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Standard Test Method for Determination of Carbohydrates in Biomass by Gas Chromatography¹

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INTRODUCTION

This test method gives a reproducible way to quantitatively determine in lignocellulosic materials the kind and amount of the structural carbohydrates made from arabinose, xylose, mannose, galactose, and glucose. This way is accomplished by first hydrolyzing the carbohydrates to their constituent monosaccharides. Subsequent derivatization produces the corresponding alditol acetates that are quantified using capillary gas chromatography.

1. Scope

1.1 This test method describes the determination of structural carbohydrates present in a biomass sample, expressed as the percent mass of an oven-dried sample basis of each anhydrosugar.

1.2 Sample materials suitable for this procedure include hard and softwoods, herbaceous materials, such as sericea and switchgrass, agricultural residues, such as corn stover, wheat straw, and bagasse, wastepaper, such as boxboard, office waste, and newsprint, acid or alkaline-pretreated biomass, washed free of any residual acid or alkali, and the solid fraction of fermentation residues.

1.3 The options for the types of samples to be analyzed in this procedure are:

1.3.1 *Prepared Biomass Samples:*

1.3.1.1 *Air Dried Material*—Results are reported as the percent by mass, based on the oven-dried mass of the air-dried sample.

1.3.1.2 *45°C Dried Material*—Results are reported as the percent by mass, based on the oven-dried mass of the 45°C dried sample.

1.3.1.3 *Freeze Dried Material*—Results are reported as the percent by mass, based on the oven-dried mass of the freeze dried sample.

1.3.2 *Extractives-Free Sample*—Results are reported as the percent by mass, based on the oven-dried mass of the extracted sample.

1.4 This standard method is generally not suitable for samples that contain soluble, nonstructural carbohydrates unless they are removed prior to analysis.

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.* See Section 8 for specific hazards statements.

2. Referenced Documents

2.1 *ASTM Standards:*²

D 1193 Specification for Reagent Water

E 1690 Test Method for Determination of Ethanol Extractives in Biomass

E 1721 Test Method for Determination of Acid-Insoluble Residue in Biomass

E 1756 Test Method for Determination of Total Solids in Biomass

E 1757 Practice for Preparation of Biomass for Compositional Analysis

¹ This test method is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion. Current edition approved June 10, 1996. Published August 1996.

² This test method is under the jurisdiction of ASTM Committee E48 on Biotechnology and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion. Current edition approved Nov. 15, 2007. Published January 2008. Originally approved in 1996. Last previous edition approved in 2001 as E 1821-01.

³ 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 Descriptions of Terms Specific to This Standard:

3.1.1 *anhydrosugars, n*—the nominal repeating unit of a polysaccharide. When polysaccharides undergo acid hydrolysis, each repeating unit adds a single molecule of water to form the free monosaccharide that is analyzed. The extra weight from this water of hydrolysis must be ~~corrected for~~ taken in to account when calculating the actual mass percent of the polysaccharide in the original biomass sample.

3.1.2 *as received biomass, n*—material as it is received in its field or process collected state.

3.1.3 *extractives-free biomass*—air-dried solids left after biomass has been treated according to Test Method E 1690.

3.1.4 *oven-dried mass, n*—the moisture-free mass of any biomass sample (as received, prepared, extractives-free, etc.) dried at 105°C as described in Test Method E 1756.

3.1.5 *prepared biomass, n*— *as received biomass* material that has been treated according to Practice E 1757 in order to raise the total solids content above 85 %, based on an oven-dried solids weight.

3.1.6 *structural carbohydrates, n*—polysaccharides that cannot be removed by extraction with solvents and are liberated from the biomass solids with dilute acid hydrolysis. For the purpose of this test method, the monosaccharides that are considered present are arabinose, xylose, mannose, galactose, and glucose.

3.2 Abbreviations: ~~Abbreviations:~~ Abbreviations:

3.2.1 $\%Anhydro_{ext}$ —the percent by mass of the anhydrosugar on an extractives-free, oven-dried mass basis.

3.2.2 $\%Anhydro_{whole}$ —the percent by mass of the anhydrosugar, on an oven-dried mass basis.

3.2.3 AR_c (Amount Ratio)—ratio of the concentration (amount) of monosaccharide c to the concentration (amount) of internal standard in the specimen.

