Designation: D7591 – 12

Standard Test Method for Determination of Free and Total Glycerin in Biodiesel Blends by Anion Exchange Chromatography[1]

This standard is issued under the fixed designation D7591; 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 and describes an anion exchange chromatography procedure for determining free and total glycerin content of biodiesel (B100) and blends (B0 to B20) with diesel fuel oils defined by Specification D975 Grades 1-D, 2-D, and low sulfur 1-D and 2-D and Specification D6751 (for B100 feedstocks). It is intended for the analysis of biodiesel and blend samples containing between 0.5 to 50 mg/kg glycerin.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *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]

D975 Specification for Diesel Fuel Oils

D1193 Specification for Reagent Water

D4057 Practice for Manual Sampling of Petroleum and Petroleum Products

D4177 Practice for Automatic Sampling of Petroleum and Petroleum Products

D6299 Practice for Applying Statistical Quality Assurance and Control Charting Techniques to Evaluate Analytical Measurement System Performance

D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels

D6792 Practice for Quality System in Petroleum Products and Lubricants Testing Laboratories

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions:*

3.1.1 *free glycerin, n*—measure of the amount of glycerin remaining in the fuel.

3.1.2 *total glycerin, n*—sum of the free glycerin and the glycerin portion of any unreacted or partially reacted oil or fat.

4. Summary of Test Method

4.1 *Free Glycerin*—A small volume of an extract of the blend sample is directly injected into an ion chromatograph consisting of appropriate ion exchange columns and into an electrochemical detector. Glycerin is separated based on its affinity for ion exchange sites of the resin with respect to the resin's affinity for the eluent. An electrochemical detector is employed for detection of glycerin. Glycerin is quantified by peak area based on an external calibration curve, and is reported as µg/g (mg/kg), or may be converted to wt%. Calibration standards are prepared from commercially available glycerin (99+% purity) in an aqueous solution.

4.2 *Total Glycerin*—A small volume extract of a saponified blend sample is directly injected into an ion chromatograph consisting of appropriate ion exchange columns and into an electrochemical detector. Glycerin is separated based on its affinity for ion exchange sites of the resin with respect to the resin's affinity for the eluent. An electrochemical detector is employed for detection of glycerin. Glycerin is quantified by peak area based on an external calibration curve, and is reported as µg/g (mg/kg), or may be converted to wt%. Calibration standards are prepared from commercially available glycerin (99+% purity) in an aqueous solution.

5. Significance and Use

5.1 Petroleum-based diesel may be blended with biodiesel. High levels of free glycerin in biodiesel can cause injector deposits ("gel effect"), as well as clogging fuel systems. High levels of unreacted glycerides can cause injector deposits and can adversely affect cold weather operation and filter plugging.

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.04.0C on Liquid Chromatography.

Current edition approved Jan. 1, 2012. Published March 2012. DOI:10.1520/D7591–12

[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.

