Designation: ~~E1758 – 01 (Reapproved 2007)~~ E1758 – 01 (Reapproved 2015)

Standard Test Method for Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography[1]

This standard is issued under the fixed designation E1758; 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.

INTRODUCTION

The carbohydrates making up a major portion of biomass samples are polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose subunits. The polysaccharides present in a biomass sample can be hydrolyzed to their component sugar monomers by sulfuric acid in a two-stage hydrolysis process. These monosaccharides can then be quantified by ion-moderated partition HPLC.

1. Scope

1.1 This test method covers the determination of carbohydrates present in a biomass sample, expressed as the percent, by mass, of each sugar on a 105°C dried mass basis.

NOTE 1—The percent sugar must be corrected for the water of hydrolysis before calculating the actual mass percent of the polysaccharide in the original biomass sample.

1.2 Sample materials suitable for this procedure include hard and soft woods, herbaceous materials (such as switchgrass and sericea), agricultural residues (such as corn stover, wheat straw, and bagasse), wastepaper (such as office waste, boxboard, and newsprint), acid or alkaline-pretreated biomass (washed free of any residual acid or alkali), and the solid fraction of fermentation residues. All results are reported relative to the 105°C oven-dried mass of the sample.

1.3 The options for the types of samples to be analyzed in this test method are as follows:

1.3.1 *Prepared Biomass Samples:*

1.3.1.1 *Air Dried ($\%T_{ad}$)*—The percent, by mass, of total solids of the air-dried sample.

1.3.1.2 *45°C Dried ($\%T_{45}$)*—The percent, by mass, of total solids of the 45°C dried sample.

1.3.1.3 *Freeze Dried ($\%T_{fd}$)*—The percent, by mass, of total solids of the freeze dried sample.

1.3.2 *Extractives-Free Sample ($\%T_{ext}$)*—The percent, by mass, of total solids of the extracted sample determined at 105°C.

1.4 The values stated in SI units are to be regarded as the standard.

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.* Specific precautionary statements are given in Note 3 *and Note 4*.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

E1690 Test Method for Determination of Ethanol Extractives in Biomass

E1721 Test Method for Determination of Acid-Insoluble Residue in Biomass

E1756 Test Method for Determination of Total Solids in Biomass

E1757 Practice for Preparation of Biomass for Compositional Analysis

[1] This test method is under the jurisdiction of ASTM Committee E48 on Bioenergy and Industrial Chemicals from Biomass and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion.

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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.

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *as received biomass*—the biomass material as it is received in its field or process collected state.

3.1.2 *oven-dried mass*—the moisture-free mass of a biomass sample dried at 105°C as described in Test Method E1756.

3.1.3 *prepared biomass*—material that has been treated according to Practice E1757 in order to raise the total solids content above 85 %, by mass, based on an oven-dried solids mass.

3.2 *Abbreviations*—Abbreviations of standards used in the procedure, and definitions of terms used in the calculations are as follows:

3.2.1 C_1—known concentration of sugar recovery standard before hydrolysis, in mg/mL.

3.2.2 C_2—concentration of sugar recovery standard detected by HPLC after hydrolysis, in mg/mL.

3.2.3 C_{corr}— concentration of sugar in hydrolyzed sample corrected for hydrolysis, in mg/mL.

3.2.4 C_{spl}— concentration of sugar in hydrolyzed sample detected by HPLC, in mg/mL.

3.2.5 *CVS (calibration verification standard)*— standards used in determining the quality of the calibration curve as well as the quality of the standard reagents used in preparing the calibration standards.

3.2.6 m_1—initial mass of sample, in mg.

3.2.7 *% extractives*—the percentage, by mass, of extractives in the prepared biomass sample as described in Test Method E1690.

3.2.8 $\%R_{srs}$— percent recovery of sugar recovery standard, as determined in 13.2.

3.2.9 $\%sugar_{extractives\text{-}free}$—the percentage, by mass, of sugar on an extractives-free 105°C dry weight basis, as determined in 13.6.1.

3.2.10 $\%\ sugar_{whole\ sample}$—the corrected mass percent sugar value on an extractives-free basis corrected to an as received (whole sample) 105°C dry mass basis.

3.2.11 $\%T_{45}$—percentage, by mass, of total solids of the sample prepared by drying at 45°C, as described by Practice E1757.

3.2.12 $\%T_{105}$—percentage, by mass, of total solids in the sample, dried at 105°C, as determined by Test Method E1756.

3.2.13 $\%T_{ad}$— percentage, by mass, of total solids of the air-dried sample determined at 105°C as described by Test Method E1756.

3.2.14 $\%T_{ext}$— percentage, by mass, of total solids of the extracted sample determined at 105°C as described by Test Method E1756.

3.2.15 $\%T_{fd}$— percentage, by mass, of total solids of the sample prepared by freeze drying, as described by Test Method E1756.

