Study of Surrogates for SW846 Method 8330

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Abstract

The compounds 1,4-dinitrobenzene (1,4-DNB), 1,2-dinitrobenzene (1,2-DNB), and 3,4-dinitrotoluene are investigated as possible surrogate compounds. Both 1,4-DNB and 1,2-DNB do not coelute with any method target compounds in a C8 column with the specification of 82% water and 18% isopropanol at 1.0 mL/min. Elution conditions using a cyanopropyl (LC–CN) confirmation column are studied for the separation of target analytes and the surrogate compound. Adequate separation on a LC–CN column can also be achieved for the 1,4-DNB and method target analytes using an eluent composed of 55% water, 40% methanol, and 5% acetonitrile at 1.2 mL/min. 1,4-DNB is suitable for use as a surrogate in the Environmental Protection Agency Solid Waste 846 Method 8330. It is shown that utilization of 1,4-DNB as a surrogate compound in Method 8330 analysis improves the defensibility of the data.

Introduction

Method 8330, described in the United States Environmental Protection Agency (EPA) Solid Waste 846 (SW846) manual, is intended for the determination of residues of nitroaromatics and nitramines in explosives-contaminated soils and water. This method is one of the most important EPA methods for environmental monitoring, especially in defense cleanup applications. The 14 target compounds are listed in Table I (1).

According to the description in method 8330, soil samples are first dried in air and extracted with acetonitrile (ACN) in a temperature-controlled ultrasonic bath for 18 h. Water samples are extracted with ACN using a salting-out technique. The ACN extract is then mixed with an equal amount of aqueous calcium chloride solution, filtered, and finally separated by a reversed-phase high-performance liquid chromatography (HPLC) column and detected by an ultraviolet (UV) detector at 254 nm.

According to SW846 method 8330, extracts are analyzed on a LC_{18} column using an eluent composed of water–MeOH (1:1, v/v) at a flow rate of 1.5 mL/min. If a positive compound is detected on the LC_{18} column, the method requires that the extracts be analyzed using a dissimilar column, cyanopropyl (LC–CN), in an attempt to confirm the earlier results. The eluent for the LC–CN confirmation column is also composed of water–MeOH (1:1, v/v) at 1.5 mL/min.

There have been several improvements to method 8330 since its publication by the EPA in 1992. An LC_8 column is commonly used in the laboratory industry as a replacement for the LC_{18} column because it has better separation for the target compounds. A solid-phase extraction method has been tested as an alternative extraction method to the relatively tedious

Table I. SW846 Method 8330 Analytes

Number	Compound	Abbreviation	CAS number
1	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
2	Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
3	1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
4	1,3-Dinitrobenzene	1,3-DNB	99-65-0
5	Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
6	Nitrobenzene	NB	98-95-3
7	2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
8	4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
9	2-Amino-4,6-dinitrotoluene	2-Am-DNT	355-72-78-2
10	2,4-Dinitrotoluene	2,4-DNT	121-14-2
11	2,6-Dinitrotoluene	2,6-DNT	606-20-2
12	2-Nitrotoluene	2-NT	88-72-2
13	3-Nitrotoluene	3-NT	99-08-1
14	4-Nitrotoluene	4-NT	99-99-0

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salting-out extraction method used for water samples (2).

Photodiode array (PDA) detectors are now available at an affordable price and are routinely utilized during the method 8330 analysis. In addition to the retention times of target analytes, information about UV absorption spectra in a preset range of wavelengths at a particular retention time can be provided by the PDA. As a result, it has been proposed that the PDA detector be used to replace the requirement of second column confirmation specified by the method (2).

Although the PDA detector can generate UV spectra that provide confirmational data for a single analysis, the UV spectra usually lack detailed structure; therefore, the confidence level of confirmation is not as high as that in mass spectroscopy analysis. Even in the final update (version III) of the SW846 manual, method 8000B, which governs all organic analysis methods in the manual including method 8330, is vague about whether PDA data can replace a second column confirmation (3). Consequently, secondary LC–CN column confirmation is often required in many federal defense cleanup projects.

