

METHOD 8332

NITROGLYCERINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 Method 8332 is used to determine the concentration of nitroglycerine (CAS No. 55-63-0) in aqueous matrices such as waste water, groundwater, and surface water. This method may also be applicable to other matrices. Method 8332 also provides a qualitative procedure for determining the presence or absence of nitroglycerine (See Appendix A).

1.2 This method is restricted to use by, or under the supervision of, analysts experienced in the use of HPLC instrumentation and skilled in the interpretation of HPLC chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Samples are injected onto a reverse phase high performance liquid chromatograph (HPLC) column and the nitroglycerine concentration is quantitated using a UV detector.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 If solid matrices are extracted using an ultrasonic bath as the extraction technique, the period of extraction should not exceed 2 hours or degradation of nitroglycerine may occur. Acetonitrile may be used as a solvent.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 HPLC (isocratic) - equipped with a pump, a direct injection port or 20 μ L loop injector and a 214 nm UV detector.

4.1.2 Column - Waters Radial - Pak CN, 10 μ m particle size (or equivalent).

4.1.3 Confirmation columns:

LC-18 Reverse-phase HPLC column (octadecyldimethylsilyl), 250 x 4.6 mm with a 5 μ m particle size (Supelco Inc. or equivalent) using 3:2 MeOH/Water @ 1.5 mL/min.

LC-CN Reverse-phase HPLC column (cyanopropylmethylmethyl-silyl), 250 x 4.6 mm with a 5 μ m particle size (Supelco Inc. or equivalent) using 1:1 MeOH/Water @ 1.5 mL/min.

4.1.4 Integrator.

4.2 Injection syringe.

4.3 Membrane filter - 0.45 µm

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall 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.

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

5.3 Acetonitrile, CH₃CN.

5.4 Nitroglycerine - "Nitrostat" tablets (nominal concentration = 0.4 mg/tablet), available from Warner Lambert/Parke Davis. Certificate of Analysis for any current lot is available from Warner Lambert, Lititz, Pa. (717) 626-2011.

NOTE: Alternatively, the analyst may purchase a solution of NG from a commercial vender such as AccuStandard, New Haven, CT or Crescent Chemical Co., Hauppauge, NY. NG SARMS may also be obtained from the Army Environmental Center, Aberdeen Proving Ground, MD.

5.5 HPLC mobile phase eluant - 60% Acetonitrile/40% Organic-free reagent water.

5.6 HPLC calibration standards - Dissolve five nitroglycerine tablets in the HPLC mobile phase, using a 500 mL volumetric flask. Dilute to volume with mobile phase. The resulting standard will contain 4 mg/L nitroglycerine; 8 mg/L and 12 mg/L nitroglycerine standard solutions can be prepared in the same manner. Other concentrations, as needed, can be prepared by appropriate dilutions. Use the concentration listed on the "Nitrostat" Certificate of Analysis to calculate the concentration of the standards to three significant figures.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Chromatographic Conditions (Recommended):

Flow rate: 1.0 mL/min.
UV Detector: 214 nm.
Injection: 20 µL (for 4 to 12 mg/L nitroglycerine).

Chromatographic conditions should be established to give a retention time for nitroglycerine of approximately 5.1 min.

7.2 Initial Calibration - Prepare a 5-point calibration curve and establish the calibration range of the method (nominally between 4 mg/L and 12 mg/L). Calculate a correlation coefficient, slope, and zero intercept from the regression analysis of the data points (peak area vs. concentration).

7.3 Samples should be diluted 1:1 with acetonitrile prior to analysis. After addition of the acetonitrile, dirty samples should be filtered through a 0.45 µm membrane filter to help prevent unwanted particulate from damaging the HPLC system.

7.4 Make 20 µL injections for each sample or sample dilution. If the response for the sample exceeds that of the initial calibration range, the sample must be diluted. Calculate sample concentrations using the slope and the intercept figures from the regression analysis of the standards.

7.5 Preliminary results indicate that solid samples may be extracted for 2 hours with acetonitrile for NG using an ultrasonic bath.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000, Sec. 7.0 and includes evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

8.3 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. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 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, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch.

