analytes appears to be achieved. Two eluents in particular look attractive. One is the eluent composed of 65/20/15 (V/V/V) water/MeOH/ACN. The only obvious problem is the fairly long reten-

tion time for HMX and tetryl, which would result in the inability to confirm the presence of these analytes at very low concentration due to peak broadening. In addition, 2,4-DNT cannot be adequately resolved from either DNA or 2,6-DNT, and the three isomers of NT are not resolved. A second useful separation appears to be possible between 20 and 25% ACN. In this region, HMX elutes much earlier, reducing the problem with detection capability. DNA, however, elutes near RDX (Table 2) and thus the ability to confirm the presence of DNA would be poor for soils containing RDX.

The best separation was achieved using an eluent of 65/12/23 (V/V/V) water/MeOH/ACN at 1.2 mL/min. (Table 4). These results are also shown on the plot in Figure 1. Chromatograms for a standard containing the eight primary target analytes (Walsh et al., 1993) is shown in Figure 2. A chromatogram showing the separation for all 15 target analytes is shown in Figure 3. The only significant problem demonstrated is the inability to separate RDX from DNA. Although the three isomers of NT are not adequately separated from TNB, these iso-

Table 3. Most frequently observed analytes in explosives contaminated soils and waters from ammunition plants, arsenals and depots (Walsh et al., 1993)

Analyte	Frequency of occurrence (%)	
	Soil	Water
TNT	80	56
TNB	44	28
RDX	35	61
2,4-DNT	28	21
DNB	22	13
2-Am-DNT	18	23
HMX	14	14
Tetryl	12	13

Table 4. Retention times for target analytes on LC-CN column eluted with water 65/12/23 (V/V/V) water/MeOH/ACN at 1.2 mL/min.

Analyte	Retention time (min)
Nitrate ion	2.10
NB	5.96
DNB	6.69
TNB	7.32
2-NT	7.41
4-NT	7.41
3-NT	7.61
DNA	8.03
RDX	8.07
2,4-DNT	8.51
2,6-DNT	8.69
2-Am-DNT	9.43
4-Am-DNT	9.44
TNT	10.36
HMX	11.66
Tetryl	15.07

mers are not commonly found in these samples (Walsh et al., 1993) and hence this does not seem to be a major problem. It is interesting that the two isomers of DNT are separated sufficiently to be able to confirm if one or both are present. Since they elute almost 2 minutes before TNT, they should be easily confirmed even when the concentrations of TNT is orders of magnitude higher than either DNT isomer.

Tests of this separation were conducted using soil extracts from Hastings East Industrial Area (Fig. 4), Hawthorne Army Ammunition Plant (Fig. 5) and Nebraska Ordnance Works (Fig. 6). The separation achieved in all cases was adequate for confirmation of the analyte present. Groundwater samples from Hastings (Fig. 7) and the Rockeye Site, Naval Surface Warfare Center (Fig. 8) were also analyzed using this separation. Again the separation achieved was adequate to confirm the presence of the analytes present in these samples.

