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Rubber and rubber products — Determination of 2-mercaptobenzothiazole content by high performance liquid chromatography (HPLC)

Caoutchouc et produits à base de caoutchouc — Détermination de la teneur en 2-mercaptobenzothiazole par chromatographie en phase liquide haute performance

Reference number
ISO 21490:2022(E)

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Contents

Page

Foreword ... **iv**
Introduction ... **v**
1 Scope ... **1**
2 Normative references ... **1**
3 Terms and definitions ... **1**
4 Principle ... **1**
5 Reagents and materials ... **1**
6 Apparatus ... **2**
7 Sampling ... **2**
8 Procedure ... **2**
8.1 Sample preparation ... 2
8.2 Chromatographic conditions ... 3
8.3 Preparation of standard working solutions and the calibration curve ... 3
8.4 HPLC-DAD testing ... 3
9 Test results ... **3**
9.1 Calibration curve ... 3
9.2 Calculation ... 4
10 Precision ... **4**
11 Test report ... **4**
Annex A (informative) **Schematic chemical reactions during curing or rubber with sulfenamide that gives 2-MBT** ... **5**
Annex B (informative) **A gradient programme of HPLC** ... **6**
Annex C (informative) **Calibration** ... **7**
Annex D (informative) **Spectrum and chromatogram of 2-MBT** ... **9**
Annex E (informative) **Precision** ... **11**
Bibliography ... **12**

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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 documents 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 ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 2, *Testing and analysis*.

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.

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Introduction

2-Mercaptobenzothiazole (sometimes also referred to as: MBT; 2-MBT; 2-benzothiazolethione, or BTSH) is used in the rubber industry as a curing agent. MBT is in the group of thiazoles and is considered as scorch fast when used as a primary accelerator.

2-Mercaptobenzothiazole as the acidic sulfur accelerator is widely used in rubber materials because of its good characteristics: stable sulfides, good vulcanization, and a low critical temperature to accelerate vulcanization so that the rubber product can reach higher tensile strength and hardness levels.

Measuring 2-mercaptobenzothiazole concentration in rubber compounds at different stages of curing the rubber product is an excellent means to define the optimal curing conditions of temperature and time in order to obtain the right properties for the products at the best cost.

During the curing of rubber compounds sulfenamides are used as accelerators, which chemically react at an early stage of the curing to produce 2-mercaptobenzothiazole and other species. 2-Mercaptobenzothiazole then contributes to the initiation of the mechanism which creates the sulfur crosslinks between the rubber macromolecules at the end (an example is given in Figure A.1). To ensure continuous progress, it is important to know the chemical mechanisms involved at each stage. Thus, it is necessary to quantify the content of 2-mercaptobenzothiazole during the decomposition of the sulfenamide and to know whether 2-mercaptobenzothiazole has disappeared in any further chemical reactions.

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Rubber and rubber products — Determination of 2-mercaptobenzothiazole content by high performance liquid chromatography (HPLC)

1 Scope

This document specifies a quantitative test method to determine the 2-mercaptobenzothiazole content in rubber and rubber products by high performance liquid chromatography (HPLC).

This document delivers a method for quantifying 2-mercaptobenzothiazole in rubber products for a better selection of curing conditions.

This document provides a method to follow the curing of rubber with sulfur- and benzothiazole-based accelerators using a chemical measurement which is complementary to the classical rheometric technique.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4661-2, *Rubber, vulcanized — Preparation of samples and test pieces — Part 2: Chemical tests*

3 Terms and definitions

No terms and definitions are listed in this document.

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/

4 Principle

The 2-mercaptobenzothiazole in rubber is ultrasonically extracted with a chloroform-methanol solution determined and confirmed with HPLC-DAD (high performance liquid chromatography equipped with a diode-array detector).

Note There is a risk of neo-formed MBT from MBTs if a thiazole accelerator is used in the formulation of the sample.

5 Reagents and materials

Unless otherwise specified, analytical grade chemicals should be used. Water shall be distilled or deionized to fulfil grade 3 in accordance with ISO 3696.

5.1 Methanol, of analytical grade.

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5.2 Chloroform, of analytical grade.

5.3 Acetonitrile, of chromatographic grade.

5.4 2-Mercaptobenzothiazole standard material, of purity ≥ 99,1 %.