3.2.4 $area_c$ — reported area counts for the monosaccharide c peak in the chromatogram, as integrated by the electronic integrator.

3.2.5 $area_{IS}$ — reported area counts for the internal standard peak in the chromatogram, as integrated by the electronic integrator.

3.2.6 C_{avg} — average concentration of monosaccharide c in specimen s , in mg/mL, averaged across multiple injections of specimen s .

3.2.7 C_{LF} — original concentration of monosaccharide c in loss factor sample, in mg/mL.

3.2.8 C_{IS} — concentration of internal standard (inositol) in the calibration standards and specimen, in mg/mL.

3.2.9 C_s — concentration of monosaccharide c in specimen s , measured by gas chromatography (GC), in mg/mL.

3.2.10 C_{STD} — concentration of monosaccharide c in the calibration standard, in mg/mL.

3.2.11 CV (*coefficient of variation*)—the estimated standard deviation divided by the average value measured.

3.2.12 $\%extractives$ —the percentage by mass of extractives in the extracted specimen as described in Test Method E 1690.

3.2.13 k —constant used to convert the mass of monosaccharide to the mass of anhydrosugar from which it is derived. For arabinose and xylose, $k = 0.88$ (m/z 132/150); for mannose, galactose and glucose, $k = 0.90$ (m/z 162/180).

3.2.14 LF —loss factor for monosaccharide c . Used to correct for the amount of monosaccharide lost through degradation during acid hydrolysis of biomass.

3.2.15 m_f — initial mass of the biomass specimen, in mg.

3.2.16 m_{corr} — mass of monosaccharide in solution, corrected for hydrolysis losses, in mg.

3.2.17 RR_{avg} — averaged response ratio of monosaccharide c to the internal standard (inositol) in the calibration standard. Derived from multiple injections of the same calibration standard.

3.2.18 RR_s — response ratio of monosaccharide c to the internal standard (inositol) in the specimen.

3.2.19 RR_{STD} — response ratio of monosaccharide c to the internal standard (inositol) in the calibration standard.

3.2.20 RRF (*Relative Response Factor of monosaccharide c*)—this is the ratio of the detector response for monosaccharide c versus the detector response for the internal standard (inositol) for a given injection of the specimen.

3.2.21 V_f — 87 mL, volume of hydrolysis solution.

3.2.22 $\%T_{45}$ — percentage by mass, of total solids of the specimen prepared by drying at 45°C, as described by Practice E 1757.

3.2.23 $\%T_{105}$ — percentage by mass, of total solids of the specimen, dried at 105°C, as determined by Test Method E 1756.

3.2.24 $\%T_{ad}$ — percentage by mass, of total solids of the air-dried specimen determined at 105°C as described by Test Method E 1756.

3.2.25 $\%T_{ext}$ — percentage by mass, of total solids of the extracted specimen determined at 105°C as described by Test Method E 1756.

3.2.26 $\%T_{fd}$ — percentage by mass, of total solids of the specimen prepared by freeze drying, as described by Practice E 1757.

3.2.27 $\%T_{prep}$ — percentage, by mass, of total solids of the specimen prepared by freeze drying, $\%T_{fd}$, or by drying at 45°C, $\%T_{45}$, as determined by Practice E 1757.

4. Significance and Use

4.1 The structural carbohydrate content is used in conjunction with other assays to determine the total composition of biomass samples.

5. Interferences

5.1 The results of structural carbohydrate analysis are affected by incomplete hydrolysis of biomass or hydrolysis conditions that are too severe. Incomplete hydrolysis will bias the results low because dimeric and oligomeric carbohydrates are not quantified. Hydrolysis conditions that are too severe degrade the liberated monosaccharides into materials that are not quantified by this procedure, again biasing the results low.

5.2 Incomplete neutralization and removal of acetic acid from the methylene chloride extract prior to GC analysis can result in ghost peaks appearing in the chromatogram or carryover of monosaccharides from one injection to the next (owing to buildup of monosaccharides in the injection port), leading to erroneous quantitation.

5.3 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.

5.4 Materials containing nonstructural carbohydrates also are unsuitable for this procedure since ~~they nonstructural carbohydrates may undergo degradation to materials that are not quantified in this procedure, and hence are lost.~~ procedure.