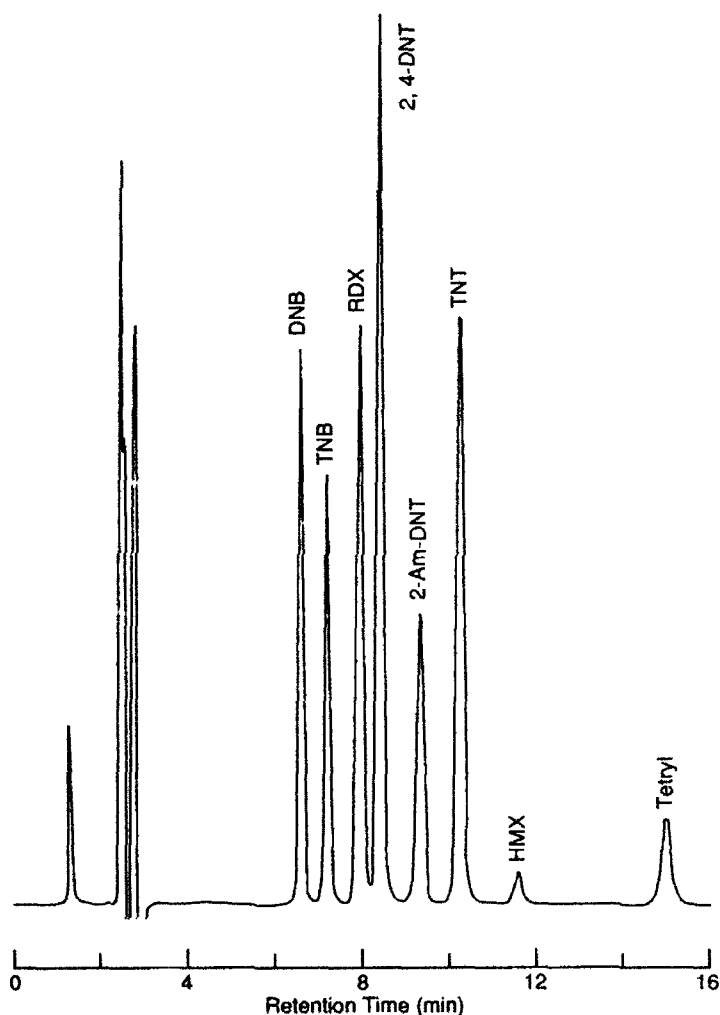


Figure 2. LC-CN chromatogram for the 8 primary target analytes with an eluent composition of 65:12:23 (V/V/V) water/MeOH/ACN at 1.2 mL/min.

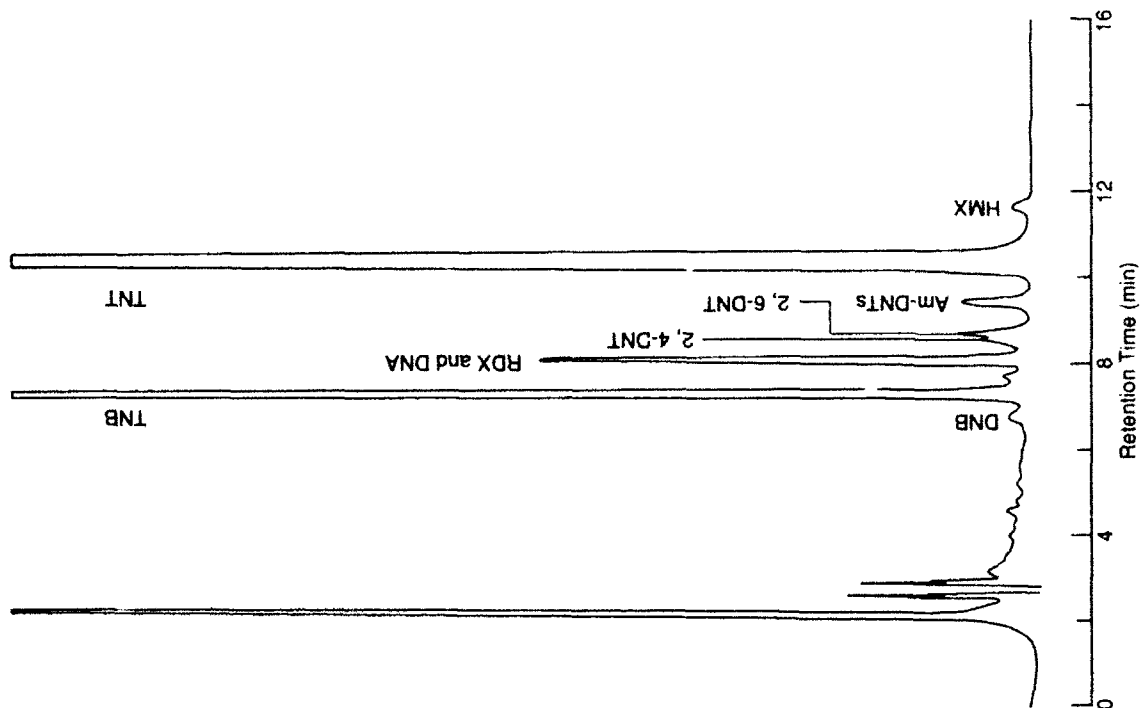


Figure 4. LC-CN chromatogram for soil extract from Hastings East Industrial Area (65:12:23 V/V/V water/MeOH/ACN at 1.2 mL/min).