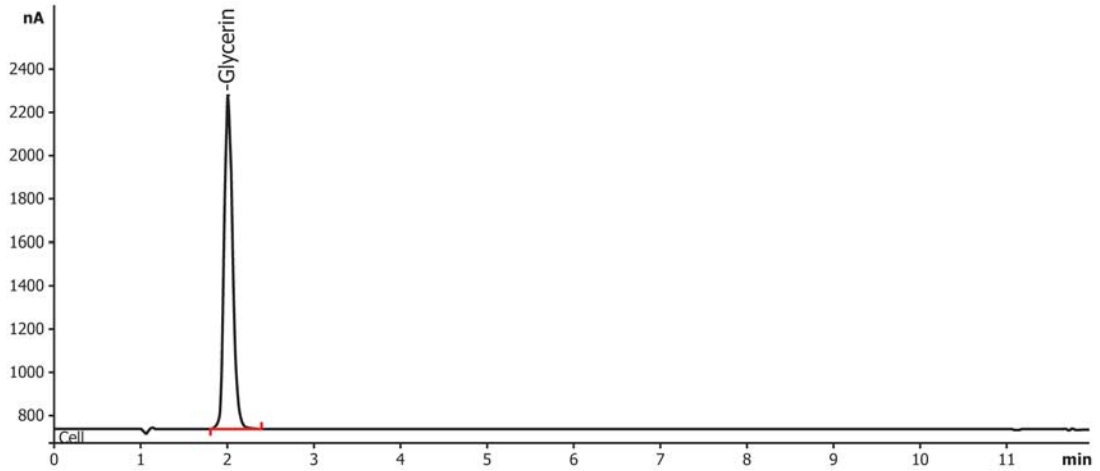

FIG. 1 Typical Chromatogram of a Solution Containing 0.7 mg/kg of Glycerin

6. Interferences

6.1 Interferences can be caused by substances with similar ion chromatographic retention times, especially if they are in high concentration compared to the analyte of interest. Sample dilution can be used to minimize or resolve most interference problems. Also, an excess of unreacted hydroxide (base) during the sample preparation step for total glycerin can cause a pH imbalance on the anion exchange column, resulting in a negative dip in front of the glycerin peak.

6.2 A water dip (system void, negative peak as shown in Fig. 1) can cause interference with some integrators. This dip can be eliminated by dilution with the eluent. The water dip should not be a problem since the glycerin peak is resolved from the void peak.

6.3 Interferences can be caused by contamination of glassware, eluent, reagents, etc. Take care to ensure that contamination is kept at the lowest possible levels. The use of nitrile gloves is highly recommended to prevent contamination during sample preparation.

6.4 There are several known additives based on natural products that might have similar retention times and detector response similar to glycerin. In the case of higher than expected values for biodiesel blends, it is highly recommended that the user needs to verify these higher than expected values for glycerin using a different analytical technique.

6.5 Pre-rinsing of the sample preparation containers with deionized water is mandatory.

7. Apparatus

7.1 *Analytical Balance*—capable of weighing up to 200 g accurately to ±0.0001 g.

7.2 *Desiccator*—containing freshly activated silica gel (or equivalent desiccant) with moisture content indicator.

7.3 *Pipettes or Volumetric Transfer Devices*— 1 and 5 mL class A volumetric pipettes or calibrated variable volume automatic pipettes fitted with disposable polypropylene tips.

7.4 *Volumetric Flasks*—25, 50, 100 and 1000 mL class A volumetric flasks.

7.5 *Container*—standard HDPE plastic 100 mL bottle with cap.

7.6 *Ion Chromatograph*—Analytical system with all required accessories including syringes, columns, high-pressure dual piston pump, and detector.

7.6.1 *Injection System*—capable of delivering 5 to 25 µL with a precision better than 1%.

7.6.2 *Pumping System*—capable of delivering mobile phase flows between 0.1 and 5.0 mL/min with a precision better than 2%. Due to the corrosive nature of the eluent, a PEEK pump head is recommended.

7.6.3 *Guard Column*—for protection of the analytical column from strongly retained constituents.

7.6.4 *Anion Exchange Column*—capable of producing satisfactory analyte separation.

7.6.5 *Electrochemical Detector*—integrated, temperature controlled to 0.1°C, capable of measuring at least 0 to 200 µA on a linear scale. Detector has a pulsed amperometric detection mode for required sensitivity. Consult with the manufacturer for optimal cell settings.

7.6.6 *Electrochemical Detector Cell*—minimum 3 mm gold working electrode surface with wall jet design, solid state reference and counter electrodes. Ensure a minimal volume in the cell for enhanced sensitivity. A platinum working electrode may also be used.

7.6.7 *Integrator or Chromatography Data System Software*—capable of measuring peak areas and retention times, and performing a baseline correction.

7.6.8 *Sample Digestion System*—capable of heating, and stirring with integrated reflux. Reflux is needed to minimize loss of petroleum diesel in biodiesel blend samples. A chiller is recommended for providing water to the reflux condenser for efficiency and to conserve water resources.

7.7 *Mechanical Wrist Shaker.*

7.8 *Gloves, nitrile.*

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade or higher purity chemicals shall be used for the preparation of all samples,

standards and eluent solutions. 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.[3] 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.

8.2 *Water Quality*—Unless otherwise indicated, reference to water shall be understood to mean reagent water as defined by Type II in Specification D1193 or better. For eluent preparation and handling, comply with all ion chromatograph instrument and column vendor requirements (for example, filtering, degassing, etc.).