5.5 Filtration membrane, with a pore size of 0,45 µm.

5.6 Chloroform-methanol solution, 2 V+1 V.

6 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used.

6.1 HPLC-DAD.

6.2 Analytical balance, accuracy ± 0,1 mg.

6.3 Ultrasonic extraction apparatus.

6.4 One-mark volumetric flask, 100 ml.

6.5 Quantitative filter paper.

6.6 Flask with stopper, 20 ml.

6.7 Pipette, 10 ml.

7 Sampling

Prepare the sample according to ISO 4661-2, cut it into pieces smaller than 0,1 cm × 0,1 cm. Store the sample pieces in a dry and cool place, and avoid their contamination.

8 Procedure

8.1 Sample preparation

Weigh approximately 0,4 g (accurate to 0,1 mg) of a homogeneous test sample and put it into a 20 ml flask with a stopper. Add accurately, by pipette, 10 ml chloroform-methanol solution (5.6) and stopper the flask. Extract for 14 h in the ultrasonic extraction apparatus (6.3) at a temperature not exceeding 40 °C.

In order to maintain the bath below 40 °C, it is necessary to periodically add cold water or another cooling agent to the bath during the extraction.

Transfer the extracted solution into a 100 ml volumetric flask (6.4) with funnel and filter paper (6.5), dilute it to the mark with methanol and mix thoroughly.

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8.2 Chromatographic conditions

Since the test results depend on the equipment that is used, there are no universal parameters for chromatographic analysis (see example in Annex B). The following parameters have been proven to be suitable for testing as reference:

— column: C18, 5 µm, 4,6 mm × 250 mm or chromatographic column with the similar performance;

— temperature of column: 35 °C;

— wavelength range: 200 nm to 400 nm;

— test wavelength: 320 nm;

— mobile phase A (water containing 1 % acetonitrile) and mobile phase B (acetonitrile);

— flow rate: 1,0 ml/min;

— injection volume: 10 µl.

8.3 Preparation of standard working solutions and the calibration curve

Weigh 100 mg of a 2-mercaptobenzothiazole standard material and put it into a 100 ml volumetric flask. Fill the flask with methanol to obtain a 1 mg/ml standard stock solution. The standard stock solution is diluted with methanol stepwise into 0,005 mg/ml, 0,010 mg/ml, 0,020 mg/ml, 0,030 mg/ml and 0,050 mg/ml standard working solutions (see example in Annex C).

Inject those solutions using the analytical conditions described in 8.2, and the calibration curve is obtained by plotting the peak area of each standard against their concentration. Because of the instability of MBT, standard working solutions shall be analysed without delay to avoid systematic errors in the calibration.

8.4 HPLC-DAD testing

Filter the solution through the filtration membrane for HPLC detection.

Analyse the sample solutions (10 µl) and standard working solutions (10 µl) by HPLC-DAD separately. To ensure that the peak of response in the extracts is indeed 2-mercaptobenzothiazole, it is necessary to verify that the retention time and the UV-visible spectrum of the peak are identical for the extracts and standard solutions (see example in Annex D).

A blank test shall be carried out in parallel with the determination. The blank test is performed according to 8.1 but omitting the test sample.

To avoid column damages, appropriate measures should be taken such as washing the column after testing.

9 Test results

9.1 Calibration curve

The linear calibration equation is determined by plotting the standard working solution concentration (ordinate) against the peak area (abscissa). An example of a calibration curve is given in Annex C. Through the least square fitting, the linear determination coefficient R^2 should be greater than 0,995.

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The 2-mercaptobenzothiazole content in standard solution can be calculated by Formula (1).

$$c = K \times A + b \quad (1)$$

where

c is the 2-mercaptobenzothiazole content in the standard solution, in mg/ml;

K is the slope of the calibration curve;

A is the chromatography area of 2-mercaptobenzothiazole in the standard solution;

b is the intercept of the calibration curve.

9.2 Calculation

The 2-mercaptobenzothiazole content in sample is calculated according to Formula (2).

$$W = \frac{(c - c_0) \cdot \mathrm{V}}{m} \quad (2)$$

where

W is the 2-mercaptobenzothiazole concentration in the sample, in g/kg;

c is the 2-mercaptobenzothiazole concentration in the extracted solution, in mg/ml;

c_0 is the 2-mercaptobenzothiazole concentration in the blank, in mg/ml;

V is the final constant volume of the extracted solution, in ml;

m is the sample mass, in g.