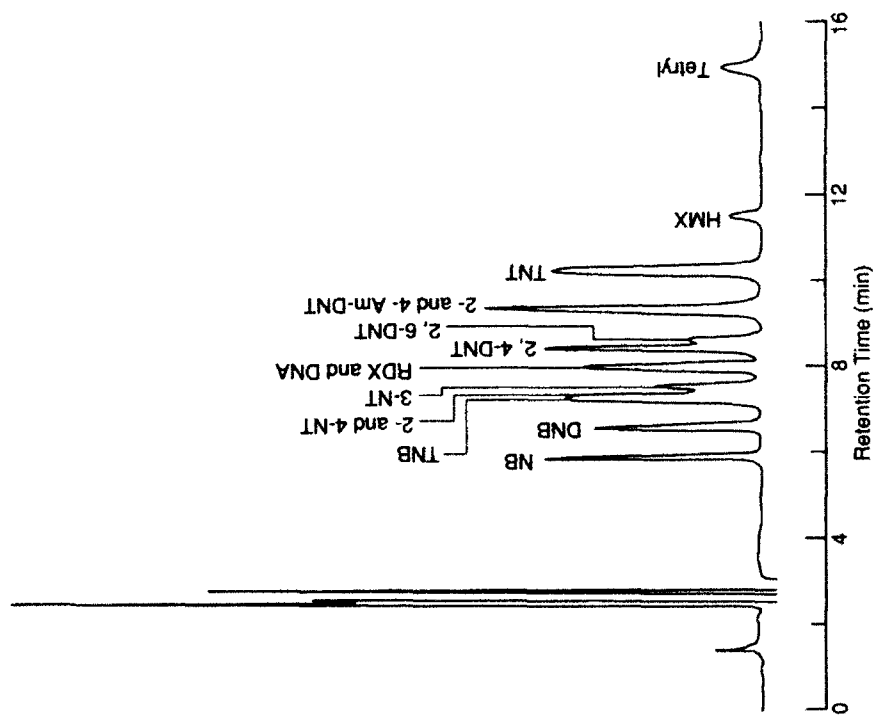


Figure 3. LC-CN chromatogram for 15 target analytes at an eluent composition of 65:12:23 (V/V/V) water/MeOH/ACN at 1.2 mL/min.