Because the elution of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is close to the void volume, quantitation of HMX in the LC_8 or LC_{18} columns can be difficult for samples containing polar interferences. The LC–CN column, on the other hand, retains HMX significantly better than either a LC_8 or LC_{18} column, making it an excellent confirmation column for method 8330. One problem associated with the LC–CN column, however, is that there are several coelution pairs among the 14 target compounds under the elution conditions specified in the method (1). In some circumstances, coelution can make confirmation impossible.

Jenkins and Golden (4) tested many elution conditions on a LC–CN column and concluded that the best separation can be achieved using an eluent composed of water–MeOH–ACN (65:12:23, v/v/v) at 1.2 mL/min. Although the separation is still not comparable with that of an LC_8 column, under the suggested elution conditions, adequate separation on an LC–CN column for the most commonly found explosive analytes can be achieved.

In spite of these improvements for method 8330, the search for a proper surrogate compound still continues. Surrogate compounds are commonly used in most SW846 organic analysis methods to monitor the entire analytical process, including extractions or purges, various cleanups, solvent exchanges, dilution, and instrument performance for each sample. Surrogate compounds should be chemically similar to the target compounds. However, they must not coelute with the target compounds for gas chromatography or HPLC analysis. Surrogate compounds are spiked into each sample before extraction. When properly chosen, good surrogate compound recovery indicates a good performance of the method on the particular sample. To further improve analysis accuracy, an internal standard can also be used. The internal standard is added after sample extraction and before sample analysis. The use of an internal standard can correct minor instrument drift and therefore increase analysis precision.

One of the major drawbacks of adding surrogate or internal standard compounds is their coelution with target compounds. Because of its complex nature of separation in method 8330, internal standards are not commonly used in the environmental analysis industry in order not to further complicate the coelution problem. However, the surrogate compound is preferred in most of the organic analysis methods in the SW846 manual. Without surrogate compound recovery, it is difficult to assess the data quality of each sample.

Because the surrogate compound is added to all samples before extraction, coelution between the surrogate and target compounds pose a more severe problem than coelutions between target compounds. Because of the fact that a proper surrogate compound was not found, method 8330 does not assign or suggest any surrogate compound as in other EPA methods.

This paper presents elution results for three surrogate compound candidates. Based on the experimental results, 1,4-dinitrobenzene (1,4-DNB) is suggested as a surrogate compound for method 8330. In addition, elution conditions for achieving the best separation of all 14 target analytes and the surrogate compounds are proposed on both LC_8 and LC–CN columns.

Experimental

Chemicals

Intermediate stock solutions for all 14 target compounds were purchased from AccuStandard (New Haven, CT). Surrogate test compounds 1,2-dinitrobenzene (1,2-DNB) and 3,4-dinitrotoluene (3,4-DNT) were also purchased from AccuStandard. 1,4-DNB was obtained from Aldrich Chemical Company (Milwaukee, WI). ASTM type II water (5) was used in the preparation of the calcium chloride solution. Acetonitrile, methanol, and water for the HPLC elution were all HPLC grade.

Instrumentation

The HPLC system was a Hewlett-Packard (Wilmington, DE) HPLC 1100 equipped with an autosampler, degasser, quaternary pump, column temperature control compartment, and a PDA detector. The data acquisition and process system was the HP ChemStation for LC 3D software running on a PC computer. The UV absorption was at 254 nm for quantitations.

Columns

The HPLC LC_8 column was a Waters (Milford, MA) Nova-Pak C_8 (3.9 × 150 mm, 4-µm particle size). The HPLC LC–CN column was a Supelco (Bellefonte, PA) LC–CN (4.6 × 250 mm, 5-µm particle size).