10 Precision

See Annex E.

11 Test report

The test report shall include the following information:

a) reference to this document, i.e. ISO 21490:2022;

b) detailed description of the test sample;

c) content of the 2-mercaptobenzothiazole in the test sample for each curing temperature and time condition, g/kg;

d) the difference between the test procedure and the specified steps;

e) any deviations during the analytical procedure;

f) date of the test.

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The 2-mercaptobenzothiazole content in standard solution can be calculated by Formula (1).

$$c = K \times A + b \quad (1)$$

where

c is the 2-mercaptobenzothiazole content in the standard solution, in mg/ml;

K is the slope of the calibration curve;

A is the chromatography area of 2-mercaptobenzothiazole in the standard solution;

b is the intercept of the calibration curve.

9.2 Calculation

The 2-mercaptobenzothiazole content in sample is calculated according to Formula (2).

$$W = \frac{(c - c_0) \cdot \mathrm{V}}{m} \quad (2)$$

where

W is the 2-mercaptobenzothiazole concentration in the sample, in g/kg;

c is the 2-mercaptobenzothiazole concentration in the extracted solution, in mg/ml;

c_0 is the 2-mercaptobenzothiazole concentration in the blank, in mg/ml;

V is the final constant volume of the extracted solution, in ml;

m is the sample mass, in g.

10 Precision

See Annex E.

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The test report shall include the following information:

a) reference to this document, i.e. ISO 21490:2022;

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d) the difference between the test procedure and the specified steps;

e) any deviations during the analytical procedure;

f) date of the test.

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Annex B
(informative)

A gradient programme of HPLC

Table B.1 shows a gradient programme of HPLC as an example.

Table B.1 — A gradient programme of HPLC

Time min	Eluent A %	Eluent B %
0 to 2	70	30
2 to 17 linearly to	10	90
17 to 22	10	90
22 to 25 linearly to	70	30
25 to 28	70	30

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Annex C
(informative)

Calibration

An example is given for preparation of 2-MBT standard solutions from 0,005 mg/ml to 0,050 mg/ml. The solutions are prepared by successive dilutions of a 2-MBT solution of 1 mg/ml obtained by dissolving 100 mg of 2-MBT (of purity ≥ 99,1 %) in 100 ml of methanol.

NOTE The order of injection of the standard solutions generally is from the less concentrated to the more concentrated.

Table C.1 provides information for preparation of the 2-MBT standard solutions. Figure C.1 provides an example of a calibration curve for 2-MBT.

Table C.1 — Steps for preparation of the 2-MBT standard solutions for the calibration range

Solutions	2-MBT concentration mg/ml
2,5 ml of 1 mg/ml solution, then complete up to 50 ml of methanol	0,050
6 ml of 0,05 mg/ml solution, then complete up to 10 ml of methanol	0,030
4 ml of 0,05 mg/ml solution, then complete up to 10 ml of methanol	0,020
2 ml of 0,05 mg/ml solution, then complete up to 10 ml of methanol	0,010
1 ml of 0,05 mg/ml solution, then complete up to 10 ml of methanol	0,005