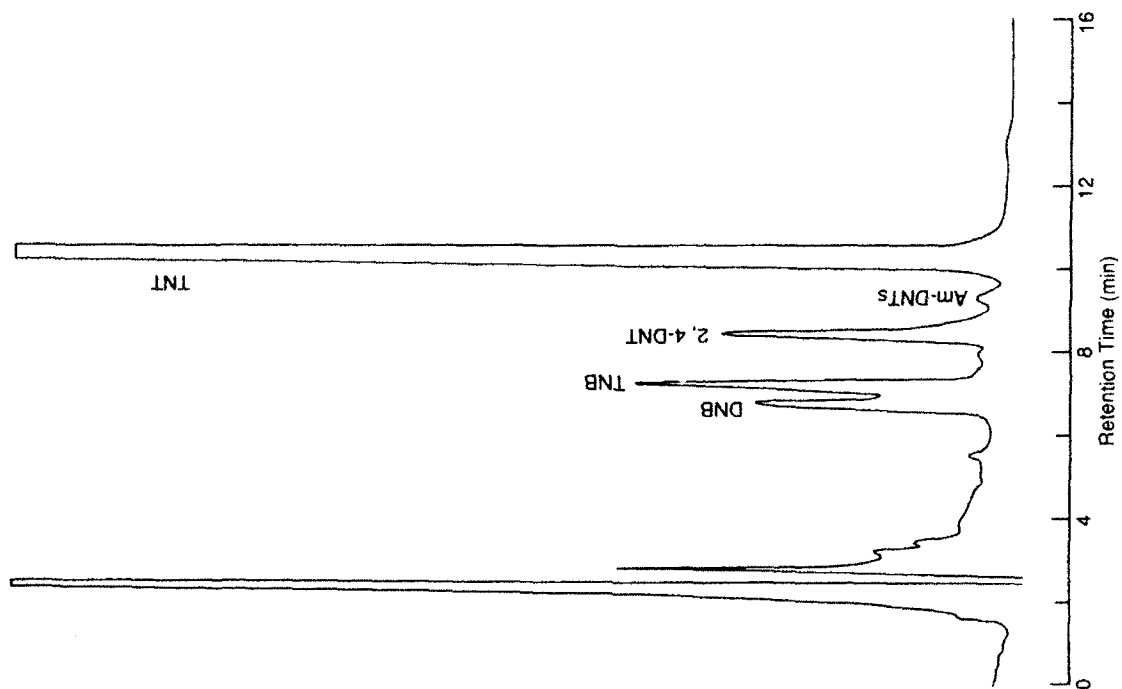


Figure 5. LC-CN chromatogram for soil extract from Hawthorne AAP (65:12:23 V/V/V water/MeOH/ACN at 1.2 mL/min).

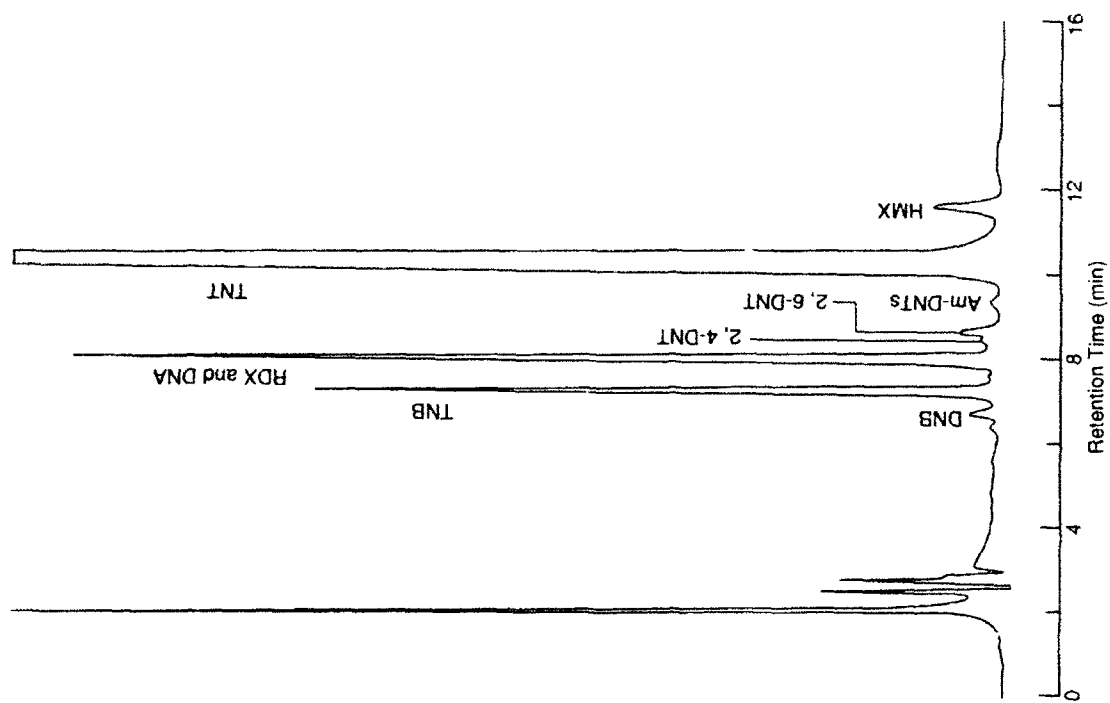


Figure 6. LC-CN chromatogram of soil extract from Nebraska Ordnance Works (65:12:23 V/V/V water/MeOH/ACN at 1.2 mL/min).

