# Method 1673

## Poly(ethylene glycol)-600 by Derivatization and High-Pressure Liquid Chromatography

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## 1. Scope and Application

**1.1** This method is designed to meet the survey and monitoring requirements of the EPA's Engineering and Analysis Division (EAD). The method is used to determine poly(ethylene glycol)-600 (PEG-600, Chemical Abstracts Service Registry Number 25322-68-3),[1] a substance used in the pharmaceutical manufacturing industry (PMI), that can be derivatized and analyzed by high pressure liquid chromatography (HPLC).

**1.2** PEG-600 is a mixture of oligomers with a molecular weight centered around 600 Da. The exact composition may vary from manufacturer to manufacturer and even between batches from the same manufacturer. This method has been developed for aqueous samples and is not, in its present form applicable to solids or sludges.

**1.3** The detection limits of the method are usually dependent on the level of interferences rather than instrumental limitations. The detection limit provided in Table 1 is the minimum level that can be reliably quantified by this method with no interferences present.

**1.4** This method is for use only by analysts experienced with HPLC or under the close supervision of such qualified persons.

## 2. Summary of the Method

**2.1** One liter of aqueous sample is placed into a liquid-liquid extractor and a known quantity of surrogate is added. Extraction with dichloromethane is carried out over an 18-hour period. The dichloromethane extracts are dried over anhydrous sodium sulfate, evaporated to a small volume and dried again. Remaining dichloromethane is removed and the water-free extract is derivatized using 3,5-dinitrobenzoyl chloride and pyridine in tetrahydrofuran. The tetrahydrofuran solution is diluted with diethyl ether, extracted to remove side products, evaporated, and solvent exchanged with acetonitrile/water and chromatographed on a reverse-phase $C_{18}$ column with a solvent gradient of 40 percent acetonitrile/water to 100 percent acetonitrile. Detection is performed at 254 nanometers. The PEG-600 derivative is identified by its retention time relative to that of the derivatized surrogate and quantified by external standard techniques. Derivatized samples must be stored at 4°C in an amber container and analyzed within 96 hours of preparation.

**2.2** The quality of the analysis is assured through reproducible calibration and testing of the derivatization/extraction procedure and the HPLC system.

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1. This Chemical Abstracts Service Registry Number (CASRN) is common to all poly(ethylene glycol) oligomers and mixtures and does not specifically identify PEG-600.

## 3. Definitions

There are no method specific definitions to be noted for this document.

## 4. Interferences

**4.1** Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3.

**4.1.1** Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained, dried, and heated in a laboratory oven at 130 degrees celsius (°C) for several hours before use. Solvent rinses with acetone may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

**4.1.2** The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all glass systems may be required.

**4.2** Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. If matrix interferences occur, some additional cleanup may be necessary.

**4.3** The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for resolution of PEG-600, other matrix components may interfere.

## 5. Safety

**5.1** The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data Sheets should also be made available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 1 through 3.

## 6. Equipment and Supplies

**6.1** Reaction vessel—5-mL screw-cap vial with V-shaped chamber (Aldrich Z18,302-4, or equivalent) with polytetrafluoroethylene (PTFE) lined cap (Aldrich Z11,511-8, or equivalent).

**6.2** Liquid-liquid extractor—Capable of extracting 1 L of sample (Aldrich Z10,156-7, or equivalent)

**6.3** Kuderna-Danish (K-D) apparatus.

**6.3.1** Concentrator tube—10-mL graduated (Kontes K-570050 or equivalent). A ground-glass stopper is used to prevent evaporation of extracts.

**6.3.2** Evaporation flask—500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

**6.3.3** Snyder column—Three-ball macro (Kontes K-503000-0121 or equivalent).

**6.3.4** Snyder column—Two-ball micro (Kontes K569001-0219 or equivalent).

**6.3.5** Springs—One-half inch (Kontes K-662750 or equivalent).

**6.4** Vials and bottles—10-mL, 25-mL, 100-mL, and 1-L amber glass with PTFE-lined screw-caps.

**6.5** Boiling chips—Solvent-extracted with methylene chloride, approximately 10/40 mesh (silicon carbide or equivalent).