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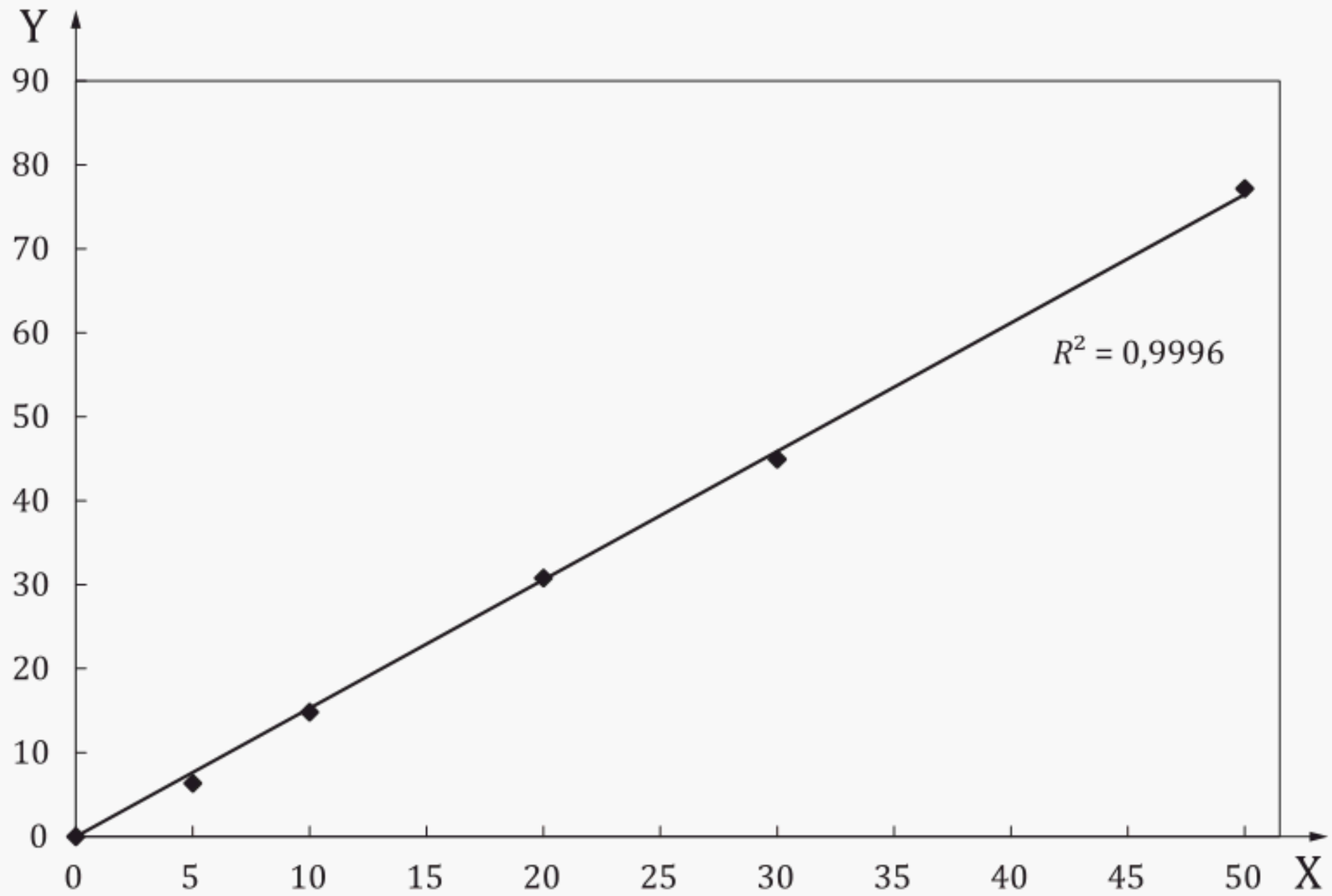

Key

X amount (mg/ml)

Y area (mV.min)