8.4.1 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.

8.4.2 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 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.

8.4.3 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 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.

9.0 METHOD PERFORMANCE

9.1 Method 8332 was tested by one laboratory using two industrial wastewater samples. The wastewaters were analyzed at two dilutions with three injections of each dilution. The results of these analyses are shown in Table 1.

10.0 REFERENCES

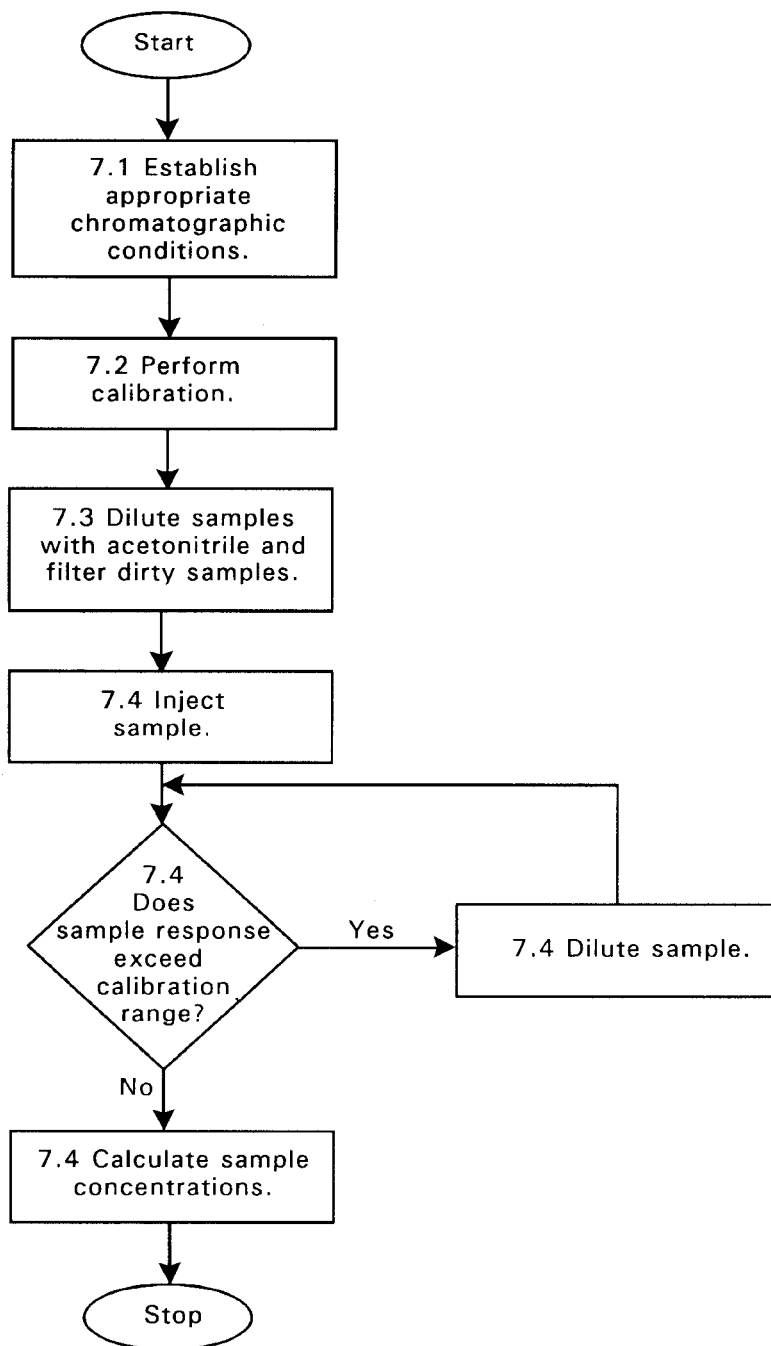
1. U.S. Department of the Treasury; Bureau of Alcohol, Tobacco and Firearms; Lab Number 88-N-0648 B.

TABLE 1
SINGLE LABORATORY PRECISION

Nitroglycerine by Weight	
<u>As parts per Million</u>	<u>As Percent</u>
1036 ± 38	0.1036 ± 0.0038
952 ± 53	0.0952 ± 0.0053

The numbers shown are the average concentration plus or minus one standard deviation calculated for six analyses on each sample.