**6.6** Balance—Analytical, capable of accurately weighing to the nearest 0.1 mg.

**6.7** High-performance liquid chromatograph (modular).

**6.7.1** Pumping system—Solvent programmable, with flow control capable of 2.00 milliliter per minute (mL/min).

**6.7.2** High-pressure injection valve with 50-microliter (µL) loop or autosampler.

**6.7.3** Column—250 millimeter (mm) long × 4.6 mm inside diameter (ID), 5 µm particle size, Betasil $C_{18}$ (or equivalent).

**6.7.4** Absorbance detector—254 nm.

**6.7.5** Strip chart recorder compatible with the detector. Use of a data system is recommended.

**6.8** Glass-fiber filter paper, 0.6–0.9 µm.

**6.9** Pipette—Capable of accurately delivering 0.1–1.0 mL of solution.

**6.10** Water bath—Heated, with concentric ring cover, capable of temperature control of ± 2°C. The bath should be used under a hood.

**6.11** Sand bath—Heated, with temperature control of ± 5°C.

**6.12** Volumetric flasks—100-mL, 1-L.

**6.13** Microsyringes—100-µL, 10-µL.

## 7. Reagents and Standards

**7.1** Reagent grade or pesticide grade chemicals shall be used in all tests. 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 determinations.

**7.2** Reagent water: Water in which the compounds of interest and interfering compounds are not detected by this method. It may be generated by any of the methods in this subsection.

**7.2.1** Activated carbon: pass tap water through a carbon bed (Calgon Filtrasorb-300, or equivalent).

**7.2.2** Water purifier: Pass tap water through a purifier (Millipore Super Q, or equivalent).

**7.2.3** Boil and purge: Heat tap water to between 90 and 100°C and bubble contaminant free inert gas through it for approximately 1 hour. While still hot, transfer the water to screw-cap bottles and seal with a PTFE-lined cap.

**7.3** Dichloromethane—Pesticide grade or equivalent.

**7.4** Acetonitrile—Pesticide grade or equivalent.

**7.5** Diethyl ether—Pesticide grade or equivalent.

**7.6** Tetrahydrofuran, anhydrous—Can be prepared by distillation from potassium benzophenone ketyl, or other, similar methods.

**7.7** Surrogate—Di(ethylene glycol) monohexyl ether (CASRN 112-59-4)

**7.8** Sodium sulfate, anhydrous.

**7.9** Hydrochloric acid solution—Dilute 100 mL of concentrated hydrochloric acid to approximately one liter with reagent water.

**7.10** Sodium bicarbonate solution—Dissolve 10 g of sodium bicarbonate in approximately 1 L of reagent water.

**7.11** Saturated sodium chlroide solution—Prepare a saturated solution in reagent water by adding reagent sodium chloride until no more will dissolve at room temperature.

**7.12** Pyridine, anhydrous (CASRN 110-86-1)(Aldrich 27,097-0, or equivalent).

**7.13** 3,5-Dinitrobenzoyl chloride (CASRN 99-33-2) in anhydrous tetrahydrofuran (10 mg/mL)—Weigh 1 g of 3,5-dinitrobenzoyl chloride into a 100-mL volumetric flask and fill to the mark with anhydrous tetrahydrofuran. Store the resulting solution in an amber bottle protected from moisture.

**7.14** Stock standard solutions.

**7.14.1** Stock PEG-600 (approximately 10.0 mg/mL)—Prepare by weighing 1.0 g of PEG-600 into a 100-mL volumetric flask and diluting to volume with tetrahydrofuran (if aliquots of this solution are to be derivatized, then anhydrous tetrahydrofuran must be used).

**7.14.2** Secondary standard—1.0, 2.5, 5.0, 7.5 and 10.0 mg/L. Measure 0.1, 0.25, 0.5, 0.75, and 1.0 mL of stock solution (Section 7.14.1) into separate 1-L volumetric flasks and dilute to volume with reagent water. Store in 1-L amber bottles.

**7.14.3** Surrogate standard—Prepare by weighing 1.0 g of di(ethylene glycol) monohexyl ether into a 100-mL volumetric flask and diluting to volume with tetrahydrofuran (if aliquots of this solution are to be derivatized, then anhydrous tetrahydrofuran must be used). Store in a 100-mL amber bottle protected from moisture.

