
**Rubber, raw, natural — Determination of
castor oil content —**

Part 1:

**Determination of castor oil glyceride
content by thin-layer chromatography**

*Caoutchouc naturel brut — Détermination de la teneur en huile de
ricin —*

*Partie 1: Détermination de la teneur en glycérides d'huile de ricin par
chromatographie en couche mince*

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Foreword

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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.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6225-1 was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 2, *Testing and analysis*.

This second edition cancels and replaces the first edition (ISO 6225-1:1984), which has been revised to update the normative references.

ISO 6225 consists of the following parts, under the general title *Rubber, raw, natural — Determination of castor oil content*:

- *Part 1: Determination of castor oil glyceride content by thin-layer chromatography*
- *Part 2: Determination of total ricinoleic acid content by gas chromatography*

Introduction

Certain grades of natural rubber are treated with castor oil to facilitate crumbing of the rubber during production. This part of ISO 6225 is intended to facilitate estimation of the amount of castor oil remaining in the rubber.

The principal constituent of castor oil, making up about 80 % to 85 % (by mass), is the triglyceride of ricinoleic acid. This glyceride may partly hydrolyse to ricinoleic acid and glycerol.

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Rubber, raw, natural — Determination of castor oil content —

Part 1:

Determination of castor oil glyceride content by thin-layer chromatography

WARNING — Persons using this part of ISO 6225 should be familiar with normal laboratory practice. This part of ISO 6225 does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 6225 specifies a thin-layer chromatographic method for the determination of the castor oil and castor oil glyceride content of raw rubber.

It is applicable to all grades of natural rubber.

The lower limit of detection is approximately 0,05 % of castor oil glycerides.

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 1407:1992, *Rubber — Determination of solvent extract*

3 Principle

A test portion of rubber is extracted with acetone and the castor oil glycerides separated from the other extracted compounds by thin-layer chromatography. The castor oil glyceride spots are developed with phosphomolybdic acid or anisaldehyde and evaluated visually or spectrometrically.

4 Reagents

During the analysis, except where otherwise specified, use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

4.1 Silica gel, TLC grade.

4.2 Developing solvent.

Prepare a mixture of light petroleum (boiling range 40 °C to 60 °C), diethyl ether and glacial acetic acid, in the proportions, by volume, of 50:50:1, respectively.

4.3 Spray reagents.

4.3.1 Phosphomolybdic acid, ethanolic solution.

Dissolve 15 g of phosphomolybdic acid in 100 cm³ of 95 % (by volume) ethanol.

4.3.2 Anisaldehyde, solution.

Mix 10 cm³ of ethanol, 0,5 cm³ of sulfuric acid ($\rho = 1,84 \text{ Mg/m}^3$) and 0,5 cm³ of anisaldehyde.

4.4 Solvents.

4.4.1 Acetone, redistilled.

4.4.2 Dichloromethane.

4.5 Standard castor oil solutions.

4.5.1 Weigh accurately 0,5 g \pm 0,01 g of castor oil (pharmaceutical grades have been found satisfactory) and prepare a stock solution by diluting to 100 cm³ with dichloromethane (4.4.2) in a one-mark volumetric flask (5.8).

4.5.2 Dilute 2 cm³; 4 cm³; 6 cm³; 8 cm³ and 10 cm³ aliquot portions of the stock solution (4.5.1) to 10 cm³ with dichloromethane (4.4.2) in one-mark volumetric flasks to give solutions corresponding to 0,2 %; 0,4 %; 0,6 %; 0,8 % and 1,0 % (by mass) of castor oil, based on the rubber, when 5 g of rubber is taken for analysis.

5 Apparatus¹⁾

Ordinary laboratory equipment, plus the following:

5.1 **Extraction unit**, all-glass (see Figure 1, 2 or 3 in ISO 1407:1992).

5.2 **Water-bath or electric heating mantle**.

5.3 **Thin-layer chromatography (TLC) plate**, of dimensions 200 mm \times 200 mm, coated with a layer approximately 0,25 mm thick of silica gel (4.1). Commercial plates may also be used.

5.4 **Thin-layer applicator**.

5.5 **Developing tank**, of sufficient size to hold the TLC plate (5.3).

5.6 **Spray apparatus**, for the spray reagent (4.3).

5.7 **Oven**, capable of being maintained at 100 °C \pm 5 °C.

5.8 **One-mark volumetric flasks**, of capacity 5 cm³; 10 cm³ and 100 cm³.

5.9 **Spectrometer**, capable of accurate measurement (± 1 % of total absorbance at 700 nm \pm 1 nm), equipped with cells of optical path length 10 mm.

