METHOD 8330A

NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method is intended for the trace analysis of explosives residues by high performance liquid chromatography (HPLC) using a UV detector. The following RCRA compounds in a water, soil, or sediment matrix have been determined by this method:

Analyte	Abbreviation	CAS Number
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	19406-51-0
2-Amino-4,6-dinitrotoluene	2-Am-DNT	35572-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

[a] Chemical Abstract Service Registry Number

1.2 This method provides a salting-out extraction procedure for low concentrations (parts per trillion, or ng/L) of explosive residues in surface or ground water. Direct injection of

diluted and filtered water samples can be used for water samples of higher concentration. Solid-phase extraction, using Method 3535, may also be applied to aqueous samples.

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See Sec. 7.0 and Sec. 11.0.

1.4 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 Use of this method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials (see Sec. 5.0). Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 This method provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosive residues in water, soil, and sediment. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-level salting-out method with no evaporation -- Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent water. An aliquot is separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column.

2.3 Solid-phase extraction method -- Aqueous samples may also be prepared using solid-phase extraction, as described in Method 3535.

2.4 High-level direct injection method -- Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column. If HMX is an important target analyte, methanol is preferred.

2.5 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and analyzed as described in Sec. 2.4.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware. Also refer to any applicable base method (e.g., Methods 5000, 3500, 3600, and 8000) for information regarding potential interferences.

4.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

4.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

4.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by this method. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Heed the warning about not drying the neat materials at elevated temperatures in Sec. 7.6.1.

5.3 It is advisable to screen soil or waste samples using Methods 8510 or 8515 to determine whether high concentrations of explosives are present. Soil samples containing as much as 2% of 2,4,6-TNT have been safely ground. Samples containing higher concentrations

should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, but the other nitroaromatics will also cause a color to be developed and provide a rough estimation of their concentrations. Method 8510 is for RDX and HMX, but mixtures of RDX (and/or related compounds with 2,4,6-TNT will cause an orange color, rather than a pink color to form. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 HPLC system

6.1.1 HPLC -- Equipped with a pump capable of achieving 4000 psi, a 100-µL loop injector and a 254-nm UV detector (Perkin-Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of maintaining a stable baseline at 0.001 absorbance units full scale.

6.1.2 Recommended columns

The columns listed in this section were the columns used to develop the method. The listing of these columns in this method is not intended to exclude the use of other columns that are available or that may be developed. Laboratories may use these columns or other columns provided that the laboratories document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that are appropriate for the intended application.

6.1.2.1 Primary column -- C-18 reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-18, or equivalent).

6.1.2.2 Secondary column -- CN reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-CN, or equivalent).

6.1.3 Strip chart recorder

6.1.4 Digital integrator (optional)

6.1.5 Autosampler (optional)

6.2 Other equipment

6.2.1 Temperature-controlled ultrasonic bath

6.2.2 Vortex mixer

6.2.3 Balance capable of weighing ± 0.0001 g

6.2.4 Magnetic stirrer equipped with PTFE stirring bars

6.2.5 Water bath -- Heated, equipped with concentric ring cover, capable of temperature control (± 5 °C). The bath should be used in a hood.

6.2.6 Oven -- Forced air, without heating.

6.3 Materials

6.3.1 High-pressure injection syringe -- 500-µL (Hamilton liquid syringe, or equivalent).

6.3.2 Disposable cartridge filters -- 0.45-µm PTFE filter.

6.3.3 Pipets -- Class A, glass, appropriate sizes.

6.3.4 Pasteur pipets

6.3.5 Scintillation vials -- 20-mL, glass.

6.3.6 Vials -- 15-mL, glass, PTFE-lined cap.

6.3.7 Vials -- 40-mL, glass, PTFE-lined cap.

6.3.8 Disposable syringes -- Plastipak, 3-mL and 10-mL or equivalent.

6.3.9 Volumetric flasks -- 10-mL, 25-mL, 100-mL, and 1-L, fitted with ground-glass stoppers, Class A.

NOTE: The 100-mL and 1-L volumetric flasks used for magnetic stirrer extraction must be round.

6.3.10 Vacuum desiccator -- Glass.

6.3.11 Mortar and pestle -- Steel.

6.3.12 Sieve -- 30-mesh.

6.3.13 Graduated cylinders -- 10-mL, 25-mL, and 1-L.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Solvents

The choice of solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups. Whatever solvent system is employed, *including* those specifically listed in this method, the analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Method 3500, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

All solvents should be HPLC-grade or equivalent. Solvents may be degassed prior to use.