**7.14.4** Stock standard solution and surrogate solution must be replaced after six months, or sooner, if comparison with check standards indicates a problem.

**7.14.5** Aqueous performance standard—An aqueous performance standard containing PEG-600 at 2.5 mg/L and surrogate at 1mg/L, shall be prepared daily, and analyzed each shift to demonstrate performance (Section 9).

## 8. Sample Collection, Preservation, and Storage

**8.1** Grab samples are collected in glass containers having a total volume greater than one liter. Fill sample bottles so that no air bubbles pass through the sample as the bottle is filled and seal each bottle so that no air bubbles are entrapped. Maintain the hermetic seal on the sample bottle until time of analysis.

**8.2** Samples are maintained at 0–4°C from the time of collection until analysis. Samples must be extracted within five days of collection, derivatized within seven days of extraction, and analyzed within four days of derivatization.

## 9. Quality Control

**9.1** Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 4). The minimum requirements of this program consist of an initial demonstration of laboratory capability and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

**9.1.1** The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

**9.1.2** In recognition of advances that are occurring in analytical technology, and to allow the analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve separations or lower the costs of measurements. These options include alternate extraction, concentration, cleanup procedures, and changes in columns and detectors. Alternative techniques, such as substitution of immunoassay, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

**9.1.2.1** Each time a modification is made to this method, the analyst is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory is required to demonstrate that the method detection limit (MDL) (40 *CFR* Part 136, Appendix B) is lower than one-third the regulatory compliance level. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.

**9.1.2.2** The laboratory is required to maintain records of modifications made to this method. These records include the information below, at a minimum.

**9.1.2.2.1** The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

**9.1.2.2.2** A listing of pollutant(s) measured, by name and CAS Registry Number.

**9.1.2.2.3** A narrative stating the reason(s) for the modification.

**9.1.2.2.4** Results from all quality control (QC) tests comparing the modified method to this method, including:

**(a)** calibration (Section 10);

**(b)** calibration verification (Section 10.1.3.1);

**(c)** initial precision and accuracy (Section 9.2);

**(d)** analysis of blanks (Section 9.3);

**(e)** accuracy assessment (Section 9.5).

**9.1.2.2.5** Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

**(a)** sample numbers and other identifiers;

**(b)** extraction dates;

**(c)** analysis dates and times;

**(d)** analysis sequence/run chronology;

**(e)** sample weight or volume;

**(f)** extract volume prior to each cleaning step (Section 11.1.2);

**(g)** final extract volume prior to injection;

**(h)** injection volume;

**(i)** dilution data, differentiating between dilution of a sample or an extract;

**(j)** instrument and operating conditions;

**(k)** column and operating conditions (nature of column, dimensions, flow rates, solvents, etc.)

**(l)** detector operating conditions (wavelength, etc.)

**(m)** chromatograms, printer tapes, and other recording of raw data; and

**(n)** quantitation reports, data system outputs, and other data necessary to link raw data to the results reported.

**9.1.3** Analyses of blanks are required to demonstrate freedom from contamination and that PEG-600 and interfering compounds have not been carried over from a previous analyses (Section 4). The procedures and criteria for analysis of a blank are described in Section 9.3.

**9.1.4** The laboratory shall, on an ongoing basis, demonstrate through the analysis of the aqueous performance standard (Section 7.14.5) that the analysis system is in control. This procedure is described in Section 10.

**9.1.5** The laboratory shall maintain records to define the quality of data that is generated.

**9.2** Initial precision and accuracy—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations for compounds to be calibrated:

**9.2.1** Analyze four aliquots of the aqueous performance standard (Section 7.14.5) according to the method beginning in Section 11.

**9.2.2** Using results from Section 9.2.1, compute the average recovery (X) in percent of spike level and the standard deviation of the recovery (s) in percent of the spike level for PEG-600 and the surrogate.

**9.2.3** For each compound, compare s and X with the corresponding limits for initial precision and accuracy found in Table 2. If s and X for all compounds meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, any individual s exceeds the precision limit or any individual X falls outside the range for accuracy, system performance is unacceptable for that compound. This is an indication that the analytical system is not performing properly for the compound(s) in question. In this event, correct the problem and repeat the entire test (Section 9.2.1).

