Designation: D5896 – 96 (Reapproved 2012)

Standard Test Method for Carbohydrate Distribution of Cellulosic Materials[1]

This standard is issued under the fixed designation D5896; 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 determination of the carbohydrate composition of cellulosic materials such as ground wood meal, chemically refined pulp, mechanical pulps, brownstocks, and plant exudates (gums) by ion chromatography. This test method is suitable for rapid, routine testing of large numbers of samples with high accuracy and precision. For a review of this technique, see Lee (**1**) .[2]

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.* For hazard statement, see Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1193 Specification for Reagent Water

D1695 Terminology of Cellulose and Cellulose Derivatives

3. Terminology

3.1 For standard terminology of cellulose and cellulose derivatives, see Terminology D1695.

3.2 *Abbreviations:*

3.2.1 IC—ion chromatography,

3.2.2 SPE—solid phase extraction,

3.2.3 PAD—pulsed amperometric detector,

3.2.4 PED—pulsed electrochemical detector,

3.2.5 mM—millimolar.

4. Summary of Test Method

4.1 IC analysis of cellulosics requires the following operations:

(1) sample preparation,

(2) total hydrolysis,

(3) dilution,

(4) SPE,

(5) ion chromatographic analysis, and

(6) calibration/calculation.

5. Significance and Use

5.1 This test method requires total hydrolysis of carbohydrate material to monosaccharides, and is thus applicable to any cellulosic or related material that undergoes substantial hydrolysis, including cellulose derivatives such as cellulose acetate.

5.2 The carbohydrate composition of a cellulosic material can be expressed on the basis of the total initial sample, or on the basis of the carbohydrate portion of the sample. The former requires quantitative handling and may require special knowledge of the other components present in order to establish the absolute carbohydrate level or determine individual wood hemicelluloses such as galactoglucomannan, etc. Since the solid portion of purified pulps is almost all carbohydrate (98 + %), the latter basis is often used to express the carbohydrate distribution as a percent.

5.3 If heated under alkaline conditions, isomeric sugars may begin to appear in the chromatogram. The major impurity present in purified pulps is saccharinic acids. These acidic components, and other anions such as sulfate, carbonate, and acetate are removed by a strong base anion exchange SPE, and would need to be determined separately to get a more exact carbohydrate distribution.

6. Apparatus

6.1 *Blender.*

6.2 *Screw Cap Culture Tubes,* 25 by 150 mm, outside diameter.

6.3 *Refrigerator.*

6.4 *Pressure Cooker.*

[1] This test method is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.36 on Cellulose and Cellulose Derivatives.

Current edition approved June 1, 2012. Published August 2012. Originally approved in 1996. Last previous edition approved in 2007 as D5896 - 96 (2007). DOI: 10.1520/D5896-96R12.

[2] The boldface numbers in parentheses refer to the list of references at the end of this test method.

[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.

6.5 *SPE Cartridges.*

6.6 *Water Bath.*

6.7 *Ion Chromatograph.*

6.8 *Moisture Balance.*

6.9 *Hot Plate.*

6.10 *Pipets.*

TOTAL HYDROLYSIS

7. Reagents and Materials

7.1 *Sulfuric Acid* (72 ± 0.1 weight %): To 1 volume of water, add slowly while stirring vigorously 2 volumes of concentrated sulfuric acid (sp gr 1.84). Standardize against an alkaline standard, and adjust to 72 ± 0.1 weight %.

8. Hazards

8.1 **Precaution:** Wear eye protection and chemical resistant gloves while working with strong acid.

9. Summary of Procedure

9.1 The total hydrolysis of cellulosic material requires a primary hydrolysis with strong mineral acid followed by a secondary hydrolysis in dilute acid. The primary hydrolysis results in the formation of a mixture of oligosaccharides; the secondary hydrolysis completes the conversion to monomeric sugars.

10. Sampling, Test Specimens, and Test Units

10.1 Extract wood samples with ethanol to remove extractives, then grind in a Wiley mill to pass a 40-mesh screen. Disintegrate (fluff) dry pulp or paper samples in a blender. Determine the moisture content using a moisture balance or similar device.

11. Procedure

11.1 Add 1 mL of cold, 72 % sulfuric acid to 100 mg of cellulose (bone dry basis) in a 25 by 150-mm screw top culture tube. (For wood samples, adjust sample size upward based on estimated polysaccharide content of the sample.)

11.2 Mix with glass rod, and place in refrigerator overnight (with glass rod in place).

11.3 Heat samples (with stirrers in place) at 30°C for 1 h.

11.4 Remove glass rod and rinse while adding 28 mL of water to each tube and, with caps on, place samples in a pressure cooker, and heat to 15 psi.

11.5 Maintain pressure at 15 psi for 1 h.

11.6 Cool to room temperature and dilute the sample to avoid overloading the analytical column (usually a dilution between 1 to 20 and 1 to 50 is adequate). Dilute with water containing a standard such that its concentration in the diluted sample is 2 ppm. D-Fucose (6-deoxy-D-galactose) or 2-deoxy-D-glucose make good internal standards.