3.2.16 $\%T_{prep}$— percentage, by mass, of total solids of the sample prepared by freeze drying, $\%\ T_{fd}$, or by drying at 45°C, $\%\ T_{45}$, as determined by Practice E1757.

3.2.17 *SRS (sugar recovery standards)*—standards used to determine sugar recovery after hydrolysis.

3.2.18 V_F—volume of filtrate, 87.0 mL.

4. Significance and Use

4.1 The percentage, by mass, of sugar content is used in conjunction with other assays to determine the total composition of biomass samples.

5. Interferences

5.1 Samples with high protein content may result in the percentage, by mass, of sugar values being biased low, as a consequence of protein binding with some monosaccharides.

5.2 Test specimens not suitable for analysis by this procedure include alkaline and acid-pretreated biomass samples that have not been washed. Unwashed pretreated biomass samples containing free acid or alkali may change visibly on heating.

6. Apparatus

6.1 *Analytical Balance,* readable to 0.1 mg.

6.2 *Autoclave,* capable of maintaining 121 ± 3°C.

6.3 *Convection Ovens,* temperature control to 45 ± 3 and 105 ± 3°C.

6.4 *Desiccator,* using anhydrous calcium sulfate.

6.5 *Guard Columns,* cartridges appropriate for the column used.

NOTE 2—Deashing guard column cartridges from BioRad,[3] of the ionic form H^+/CO_3^-, are an option when using an HPX-87P[3] column, or equivalent. These cartridges are effective in eliminating baseline ramping.

6.6 *Hewlett Packard*[4] Model 1090 HPLC, or equivalent, with refractive index detector.

6.7 *HPLC Columns, BioRad* HPX-87C[3] or HPX-87P,[3] or both, or equivalent.

6.8 *Water Bath,* set at 30 ± 1°C.

7. Reagents and Materials

7.1 *Chemicals:*

7.1.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. 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.[5] 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.

7.1.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type 1 of Specification D1193.

7.1.3 *Calcium Carbonate.*

7.1.4 *High-Purity Sugars (98 % +, By Mass)*—Two sets of glucose, xylose, galactose, arabinose, and mannose, meeting the requirements described above, dried at 45°C. The sugars are used in preparing calibration standards, calibration verification standards (CVS), and sugar recovery standards (SRS). The sugars used in preparing the calibration standards should be from a source (manufacturer or lot) other than that used in preparing the CVS. Either set of sugars may be used for preparing the SRS solutions used in determining sugar recoveries after hydrolysis.

7.1.5 *Sulfuric Acid Solution (72 % w/w or 12.00 ± 0.02 M)*—Slowly add 665 mL of concentrated sulfuric acid (H_2SO_4) to 300 mL of water while cooling in an ice bath and stirring. Allow to come to room temperature. Adjust the relative density to 1.6389 ± 0.0012 at 15.6°C/15.6°C.

7.2 *Materials:*

7.2.1 *Autosampler Vials,* with crimp top seals to fit.

7.2.2 *Disposable Syringes,* 3 mL.

7.2.3 *Disposable Syringe Filters,* nylon, 0.2 µm.

7.2.4 *Glass Serum Bottles,* crimp top style, 125 mL, with rubber stoppers and aluminum seals to fit.

8. Hazards

8.1 Handle the sulfuric acid carefully to avoid contact with skin or clothing, as it is corrosive.

8.2 The glass bottles are hot and may be pressurized after the autoclave step. Use caution when handling.

9. Sampling, Test Specimens, and Test Units

9.1 Test specimens suitable for analysis by this procedure are:

9.1.1 Prepared biomass prepared according to Practice E1757, and

9.1.2 Extractives-free material prepared according to Test Method E1690.

10. Calibration and Standardization

10.1 Prepare a series of three to six sugar standards in deionized water at concentrations appropriate for preparing calibration curves to quantitfy each sugar of interest. An HPX-87C[3] column, or equivalent, is used to analyze glucose, xylose, and arabinose. If mannose and galactose are also to be quantified, an HPX-87P[3] column, or equivalent, must be used instead. Typically, the concentrations of these sugar standards cover the range starting at the detection limit of the instrument and extending up to 4.0 mg/mL.

10.2 Prepare an independent CVS, as described in 8.1.2, for each set of calibration standards, using sugars obtained from a source other than that used in preparing the calibration standards. The CVS will contain precisely known amounts of each sugar contained in the calibration standards, at a concentration in the middle of the validated range of the calibration curve. The CVS will be analyzed after each calibration curve and at regular intervals in the HPLC sequence, as dictated by good laboratory practice. The CVS is used in confirming the quality of the calibration curve(s) and the standard reagents used in preparing the calibration

[3] The sole source of supply of the apparatus known to the committee at this time is BioRad Aminex®, HPX-87C and Aminex® HPX-87P, available from BioRad, Main Office, 3300 Regatta Boulevard, Richmond, CA 94804. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[4] Available from Hewlett-Packard, HP Analytical Direct, 2850 Centerville Road, Wilmington, DE 19808.

[5] *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 *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.