**9.3** Blanks—Reagent water blanks are analyzed to demonstrate freedom from contamination.

**9.3.1** With each sample batch (samples analyzed on the same 8-hour shift), a blank shall be carried through the extraction and derivatization procedure and be analyzed immediately after analysis of the aqueous performance standard (Section 9.1.4) to demonstrate freedom from contamination. If PEG-600 or any potentially interfering compound is found in a blank at greater than 200 µg/L, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination at this level.

**9.4** The specifications contained in this method can be met if the apparatus used is calibrated properly, then maintained in a calibrated state. The standards used for calibration (Section 10.1), calibration verification (Section 10.1.3.1) and for initial (Section 9.2) and ongoing (Section 9.1.4) precision and accuracy should be identical, so that the most precise results will be obtained.

**9.5** Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis.

## 10. Calibration

**10.1** Establish liquid chromatographic operating parameters to produce a retention time equivalent to that given in Section 12.2.1. Prepare derivatized calibration standards according to the procedure in Section 10.1.1. Calibrate the chromatographic system using the external standard technique (Section 10.1.3).

**10.1.1** Preparation of calibration standards. Prepare calibration standards by adding one mL of surrogate standard (Section 7.14.3) to each of the secondary standards (Section 7.14.2).

**10.1.2** Process each calibration standard solution through the extraction, concentration, and derivatization procedures described in Section 11.

**10.1.3** External standard calibration procedure. Analyze each derivatized calibration standard using the chromatographic conditions specified in Section 12.1, and tabulate peak area against concentration injected. The results may be used to prepare calibration curves for PEG-600

**10.1.3.1** The working calibration curve must be verified at the beginning of each 8-hour shift by the measurement of one or more calibration standards. If the response for any analyte varies from the previously established responses by more than 10 percent, the test must be repeated using a fresh calibration standard after it is verified that the analytical system is in control. Alternatively, a new calibration curve may be prepared. If an autosampler is available, it is convenient to prepare a calibration curve daily by analyzing standards along with test samples.

## 11. Sample Extraction and Derivatization

**11.1** Extraction of samples and standards.

**11.1.1** Place one liter of sample and 1 mL of surrogate standard (Section 7.14.3) or 1 L of calibration standard (Section 10.1.1) in the liquid-liquid extractor and extract with pesticide grade dichloromethane for 18 hours.

**11.1.2** Dry the dichloromethane solution over anhydrous sodium sulfate and evaporate off the solvent using the Kuderna-Danish procedure. Dry again over anhydrous sodium sulfate when the volume reaches 10–25 mL and use a gentle stream of dry nitrogen to removemost of the remaining solvent. Quantitatively transfer the residue to a V-shaped reaction vial using anhydrous dichloromethane or anhydrous tetrahydrofuran and remove the last of the solvent with a stream of dry nitrogen.

**11.2** Derivatization

**11.2.1** After ensuring that the extract is free of water, add 5 mL of derivatization solution (Section 7.13) to the vial and two drops of anhydrous pyridine (Section 7.12).

**11.2.2** Heat the vial and contents in a sand bath at 60°C (±5°C) for 1 hour.

**11.2.3** Cool the vial and quantitatively transfer the contents to a 125-mL separatory funnel. Add 50 mL of diethyl ether (ether) and sequentially extract with two 25-mL portions of dilute hydrochloric acid (Section 7.9), then two 25-mL portions of reagent water, then two 25-mL portions of sodium bicarbonate solution (Section 7.10) and finally with two 25-mL portions of saturated sodium chloride solution (Section 7.11). Take care not to lose any ether solution during the extraction procedure.

**11.2.4** Place a small plug of glass wool in a funnel and add approximately 10 g of anhydrous sodium sulfate to the funnel. Drain the ether solution through the sodium sulfate. If necessary use a clean spatula to break up any lumps of sodium sulfate in the funnel, then rinse the separatory funnel with two 10-mL portions of ether and drain through the anhydrous sodium sulfate in the funnel.

**11.2.5** Quantitatively transfer the ether solution to a clean Kuderna-Danish apparatus and evaporate most of the solvent (alternatively, dry nitrogen can be used to evaporate the ether while warming on a steam bath).

