



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

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1. Scope

1.1 This test method covers the chemical analysis of engine coolant for denatonium benzoate (DNB) by high-performance liquid chromatography (HPLC). DNB is added to potentially render engine coolant unpalatable to animals and humans. This analytical method was designed for the analysis of DNB and is not valid for any other bittering agents such as denatonium saccharide.

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](#)

[E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods](#)

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.

[E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method](#)

3. Summary of Test Method

3.1 The denatonium benzoate analysis is achieved by an HPLC method, where a weight of engine coolant is placed in an auto-sampler vial and mixed with a known volume of de-ionized water. The auto-sampler vial is placed in a HPLC autosampler and the measurement of denatonium benzoate is performed using a C-18 reverse phase column attached to an ultraviolet detector. The ultraviolet detector is used to measure the response of the DNB active ingredients (denatonium and benzoate) in the engine coolant after they have been separated in the reverse phase column. The denatonium and benzoate responses are compared to responses of known concentrations and the HPLC's computer calculates the amount of DNB present in the coolant.

4. Significance and Use

4.1 This test method provides for the qualitative and quantitative determination of denatonium benzoate in engine coolant in milligrams per litre to low percent range and requires approximately 1 mL per test, with results available in less than 10 min. Denatonium benzoate is a compound composed of a quaternary ammonium cation, denatonium and an inert anion, benzoate. In solution the denatonium benzoate exists in equilibrium between the denatonium benzoate compound, the denatonium cation and benzoate anion. By slightly adjusting the pH of the solution to be more acidic ($\approx$ pH 4.6) the equilibrium will be shifted to the direction of forming more denatonium and benzoate ions in the solution.

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. Known chromatographic interferences have been determined and the analysis modified to minimize any co-elution of interfering peaks. The eluent strength and flow rate can be used to reduce or solve most interference problems.