Figure C.1 — Example of a calibration curve for 2-MBT

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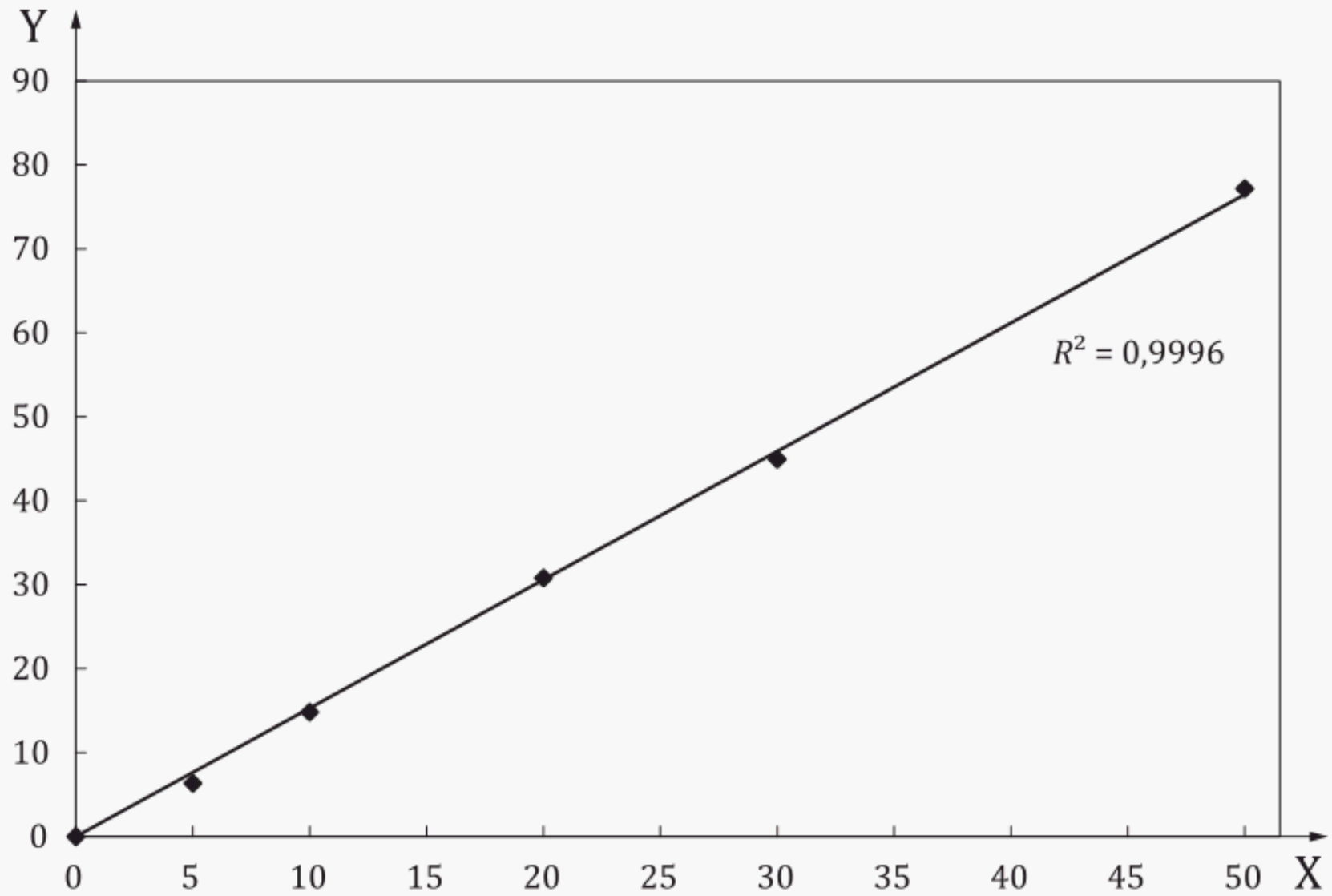

Key

X amount (mg/ml)

Y area (mV.min)

