



**International
Standard**

ISO 6350

**Lignins — Determination of dry
matter content — Oven-drying and
freeze-drying methods**

*Lignines — Détermination de la teneur en matières sèches —
Méthodes par séchage à l'étuve et par lyophilisation*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document describes methods for the determination of dry matter content in lignins by oven-drying or freeze drying.

In general, freeze-drying is preferred over oven-drying at 105 °C, especially for kraft lignins in the sodium form, in order to preserve the integrity of the sample. In a study on the effect of drying on lignin solids^[1], kraft lignins - particularly hardwood lignins - in the base form (also referred to as sodium form) were most affected by oven-drying. In some cases, the solids contents of oven-dried samples were over four percentage points lower than those of the corresponding freeze-dried samples.

When drying samples prior to the determination of other lignin properties, only the freeze-drying method is acceptable. Water interferes with certain lignin analyses^[2,3], and oven-drying might lead to changes in lignin structure as a result of, for example, decomposition or condensation reactions. Thus, any residual water is removed from the test specimen through lyophilization (freeze-drying). In addition, freeze-drying prevents the loss of volatile organic compounds (VOC's) which can be determined separately if required.

Several related procedures for the preparation of biomass for compositional analysis and for the determination of solids and extractives in biomass have been published by the National Renewable Energy Laboratory (NREL)^[4-7]. However, the procedures described in this document are specifically applicable to lignins.

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Lignins — Determination of dry matter content — Oven-drying and freeze-drying methods

1 Scope

This document describes methods for the determination of the dry matter content of lignins by oven-drying and freeze-drying. The methods are applicable to all types of lignins isolated from kraft, organosolv, soda, and sulfite pulping processes, and to lignin obtained by enzymatic or acid hydrolysis of biomass. However, the oven-drying method is not applicable to kraft lignins in the base form, also referred to as sodium form. Both methods are applicable only to lignins in the solid form.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

lignins

class of complex organic macromolecules, containing aromatic sub-units, that plays a key role in the formation of cell walls in wood and bark, conferring mechanical strength and rigidity to the cell walls and to plants as a whole

Note 1 to entry: Lignin is the main non-carbohydrate constituent of wood.

3.2

kraft lignin

depolymerized and chemically modified lignin isolated from a kraft pulping process, such as that originating from kraft black liquor

3.3

soda lignin

depolymerized and chemically modified lignin isolated from a soda pulping process, such as that originating from soda liquor

3.4

organosolv lignin

depolymerized and chemically modified lignin isolated from an organosolv pulping process, such as that originating from organosolv liquor

3.5

hydrolysis lignin

lignin produced by conversion of biomass, through enzymatic or acid hydrolysis, into sugars and lignin streams, followed by separation of the lignin fraction

3.6

biomass

biological material derived from living, or previously living organisms, such as wood, agricultural crops and other plant-based biodegradable material

3.7

constant mass

mass of the test specimen determined at the equilibrium condition after drying until the difference between two successive drying and weighings, separated in time by at least half the initial drying period, does not exceed 0,1 % mass fraction of the test specimen before drying

4 Principle

The dry matter content is calculated from the difference in the mass of the specimen before and after drying.

5 Apparatus

5.1 Containers, appropriate for freeze-drying or oven-drying, as recommended by the manufacturer. Containers shall be water-vapour-proof with tightly fitting lids, and made from a material not affected by the conditions of the test, previously dried to constant mass and weighed.

5.2 Drying oven, capable of maintaining the air temperature at $105\text{ °C} \pm 2\text{ °C}$, and suitably ventilated.

5.3 Freeze-dryer, typically available with condenser refrigeration of -80 °C , and capable of maintaining a pressure (vacuum) of about 25 mTorr.

5.4 Analytical balance, accurate to 0,1 mg.

5.5 Desiccator, using Drierite^{TM1)} or equivalent desiccant.

6 Sampling

Obtain a representative sample of lignin equivalent to about 2 g to 3 g on an air-dry basis. Report the origin of the sample and the sampling procedure. For example, in the case of kraft lignin samples, it shall be reported whether they were collected in their base form, or after acid-washing; or as they come out of the press, partly dried, flash dried, or otherwise.