Operate the spectrometer in accordance with the manufacturer's instructions for optimum performance.

1) The term millilitre (ml) is commonly used as a special name for the cubic centimetre (cm³), in accordance with a decision of the Twelfth Conférence Générale des Poids et Mesures. The term millilitre is acceptable, in general, for references in International Standards to capacities of volumetric glassware and to liquid volumes. Apparatus with either type of marking is satisfactory for use with this part of ISO 6225.

6 Preparation of test sample

Take a piece of rubber, weighing at least 10 g, from the bale and cut it into small pieces (about 1 mm × 3 mm).

If there is an excess of castor oil, the surface of the bale is liable to feel oily. In this case, select a sufficient number of pieces, each of at least 10 g, to provide adequate representation. Prepare and analyse each piece separately, making sure that cross-contamination does not occur during the preparation.

7 Procedure

7.1 Test portion

Weigh out accurately $5 \text{ g} \pm 0,1 \text{ g}$ of the test sample and place it in the extraction thimble of the extraction unit (5.1). If the sample is sheeted out, roll it into a cylinder between filter paper or cloth to prevent sticking.

7.2 Determination

7.2.1 Introduce the thimble into the syphon cup and, using 100 cm^3 of acetone (4.4.1) in the extraction flask, extract the test portion for 16 h using the water-bath or electric heating mantle (5.2) to maintain a temperature sufficient to reflux the acetone and fill the extraction cup 10 to 20 times per hour.

7.2.2 Evaporate the acetone from the extract in the extraction flask, for example by distillation over a water-bath, until about 2 cm^3 remains.

7.2.3 Transfer the extract to a 10 cm^3 one-mark volumetric flask (5.8), rinse with dichloromethane (4.4.2) and make up to the mark with dichloromethane.

7.2.4 Spot $5 \mu\text{l}$ of the test solution prepared in 7.2.3 and $5 \mu\text{l}$ of each of the diluted standard castor oil solutions prepared in 4.5.2 onto the TLC plate (5.3).

7.2.5 Develop the TLC plate to a height of 100 mm in the developing tank using the developing solvent (4.2).

7.2.6 Remove the plate, allow to air-dry and spray with either phosphomolybdic acid solution (4.3.1) or anisaldehyde solution (4.3.2). Bake in the oven (5.7) until the spots have developed colour on a pale background. This takes about 10 min.

NOTE Phosphomolybdic acid is adequate for relatively "clean" rubbers (i.e. those having low contents of extractables); however, in some less "clean" rubbers, the main castor oil spot is overlapped by another one, which also gives a blue coloration. Interference can be avoided by spraying with anisaldehyde solution (4.3.2) which gives spots which are initially mauve and soon turn green.

7.2.7 If using the phosphomolybdic acid solution, proceed as specified in 8.1 to 8.2.4 for visual and spectrometric determination of the castor oil content. If using the anisaldehyde solution, proceed as specified in 8.1 for visual determination of the castor oil content.

NOTE The method using anisaldehyde is not suitable for the spectrometric determination of castor oil glycerides.

8 Evaluation

8.1 Compare the area of the larger of the two blue spots which corresponds to the glyceride of ricinoleic acid (R_f value of about 0,2) with those of the spots from the standard solutions and visually estimate the castor oil content, expressed as a percentage by mass, of the test portion.

8.2 Alternatively, if additional accuracy is desired, the larger spots may be scraped from the plate and the blue colour evaluated spectrometrically as follows.

8.2.1 Quantitatively scrape off the larger of the two blue spots (see 8.1) from the test solution and each of the standard solutions and macerate each with 1 cm³ of water. Centrifuge to obtain clear solutions. Transfer the supernatant liquid to a 5 cm³ one-mark volumetric flask (or larger flask if the resulting solution is too concentrated) and make up to the mark with water. Ensure that no silica gel is transferred to the volumetric flask.

8.2.2 Measure the absorbance (optical density) at 700 nm of each solution (see 8.2.1), using the spectrometer (5.9). Use water as the reference liquid.

8.2.3 Construct a calibration curve of absorbance against the castor oil content, expressed as a percentage by mass, using the values from the spots from the standard solutions.

8.2.4 From the calibration curve, read the castor oil content, expressed as a percentage by mass, of the test portion.

8.3 Report the result to the nearest 0,05 % (by mass).

9 Test report

The test report shall include the following information:

- a) a reference to this part of ISO 6225;
- b) all details necessary for complete identification of the sample;
- c) the results obtained and the method of evaluation used;
- d) any unusual features which may have affected the results;
- e) the date of the test.

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