Results and Discussion

Selection of surrogate compound

An ideal candidate for the surrogate compound, when compared to analytes, has a similar chemical composition and similar chemical behavior in the analytical process but is not

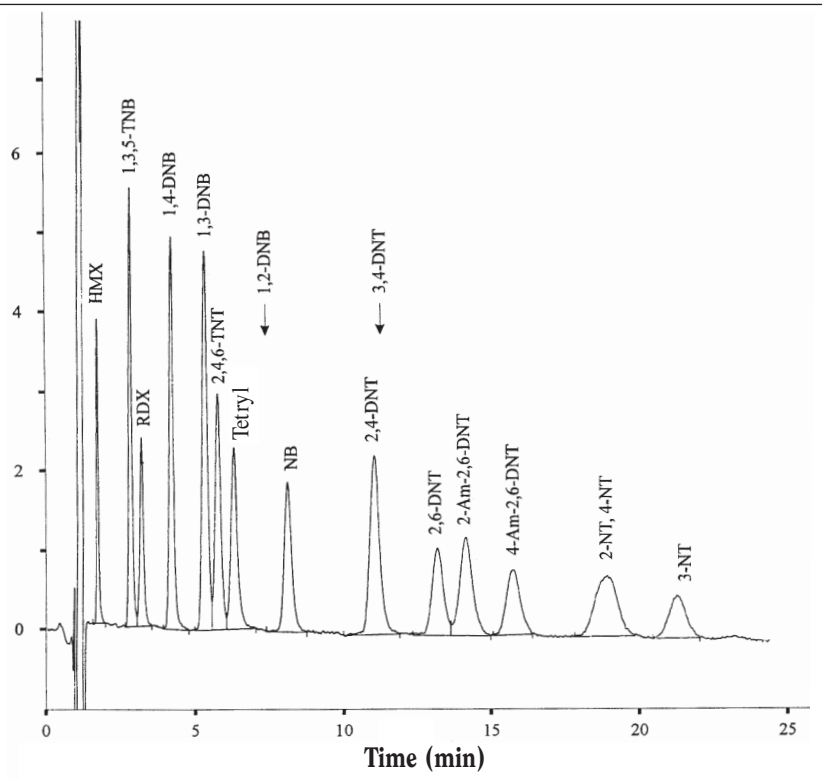

Figure 1. LC_8 chromatogram for method 8330 analytes with an eluent composed of water–isopropanol (82:18, v/v) at 1.0 mL/min. Retention times for surrogate compounds 1,2-DNB, 1,4-DNB, and 3,4-DNT are also indicated (although no chromatographic peaks are present for 1,2-DNB and 3,4-DNT).