7.2.1 Acetonitrile, CH_3CN -- HPLC-grade, or equivalent.

7.2.2 Methanol, CH_3OH -- HPLC-grade, or equivalent.

7.3 Calcium chloride, $CaCl_2$ -- Prepare an aqueous solution containing 5 g/L of calcium chloride.

7.4 Sodium chloride, NaCl, shipped in glass bottles.

7.5 Organic-free reagent water -- All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

7.6 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

CAUTION: Calibration standards are commercially available from several sources including Supelco, AccuStandard and Radian, as both solutions and neat materials. It is highly recommended that commercially-prepared stock standard solutions be obtained rather than neat materials be handled.

7.6.1 Stock standard solutions

Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100-mL volumetric flask and dilute to volume with acetonitrile. Invert flask several times until dissolved. Store in refrigerator at ≤ 6 °C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

WARNING: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See Sec. 5.0 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature takes several days. DO NOT DRY AT ELEVATED TEMPERATURES!

7.6.2 Intermediate standard solutions

7.6.2.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

7.6.2.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

7.6.2.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

7.6.3 Working standard solutions

Calibration standards at a minimum of five concentration levels should be prepared by the dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L of calcium chloride solution (Sec. 7.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

7.7 Surrogate spiking solution

The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

7.8 Matrix spiking solutions

Prepare matrix spiking solutions in methanol. All target analytes should be included.

7.9 HPLC mobile phase

To prepare 1 L of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to Chapter Four, "Organic Analytes."

8.2 Samples and sample extracts should be stored in the dark at $\leq$6 °C. Holding times are the same as for semivolatile organics.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific quality control (QC) procedures. Refer to Method 3500 for QC procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 3500, 3600, or 5000.

9.3 The quality control procedures necessary to validate the HPLC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would prevent the determination of that analyte, determine the source and eliminate it, if possible, before processing samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch, the addition of surrogates to each field sample and QC sample, and routine analyses of matrix spike and matrix spike duplicate aliquots. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedure (Sec. 11.0) as those used on actual samples.

9.6.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

9.6.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

9.6.3 Also see Method 8000 for the details on carrying out sample quality control procedures for preparation and analysis. In-house method performance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries

The laboratory should evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating the recoveries of multiple surrogates and the associated corrective actions should be defined in an approved project plan.

9.8 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.3 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Sample preparation

This method addresses both aqueous and solid samples. There are three extraction procedures that may be applied to aqueous samples, depending on the expected level of explosive residue in the sample and the available equipment. The extraction procedure options include a low-level salting-out extraction, a high-level extraction, and solid-phase extraction. It is highly recommended that aqueous process waste samples be screened with the high-level method to determine if use of the low-level method (1 - 50 µg/L) is necessary. Most groundwater samples will fall into the low-level method.

11.1.1 Aqueous low-level method (salting-out extraction)

11.1.1.1 Add 251.3 g of sodium chloride to a 1-L volumetric flask (round). Measure 770 mL of a water sample (using a 1-L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

11.1.1.2 Add 164 mL of acetonitrile (measured with a 250-mL graduated cylinder) while the solution is being stirred and stir for an additional 15 min. Turn off the stirrer and allow the phases to separate for 10 min.

11.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100-mL volumetric flask (with a round bottom). Add 10 mL of fresh acetonitrile to the water sample in the 1-L flask. Again stir the contents of the flask for 15 min followed by standing for 10 min for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

11.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100-mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 min, followed by standing for 10 min for phase separation. Carefully transfer the acetonitrile phase to a 10-mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

11.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100-mL volumetric flask. Again stir the contents of the flask for 15 min, followed by standing for 10 min for phase separation. Combine the second acetonitrile portion with the initial extract in the 10-mL graduated cylinder (transfer to a 25-mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [V_t] in the calculation of concentration after converting to µL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

11.1.1.6 If the diluted extract is turbid, filter it through a 0.45-µm PTFE filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 11.4.

11.1.2 Aqueous high-level method

11.1.2.1 Sample filtration

Place a 5-mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45-µm PTFE filter using a disposable syringe.

11.1.2.2 Discard the first 3 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 11.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

11.1.3 Solid-phase extraction

Aqueous samples containing nitroaromatics and nitramines may also be extracted using solid-phase extraction (SPE) in both disk and cartridge formats. See Method 3535 for the procedures to be employed and the apparatus and materials that are necessary.