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APPENDIX A

THIN-LAYER CHROMATOGRAPHY PROCEDURE FOR QUALITATIVE DETERMINATION OF THE PRESENCE OF NITROGLYCERINE

A.1.0 APPARATUS

A.1.1 Thin-layer chromatography (TLC) system

A.1.1.1 TLC plate, Analtech silica gel GHL (Catalog # 11511), or equivalent.

A.1.1.2 Device (syringe, capillary pipet or other) for spotting TLC plate.

A.1.1.3 TLC developing tank.

A.1.1.4 Sprayer.

A.2.0 REAGENTS

A.2.1 Solvents

A.2.1.1 Acetonitrile, CH_3CN .

A.2.1.2 1,2-Dichloroethane, $\text{ClCH}_2\text{CH}_2\text{Cl}$.

A.2.1.3 Carbon tetrachloride, CCl_4 .

A.2.1.4 Ethanol (absolute), $\text{CH}_3\text{CH}_2\text{OH}$.

A.2.1.5 Acetone, CH_3COCH_3 .

A.2.1.6 Tetrahydrofuran, $\text{C}_4\text{H}_8\text{O}$.

A.2.2 Diphenylamine, $(\text{C}_6\text{H}_5)_2\text{NH}$.

A.2.3 alpha-Naphthylamine, $\text{C}_{10}\text{H}_7\text{NH}_2$.

A.2.4 Sulfanilic acid, $4\text{-(H}_2\text{N)C}_6\text{H}_4\text{SO}_3\text{H}$.

A.2.5 Sulfuric acid, H_2SO_4 .

A.2.6 Potassium Hydroxide, KOH .

A.2.7 Acetic acid (30%), $\text{CH}_3\text{CO}_2\text{H}$.

A.2.8 TLC developing solvent - 20% dichloroethane/80% carbon tetrachloride.

A.2.9 TLC overspray - Prepare a solution of 5% diphenylamine in ethanol. For very weak spots, a solution of 5% diphenylamine in concentrated sulfuric acid may be required.

A.2.10 Alternative TLC overspray (Greiss reagent) -

1. Alcoholic KOH (2%)
2. 1% sulfanilic acid in acetic acid (30%)
3. 1% alpha-naphthylamine in acetic acid (30%)

A.2.11 TLC standard - Use double-base smokeless powder (e.g. Hercules Red Dot, Bullseye, or Winchester-Western ball powder) available at any gun shop. Dissolve in acetone, acetonitrile, or THF.

A.3.0 PROCEDURE

A.3.1 Add a quantity of the developing solvent mixture to the developing tank that will be sufficient to continuously wet the edge of the TLC plate. Cover the tank tightly, and allow the vapor phase to equilibrate (several hours).

A.3.2 Streak or spot the sample near the edge of the plate, but above the area that will be immersed in the developing solvent. Allow streaked area to dry completely.

A.3.3 Streak or spot the standard solution next to the sample spot, at the same distance from the edge of the plate as the sample spot. Allow streaked area to dry completely.

A.3.4 Stand the plate in the equilibrated developing tank and cover tightly. Let the plate develop until capillary action has carried the solvent nearly to the top of the plate.

NOTE: It may be necessary to adjust the developing time to achieve suitable separation of the nitroglycerine from interfering sample components.

A.3.5 Remove the plate from the tank and air dry.

A.3.6 Spray the developed plates with a 5% solution of diphenylamine in ethanol. After spraying developed TLC plates, expose the plates to U.V. light (longwave, shortwave, or both simultaneously) for 15-30 minutes, until spots appear. For very weak spots, spray again with 5% diphenylamine in concentrated sulfuric acid.

A.3.7 Spray developed plates with Greiss reagent (Section A.2.10) and warm the plates in a 100°C oven for 5-10 minutes.

A.3.8 The presence of a spot at the same distance from the origin as the standard (R_f of approximately 0.4) indicates that nitroglycerine may be present. The absence of a spot in this location indicates that nitroglycerine is not present above the detection limit of the test.