



Designation: D7304 – 06

Standard Test Method for Determination of Denatonium Ion in Engine Coolant by HPLC¹

This standard is issued under the fixed designation D7304; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the chemical analysis of engine coolant for denatonium ion by high-performance liquid chromatography (HPLC).

1.2 This test method is applicable to both new and used coolants.

1.3 Coelution of other ions may cause interferences in the detection of the denatonium cation. In the case of unfamiliar formulations, identification verification should be performed by either or both fortification and dilution of the sample matrix with denatonium ion.

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*²

D1193 Specification for Reagent Water

D1176 Practice for Sampling and Preparing Aqueous Solutions of Engine Coolants or Antirusts for Testing Purposes

3. Summary of Test Method

3.1 A sample volume of working sample is prepared by dilution of the sample with water. A high-pressure pump forces the mobile phase, eluant, through the HPLC columns (a guard and analytical column) at typical flow rates of 0.1– 2 mL/min. A sample to be separated is introduced in the mobile phase by

an injection device prior to the column. The analytes are separated as they pass through the column. An optical sensor detects the changes in characteristics of the eluant stream and converts the signal into an absorbance spectrum. The data system compares this response with an external calibration curve and the results of the concentration of analyte reported as ppm or milligrams per litre (mg/L). Refer to [Appendix X1](#) for a HPLC flow diagram.

4. Significance and Use

4.1 This test method provides for the qualitative and quantitative determination of denatonium ion in engine coolant in milligrams per liter to low percent range and requires approximately 100 mL per test, with results available in less than 30 min. Acceptable levels of denatonium vary with manufacturer's blending specifications and applicable minimum or maximum industry and state specifications. Typically specification ranges from 30 to 50 ppm denatonium ion.

5. Interferences

5.1 Interferences can be caused by substances with similar retention times, especially if they are in high concentration compared to the analyte of interest, denatonium ion. Sample dilution and optimized gradient elution can be used to minimize or solve most interference problems.

5.2 A water dip (solvent system peak) can cause interference with some integrators. This is eliminated by dilution with the eluant if the sample dilution factor is 4+1 (v/v) or greater.

5.3 Method interferences can be caused by the contamination of glassware, eluant, reagents, etc. Great care must be taken to ensure that contamination is kept at the lowest possible level

6. Apparatus

6.1 *HPLC System*—Analytical system equipped with all required accessories including syringes, gasses, columns, pumps, detectors and software.

6.1.1 *Gradient Pump.*

6.1.2 *UV/VIS-Multiple Wavelength Detector.*

6.1.3 *Eluant Degas System.*

¹ This test method is under the jurisdiction of ASTM Committee D15 on Engine Coolants and Related Fluids and is the direct responsibility of Subcommittee D15.04 on Chemical Properties.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

6.1.4 *Analytical Column* ZORBAX RX-C8 or equivalent column, capable of producing analyte separation equivalent to or better than that shown in Fig. 1.

6.1.5 *Guard Column* for ZORBAX RX-C8 or equivalent, for protection of the analytical column from strongly retained constituents.

6.1.6 *Integrator or Chromatography Data System Software* capable of obtaining approximately the same detection limits as listed in Table 1.

6.2 *Analytical Balance*, 0.0001 g precision.

6.3 *Volumetric Flasks*, 50 mL, 100 mL, 200 mL.

7. Reagents and Materials

7.1 Acetonitrile, (ACN), Reagent Grade.

7.2 Phosphoric acid, H₃PO₄, 85 %, Reagent Grade.

7.3 Potassium Phosphate, Monobasic, KH₂PO₄, 99.995 % purity.

7.4 Denatonium Benzoate, Reagent Grade.

7.5 Ethylene Glycol, Reagent Grade.

7.6 Deionized water, Type II water.

7.7 *Purity of Reagents*—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.

7.8 Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D1193, Type II. It is recommended that all water be filtered through a 0.45 μm filter. For eluant preparation, degas the water by sparging with helium or vacuum degassing and sonication.

TABLE 1 Minimum Detection Limits

Analyte	Detection Limits, ppm ^A
Denatonium ion	1.0

^A Determination using 100-μL sample volume. Sample diluted 1+3 (wt/v) with type II water, full scale, UV/Vis detector set at 210 nm wavelength, Zorbax RX-C8 analytical column with Zorbax RX-C8 guard columns. Other systems will require MDL determination using chosen dilution factors, eluants, columns and detectors.

7.9 *Phosphate Buffer Stock Solution*—Weigh 650 mg of Monobasic Potassium Phosphate and transfer it into a 1 L volumetric flask, add 0.5 mL Phosphoric acid and fill to the mark with Type II water. Mix this solution thoroughly. Filter the solution with 0.45 μm filter before use.

7.10 *Denatonium Ion Standard Stock Solution*—Weigh 0.1372 g of Denatonium Benzoate into 100-mL volumetric flask and dilute with ethylene glycol to 100 g. This stock solution is 1000 ppm of denatonium and is stable for one month. Make-up working standard solutions in 25% v/v ethylene glycol/Type II water to desired concentrations. Example: 5 ppm denatonium standard solution - weigh 0.5000 g of stock denatonium standard solution into a 100 mL volumetric flask and fill to 100 mL mark with 25 % v/v ethylene glycol water solution.

8. Hazards

8.1 Personnel protective equipment (such as eye protections, gloves, laboratory coat, etc) should be used in the handling of all chemicals. Special care should be taken when handling solutions around electrical equipment. All solution should be prepared in a hood.

8.2 Read all equipment manuals before attempting to operate HPLC instrumentation. Special attention should be given to all warnings.

8.3 Be familiar with the MSDS for all chemicals used in this procedure. A dust mask is recommended for the handling of

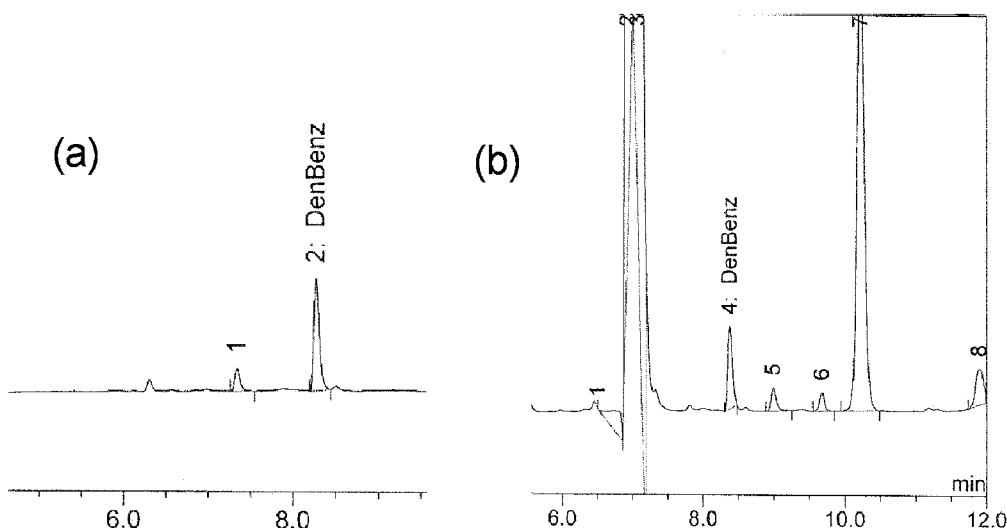


FIG. 1 (a) Denatonium Benzoate Standard (b) Denatonium Benzoate in an Engine Coolant