

BS EN ISO 15320:2011



BSI Standards Publication

**Pulp, paper and board
— Determination of
pentachlorophenol in
an aqueous extract (ISO
15320:2011)**

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National foreword

This British Standard is the UK implementation of EN ISO 15320:2011. It supersedes BS EN ISO 15320:2003 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee PAI/11, Methods of test for paper, board and pulps.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English Version

Pulp, paper and board - Determination of pentachlorophenol in
an aqueous extract (ISO 15320:2011)

Pâtes, papiers et cartons - Dosage du pentachlorophénol
dans un extrait aqueux (ISO 15320:2011)

Zellstoff, Papier und Pappe - Bestimmung von
Pentachlorphenol in einem wässrigen Extrakt (ISO
15320:2011)

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Foreword

This document (EN ISO 15320:2011) has been prepared by Technical Committee ISO/TC 6 "Paper, board and pulps" in collaboration with Technical Committee CEN/TC 172 "Pulp, paper and board" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2012, and conflicting national standards shall be withdrawn at the latest by February 2012.

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The text of ISO 15320:2011 has been approved by CEN as a EN ISO 15320:2011 without any modification.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 15320 was prepared by Technical Committee ISO/TC 6, *Paper, board and pulps*.

This second edition cancels and replaces the first edition (ISO 15320:2003), which has been technically revised.

Pulp, paper and board — Determination of pentachlorophenol in an aqueous extract

WARNING — The use of this International Standard may involve hazardous materials, e.g. methanol and pentachlorophenol, which are toxic substances, as well as acetic anhydride, which is corrosive. This International Standard does not address all the safety and environmental problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety, health and environmental practices and determine the applicability of safety regulations prior to use.

1 Scope

This International Standard specifies a test method for the determination of pentachlorophenol (PCP) in an aqueous extract of pulp, paper and board. Although it was developed for paper and board intended to come into contact with foodstuffs, it is applicable to all kinds of pulp, paper and board.

The working range for acetylation is 0,05 mg/kg to 0,5 mg/kg.

NOTE The upper limit of the working range could be increased if the aqueous extract is diluted.

2 Normative references

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 186, *Paper and board — Sampling to determine average quality*

ISO 536, *Paper and board — Determination of grammage*

ISO 638, *Paper, board and pulps — Determination of dry matter content — Oven-drying method*

ISO 7213, *Pulps — Sampling for testing*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

3 Principle

A specimen of the material to be tested is extracted with either cold or hot water. The pentachlorophenol extract is concentrated by adsorption onto a phenyl silica column using solid-phase extraction. The pentachlorophenol is then eluted from the phenyl silica column with *n*-hexane and an acetylated derivative formed with acetic anhydride. The amount of pentachlorophenol present is then determined using gas chromatography employing an electron-capture detector (ECD) or mass spectrometer (MS) detector. The result is expressed as milligrams per kilogram of material.

4 Apparatus

- 4.1 Conical flask**, 500 ml, wide necked with a ground-glass stopper with tap (see ISO 1773).
- 4.2 Filtration equipment**, fritted-glass filter of porosity 4 (nominal size 90 μm) with a filter flask of 500 ml (see ISO 6556).
- 4.3 Marked volumetric flask**, 250 ml (see ISO 1042).
- 4.4 Solid-phase extraction (SPE) system and SPE columns**, phenyl silica columns, 500 mg/3 ml.
- 4.5 Gas chromatograph**, a conventional split/splitless injector gives suitable sensitivity when used in the splitless mode. Alternatively, an on-column injection mode may be used.
- 4.6 Capillary column**, suitable for determination of acetylated derivatives of pentachlorophenol. A column with the following features is given as an example:
- stationary phase: polydimethyl-siloxan with 5 % phenyl groups;
 - film thickness: 0,25 μm ;
 - length: 30 m;
 - internal diameter: 0,32 mm.
- 4.7 Detector**, electron-capture detector (ECD) or mass spectrometer (MS) suitable for carrying out the measurement. The following features are given as an example:
- a) ionization: EI 70 eV (electron impact);
 - b) resolution: 1 amu (atomic mass unit);
 - c) runnability: SIM mode (selected ion monitoring).

NOTE SIM is called SIR (selected ion recording) for some instruments.

5 Reagents

All reagents shall be of a grade "pro analysis" (p.a.) or equivalent quality.

