SCANDINAVIAN PULP, PAPER AND BOARD
TESTING COMMITTEE

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Pulps

Carbohydrate composition

0 Introduction

This SCAN-test Method describes a method for the determination of the contents of the five principal, neutral monosaccharides arabinose, galactose, glucose, xylose and mannose, as they appear in the polysaccharides in wood pulps.

The procedure is based on the sulphuric acid hydrolysis of the samples. The monosaccharides are determined either by using high performance anion exchange chromatography with a pulsed amperometric detector (HPAEC-PAD) – subsequently referred to as ion chromatography (IC), or by using gas chromatography with a flame ionization detector (GC-FID) – subsequently referred to as gas chromatography (GC).

1 Scope

This SCAN-test Method describes the determination of the carbohydrate composition in wood pulp samples.

This method makes it possible to determine concentrations of individual anhydrous monosaccharides down to 1 mg/g oven-dry pulp.

2 Normative reference

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 638 Pulps – Determination of moisture content – Oven drying method (revised version will be applicable to pulp, paper and board)

3 Terms and definitions

For the purposes of this SCAN-test Method, the following definition apply:

3.1 Carbohydrate composition

amounts of the five principal, neutral monosaccharides; arabinose, galactose, glucose, mannose and xylose, in a sample, in milligrams per gram.

4 Principle

The samples are hydrolysed with sulphuric acid using a two-step technique. The amounts of the different monosaccharides are determined using either ion chromatography (IC) or gas chromatography (GC)

If GC is used, the hydrolysed sample is reduced and acetylated, and the resulting alditol acetates of the monosaccharides are then separated and determined by GC.

5 Reagents

All chemicals must be of analytical grade.

5.1 Water

Water, of high purity, distilled or deionized.

5.2 Monosaccharide standards

Monosaccharide standards, for calibration: arabinose, galactose, glucose, mannose and xylose.

Prepare standard solutions of appropriate concentrations.

5.3 Sulphuric acid

72 % H_2SO_4, Add 300 ml of water to 1000 ml volumetric flask. Add slowly 670 ml of concentrated sulphuric acid (H_2SO_4 sp gr 1,84) while cooling under a cold water tap. When the temperature has reached equilibrium with the ambient temperature, dilute to the mark and mix.

5.4 Eluent solution (for IC determination)

The composition of this solution depends on the type of IC column to be used. Therefore, follow the recommendations given by the IC column supplier.

The following reagents are required only for GC determinations:

5.5 Ammonia, NH_3 conc. 25 %

5.5.1 Ammonia 12 M

Mix 9 parts ammonia (25 %) with 1 part of water.

5.6 Potassium hydroxide, KOH, p.a

5.6.1 Potassium hydroxide 7,5 M

Weigh 123 g KOH pellets into a 250 ml volumetric flask. Add approx. 200 ml distilled water while stirring.

The reaction is exothermic, heat is evolved. Allow the solution to cool to ambient temperature and dilute it to the mark with water.

5.7 Potassium borohydride, KBH4 ,p.a

5.7.1 Potassium borohydride solution

Dissolve 150 mg KBH_4 in 250 µl 12 M NH_3 and 750 µl distilled water in a septum vial (4 ml).

This solution shall be freshly prepared every day before use.

5.8 Acetic acid, CH_3COOH, p.a conc.

5.9 Acetic acid anhydride, p.a conc.

5.10 1-methylimidazole, p.a

5.11 Ethanol, 95-99 %

5.12 Sodium sulphate, Na_2SO_4, p.a., water-free

5.13 2-Deoxy-galactose

5.13.1 Internal standard solution, 2-deoxy-galactose 20 mg/ml

Weigh 1,00 g 2-deoxy-galactose to the nearest 0,1 mg and transfer it quantitatively to a 50 ml volumetric flask and diluted to the mark with distilled water.

6 Apparatus

Ordinary laboratory equipment and the following:

6.1 *Grinder* with a 40 mesh screen or equivalent equipment.

6.2 *Water bath* at a temperature of (30 ± 0,5) ˚C .

6.3 *Autoclave* at a temperature of (120 ± 5) ˚C

6.4 *Drying oven*, (105 ± 3) ˚C for determining dry matter content in accordance with ISO 638

The apparatus in 6.5 is used for IC determination only:

6.5 *Ion-chromatograph* (IC) with a suitable column and detector for monosaccharide determination

Apparatuses in 6.6-6.7 are used for GC-determinations only:

6.6 *Water bath* at a temperature of (40 ± 0,5) ˚C

6.7 *Gas chromatograph* (GC) with suitable column and detector for monosaccharide determination

7 Sampling

The sampling procedure is not covered by this method. Make sure that the test portions taken are representative of the sample received. Pulp

samples must be ground before analysis using an appropriate grinder (6.1).

8 Procedure

8.1 Determination of dry matter content

Weigh a portion of the sample material and determine the dry matter content in accordance with ISO 638.

8.2 Test material preparation

Carry out the preparation and testing in duplicate.