11.1.4 Soil and sediment samples

11.1.4.1 Sample homogenization

Dry soil samples in air at room temperature (or less) to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30-mesh sieve.

WARNING: Soil samples should be screened by Method 8510 or Method 8515 prior to grinding in a mortar and pestle (see Sec. 5.3).

11.1.4.2 Sample extraction

11.1.4.2.1 Place a 2.0-g subsample of each soil sample in a 15-mL glass vial. Add 10.0 mL of acetonitrile, cap with a PTFE-lined cap, vortex swirl for one min, and place in a cooled ultrasonic bath for 18 hr.

11.1.4.2.2 After sonication, allow sample to settle for 30 min. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 7.3) in a 20-mL vial. Shake, and let stand for 15 min.

11.1.4.2.3 Place supernatant in a disposable syringe and filter through a 0.45-µm PTFE filter. Discard first 3 mL and retain remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 11.4.

11.2 Chromatographic conditions (recommended)

Primary column:	C-18 reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-18 or equivalent).
Secondary column:	CN reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-CN or equivalent).
Mobile phase:	50/50 (v/v) methanol/organic-free reagent water.

Flow rate:	1.5 mL/min
Injection volume:	100-µL
UV detector:	254 nm

11.3 Calibration of HPLC

11.3.1 Allow all electronic equipment to warm up for 30 min. During this period, pass at least 15 void volumes of mobile phase through the column (approximately 20 min at 1.5 mL/min) and continue until the baseline is level at the UV detector's greatest sensitivity.

11.3.2 Initial calibration -- Sequentially inject each of at least five calibration standards over the concentration range of interest into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Employ one of the linear calibration options described in Method 8000.

11.3.3 Calibration verification -- Analyze one mid-point calibration standard, at a minimum, at the beginning of the day, and after every 20 sample extracts (*recommended* after every 10, in order to minimize the number of samples that may be affected by a failing standard), and after the last sample of the day. Calculate the calibration factor for each analyte from the peak height or peak area and compare it with the mean calibration factor obtained for the initial calibration, as described in Method 8000. The calibration factor for the calibration verification must agree within ± 20% of the mean calibration factor of the initial calibration. If this criterion is not met, a new initial calibration must be performed.

11.4 HPLC analysis

11.4.1 Analyze the samples using optimized chromatographic conditions. Use the conditions given in Sec. 11.2 either directly or as a basis for the optimization. Tentative identification of an analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation is necessary when the sample composition is not well characterized. All positive measurements observed on the C-18 column should be confirmed by injection onto the CN column, or by another appropriate technique, e.g., diode array or mass spectral detection.

When results are confirmed using a second HPLC column of dissimilar stationary phase, such as the CN column, the analyst should check the agreement between the quantitative results on both columns once the identification has been confirmed. See Method 8000 for a discussion of such a comparison and appropriate data reporting approaches.

11.4.2 Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 20 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

11.4.3 Table 1 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1. The retention times listed in Table 1 are provided for illustrative purposes only. Each laboratory must determine retention times and retention time widows for their specific application of the method.

11.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

11.4.5 The calculation of sample concentrations is described in Method 8000.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 See Method 8000 for information regarding data analysis and calculations.

12.2 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance for purposes of laboratory accreditation.

13.2 Table 2 provides the single-laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories. These data are provided for guidance purposes only.

13.3 Table 3 provides the multi-laboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories. These data are provided for guidance purposes only.

13.4 Table 4 provides the multi-laboratory variance of the high-level method for water based on data from nine laboratories. These data are provided for guidance purposes only.

13.5 Table 5 provides multi-laboratory recovery data from the analysis of spiked soil samples by seven laboratories. These data are provided for guidance purposes only.

13.6 Table 6 provides a comparison of method accuracy for soil and aqueous samples (high-level method). These data are provided for guidance purposes only.

13.7 Table 7 provides precision and accuracy data for the salting-out extraction method. These data are provided for guidance purposes only.

13.8 Table 8 provides data from a comparison of direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques. These data are provided for guidance purposes only.

13.9 Table 9 provides data comparing the precision of duplicate samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques. These data are provided for guidance purposes only.