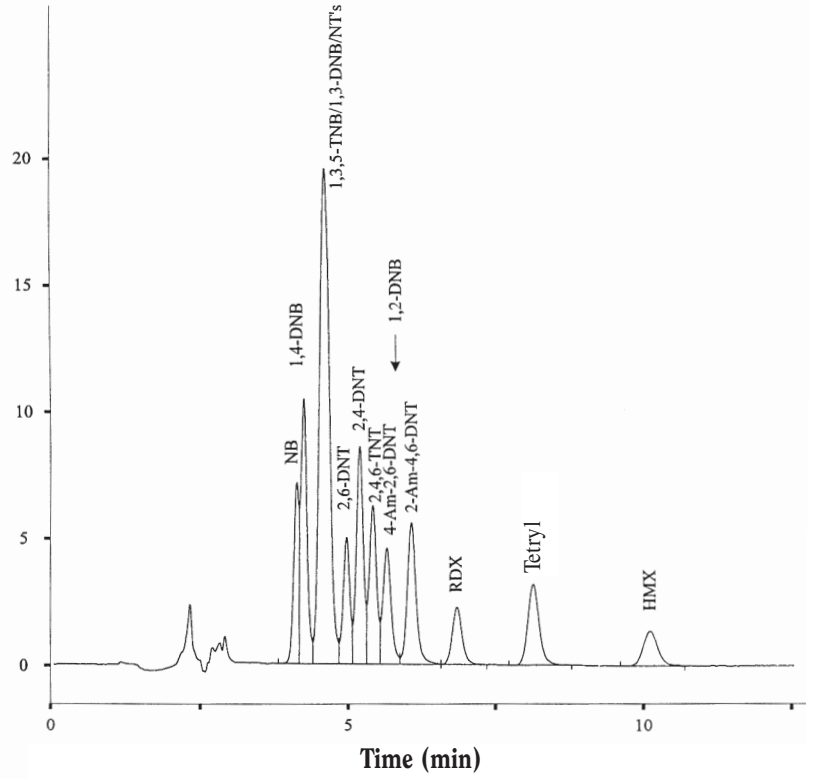

Figure 2. LC–CN chromatogram for method 8330 analytes with an eluent composed of water–MeOH (50:50, v/v) at 1.5 mL/min. Retention times for surrogate compounds 1,2-DNB and 1,4-DNB are also indicated.

normally found in environmental samples. In addition, the surrogate compound should not coelute with target compounds in both the primary and confirmatory columns. Because most of the analytes are nitroaromatics, the immediate candidates would be nitrobenzenes or nitrotoluenes (NTs), which are similar in chemical structure and behavior to the analytes. Nitrobenzene and all isomers of NT are method target analytes; trinitrobenzenes (TNBs) and trinitrotoluenes (TNTs) are very difficult to synthesize, except 1,3,5-TNB and 1,3,5-TNT, which are method target compounds. This leaves only dinitrobenzenes (DNBs) and dinitrotoluenes (DNTs) as possible surrogate compounds.

The only two DNB compounds that can be used for surrogates are 1,2-DNB and 1,4-DNB, because 1,3-DNB is a method target compound. Similarly, the only surrogate candidates in the NT series are 2,3-, 2,5-, 3,4-, and 3,5-DNT; 2,4- and 2,6-DNT are excluded for being method target compounds. However, 2,3-, 2,5-, and 3,5-DNT are not readily available, leaving only 3,4-DNT as a surrogate candidate.

Based on this reasoning, the present experiment is intended to search the best surrogate compound for explosive analysis among the following three compounds: 1,2-DNB, 1,4-DNB, and 3,4-DNT.

LC_8 column results

The three surrogate compounds were injected into a LC_8 column using the manufacturer's suggested elution conditions of water–isopropanol (82:18) at 1.0 mL/min (6). Figure 1 shows the LC_8 chromatogram of the 14 target compounds and the surrogate compound 1,4-DNB. Also indicated in Figure 1 are the retention times for 1,2-DNB and 3,4-DNT. As the figure implies, 3,4-DNT coelutes with the analyte 2,4-DNT, but 1,2-DNB and 1,4-DNB have good separation from the target compounds. Because the LC_8 column is the primary column in which all target compounds are separated (except 2-NT and 4-NT), 3,4-DNT is not tested on any other LC–CN column. Therefore, the focus will be on selecting a surrogate from the remaining two candidates.

The incomplete resolution of the TNT manufacturing byproducts, 2-NT and 4-NT, is not critical, because they are rarely present in contaminated sites.