**11.2.6** Perform a solvent exchange with 40% acetonitrile in water, adjust the volume to 2 mL and filter, if necessary, for analysis.

## 12. High-Pressure Liquid Chromatography

**12.1** Chromatographic conditions.

Column: Betasil $C_{18}$, 250 mm long × 4.6 mm ID, 5-µm particle size (Keystone 255-701, or equivalent).

Mobile Phase: 40% acetonitrile/water to 100% acetonitrile over a period of 20 minutes.

Flow Rate: 2.0 mL/min

UV Detector: 254 nm

Injection Vol.: 50 µL

**12.2** Analysis.

**12.2.1** Analyze samples by HPLC, using conditions described in Section 12.1. The retention time of the PEG-600 derivative relative to the surrogate derivative is centered about 0.63. The ML achievable in reagent water is 1 mg/L. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 9 are met.

**12.2.2** Because PEG-600 is a mixture of poly(ethylene glycol) oligomers, the exact nature of PEG-600 samples from various manufacturers and different batches from a single manufacturer, may vary. For this reason, concentrations of PEG-600 in a specific waste stream are best determined when standards are prepared using the same batch of PEG-600 in use by the pharmaceutical manufacturer at the time of discharge of the waste stream under analysis. Where it is not possible to obtain such a sample, adequate results can be obtained by the use of a PEG-600 product as a standard that is unrelated to the one in use by the pharmaceutical manufacturer, and careful definition of an "elution range" for derivatized PEG-600 in both the external standards and the samples. (See Section 12.2.3)

**12.2.3** An "elution range" or retention time window is defined as a characteristic period of time during which the derivatized PEG-600 elutes from the chromatographic column. This range should encompass at least 90 percent of the PEG-600 derivative in both the standard and the sample. The width of the retention time window used for quantitation should be based upon

measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of the retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

**12.2.4** If the peak area exceeds the linear range of the calibration curve, a smaller sample volume may be injected. Alternatively, the final solution (Section 11.2.6) may be diluted with 40% acetonitrile/water, as appropriate, and reanalyzed.

**12.3** Calculations.

**12.3.1** Calculate each response factor (RF) as follows (mean value based on 5 points):

$$RF = \frac{concentration\ of\ standard}{area\ of\ the\ signal}$$

$$mean\ RF = \overline{RF} = \frac{\sum_{i=1}^{5} RF_i}{5}$$

**12.3.2** Calculate the concentration of PEG-600 as follows:

$$\mu g/mL = \overline{RF} \times area\ of\ signal \times concentration\ factor$$

*where:*

$$concentration\ factor = \frac{final\ volume\ of\ extract}{initial\ sample\ volume}$$

## 13. Method Performance

**13.1** The ML for PEG-600 was obtained using reagent water.

**13.2** This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the range from the ML to 30 times the ML.

**13.3** A representative chromatogram is presented as Figure 1.

## 14. Waste Management

**14.1** It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions. In addition it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

**14.2** Samples containing acids at a pH of less than 2 are hazardous and must be neutralized before being poured down a drain or must be handled as hazardous waste.

**14.3** For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel," available from the American Chemical Society's Department of Government Relations and Science Policy, 115 16th Street, NW, Washington, DC 20036

## References


1. “Working with Carcinogens," DHEW, PHS, NIOSH, Publication 77-206 (1977).

2. “OSHA Safety and Health Standards, General Industry," 29 *CFR* 1910, OSHA 2206, (1976).

3. “Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety (1979).

4. “Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL Cincinnati, OH 45268, EPA-4-79-019 (March 1979).

Table 1. PMI Analyte

| PMI Analyte | CASRN[1] | Minimum Level[2] |
|---|---|---|
| Poly(ethylene glycol)-600 (PEG-600) | 25322-68-3 | 1 mg/L |

[1] Chemical Abstracts Service Registry Number.

[2] This is the minimum level at which the entire analytical system shall give a recognizable signal and an acceptable calibration point.