- 5.1 Water**, grade 2 in accordance with ISO 3696.
- 5.2 Methanol**, CH_3OH .
- 5.3 Hydrochloric acid**, HCl (0,1 mol/l).
- 5.4 *n*-Hexane**, C_6H_{14} .
- 5.5 Sulfuric acid**, H_2SO_4 (diluted 1+1).
- 5.6 Acetic anhydride**, $\text{C}_4\text{H}_6\text{O}_3$ (99 %).
- 5.7 Potassium carbonate solution**, K_2CO_3 (0,1 mol/l). Weigh 13,8 g of potassium carbonate with an accuracy of 0,1 g into a beaker and dissolve it in a small amount of water. Transfer the solution to a 1 000 ml volumetric flask and dilute to volume with water.

5.8 Sodium sulfate, anhydrous, Na₂SO₄.

5.9 Reference standard solutions

5.9.1 Pentachlorophenol reference stock standard solution, 100 µg/ml. This solution is commercially available.

5.9.2 Pentachlorophenol dilute reference stock standard solution, 5 µg/ml. Pipette 1 ml of the stock solution (5.9.1) into a 20 ml volumetric flask and dilute to volume with methanol (5.2). This solution is stable for at least 6 months when stored in a refrigerator at 4 °C.

5.9.3 Pentachlorophenol reference standard solution, 0,5 µg/ml. Pipette 1 ml of the dilute stock solution (5.9.2) into a 10 ml volumetric flask and dilute to volume with methanol (5.2). This solution is stable for at least 6 months when stored in a refrigerator at 4 °C.

5.10 Internal standard solutions for ECD

5.10.1 2,3,6-Trichlorophenol internal standard stock solution, 10 µg/ml. This solution is commercially available.

5.10.2 2,3,6-Trichlorophenol internal standard solution, 2 µg/ml. Pipette 2 ml of the internal standard stock solution (5.10.1) into a 10 ml volumetric flask and dilute to volume with methanol (5.2). This solution is stable for at least 3 months when stored in a refrigerator at 4 °C.

5.11 Internal standard solutions for MS

5.11.1 ¹³C₆ labelled pentachlorophenol (labelled at all six carbons) internal standard stock solution, 10 µg/ml. This solution is commercially available. It is an alternative to the 2,3,6-trichlorophenol solution mentioned in 5.10.1 and can be employed only when a mass spectrometer is used as a detector.

5.11.2 ¹³C₆ labelled pentachlorophenol internal standard solution, 1 µg/ml. Pipette 1 ml of the internal standard stock solution (5.11.1) into a 10 ml volumetric flask and dilute to volume with methanol (5.2). This solution is stable for at least 6 months when stored in a refrigerator at 4 °C.

6 Sampling

If the analysis is being made to evaluate a lot of paper, board or pulp, the sample shall be selected in accordance with ISO 186 or ISO 7213, as relevant. If the analysis is made on another type of sample, report the source of the sample, and, if possible, the sampling procedure. Select the specimens so that they are representative of the sample received.

Do not touch the test area of the sample or test specimen with fingers; use protective gloves. A minimum of 10 g of sample is required.

Take a separate sample for the determination of the dry matter content in accordance with ISO 638.

7 Extraction

Determine the dry matter content of the sample in accordance with ISO 638.

7.1 Cold-water extraction

Using protective gloves, tear or cut the sample as taken into pieces of approximately 1 cm² to 2 cm².

Weigh ($10 \pm 0,1$) g (oven-dried) of the test pieces to an accuracy of 0,01 g, put them into the conical flask (4.1), add 200 ml of water and stopper the flask. Leave this preparation to stand for 24 h at (23 ± 2) °C, shaking occasionally.

Decant the solution and wash the test pieces remaining in the flask twice. If necessary, filter the preparation (see 4.2). Transfer the extract and washings or the filtrate to a marked volumetric flask (4.3) and fill up to the mark with water. Use the contents of the flask for the PCP measurement.

7.2 Hot-water extraction

Using protective gloves, tear or cut the sample as taken into pieces of approximately 1 cm² to 2 cm².

Weigh ($10 \pm 0,1$) g (oven-dried) of the test pieces to an accuracy of 0,01 g, put them in the conical flask (4.1), add 200 ml of boiling water and stopper the flask. Leave this preparation to stand for 2 h \pm 5 min in a water bath of temperature (80 ± 2) °C, shaking occasionally.

Decant the solution and wash the test pieces in the flask twice with water at 80 °C. If necessary filter the hot preparation (see 4.2). Cool to (23 ± 2) °C, then transfer the extract and washings or the filtrate to a marked volumetric flask (4.3), and fill up to the mark with water. Use the contents of the flask for the PCP measurement.

8 Procedure

Analyse the water extract received from 7.1 or 7.2 as described below. Carry out duplicate determinations.