6. Apparatus

6.1 *Analytical Balance*, readable to 0.1 mg.

6.2 *Autoclave*, capable of maintaining $121 \pm 3^\circ\text{C}$.

6.3 *Convection Ovens*, temperature controlled to $45 \pm 3^\circ\text{C}$ and $105 \pm 3^\circ\text{C}$.

6.4 *Desiccator*, containing anhydrous calcium sulfate.

6.5 *Gas Chromatograph*, equipped with electronic integrator, capillary split injection port, flame ionization detector with make-up gas, $250 \mu\text{m} \times 15 \text{m}$ fused-silica capillary column coated with 50 % cyanopropylphenyl methylpolysiloxane, 0.25 μm film thickness (DB[®]-225³ or equivalent).

6.6 *Ice Bath*.

6.7 *Ultrasonic Bath*.

6.8 *Vortex Mixer*, or equivalent method to rapidly mix solutions in a test tube.

6.9 *Water Bath*, setable to $30 \pm 1^\circ\text{C}$ and $40 \pm 1^\circ\text{C}$.

7. Reagents and Materials

7.1 *Chemicals*:

7.1.1 *Purity of Reagents*—Use reagent grade chemicals 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.⁴ Monosaccharides used to prepare the monosaccharide stock solutions and loss factor standard solutions shall be 98+ mass % purity. Other chemical grades may be substituted, 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 mean reagent water as defined by Type 1 of Specification D 1193.

7.1.3 *Acetic Acid* (CH_3COOH), glacial.

7.1.4 *Acetic Anhydride* ($(\text{CH}_3\text{CO})_2\text{O}$).

7.1.5 *Ammonium Hydroxide*, (NH_4OH), concentrated (28–30 wt % NH_3).

7.1.6 *Ammonium Hydroxide Solution (~3 M)*—Dilute $5.0 \pm 0.1 \text{mL}$ of concentrated ammonium hydroxide (NH_4OH) with $20.0 \pm 0.1 \text{mL}$ of water. Prepare fresh before each use.

7.1.7 *Monosaccharide Stock A Solution*— Combine the following monosaccharides. Weigh each monosaccharide in the following nominal amounts (record each actual mass to the nearest 0.1 mg). Dissolve in water and dilute to 100 mL. Store at 4°C and discard after four weeks.

Arabinose ($\text{C}_5\text{H}_{10}\text{O}_5$)	90–110 mg
Xylose ($\text{C}_5\text{H}_{10}\text{O}_5$)	650–750 mg
Mannose ($\text{C}_6\text{H}_{12}\text{O}_6$)	90–110 mg
Galactose ($\text{C}_6\text{H}_{12}\text{O}_6$)	90–110 mg
Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$)	1900–2100 mg

7.1.8 *Monosaccharide Stock B Solution*— Prepare in manner identical to monosaccharide stock A solution.

7.1.9 *Dichloromethane*, (CH_2Cl_2).

7.1.10 *Inositol Solution (20 mg/mL)*—Dissolve $5.000 \pm 0.0025 \text{g}$ of inositol ($\text{C}_6\text{H}_{12}\text{O}_6$, 98 + wt %) in water and dilute to 250 mL. Store at 4°C and discard after one week.

³ Annual Book of ASTM Standards, Vol 11.05.

³ DB[®]-225 is a trademark of J&W Scientific Inc., 91 Blue Ravine Road, Folsom, CA 95630.

⁴ DB[®]-225 is a trademark of J&W Scientific Inc., 91 Blue Ravine Road, Folsom, CA 95630.

⁴ *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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

7.1.11 *Loss Factor Standard Stock Solution*—Combine together each of the following monosaccharides. Weigh each monosaccharide in the following nominal amounts (record each actual weight to the nearest 0.1 mg). Dissolve in water and dilute to 100 mL. Store at 4°C and discard after four weeks.

Arabinose (C ₅ H ₁₀ O ₅)	900–1100 mg
Mannose (C ₆ H ₁₂ O ₆)	900–1100 mg
Galactose (C ₆ H ₁₂ O ₆)	900–1100 mg
Xylose (C ₅ H ₁₀ O ₅)	900–1100 mg
Glucose (C ₆ H ₁₂ O ₆)	900–1100 mg

7.1.12 *1-Methylimidazole*, ((C₃H₃N₂-)(CH₃)).