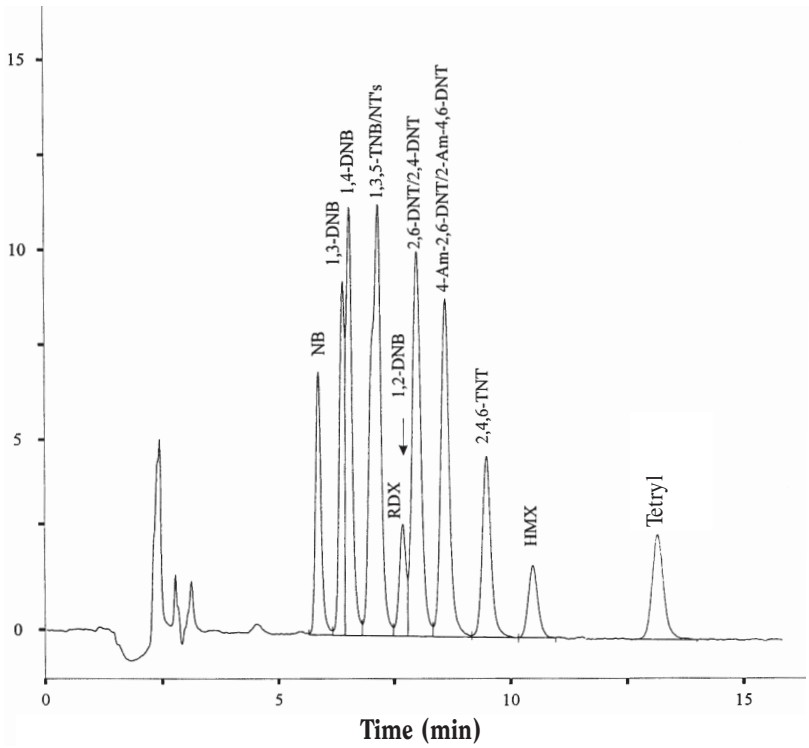

Figure 3. LC–CN chromatogram for method 8330 analytes with an eluent composed of water–MeOH–ACN (65:12:23, v/v/v) at 1.2 mL/min. Retention times for surrogate compounds 1,2-DNB and 1,4-DNB are also indicated.

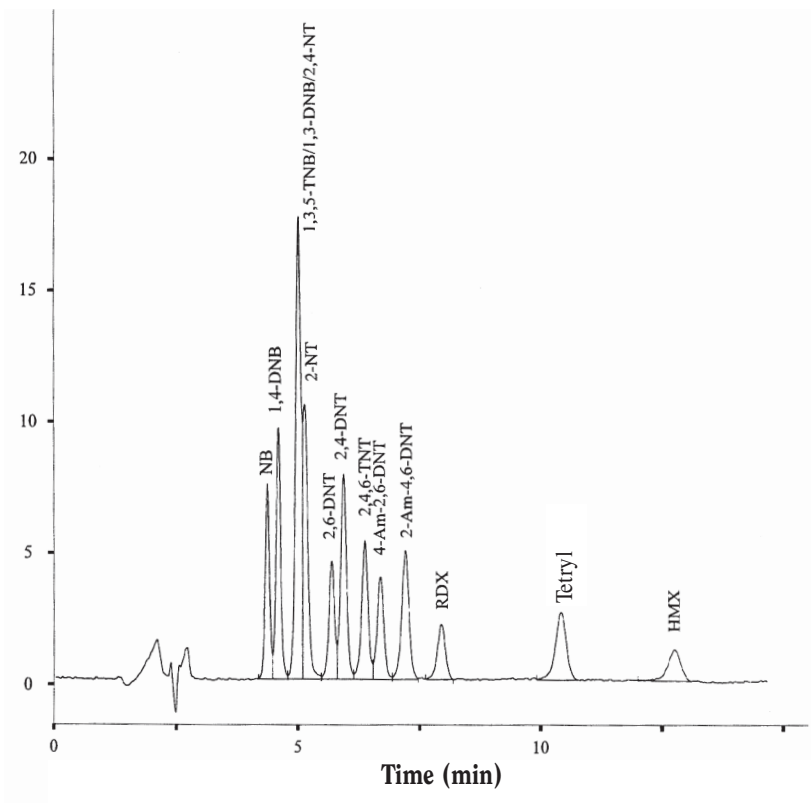

Figure 4. LC–CN chromatogram for 8330 analytes and 1,4-DNB with an eluent composed of water–MeOH–ACN (55:40:5, v/v/v) at 1.2 mL/min.

LC–CN column results

Method 8330 conditions

Figure 2 is an LC–CN chromatogram using the elution conditions of water–MeOH (50:50) at 1.5 mL/min, as suggested in the SW846 method 8330 protocol (1). Under those conditions, 1,2-DNB coelutes with TNT, and 1,4-DNB elutes slightly after NB with only approximately 10% resolution.