8.1 Preparation

Sample 50 ml of the water extract. Add 200 μ l of the internal standard solution (5.10.2 or 5.11.2) and acidify with 1 ml of sulfuric acid (5.5).

8.2 Solid phase concentration

8.2.1 Conditioning

Pre-rinse a phenyl column (4.4) with 2 ml of methanol (5.2) and 5 ml of 0,1 mol/l HCl (5.3) in sequence. Do not allow the column to run dry.

8.2.2 Concentration

Pour the acidified extract onto the column, elute at 2 ml/min to 3 ml/min. Subsequently, rinse the column with 5 ml of water and suck it dry (for approximately 5 min to 10 min) using a vacuum.

8.2.3 Elution

For the receiver of *n*-hexane eluent, use, for example, a 50 ml conical flask or a separatory funnel, with 35 ml of potassium carbonate solution (5.7). Pour 2,5 ml of *n*-hexane onto the phenyl column and wait until the first drop is seen in the lower end. Close the valve and elute after 2 min. Rinse with another 2,5 ml of *n*-hexane.

8.3 Derivatization

Add 1 ml of acetic anhydride (5.6) to the eluted solution (see 8.2.3) and mix vigorously for approximately 3 min to allow the release of all the carbon dioxide. Allow the two phases to separate. Fill the receiver with water until the *n*-hexane phase reaches the neck of the receiver. Transfer the organic layer into a vial in order to avoid a reverse reaction. Dehydrate the extract with anhydrous sodium sulfate (5.8). This extract has to be analysed by gas chromatography (GC) ECD or GC/MS.

NOTE If the *n*-hexane solution is dried, for example, with anhydrous sodium sulfate (5.8), the solution can be stored for at least 2 weeks in a refrigerator at 4 °C.

9 Calibration

Pour 35 ml of 0,1 mol/l potassium carbonate solution (5.7) into a 50 ml conical flask or a separatory funnel (see 8.2.3). Add 200 µl of the internal standard solution (5.10.2 or 5.11.2) depending on the use of ECD or MS. Prepare four solutions by adding various amounts of PCP (use the reference solutions 5.9.2 and 5.9.3). Shake for 3 min. Add 5 ml of *n*-hexane (5.4) and 1 ml of acetic anhydride (5.6) and shake until the gas evolution has stopped (approximately 3 min). Fill up with pure analytical water (5.1) until the organic phase reaches the neck of the conical flask and can be removed with a pipette. Dehydrate the extract with sodium sulfate. Analyse the extracts by GC/ECD or GC/MS in accordance with Clause 10.

NOTE If the *n*-hexane solution is dried, for example, with anhydrous sodium sulfate (5.8), the solution can be stored for at least 2 weeks in a refrigerator at 4 °C.

10 Gas chromatographic analysis

10.1 Gas chromatographic condition

Choose the conditions suitable for determination of ¹³C₆ labelled pentachlorophenol. Follow the instructions provided by the manufacturer of the instrument. The following conditions are given as an example:

- splitless injection: 1 µl;
- injector temperature: 250 °C;
- electron-capture-detector temperature: 350 °C;
- carrier gas, flow: 1 ml/min;
- temperature programme: 1 min at 50 °C, then at 20 °C/min to 180 °C and hold for 2 min. After that at 2 °C/min to 200 °C and hold for 1 min; or 20 °C/min to 280 °C and hold for 5 min.

Carry out the detection either by the electron-capture detector or by the use of a mass spectrometer. In both cases, follow the instructions provided by the manufacturer of the instrument.

If a mass spectrometer is used, with ¹³C₆ labelled pentachlorophenol as the internal standard, the base peak, *m/v* 266, is used for quantification of PCP, (*m/v* 266 = *M* + 2 minus ketene, CH₂=C=O, from the acetate). The corresponding ion from the ¹³C₆ labelled pentachlorophenol is *m/v* 272 (266 + 6). Verification of ¹³C₆ labelled pentachlorophenol should be made from the retention time and the ratio between *m/v* 264 and *m/v* 266, which shall be close to 61/100 due to the chlorine isotope ratio.

If the value of pentachlorophenol obtained exceeds the range covered by the calibration solutions, repeat the analysis in 8.1 with a more dilute sample solution.

10.2 Establishing retention time of pentachlorophenol

Identify the retention time of pentachlorophenol relative to the retention time of the internal standard.

10.3 Calculation of the area ratio versus mass ratio

Measure the areas of the pentachlorophenol peaks and the peak of the internal standard.