If the sample is not analysed immediately after collection, it shall be stored in an airtight container or sealable polyethylene bag. If it is necessary to store the samples for longer than 2 days to 3 days, they shall be kept in a refrigerator or cold room at $5\text{ °C} \pm 1\text{ °C}$ prior to use. Samples shall be brought back to room temperature before opening the container or bag.

All samples shall be air-dried to over 75 % solids prior to the determination of dry matter content or analysis of other properties. This is necessary to minimize the extent of lignin degradation reactions during the subsequent drying period.

1) DrieriteTM is an example of a suitable product(s) available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

7 Determination of dry matter content

7.1 General

The choice of method for the determination of dry matter content depends on the type of lignin sample being analysed. Freeze-drying is preferred over oven-drying at 105 °C [8], especially for kraft lignins in the base or sodium form, in order to preserve the integrity of the sample.

In a study on the effect of drying on lignin solids[1], kraft lignins – particularly hardwood lignins – in the base or sodium form were most affected by drying. In some cases, the solids contents of oven-dried samples were over four percentage points lower than those of the corresponding freeze-dried samples. Thus, oven-drying is not recommended for kraft lignin samples in the sodium or base form.

The same study also showed that vacuum oven-drying at 60 °C was not an acceptable alternative to freeze-drying as the solids contents of vacuum oven-dried kraft lignin samples in the base form were lower than the corresponding freeze-dried samples.

NOTE Milder conditions of vacuum-drying can still affect the results, when the specimens are composed of extremely thermally-labile components and/or under some conditions susceptible to secondary reactions

7.2 Oven-drying

7.2.1 Weigh 1 g to 2 g of air-dried test specimen in a closed container (5.1) that has been previously dried to constant mass and weighed.

7.2.2 Place the open container with the test piece and lid in an oven (5.2) at 105 °C ± 2 °C for 4 h.

7.2.3 After drying, place the lid on the container and allow the test piece to cool in the desiccator (5.5).

7.2.4 Weigh the closed container with the test piece.

7.2.5 Repeat steps 7.2.2 to 7.2.4 with a drying period of 1 h until the test piece reaches constant mass, when the difference between two successive weighings does not exceed 0,1 % of the test piece mass before drying. The total drying period shall not exceed 7 h.

NOTE In a study on the effect of drying on lignin solids[1], oven-dried lignin samples reached constant weight after seven hours. Longer periods are unnecessary and are not recommended as they could result in lignin degradation. For example, it was reported that, when subjected to high temperature, lignin in the base form produces a significant quantity of monomers[9].

7.2.6 Repeat this procedure on a new test specimen. The results of duplicate determinations shall not deviate by more than 0,5 % from their mean. Otherwise, repeat the determination with a larger test specimen.

7.2.7 Calculate the dry matter content of the sample as described in Clause 8.

7.3 Lyophilization (Freeze-drying)

7.3.1 General

Lyophilization (freeze-drying) is preferred over oven-drying for the determination of dry matter content in order to preserve the integrity of the sample.

For drying samples prior to determination of lignin properties other than dry matter content, only the freeze-drying method is acceptable. Water interferes with certain lignin analyses[2,3], and oven-drying might lead to changes in lignin structure as a result of, for example, decomposition or condensation reactions. Thus, any residual water shall be removed from the test specimen through lyophilization (freeze-drying). In addition,

freeze-drying prevents the loss of volatile organic compounds (VOC's) which can be determined separately if required.

7.3.2 Weigh 1 g to 2 g of air-dried test specimen in a closed container ([5.1](#)) that has been previously dried to constant mass and weighed.

NOTE If lignin properties other than dry matter content are determined, a larger amount of sample is needed. An appropriate amount of test specimen can then be collected from the freeze-dried sample for use in other analyses. In such cases, grinding the sample with a mortar and pestle prior to freeze-drying can be needed in order to homogenize the sample to a uniform powder.

7.3.3 Freeze the test specimen in a freezer overnight or for at least 4 h. If the sample is in slurry form, place a well-mixed sample in a container in a freezer overnight.