7.1.13 *Potassium Borohydride Solution*, (0.15 g/mL)—Dissolve 7.50 ± 0.05 g potassium borohydride (KBH₄) in 40 mL of ~3 M ammonium hydroxide (NH₄OH) solution. Use an ultrasonic bath to get the salt to dissolve in a reasonable amount of time. Dilute to 50.0 ± 0.1 mL with ~3 M ammonium hydroxide (NH₄OH) solution. Prepare immediately before use. Discard after 6 h. This quantity is sufficient for 50 specimens and calibration standards.

7.1.14 *Potassium Hydroxide Solution (3.5 M)*—Dissolve 58.0 ± 0.5 g of potassium hydroxide (KOH, 85 wt %) in 200 mL water. Allow to cool to room temperature before diluting to 250 mL with water.

7.1.15 *Sulfuric Acid Solution (12 M)*—Slowly add 665 mL of 96 wt % sulfuric acid (H₂SO₄) to 300 mL of water cooled in an ice bath with stirring. Allow solution to come to room temperature and dilute to 1 L. Check the concentration by titration and adjust the concentration to 12.0 ± 0.1 M (24.0 ± 0.2 N).

7.2 *Materials:*

7.2.1 *Glass Filtering Crucibles*, 50 mL, medium porosity, nominal pore size of 10 μm.

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

7.2.3 *Vacuum Adaptor for Filtering Crucibles*.

7.2.4 *Vials*, 13 × 32 mm crimp-top style with polytetrafluoroethylene-faced rubber septum and aluminum crimp seals or equivalent.

8. Hazards

8.1 Do not permit sulfuric acid, glacial acetic acid, acetic anhydride, or potassium hydroxide to contact skin or clothing. They are corrosive. Wear protective clothing.

8.2 After the autoclave step, the glass bottles are hot and may be pressurized. Handle with caution.

8.3 Solutions of potassium borohydride will evolve spontaneously evolve hydrogen gas on standing. To prevent pressurization, do not seal bottles. Ensure adequate ventilation around such solutions to avert the accumulation of flammable hydrogen gas. Wet potassium borohydride is highly flammable.

8.4 Methylene chloride is a very volatile solvent and is both toxic and a suspected carcinogen. Handle only with adequate ventilation.

9. Sampling, Test Specimens and Test Units

9.1 Test specimens suitable for analysis by this procedure are listed below:

9.1.1 *As-Received Biomass*.

9.1.2 *Biomass*, prepared according to Practice E 1757.

9.1.3 *Extractives-Free Material*, prepared according to Test Method E 1690.

10. Calibration and Standardization

10.1 GC Calibration Standards. Prepare one of each.

10.1.1 *GC Calibration Standard A Solution*—Measure 10.00 mL of monosaccharide stock A solution. Add 69.0 mL of water, 5.0 mL of Inositol solution and 3.0 mL of 12 M sulfuric acid. Mix. Store at 4°C and use within four weeks of preparation.

10.1.2 *GC Calibration Standard B Solution*—Measure 10.00 mL of monosaccharide stock B solution. Add 69.0 mL of water, 5.0 mL of Inositol solution and 3.0 mL of 12 M sulfuric acid. Mix. Store at 4°C and use within four weeks of preparation.

NOTE 1—If the analyst desires a multipoint calibration curve, differing amounts of the respective stock solution can be diluted to 10.00 mL and the calibration solutions made up as described in 10.1.1 or 10.1.2.

10.2 *Loss Factor Calibration Solution*—Prepare once. Measure 10.00 mL of loss factor standard stock solution. Add 69.0 mL of water, 5.0 mL of Inositol solution and 3.0 mL of 12 M sulfuric acid. Mix. Store at 4°C and use within one week of preparation.

10.3 *Loss Factor Sample Solution*—Prepare in duplicate. Measure 10.00 mL of loss factor standard stock solution. Add 69.0 mL of water, 5.0 mL of inositol solution and 3.0 mL of 12 M sulfuric acid. Mix. Store at 4°C and use within one week of preparation.

11. Procedure

11.1 An overview of the analytical sequence is as follows:

11.1.1 Pretreatment of specimens with 12 M sulfuric acid.

11.1.2 Dilution and autoclaving of specimens and loss factor sample solutions.