Plot the area ratio, A_r , versus the mass ratio, w_r , in accordance with Equations (1) and (2)

$$A_r = \frac{A_{\text{pcp}}}{A_{\text{ist}}} \quad (1)$$

$$w_r = \frac{m_{\text{pcp}}}{m_{\text{ist}}} \quad (2)$$

where

A_{pcp} is the area of the pentachlorophenol base peak;

A_{ist} is the area of the peak representing the internal standard base peak;

m_{pcp} is the mass of pentachlorophenol in the solution, in micrograms;

m_{ist} is the mass of the internal standard, in micrograms, in this case 0,4 µg.

Read the slope of the curve and obtain the response factor, f , which is a dimensionless number, independent of the injected volume and the acetylation yield.

$$f = \frac{m_{\text{pcp}} \times A_{\text{ist}}}{m_{\text{ist}} \times A_{\text{pcp}}} \quad (3)$$

Alternatively, read the response factor from the output of the gas chromatograph.

The response for the ECD can change with time and the response factor should be checked regularly.

10.4 Calibration for mass spectrometer using $^{13}\text{C}_6$ labelled pentachlorophenol

The response factor between PCP and the $^{13}\text{C}_6$ labelled pentachlorophenol is 1. That means that $A_r = m_r$ and $f = 1$.

— A_{pcp} is the area of the pentachlorophenol base peak (266);

— A_{ist} is the area of the peak representing the internal standard base peak (272).

11 Calculation

Identify the pentachlorophenol peak and measure its area, A_{pcp} . Measure also the area of the peak representing the internal standard, A_{ist} .

Calculate the result by the equation

$$w_{\text{pcp}} = 5 \times \frac{f \times A_{\text{pcp}} \times m_{\text{ist}}}{m_{\text{s}} \times A_{\text{ist}}} \quad (4)$$

where

w_{pcp} is the mass fraction of pentachlorophenol in the sample, expressed as milligrams per kilogram;

f is the response factor as determined in 10.3 if using ECD, or $f = 1$ in accordance with 10.4 if using MS;

m_{ist} is the mass of the internal standard, in micrograms; in this case 0,4 μg 2,3,6-trichlorophenol or 0,2 μg $^{13}\text{C}_6$ labelled pentachlorophenol;

m_{s} is the mass of the sample used in the water extraction, as oven-dry, expressed as grams, normally 10 g;

A_{pcp} is the area of the pentachlorophenol base peak;

A_{ist} is the area of the peak representing the internal standard base peak.

Calculate the mean of the two determinations to two significant figures.

12 Test report

The test report shall include the following information:

- a) a reference to this International Standard;
- b) date and place of testing;
- c) complete identification of the sample tested;
- d) reference to the extraction procedure, hot-water extract or cold-water extract;
- e) the result, expressed as milligrams per kilogram of pulp, paper or board;
- f) any departure from the specified procedure, or other circumstances that may have affected the results.

Annex A (informative)

Precision

A.1 General

In January 2009, an international round-robin was performed on two pulp samples by 7 different laboratories according to this International Standard.

The pulp samples were spiked samples and the PCP content was lower in Sample 1 and higher in the Sample 2. Sample extractions were carried out both in cold water and in hot water. PCP contents were then measured.

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

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

A.2 Repeatability

Table A.1 — Repeatability

Sample	Number of labs	Extraction	Mean mg/kg	Standard deviation, s_r (mg/kg)	Coefficient of variation, $C_{V,r}$ (%)	Repeatability limit, r (mg/kg)
Sample 1	3	Cold water	0,054	0,005 5	10,1	0,015
Sample 2	3		0,550	0,006 2	1,1	0,017
Sample 1	3	Hot water	0,078	0,025 0	32,3	0,069
Sample 2	4		0,540	0,024 0	4,4	0,066

A.3 Reproducibility

Table A.2 — Reproducibility

Sample	Number of labs	Extraction	Mean mg/kg	Standard deviation, s_R (mg/kg)	Coefficient of variation, $C_{V,R}$ (%)	Reproducibility limit, R (mg/kg)
Sample 1	3	Cold water	0,054	0,017	31,6	0,047
Sample 2	3		0,550	0,15	28,4	0,416
Sample 1	3	Hot water	0,078	0,06	73,1	0,166
Sample 2	4		0,540	0,20	37,0	0,554

Bibliography

- [1] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*
- [2] ISO 1773, *Laboratory glassware — Narrow-necked boiling flasks*
- [3] ISO 6556, *Laboratory glassware — Filter flasks*
- [4] EN 645, *Paper and board intended to come into contact with foodstuffs — Preparation of a cold water extract*
- [5] EN 647, *Paper and board intended to come into contact with foodstuffs — Preparation of a hot water extract*

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