Weigh a test portion of (300 ± 10) mg to the nearest 0,1 mg into a glass beaker with a volume of at least 150 ml.

Calculate and record the oven-dry weight W of the test portion, in grams

8.3 Hydrolysis

To the test material in the beaker, add exactly 3 ml of 72 % sulphuric acid (5.3) with a pipette. Stir the contents of the beaker with a glass rod until the test material begins to dissolve. Place the beaker in a (30 ± 0,5) ˚C water bath for 1 h. Stir occasionally. Add 84 ml of water (5.1).

Cover the beaker with aluminium foil and place it in autoclave (6.3) at (120 ± 5) ˚C for 1 h. Allow the beaker and its contents to cool to approx. 80 ˚C.

8.4 Determination of saccharides

Carry out the determination according to either 8.3.1 or 8.3.2.

8.4.1 Determination using an IC instrument

8.4.1.1 Solution preparation

Transfer the test solution from the beaker to a 250 ml volumetric flask, allow it cool to room temperature and fill up to the mark with water.

8.4.1.2 Calibration

Calibrate the device using the monosaccharide standard solutions. Use the conditions recommended by the manufacturer or determine the optimum conditions empirically. The optimum conditions depend on the apparatus and the column.

Determine the calibration factor k_i for each monosaccharide as the chromatographic area per milligram of monosaccharide

8.4.1.3 Determination

Filter the test solution and inject an aliquot into the instrument. Record the dilution factor D. Run the determination according to the manufacturer's instructions.

Check from the chromatogram that the separation is adequate. If necessary, dilute the sample further until the concentration is within the calibration range and record the new dilution factor, D. Run a new determination.

Determine the chromatographic area A_i for each monosaccharide.

8.4.2 Determination using a GC instrument

To the solution in the beaker, add 2 ml of the standard deoxy-galactose solution (5.13.1) and stir with a glass rod.

Transfer the hydrolysate to a 100 ml volumetric flask immediately. Wash the beaker with 2 x 5 ml hot distilled water and transfer to the volumetric flask. Allow the sample to cool and dilute with distilled water to the mark.

8.4.2.1 Neutralisation

Transfer 1 ml of the solution to a test tube.

Add 100 µl of 12 M ammonia (5.5.1). Check with a pH indicator paper and a glass capillary that the pH is > 7.

8.4.2.2 Reduction

Add 100 µl of freshly prepared KBH_4-solution (5.7.1) and place the test tube in a water bath at 40 °C for 1 h.

Then add 100 µof conc. acetic acid (5.8) to eliminate the surplus of KBH_4.

8.4.2.3 Derivatisation

Transfer 500 µl of the sample to a 30 ml test tube with a screw cap. Add 500 µl of 1-methylimidazole (5.10) and 5 ml of acetic acid anhydride (5.9).

Cool the tube with cold water during the addition of the acetic acid anhydride.

Tighten the cap and mix the solution carefully. Allow the tube to stand in a cold water bath for 10 minutes. Add 1,0 ml of ethanol (5.11) while cooling the tubes. Mix the solution carefully.

Allow the mixture to react for 10 minutes (ethanol + acetic acid anhydride $\Rightarrow$ ethyl acetate).

Add 5 ml of distilled water, mix and place the tube in a water bath with cold water. The level of the water in the water bath shall exceed the level

of the mixture in the tube during the following step.

Add 5 ml of 7,5 M KOH-solution (5.6.1), tighten the cap and mix. After a few minutes, add another portion 5 ml 7,5 M KOH. Tighten the cap carefully and shake the tube vigorously. Allow the tubes to stand for at least 10 minutes until two clear phases have separated in the tube.

> WARNING! It is important that the tube is cooled when the KOH-solution is being added, otherwise the ethyl acetate formed will evaporate.

Transfer the upper phase (ethyl acetate) into a test tube containing a small amount of dry sodium sulphate (5.12). Do this carefully so that no water phase is transferred.

Shake the tube and allow it to stand for 5-10 minutes. Transfer the clear solution to a septum vial and seal the vial.

8.4.2.4 Calibration

Calibrate the device using the monosaccharide standard solutions. Use the conditions recommended by the manufacturer or determine the optimum conditions empirically. The optimum conditions depend on the apparatus and the column.

8.4.2.5 Determination

Inject an aliquot into the instrument. Run the determination according to the manufacturer's instructions.

Check the integration of the chromatogram and also the retention times for the different monosaccharides to be sure that they are adequate. If necessary, dilute the sample further until the concentration is within the calibration range and record the new dilution factor, D. Run a new determination.

9 Calculation

9.1 IC procedure

Calculate the anhydrous content of each monosaccharide from the expression:

$$X_i = A_i \cdot C_i \cdot D/(W \cdot k_i) \qquad (1)$$

where:

X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in milligrams per gram;
A_i is the chromatographic area of monosaccharide *i*, in area units (i.e. signal ·time);
C_i is the anhydrous factor for monosaccharide *i* (0,88 for xylose and arabinose, 0,90 for glucose, mannose and galactose);
D is the dilution factor (depending on procedure chosen);
W is the oven-dry weight of the sample, in grams;
k_i is the calibration factor for monosaccharide *i*, in chromatographic area per milligram of monosaccharide.

