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International Standard



6225/1

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INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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**Rubber, raw, natural — Determination of castor oil content —  
Part 1 : Determination of castor oil glycerides content —  
Thin layer chromatographic method**

**iTeh STANDARD PREVIEW**

*Caoutchouc naturel brut — Détermination de la teneur en huile de ricin — Partie 1 : Détermination de la teneur en glycérides d'huile de ricin — Méthode par chromatographie en couche mince*

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[ISO 6225-1:1984](#)

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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 developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been authorized 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 6225/1 was developed by Technical Committee ISO/TC 45, *Rubber and rubber products*, and was circulated to the member bodies in August 1982.

It has been approved by the member bodies of the following countries:

Austria	India	Spain
Belgium	Indonesia	Sri Lanka
Canada	Italy	Sweden
Czechoslovakia	Korea, Rep. of	Thailand
Denmark	Malaysia	Turkey
Egypt, Arab Rep. of	Netherlands	United Kingdom
France	Poland	USA
Germany, F.R.	Romania	
Hungary	South Africa, Rep. of	

No member body expressed disapproval of the document.

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

## Part 1 : Determination of castor oil glycerides content — Thin layer chromatographic method

### 0 Introduction

Certain grades of natural rubber are treated with castor oil to facilitate crumbing of the rubber during production. This International Standard 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 % (*m/m*), is the triglyceride of ricinoleic acid. This glyceride may partly hydrolyse to ricinoleic acid and glycerol.

### 1 Scope and field of application

This part of ISO 6225 specifies a thin layer chromatographic method for the determination of the castor oil and castor oil glycerides 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.

NOTE — ISO 6225/2 specifies a method for the determination of total ricinoleic acid content of raw rubber.

### 2 References

ISO 1407, *Rubber — Determination of solvent extract.*

ISO 1795, *Raw rubber in bales — Sampling.*

ISO 1796, *Rubber, raw — Sample preparation.*

ISO 6225/2, *Rubber, raw, natural — Determination of castor oil content — Part 2 : Determination of total ricinoleic acid content — Gas chromatographic method.*

### 3 Principle

Extraction of a test portion with acetone, separation of the castor oil glycerides from other extractables by thin layer

chromatography, development of the castor oil glycerides spots with phosphomolybdic acid or anisaldehyde, and visual or spectrometric evaluation.

### 4 Reagents

All recognized health and safety precautions shall be taken when carrying out the procedure specified in this part of ISO 6225.

During the analysis, 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 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 cm<sup>3</sup> of 95 % (*V/V*) ethanol.

**4.3.2 Anisaldehyde**, solution.

Mix 10 cm<sup>3</sup> of ethanol, 0,5 cm<sup>3</sup> of sulfuric acid ( $\rho = 1,84 \text{ Mg/m}^3$ ) and 0,5 cm<sup>3</sup> 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  $0,5 \pm 0,01$  g of castor oil (pharmaceutical grades have been found satisfactory) and prepare a stock solution by diluting to  $100 \text{ cm}^3$  with dichloromethane (4.4.2) in a one-mark volumetric flask (5.8).

**4.5.2** Dilute 2; 4; 6; 8; and  $10 \text{ cm}^3$  aliquot portions of the stock solution (4.5.1) to  $10 \text{ cm}^3$  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 % (*m/m*) of castor oil, based on the rubber, when 5 g of rubber is taken for analysis.

### 5 Apparatus<sup>1)</sup>

Usual laboratory equipment, and

**5.1 Extraction unit**, all-glass (see figure 1 or figure 2 of ISO 1407).

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

**5.3 Thin layer chromatography (TLC) plate**, of dimensions  $200 \text{ mm} \times 200 \text{ mm}$ , coated with a layer approximately 0,25 mm thick of the 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  $100 \pm 5$  °C.

**5.8 One-mark volumetric flasks**, of capacity 5; 10; and  $100 \text{ cm}^3$ .

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

Operate the spectrometer according to the manufacturer's instructions for optimum performance.

### 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  $1 \text{ mm} \times 3 \text{ mm}$ ).

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.

NOTE — As there could be a high concentration of castor oil on the surface of a piece, some of which might be lost if the rubber is milled, the homogenization procedure described in ISO 1796 cannot be used.

## 7 Procedure

### 7.1 Test portion

Weigh accurately  $5 \pm 0,1$  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  $100 \text{ cm}^3$  of the 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  $2 \text{ cm}^3$  remains.

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

**7.2.4** Spot  $5 \mu\text{l}$  of the test solution (7.2.3) and  $5 \mu\text{l}$  of each of the diluted standard castor oil solutions (4.5.2) on to 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 the phosphomolybdic acid solution (4.3.1) or the 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 the anisaldehyde solution (4.3.2) which gives spots which are initially mauve and soon turn green.

1) The term millilitre (ml) is commonly used as a special name for the cubic centimetre ( $\text{cm}^3$ ), 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.

**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 cm<sup>3</sup> water. Centrifuge to obtain clear solutions. Transfer the supernatant liquid to a 5 cm<sup>3</sup> 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 % (*m/m*).

## 9 Test report

The test report shall include the following information :

- a) a reference to this part of ISO 6225;
- b) 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 test.

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