11.7 Neutralization of the sample is not required, but improved resolution may occur if the sample is adjusted to pH 6–6.5 during the dilution step. Neutralization is recommended if the sample is to be stored before analysis.

11.8 Prepare an anion exchange SPE cartridge with 5 mL of water, pass 5 mL of sample through the cartridge, discarding the first 3 mL, and use the remaining 2 mL to fill a 0.5-mL injection vial. Additional 0.5-mL injection vials may be filled if multiple injections are planned.

11.9 Inject the samples onto an ion chromatograph operating as described in the following text.

HIGH-PERFORMANCE ION CHROMATOGRAPHY

12. Apparatus

12.1 *Ion Chromatograph*—This equipment can be assembled from the individual components, or purchased as a system.[4]

12.2 *Column*—The column must be suitable for separating monosaccharides and is generally protected by a suitable guard column. A column packing material that works well is composed of 10 µm beads of surface-sulfonated polystyrene/divinylbenzene (2 % crosslinked), covered with porous latex beads containing alkyl quaternary amine functionality.

13. Procedure

13.1 Perform the analysis using an ion chromatograph.

13.2 Inject 100 µL of sample onto the analytical column.

13.3 Detection is by PAD or PED in a pulsed amperometric mode using a gold working electrode.

13.4 Standard pulp samples are generally run isocratically at 1 mL/min using an eluant of 2.5 mM sodium hydroxide to obtain baseline resolution of fucose (internal standard), arabinose, galactose, glucose, xylose, and mannose in less than 30 min. If other sugars are present, it may be necessary to alter the eluant strength, or try a gradient approach.

13.5 Eluant is degassed and kept under helium (nitrogen may be substituted for helium).

13.6 A 0.5-mL/min flow of 0.3-M NaOH is added after the column, but prior to the detector to improve response.

14. Calibration and Standardization

14.1 Prepare standards of the individual sugars of interest, such as those listed in 13.4, from reagent grade standards. Run the test mixture at various concentrations (≥5) such that all real samples will have peaks that fall on the calibration lines derived from this data. Note that sample concentrations are set by the dilution ratio used in 11.6, and make sure that they are given in ppm.

14.2 Prepare a mixture of the sugars of interest, in relative ratios similar to that expected from the sample, such that it will fall within the calibration range established in 14.1. Run this sample routinely as a control that is used to establish the standard error and control chart for the method.

[4] Lists of companies that supply this equipment can be found in buyer's guides such as those published yearly by *American Laboratory* or *Analytical Chemistry*.

15. Calculation or Interpretation of Results

15.1 Since cellulose is composed totally of anhydroglucose units, the repeat unit weight is 162. Hydrolysis of 100 mg of cellulose would theoretically give 111.1 mg of glucose (formula weight (FW) = 180). Other hexoses have the same relationship. Thus, hemicelluloses such as mannans, galactoglucomannans, and glucomannans can be backcalculated in a similar manner.

15.2 Hemicelluloses or gums that contain only pentoses have a repeat unit weight of 132. Thus, hydrolysis of 100 mg of xylan would theoretically give 113.6 mg of xylose (FW = 150). Hemicelluloses that contain both 6-carbon and 5-carbon sugars would have a repeat unit weight between 132 and 162, depending on composition.

15.3 In a similar manner, the composition of triacetates could be determined and the recovery calculated based on a repeat unit weight of 288 for cellulose triacetate, and 258 for xylan triacetate.

16. Report

16.1 Report the following information:

16.1.1 The amount of each sugar detected is reported in ppm. In addition, a distribution can be reported based on the percent of each sugar relative to the total, omitting the internal standard. Information on detection limits is given in Refs **(2)** , **(3)**, and **(4)**,

16.1.2 For relatively clean samples, such as bleached pulp, the percent recovery should be calculated and reported. The percent recovery should be between 85 to 95 %.

17. Precision and Bias

17.1 Interlaboratory data has not been obtained.

17.2 Precision and bias (see **(2)** and **(5)**) will vary with the raw materials tested. For a bleached kraft Southern pine paper pulp, the following intralaboratory results were obtained from 10 replicate tests:

Sugar	ppm	SD	Percent	SD, %
arabinose	4.33	0.35	0.11	0.008
galactose	3.51	0.44	0.09	0.010
glucose	3305.93	94.86	86.95	0.184
mannose	206.93	6.67	5.44	0.107
xylose	281.49	6.04	7.41	0.131

where SD is the sample standard deviation.

17.3 *Bias*—Bias introduced by the hydrolysis procedure is not known. Since calibration is by known standards of known concentration, bias has been removed from the IC determination.

18. Keywords

18.1 carbohydrate; carbohydrate distribution; chromatography; distribution; hemicellulose; hydrolysis; ion chromatography; monosaccharides; PAD; sugars

REFERENCES

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(3) Dionex Corp., "Dionex Technical Note," *TN20*, Dionex Corp., Sunnyvale, CA, 1989 .

(4) Johnson, D. C. and LaCourse, W. R., *Analytical Chemistry*, Vol 62, 1990, p. 589A.

(5) Sullivan, J. and Douck, M., *Journal of Chromatography*, Vol 671, No. 6, 1994, p. 339.