Jenkins and Golden's condition

Figure 3 is a LC–CN chromatogram using the elution conditions of water–MeOH–ACN (65:12:23) at 1.2 mL/min, in accordance with Jenkins and Golden (4). Under those conditions, 1,2-DNB coelutes with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and 1,4-DNB elutes slightly after 1,3-DNB with only approximately 5% resolution.

Here, the resolution for the adjacent peaks was calculated according to the resolution definition of the U.S. EPA Contact Laboratory Program Statement of Work for Organic Analysis (7):

$$\%\text{Resolution} = V/H \times 100 \qquad \text{Eq 1}$$

where V is the depth of the valley between the two peaks and H is the height of the shorter of the adjacent peaks.

Proposed improvements to separate surrogates

Clearly, the elution conditions on the LC–CN column were not adequate to separate the two surrogate compounds from the method analytes using either the binary water–MeOH (50:50) (1) or ternary water–MeOH–ACN (65:12:23) (4) eluents. A valuable contribution of Jenkins and Golden's work is that they used acetonitrile to extensively reduce the retention time for HMX and methyl-2,4,6-trinitrophenylnitramine (Tetryl) from more than 22 min to less than 16 min and therefore increased the ability of confirmation at very low concentrations for these two compounds. Their extensive studies on retention time were conducted at a fixed water content of 65%, and the remaining 35% changed between ACN and MeOH. The authors explored eluent compositions not only at 65% water content but also with other possible compositions.

During the present experiment, it was difficult to separate 1,2-DNB and other candidates, because 1,2-DNB elutes in a region surrounded by many analytes. The best sep-

aration was achieved when 1,4-DNB was used as the surrogate compound. With an eluent composition of water–MeOH–ACN (55:40:5, v/v/v) at 1.2 mL/min, 1,4-DNB elutes 0.33 min after NB with approximately 90% resolution. HMX elutes last with a retention time of 13.04 min.

These chromatographic conditions provide a similar retention pattern compared with that in the SW846 method 8330. One improvement is that the surrogate 1,4-DNB is resolved approximately 90% from NB, and the separation of peaks between 2,6-DNT to 2-amino-4,6-DNT are improved.

Compared to the ternary elution conditions described by Jenkins and Golden (4), the reduced amount of water in this work (from 65% to 55%) is more than compensated for by the reduced amount of ACN (from 23% to 5%) but results in a shorter retention time for Tetryl (from 14.13 to 10.66 min), whereas the HMX retention time was delayed for only 1.7 min (from 11.30 to 13.04 min). Thus, the total elution time was reduced by 1 min (from 14 to 13 min). In order to compare retention times under the same conditions, Figures 3 and 4 should be referenced.

Table II. Retention Times for Target Analytes 1,4-DNB and 1,3-DNB on an LC–CN Column

	Retention time (min)		
Analyte	A*	B	C
NB	3.95	6.39	4.48
1,4-DNB§	4.056	7.09	4.71
1,3-DNB	4.37	6.94	5.13
TNB	4.37	7.75	5.13
2-NT	4.37	7.75	5.13
4-NT	4.37	7.75	5.13
3-NT	4.37	7.75	5.27
2,6-DNT	4.75	8.64	5.84
2,4-DNT	4.96	8.64	6.09
2,4,6-TNT	5.17	10.25	6.53
4-Am-2,6-DNT	5.40	9.29	6.86
2-Am-4,6-DNT	5.79	9.29	7.39
RDX	6.54	8.33	8.15
Tetryl	7.77	14.13	10.66
HMX	9.65	11.30	13.04

* Elution condition A: water–MeOH (50:50) at 1.5 mL/min, in accordance with SW846 Method 8330 protocol.
† Elution condition B: water–MeOH–ACN (65:12:23) at 1.2 mL/min (4).
‡ Elution condition C: water–MeOH–ACN (55:40:5) at 1.2 mL/min.
§ A good surrogate under elution condition C.