Figure C.1 — Example of a calibration curve for 2-MBT

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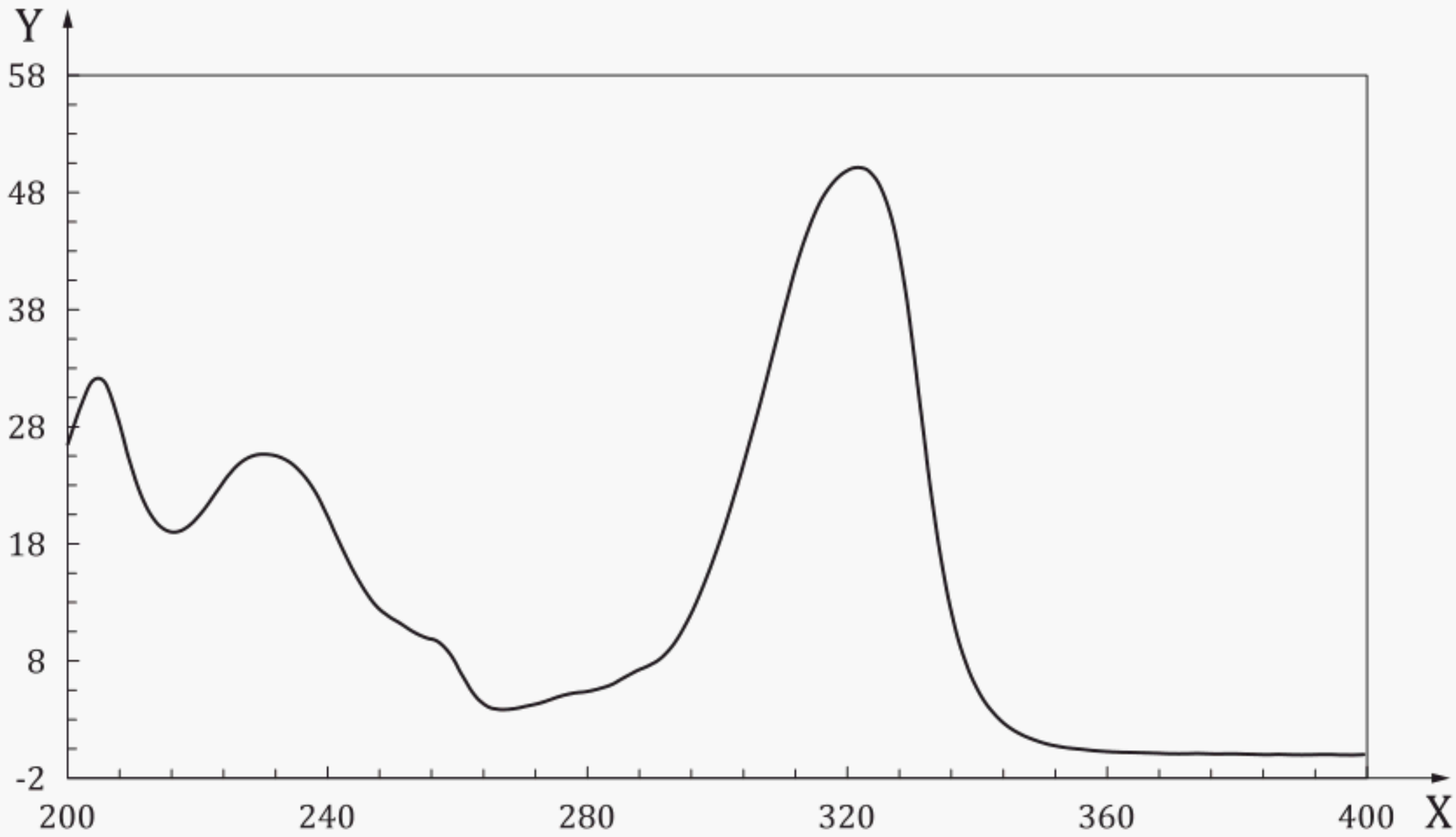

Key

X wavelength (nm)

Y transmittance (%)

Figure D.2 — Example of a spectrum of 2-MBT

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Annex E
(informative)

Precision

E.1 General

An interlaboratory test programme (ITP) was organized in October 2020. The precision evaluated was a type 1 precision in accordance with method B of ISO 19983:2017.

Eight laboratories participated in the ITP and two different materials were used. Two tests were carried out on two different days of two weeks, at intervals of one week. Two measurements were repeated each day. The data from one laboratory were discarded as outliers.

E.2 Precision results

The precision results for this document are given in Table E.1. These results were obtained using outlier deletion procedures as described in ISO 19983.

Repeatability: The difference between the two-days test (value) averages, found on nominally identical material samples under correct application of this test method, does not exceed the tabulated day-to-day repeatability in more than one in 20 cases on average.

Reproducibility: The difference between two independently measured test averages (values), found in two laboratories using the correct application of this test method on nominally identical material samples, does not exceed the tabulated reproducibility in more than one in 20 cases.

Table E.1 — Precision data

Sample	Mean content	Within laboratories			Between laboratories			Number of laboratories
		S_{rD}	r_D	(r_D)	S_R	R	(R)	
A	2,41	0,15	0,43	17,97	0,38	1,08	44,81	7
B	4,72	0,26	0,74	15,74	0,58	1,64	34,66	7

The symbols used in this table are defined as follows:

S_{rD} is the day-to-day repeatability standard deviation;

r_D is the day-to-day repeatability, in measurement units;

(r_D) is the relative day-to-day repeatability, in percent;

S_R is the reproducibility standard deviation;

R is the reproducibility, in measurement units;

(R) is the relative reproducibility, in percent.

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Bibliography

[1] ISO 19983:2017, *Rubber — Determination of precision of test methods*

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