13.10 Table 10 provides a comparison of recovery data for spiked samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

1. C. F. Bauer, T. F. Jenkins, S. M. Koza, P. W. Schumacher, P. H. Miyares and M. E. Walsh, "Development of an Analytical Method for the Determination of Explosive Residues in Soil, Part 3, Collaborative Test Results and Final Performance Evaluation," USACE Cold Regions Research and Engineering Laboratory, CRREL Report 89-9, 1989.

2. C. L. Grant, A. D. Hewitt and T. F. Jenkins, "Comparison of Low Concentration Measurement Capability Estimates in Trace Analysis: Method Detection Limits and Certified Reporting Limits," USACE Cold Regions Research and Engineering Laboratory, Special Report 89-20, 1989.

3. T. F. Jenkins, C. F. Bauer, D. C. Leggett and C. L. Grant, "Reverse-phased HPLC Method for Analysis of TNT, RDX, HMX and 2,4-DNT in Munitions Wastewater," USACE Cold Regions Research and Engineering Laboratory, CRREL Report 84-29, 1984.

4. T. F. Jenkins, and M. E. Walsh, "Development of an Analytical Method for Explosive Residues in Soil," USACE Cold Regions Research and Engineering Laboratory, CRREL Report 87-7, 1987.

5. T. F. Jenkins, P. H. Miyares and M. E. Walsh, "An Improved RP-HPLC Method for Determining Nitroaromatics and Nitramines in Water," USACE Cold Regions Research and Engineering Laboratory, Special Report 88-23, 1988.

6. T. F. Jenkins, and P. H. Miyares, "Comparison of Cartridge and Membrane Solid-Phase Extraction with Salting-out Solvent Extraction for Preconcentration of Nitroaromatic and Nitramine Explosives from Water," USACE Cold Regions Research and Engineering Laboratory, Draft CRREL Special Report, 1992.

7. T. F. Jenkins, P. W. Schumacher, M. E. Walsh and C. F. Bauer, "Development of an Analytical Method for the Determination of Explosive Residues in Soil. Part II: Further Development and Ruggedness Testing," USACE Cold Regions Research and Engineering Laboratory, CRREL Report 88-8, 1988.

8. D. C. Leggett, T. F. Jenkins and P. H. Miyares, "Salting-out Solvent Extraction for Preconcentration of Neutral Polar Organic Solutes from Water," *Analytical Chemistry*, 62: 1355-1356, 1990.

9. P. H. Miyares and T. F. Jenkins, "Salting-out Solvent Extraction for Determining Low Levels of Nitroaromatics and Nitramines in Water," USACE Cold Regions Research and Engineering Laboratory, Special Report 90-30, 1990.

10. T. F. Jenkins, P. G. Thorne, K. F. Myers, E. F. McCormick, D. E. Parker, and B. L. Escalon, "Evaluation of Clean Solid Phases for Extraction of Nitroaromatics and Nitramines from Water," USACE Cold Regions Research and Engineering Laboratory, Special Report 95-22, 1995.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figure referenced by this method.

TABLE 1

EXAMPLE ESTIMATED RETENTION TIMES AND CAPACITY FACTORS
ON LC-18 AND LC-CN COLUMNS

	Retention Time (min)		Capacity Factor (k)*	
Analyte	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

*Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.

Retention times are provided for guidance purposes only. Each laboratory must determine retention times and retention time widows for their specific application of the method.

TABLE 2

SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3
Tetryl	17	3.1	17.9	2.3	0.41	18.0

Source: Reference 1.
These data are provided for guidance purposes only.

TABLE 3

MULTI-LABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14	3.7	26.0
				153	37.3	24.0
RDX	60	2.6	4.4	104	17.4	17.0
				877	67.3	7.7
1,3,5-TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
2,4,6-TNT	40	1.88	4.7	7.0	1.27	18.0
				669	63.4	9.5
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0
Tetryl	17	5.22	30.7	2.3	0.49	21.3

Source: Reference 1.
These data are provided for guidance purposes only.

TABLE 4

MULTI-LABORATORY VARIANCE OF METHOD FOR WATER SAMPLES[a]

Analyte	Mean Conc. (µg/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

[a] Nine Laboratories
These data are provided for guidance purposes only.

TABLE 5

MULTI-LABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

	Concentration (µg/g)						
Laboratory	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean Conc	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std. Dev.	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff.*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

* Between true value and mean determined value.
Source: Reference 1.
These data are provided for guidance purposes only.