Table III. Resolution Summary of Potential Surrogates for Method 8330

	Resolution	
Surrogate candidate	LC_8	LC–CN
3,4-dinitrotoluene	coelution	coelution
1,2-dinitrobenzene	good	coelution
1,4-dinitrobenzene	good	good

Retention times for all 14 method analytes and the surrogate 1,4-DNB are listed in Table II with eluents composed of water–MeOH–ACN (55:40:5, v/v/v) at 1.2 mL/min. The LC–CN chromatogram obtained under the same conditions is shown in Figure 4. As can be seen, there is a coelution at 5.13 min for compounds 1,3-DNB, 1,3,5-TNB, 2-NT, and 4-NT. 3-NT eluted next at 5.27 min. The three isomers of NT are not commonly found in environmental samples and therefore do not create a problem (8). This set of elution parameters separated a coelution of the biodegradation products of TNT, 2-Am-DNT, and 4-Am-DNT, as well as 2,4-DNT and 2,6-DNT in the work of Jenkins and Golden (2,4-DNT and 2,6-DNT were somewhat separated by 0.19 min in their work but not in the present experiment using identical conditions), but produced a pair of coelutions between 1,3,5-TNB and 1,3-DNB. Although it is not critical to identify which biodegradation products were in the samples, a coelution of 1,3,5-TNB and 1,3-DNB does cause a confirmation problem when they occur in environmental samples (4). With the addition of a surrogate recovery, the defensibility of the data is greatly enhanced, which leads us to believe that it is worthwhile to sacrifice the coelution of 1,3-DNB with 1,3,5-TNB for the surrogate. This is especially true if a PDA detector is used, because the UV absorption spectra provided in the primary column analysis (LC_8) have already provided part of the confirmation information.

Conclusion

1,4-DNB can be used as a surrogate compound in the analysis of explosives by SW846 method 8330. This compound has a similar chemical composition and behavior in comparison with the method analytes but is not likely to be present in environmental samples. Using the manufacturer's suggested elution conditions on the primary LC_8 column, 1,4-DNB does not coelute with any method analyte (Table III). Using an LC–CN column for confirmation, adequate separation can be achieved with an eluent composed of water (55%), methanol (40%), and acetonitrile (5%) at 1.2 mL/min. The separation of analytes on an LC–CN column under these conditions was better than with the conditions recommended in the SW846 method 8330 and similar to that with the previous improvements, and the surrogate 1,4-DNB does not coelute with any of the method analytes.

References

1. Method 8330, *Nitroaromatics and Nitramines by HPLC.* Solid Waste 846, Revision 0, United States Environmental Protection Agency, Cincinnati, OH, September 1994.
2. E.S.P. Bouvier and S.A. Oehrle. Analysis and identification of nitroaromatic and nitramine explosives in water using HPLC and photodiode-array detector. *LC–GC* **13(2):** 120–30 (1995).

3. Method 8000B, *Determinative Chromatographic Separations.* Solid Waste 846, 3rd ed., final update III, United States Environmental Protection Agency, Washington, D.C., December 1996.
4. T. Jenkins and S.M. Golden. *Development of an Improved Confirmation Separation Suitable for Use With SW846 Method 8330.* CRREL special report 93-14, United States Army Cold Regions Research and Engineering Laboratory, Hanover, NH.
5. ASTM Standard D-1193-77, Vol. 11.01, American Society of Testing and Materials, Philadelphia, PA.
6. Analyze nitroaromatic and nitramine explosives. Literature code WD24, *Columns and Supplies Catalog,* Waters Chromatography, Milford, MA, 1996–1997.
7. Statement of Work for Organic Analysis, OLM03.1, United States Environmental Protection Agency Contract Laboratory Program, August 1994.
8. M.E. Walsh, T.F. Jenkins, P.H. Miyares, P.S. Schnitker, J.W. Elwell, and M.H. Stutz. Evaluation of analytical requirements associated with sites potentially contaminated with residues of high explosives. CRREL report 93-5, United States Army Cold Regions Research and Engineering Laboratory, Hanover, PA, 1993.

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