Table 2. Requirements for Initial Precision and Accuracy

| PMI Analyte and Surrogate | Spike Level (µg/L) | Average Percent Recovery (X) | Percent Standard Deviation (s) |
|---|---|---|---|
| PEG-600 | 1058 | 22–79 | 28 |
| Di(ethylene glycol) monohexyl ether | 215 | d–102 | 53 |

d = Detected (analyte must be detected)

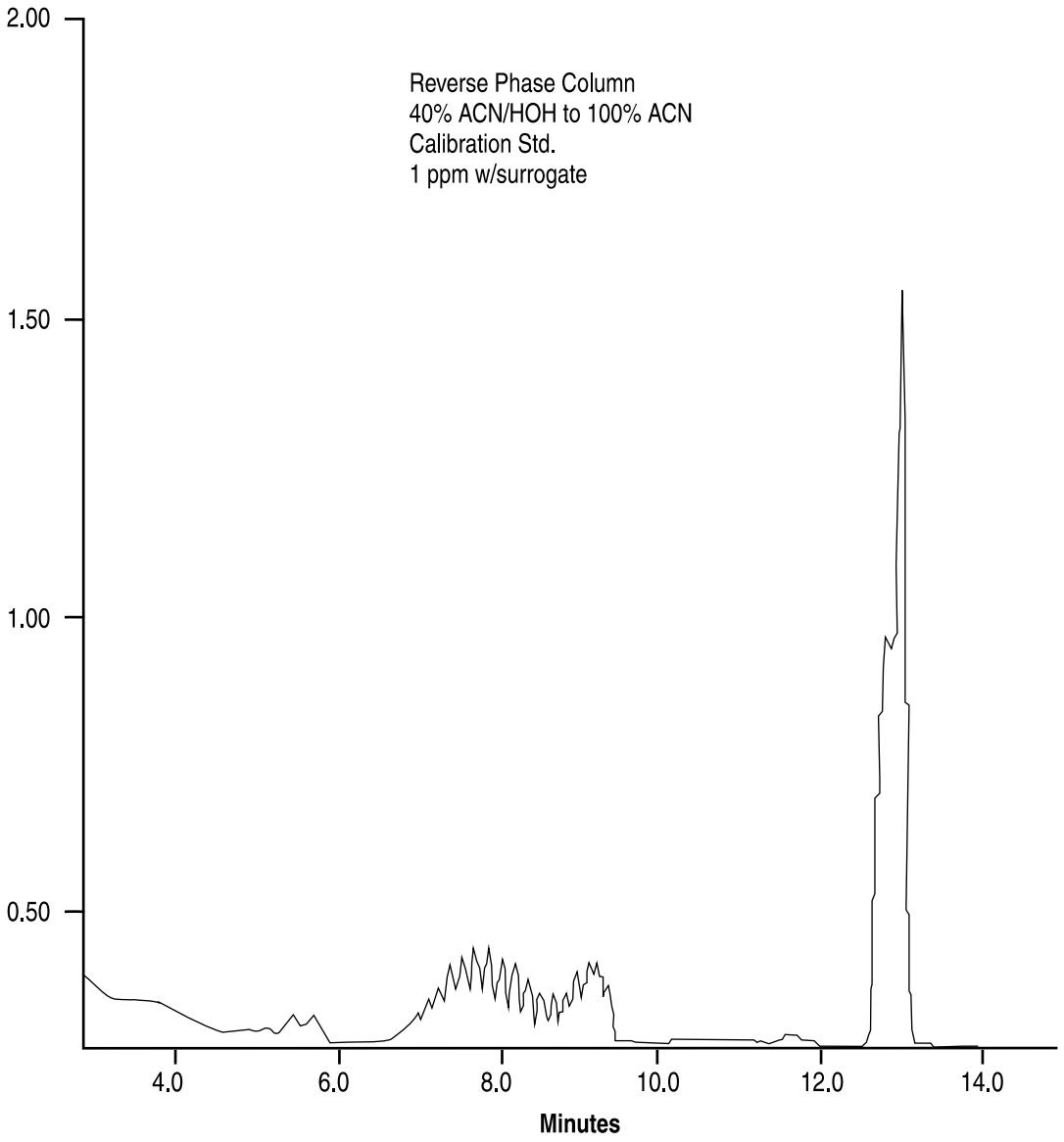


Figure 1. Chromatogram of the Aqueous Performance Standard

52-025-18