TABLE 6

COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES
(HIGH CONCENTRATION METHOD)

	Recovery (%)	
Analyte	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Data from Reference 1.
** Data from Reference 3.
These data are provided for guidance purposes only.

TABLE 7

EXAMPLE PRECISION AND ACCURACY DATA
FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	# Samples	%RSD	Mean Recovery (%)	Highest Concentration Tested
HMX	20	10.5	106	1.14
RDX	20	8.7	106	1.04
1,3,5-TNB	20	7.6	119	0.82
1,3-DNB	20	6.6	102	1.04
Tetryl	20	16.4	93	0.93
2,4,6-TNT	20	7.6	105	0.98
2-Am-DNT	20	9.1	102	1.04
2,4-DNT	20	5.8	101	1.01
1,2-NT	20	9.1	102	1.07
1,4-NT	20	18.1	96	1.06
1,3-NT	20	12.4	97	1.23

All tests were performed in reagent water.
Source: Reference 6.
These data are provided for guidance purposes only.

TABLE 8

COMPARISON OF DIRECT ANALYSIS OF GROUNDWATER SAMPLES CONTAINING NITROAROMATICS WITH SALTING-OUT AND SOLID-PHASE EXTRACTION TECHNIQUES

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
1	Direct									
	Salting-out	1.04	2.45				0.47		0.36	0.32
	SPE-Cart.	1.00	1.33				0.44		0.29	0.30
	SPE-Disk	0.93	1.35				0.57		0.28	0.56
2	Direct	94	79							
	Salting-out	54.2	63.8			0.3	0.33		3.08	1.36
	SPE-Cart.	64.0	83.1			0.3	0.34		3.34	2.27
	SPE-Disk	57.1	71.8			0.3	0.29		2.89	2.05
3	Direct	93	91							
	Salting-out	85.7	75.3			0.2	0.19		2.43	1.31
	SPE-Cart.	93.1	88.8			0.2	0.17		2.49	1.65
	SPE-Disk	78.9	74.7			0.2	0.13		1.99	1.89
4	Direct	45	14							
	Salting-out	45.7	16.4		0.17	0.3	0.13		2.18	1.21
	SPE-Cart.	48.0	21.6			0.2	0.19		2.31	1.42
	SPE-Disk	40.8	18.9			0.2	0.13		2.07	1.64
5	Direct									
	Salting-out	0.76	5.77						0.13	0.05
	SPE-Cart.	1.16	6.48						0.16	0.05
	SPE-Disk	1.19	6.11						0.16	0.14
6	Direct									
	Salting-out	10.5	6.17				0.10		0.71	0.33
	SPE-Cart.	11.5	7.03				0.10		0.79	0.40
	SPE-Disk	10.3	6.34				0.07		0.82	0.70

TABLE 8
(continued)

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
7	Direct	134	365							
	Salting-out	75.4	202				0.98		8.12	1.80
	SPE-Cart.	115	308				1.51		11.3	3.44
	SPE-Disk	109	291				1.41		9.81	3.30
8	Direct									
	Salting-out	0.61	10.9							
	SPE-Cart.	0.64	11.9							
	SPE-Disk	0.64	11.0							
9	Direct	25	13							
	Salting-out	30.2	12.1						1.14	0.56
	SPE-Cart.	31.2	12.7						1.50	0.79
	SPE-Disk	27.5	11.0						1.34	0.79
10	Direct									
	Salting-out	0.33	7.12							
	SPE-Cart.	0.62	8.23							
	SPE-Disk	0.26	7.60							
14	Direct		13							
	Salting-out		5.98							
	SPE-Cart.		12.0							
	SPE-Disk		11.6							
16	Direct		40							
	Salting-out	0.58	28.7			0.04			0.39	0.13
	SPE-Cart.	0.77	33.8			0.03			0.43	0.17
	SPE-Disk	0.66	32.7			0.03			0.44	0.22
18	Direct	165	58						9	7
	Salting-out	141	39.1			0.80	0.96		8.5	5.62
	SPE-Cart.	152	44.4			0.93	0.88		9.5	7.01

TABLE 8
(continued)