8.3 *Eluent Stock Solution,* sodium hydroxide (NaOH, 50% certified, ACS).

8.3.1 *Eluent Preparation,* 0.10 *M* NaOH. Weigh 8.00 ± 0.02 g of 50% NaOH in reagent water in a 1-L volumetric flask and dilute to volume with degassed reagent water. The eluent solution used may be different if other systems or analytical columns are used. Other volumes of stock solution may be prepared using appropriate ratios of reagents. Ready to use reagents may be used. Consult with the instrument manufacturer for guidance and use. Do not store sodium hydroxide solutions in glass.

8.4 *Potassium Hydroxide Solution for Total Glycerin,* 1.0 *M* KOH. Weigh out 56.1 g of ACS grade potassium hydroxide pellets. Dissolve the pellets in approximately 250 mL DI water in a 1 L volumetric flask. Use caution when handling the flask due to the heat produced during the dissolution of the potassium hydroxide. Dilute to the mark with DI water. Prepared ready to use 1.0 *M* potassium hydroxide solutions made with acceptable purity materials may also be used. Keep containers tightly closed when not in use to minimize carbonate formation from atmospheric carbon dioxide.

9. Preparation of Standard Solutions

9.1 Stock and working solutions.

9.1.1 *Glycerin Stock Solution, 1000 mg/L*—Accurately weigh 1 g of 99.5+% glycerin to the nearest tenth of a milligram (0.0001 g) and transfer to a 1 L volumetric flask. Dilute to the mark with water. Shake or swirl to mix the standard for homogeneity. Other volumes of stock solution may be prepared using the appropriate ratio of reagents.

9.2 *Working Standards*—Prepare glycerin working standards according to Table 1.

9.2.1 Alternatively, commercial stock calibration solutions can be used, provided that the solutions are traceable to primary stock solutions or certified reference materials, and are free from other analytes.

TABLE 1 Preparation of Glycerin Standards in Water

Glycerin Standard, mg/kg	Water (final weight), g	Glycerin Stock Solution, g
50	100	5.0
20	100	2.0
10	100	1.0
5	100	0.5
1	100	0.1
0.5	100	0.05

10. Calibration

10.1 Set up the ion chromatograph according to the manufacturer's instructions. No specific parameters are given here since different manufacturer's equipment might require changes in eluent, flow conditions, and instrument settings to perform the separation and obtain the results. Calibrate the ion chromatograph with at least five concentration levels of glycerin, starting near but above the minimum detection limit, and covering the expected working range of samples subsequently to be analyzed. Select concentrations of calibrant solutions used that bracket the expected range for the samples to be analyzed. Use one or more mid-range standards to verify the linearity of the calibration plot.

10.1.1 Typical ion chromatographic conditions:

Flow: 1.0 mL/min

Sample loop: 10 µL

Other analytical conditions may be used per the manufacturer's instructions.

NOTE 1—The sample loop volume will vary based on the column capacity, sensitivity, and other factors. Refer to ion chromatography equipment manuals and column information for instrument/column-specific details.

10.1.2 Establish analytical curves with only one detector scale setting. This will prevent a change of slope affecting the analytical curve.

10.2 Verify the analytical calibration plot daily or whenever samples are to be run, prior to the analysis of samples to verify the system resolution, calibration, and sensitivity as part of the quality verification process (see Section 14).

10.3 Repeat calibration after any change of the ion chromatography eluent solution from 8.3, to reestablish ion retention times and resolution. Use a check standard to verify calibration, retention times, and resolution after any change in the IC eluent solution from 8.3. Recalibrate if needed.

10.4 *Measurement of the Calibration Standards*—Inject 10 µL of each calibration solution from 9.2 into the ion chromatograph, and measure the areas of the peaks corresponding to glycerin. Generally, one injection per sample is sufficient. Refer to Section 14 for quality control discussion.

10.5 Construct the glycerin calibration plots by plotting the peak areas against the glycerin concentrations. Use linear regression to determine the best straight-line calibration. A linear least squares correlation coefficient of 0.99 or greater is required (see Fig. 2). The response factor for glycerin, Rf, is the slope of the calibration plot straight line, in mg/kg/(area count).

[3] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.