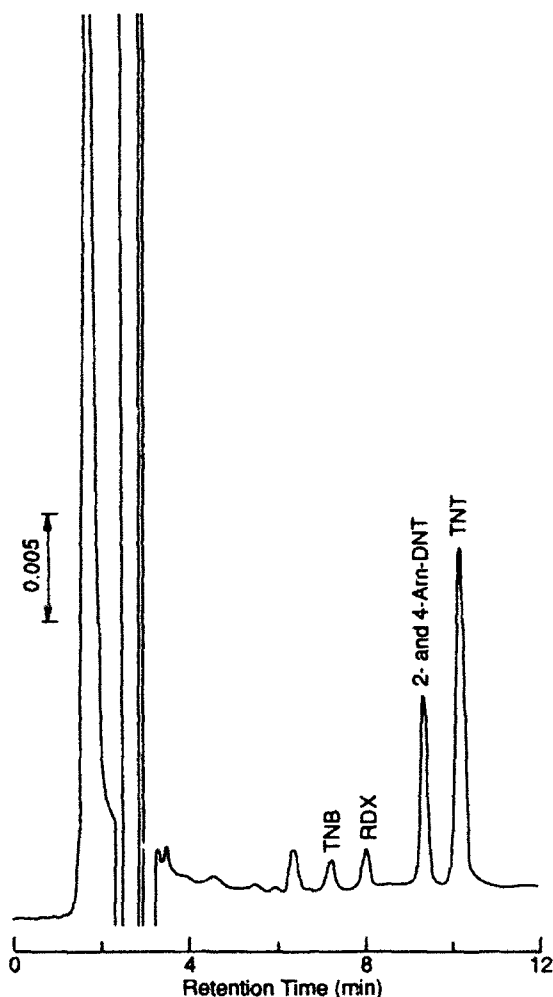


Figure 7. LC-CN chromatogram of groundwater sample from Hastings East Industrial Area (65:12:23 water/MeOH/ACN at 1.2 mL/min).

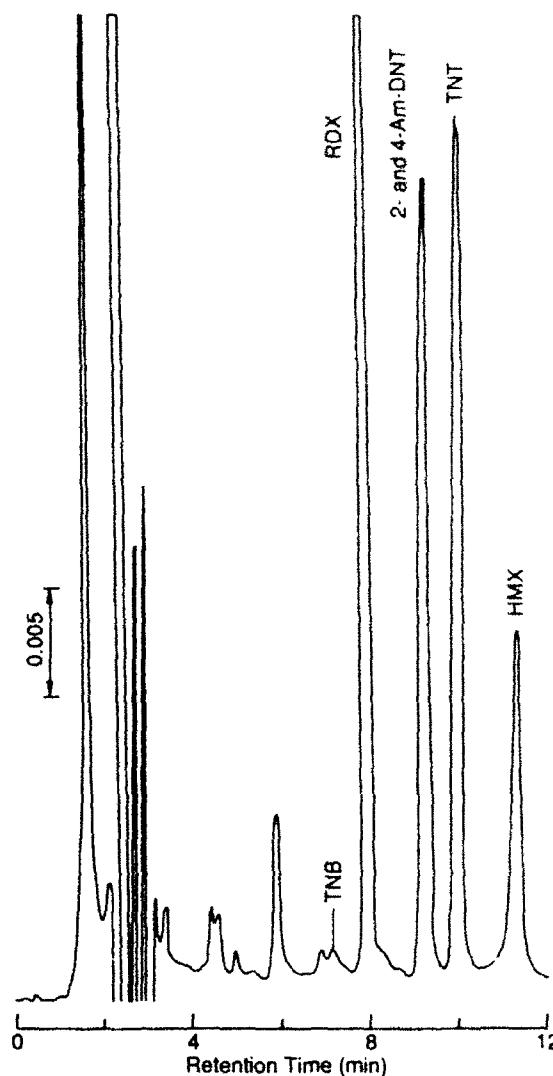


Figure 8. LC-CN chromatogram from groundwater sample from the Rockeye site, Naval Surface Warfare Center (65:12:23 V/V/V water/MeOH/ACN at 1.2 mL/min).

CONCLUSIONS

An improved RP-HPLC confirmation separation was developed that is suitable for use with EPA SW846 Method 8330. This separation provides adequate resolution of the analytes most commonly found in explosives-contaminated waters and soils. The separation is achieved on an LC-CN (cyanopropyl) column eluted with an eluent composed of water (65%), methanol (12%) and acetonitrile (23%) at 1.2 mL/min. Analysis of field-

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