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
	SPE-Disk	138	40.9			0.90	0.99		9.3	6.03
19	Direct	173	76				17		59	54
	Salting-out	172	69.5			2.6	23.1	1.20	65.2	56.4
	SPE-Cart.	142	75.6		0.11	2.5	20.9	1.08	57.7	50.5
	SPE-Disk	136	72.7		0.11	2.4	20.3	1.23	55.0	48.0
21	Direct	252	157	5			110		47	65
	Salting-out	227	132	6.62	0.30		102		42.6	56.5
	SPE-Cart.	238	146	6.90	0.33		104		48.0	63.5
	SPE-Disk	226	141	6.45	0.31		102		47.0	61.8
22	Direct	218	40							
	Salting-out	201	35.9						2.20	1.90
	SPE-Cart.	203	36.5						2.74	2.24
	SPE-Disk	199	35.8						2.78	2.08
24	Direct									
	Salting-out	2.15	7.54							
	SPE-Cart.	2.47	8.91							
	SPE-Disk	2.34	8.84							
25	Direct									
	Salting-out									
	SPE-Cart.		0.59							
	SPE-Disk		0.63							
27	Direct	112	608	8			180		10	8
	Salting-out	82.8	429	4.45	0.79		137		7.71	6.20
	SPE-Cart.	91.0	510	9.53	0.90		149		8.25	7.67
	SPE-Disk	77.3	445	7.37	0.79		128		8.16	6.33
28	Direct	325	102				14		51	40
	Salting-out	290	87.5	0.37	0.10		13.9		42.3	33.5

TABLE 8
(continued)

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
	SPE-Cart.	319	109	0.87	0.17		22.0		56.2	45.0
	SPE-Disk	249	85.7	0.65	0.13		17.2		43.0	34.5
29	Direct									
	Salting-out									
	SPE-Cart.		0.43							
	SPE-Disk		0.28							
31	Direct									
	Salting-out									
	SPE-Cart.		0.21							
	SPE-Disk		0.23							
32	Direct									
	Salting-out									
	SPE-Cart.									
	SPE-Disk	0.38								

An additional 11 samples (11, 12, 13, 15, 17, 20, 23, 26, 30, 31, and 33) were analyzed in which none of the analytes were detected by any of the techniques. Therefore, the non-detect results are not shown here. Similarly, for those samples that are shown here, the fields are left blank for the analytes that were not detected.

All data are taken from Reference 10.
These data are provided for guidance purposes only.

TABLE 9

EXAMPLE RELATIVE PERCENT DIFFERENCE BETWEEN DUPLICATE SAMPLE ANALYSES

		Relative Percent Difference (%)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
4	Direct	0	24							
	Salting-out	0	15		6	100	8		18	11
	SPE-Cart.	1	12			0	45		8	5
	SPE-Disk	3	8			0	17		2	1
29	Direct									
	Salting-out									
	SPE-Cart.		26							
	SPE-Disk		7							
LCS	Direct	1	0	0			1	1		
	Salting-out	4	4	4			3	3		
	SPE-Cart.	6	1	7			6	6		
	SPE-Disk	5	7	7			13	6		

All data are taken from Reference 10.
These data are provided for guidance purposes only.

TABLE 10

EXAMPLE RECOVERY OF ANALYTES FROM SPIKED SAMPLES

		Percent Recovery (%)				
Sample	Technique	HMX	RDX	TNB	TNT	24D
LCS1	Direct	99.5	98.5	95.6	96.5	98.1
	Salting-out	94.2	91.2	92.9	83.2	92.1
	SPE-Cart.	99.0	101.0	96.6	94.1	95.1
	SPE-Disk	92.5	95.6	89.3	88.6	86.9
LCS2	Direct	98.8	98.2	95.9	97.2	99.2
	Salting-out	91.0	95.0	89.0	81.0	89.0
	SPE-Cart.	93.5	100.0	83.0	89.1	89.3
	SPE-Disk	88.0	102.0	83.0	78.0	82.0
29	Direct	95.0	95.5	95.2	92.8	93.0
	Salting-out	107.0	89.0	85.0	89.0	65.0
	SPE-Cart.	103.0	107.0	104.0	05.0	102.0
	SPE-Disk	80.0	78.0	76.0	78.0	77.0
4	Direct	105.5	105.0	103.0	104.0	105.0
	Salting-out	23*	191*	76.0	83.0	76.0
	SPE-Cart.	351*	95*	92.2	91.1	93.7
	SPE-Disk	308*	49.5*	87.4	85.6	90.8

All data are taken from Reference 10.

* Results for these analytes in Sample 4 are believed to result from spiking levels that are very similar to the background concentrations of these analytes in this sample (see Reference 10).

These data are provided for guidance purposes only.

FIGURE 1
EXAMPLE CHROMATOGRAMS