Report the results to the nearest whole number.

Calculate the relative content of each monosaccharide from the expression:

$$Y_i = 100\% \cdot X_i / X_{tot} \qquad (2)$$

where:

Y_i is the relative content of anhydrous monosaccharide *i*, in per cent;
X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in milligrams per gram;
X_{tot} is the total content of anhydrous monosaccharides in the oven-dry sample, in milligrams per gram.

Report the results to the first decimal place.

9.2 GC-FID procedure

Calculate the anhydrous content of each monosaccharide from the expression:

$$X_i = 0{,}1 \cdot A_i \cdot W_s \cdot C_i \cdot D/(A_s \cdot W \cdot k_i) \qquad (3)$$

where:

X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in milligrams per gram;
0,1 is a factor in mg/g in order to change the result to mg/g.
A_i is the chromatographic area of monosaccharide *i*, in area units (ie. signal ·time);
A_s is the chromatographic area of the internal standard;
W_s is the weight of the internal standard, in

grams;

W is the oven-dry weight of the sample, in grams;

C_i is the anhydrous factor for monosaccharide *i* (0,88 for xylose and arabinose, 0,90 for glucose, mannose and galactose);

D is the dilution factor (depending on the procedure chosen);

k_i is the calibration factor for monosaccharide *i*, (dimensionless).

Report the results to the nearest whole number.

Calculate the relative content of each monosaccharide from the expression:

$$Y_i = 100\% \cdot X_i / X_{tot} \qquad (4)$$

where:

Y_i is the relative content of anhydrous monosaccharide *i*, in per cent;

X_i is the content of anhydrous monosaccharide *i* in the oven-dry sample, in milligrams per gram;

X_{tot} is the total content of anhydrous monosaccharides in the oven-dry sample, in milligrams per gram.

Report the results to the first decimal place.

10 Report

The test report shall include the following information:

a) A reference to this SCAN-test method;
b) Date and place of testing;
c) Identification of the sample tested;
d) The results expressed as the contents of the individual anhydrous mono-saccharides in the oven-dry sample (in milligrams per gram);
e) The relative contents of the anhydrous monosaccharides (in per cent);
f) The reference moncsaccharides used for calibration;
g) Information regarding any departure from the procedure described in this SCAN-test method and/or any other circumstances that may have affected the result.

11 Precision

11.1 Repeatability

Five laboratories determined the carbohydrate compositions of two different samples, bleached hardwood pulp and bleached softwood pulp, using either the GC or the IC determination alternative. The mean values and the mean coefficients of variation reported by each laboratory are given in Table 1 and Table 2.

Note that the bleached hardwood pulp contained only small amounts of arabinose, galactose and mannose, and that the bleached softwood pulp contained a small amount of galactose (see 11.2), thus yielding high mean relative standard deviations.

Table 1 Carbohydrate composition in bleached hardwood pulp

Monosaccharide	**Mean content, mg/g**	**CoV*, %**
Arabinose	0,6	17,0
Galactose	0,4	65,2
Glucose	773,0	1,6
Xylose	230,5	1,3
Mannose	4,2	12,7
Total monosaccharide content	968,6	1,4

* CoV = Coefficient of variation

Table 2 Carbohydrate composition in bleached softwood pulp

Monosaccharide	**Mean content, mg/g**	**CoV*, %**
Arabinose	7,8	2,2
Galactose	3,0	3,7
Glucose	825,8	0,8
Xylose	86,6	1,5
Mannose	66,3	1,9
Total monosaccharide content	989,4	0,9

* CoV = Coefficient of variation

11.2 Reproducibility

Five laboratories determined the carbohydrate compositions of two different samples, bleached hardwood pulp and bleached softwood pulp, using either the CG or the IC determination alternative. The mean values and coefficients of variation of the reported contents (here, based on five parallel

determinations) are given in Table 3 and Table 4, as well as the corresponding mean relative contents.

Note that the bleached hardwood pulp contained only small amounts of arabinose, galactose and mannose, and that the bleached softwood pulp contained a small amount of galactose, thus yielding high mean coefficient of variation.

Table 3 Carbohydrate composition in bleached hardwood pulp

Monosaccharide	**Mean content, mg/g**	**CoV*, %**
Arabinose	0,6	115
Galactose	0,4	83
Glucose	773,0	6
Xylose	230,5	9
Mannose	4,2	24
Total monosaccharide content	968,6	7

* CoV = Coefficient of variation

Table 4 Carbohydrate composition in bleached softwood pulp

Monosaccharide	**Mean content, mg/g**	**CoV*, %**
Arabinose	7,8	11
Galactose	3,0	17
Glucose	825,8	7
Xylose	86,6	9
Mannose	66,3	7
Total monosaccharide content	989,4	7

* CoV = Coefficient of variation

SCAN-test Methods are issued and recommended by FFIF, PFI and Innventia for the pulp, paper and board industries in Finland, Norway and Sweden.
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