7.3.4 Dry the test specimen in a freeze-dryer ([5.3](#)) at -80 °C and at a pressure (vacuum) of about 25 mTorr for 24 h. Gradually increase the vacuum at the start of freeze-drying, and gradually release the vacuum to atmospheric pressure at the end of the freeze-drying period, in order to avoid scattering of the powder inside the container which can result in loss of test specimen material.

It is also recommended to attach a tissue paper to the adapter side of the vacuum dryer to avoid any scattered test specimen from entering the vacuum dryer.

NOTE The use of higher freeze-drying temperatures and/or different vacuum levels can necessitate longer drying times. However, these changes are acceptable provided the samples are dried to constant mass.

7.3.5 Weigh the closed container with the test piece.

7.3.6 Repeat steps [7.3.3](#) to [7.3.5](#) for a drying period of another 24 h or more, until the test piece reaches constant mass, when the difference between two successive weighings does not exceed 0,1 % of the test piece mass before drying.

7.3.7 Repeat this procedure on a new test specimen. The results of duplicate determinations shall not deviate by more than 0,5 % mass fraction from their mean. Otherwise, repeat the determination with a larger test specimen.

7.3.8 Calculate the dry matter content of the test specimen as described in [Clause 8](#).

8 Calculation

Determine the dry matter content of the sample from [Formula \(1\)](#):

$$w_0 = \left(\frac{m_1}{m_0} \right) \times 100 \quad (1)$$

where

w_0 is the dry matter content (%);

m_0 is the mass before drying (g);

m_1 is the mass after drying (g).

9 Precision

The precision of the method was determined by conducting a round robin study with several types of lignin samples. A description of the samples used in this study, and the repeatability and reproducibility results are presented in [Annex A](#).

10 Report

The test report shall include the following:

- a) a reference to this document i.e. ISO 6350:2024;
- b) the date and place of testing;
- c) the lignin type (kraft lignin, organosolv lignin, soda lignin, sulfite lignin, hydrolysis lignin, or other), sampling location (e.g. in the base form before acid-washing; after acid-washing; out of the press; or other); drying method, if applicable (partly dried, flash dried, or other) of the lignin sample; and any other relevant information for complete identification of the sample;
- d) the dry matter content determined as specified in [Clause 8](#);
- e) any unusual features observed in the course of the test;
- f) any departure from the procedures described in this document, or any other circumstances which may have affected the result

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Annex A (informative)

Precision

A.1 General

In January 2022, an international round robin study was performed in which twelve laboratories from eight countries – Brazil; Belgium; Canada (3 laboratories); Finland; France; Japan; Sweden (2 laboratories); and the U.S (2 laboratories) - participated.

A total of four samples representing different types of lignins were included in the study, including a kraft softwood lignin, acid form, air-dried; a kraft hardwood lignin, base form, air-dried; a hydrolysis hardwood lignin, flash-dried; and a soda lignin, acid form, flash-dried. The samples were submitted to the participating laboratories for testing according to this document.

All samples were shipped in sealed vials to prevent moisture uptake. If the samples could not be analysed upon receipt, participants were requested to store them in a cold room or refrigerator until the day of analysis.

Participants were also instructed to obtain test specimens directly from the test vials, and test them as-is, without air-drying or further conditioning. Samples vials were resealed immediately after collecting the test specimens to prevent moisture uptake.

Test specimens for determining dry matter content by oven and freeze-drying were weighed simultaneously (with as little time as possible between them), and promptly after collecting from sample vials.

Repeatability and reproducibility data for dry matter content determined by oven-drying are shown in [Tables A.1](#) and [A.3](#), respectively, and by freeze-drying in [Tables A.2](#) and [A.4](#), respectively. The calculations were made in accordance with ISO/TS 24498^[10].

The repeatability and reproducibility limits reported are estimates of the maximum difference which would be expected in 19 of 20 instances, when comparing two test results for materials similar to those described under similar test conditions. These estimates might not be valid for different materials or different test conditions.

NOTE 1 Repeatability and reproducibility limits are calculated by multiplying the repeatability and reproducibility standard deviations by 2,77, where $2,77 = 1,96 \sqrt{2}$.

NOTE 2 In a number of cases, the number of laboratories listed in [Tables A.1](#) to [A.4](#) is smaller than 12. This is due to the fact that either the labs did not report the freeze-drying results, or that the results were considered as outliers and were not included in the average.