International
Standard

ISO 7836

Natural rubber — Identification of phytosterols, including β-sitosterol

Caoutchouc naturel — Identification des phytostérols, en particulier du β-sitostérol

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Foreword

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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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This document was prepared by Technical Committee ISO/TC 45, *Rubber and rubber products*, Subcommittee SC 3, *Raw materials (including latex) for use in the rubber industry*.

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.

Natural rubber — Identification of phytosterols, including β-sitosterol

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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 prior to use.

1 Scope

This document specifies a method to identify the presence of phytosterol including β-sitosterol in natural rubber in raw and vulcanised forms.

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 124, *Latex, rubber — Determination of total solids content*

ISO 1407, *Rubber — Determination of solvent extract*

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
phytosterol
sterol derived from plants

3.2
β-sitosterol
major *phytosterol* (3.1) (65 % to 85 %) contained in natural rubber

4 Principle

β-sitosterol is extracted from the sample with acetone. The extracted β-sitosterol is qualitatively detected by thin layer chromatography (TLC) method through comparison of the ratio of the distance the spot has moved compared to the solvent front, *Rf*, and colours of the TLC spot against standard β-sitosterol prepared and analysed under the same analysis conditions.

NOTE 1 When β-sitosterol is mentioned, it implies the presence of other phytosterols alongside β-sitosterol.

NOTE 2 Cis-1,4-polyisoprene can be found both in natural rubber or synthetically prepared polyisoprene rubber, but β-sitosterol is only present in natural rubber. The presence of β-sitosterol can be used to confirm the presence of natural rubber in a cis-1,4- polyisoprene rubber as an addition to other polymer characterization techniques.

NOTE 3 Polymer characterisation technique includes Fourier transform infrared (FTIR) spectrophotometry and protein test.

5 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

5.1 Acetone, (CAS RN®[1] 67-64-1).

5.2 Spray reagent.

Place 3 g of cupric acetate (CAS RN 142-71-2) and 8 ml of phosphoric acid (CAS RN 7664-38-2) into 100 ml volumetric flask. Dilute with distilled water up to the mark.

NOTE Cupric acetate monohydrate (CAS RN 6046-93-1) can also be used to replace cupric acetate (CAS RN 142-71-2).

5.3 Developing solvent, 60 parts by volume of petroleum ether (CAS RN 64742-49-0) and 40 parts by volume of diethyl ether (CAS RN 60-29-7).

5.4 Standard β-sitosterol (>95 %) (CAS RN 83-46-5).

Dissolve 0,03 g of β-sitosterol in 10 ml volumetric flask and make up with acetone (5.1).

6 Apparatus

Use ordinary laboratory apparatus and the following.

6.1 Extraction apparatus.

6.2 TLC plates, covered with a silica gel layer.

NOTE TLC plates silica gel of dimensions 20 cm × 20 cm are appropriate.

6.3 Desiccator, for storing activated TLC plates.

6.4 Sprayer, for spraying the spray reagents.

6.5 Micropipettes or graduated capillary tubes, for TLC, capacity 1 µl to 5 µl.

6.6 Developing chamber/tank.

6.7 Hairdryer, for drying the TLC plate.

6.8 Oven.

6.9 Analytical balance, accurate to 0,01 g.

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[1] CAS Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

7 Procedure

7.1 Sample preparation

7.1.1 For raw or un-vulcanised rubber, sheet a test portion thinly using a laboratory mill with a tight nip and running at even speed and cut it into very small pieces (length of edges ≤2 mm). For vulcanised rubber, cut the sample into very small pieces.

7.1.2 For rubber latex or rubber latex compound, take a portion of thoroughly mixed latex containing about 20 g of total solids, make into a thin film by pouring the portion onto a glass plate, dry to constant mass as specified in ISO 124. Cut the sample into very small pieces.

7.1.3 Place 2 g to 5 g between two filter papers. Transfer to the extraction apparatus (6.1). Extract with acetone as specified in ISO 1407 for 4 h with the test portion in the extraction cup, or 1 h to 2 h with the rubber immersed in the solvent.

7.1.4 Evaporate the extract (7.1.3) using rotary evaporator at a temperature not more than 60 °C. Dry in the oven (6.8) for 1 h at 70 °C. Dissolve the dried extract either in 5 cm^3 or 10 cm^3 of acetone with gentle heat to obtain a clear solution and then proceed directly with spotting of the thin layer plate (6.2).

7.1.5 If the acetone extract value is less than 10 %, dissolve the residue in 5 cm^3 acetone. If the acetone extract in value is more than 10 %, dissolve in 10 cm^3 acetone.

7.2 Thin layer chromatography technique

7.2.1 Preparation of the TLC plates

Activate the TLC plates (6.2) by heating in an oven at 105 °C for 2 h or at 80 °C overnight. Cool the activated plate in desiccator (6.3).

NOTE The activated plate can be stored in the desiccator for 10 days without further activation.

7.2.2 Marking the TLC plates

Inscribe a start line on the TLC plate 1,5 cm from the edge of the plate (6.2). Subdivide the line with marks 1,5 cm apart to serve as a guide for placing the standard β-sitosterol and test solution and as a reference point to measure the Rf value. Subsequently inscribe a second line parallel to the start line 15 cm above. This line serves as the solvent front.

7.2.3 Spotting the TLC plates

Place the end of the micropipettes or graduated capillary tubes (6.5) into sample solution to allow for the solution to rise in the micropipettes. Hold it vertically just above the subdivided pencil line on the plate in order to spot approximately 2 µl to 5 µl of the sample. Allow the solvent to completely evaporate from the spot. Repeat the same procedure with standard solution of β-sitosterol (5.4).

7.2.4 Developing the TLC plates

Develop the TLC plate by placing it into a developing chamber (6.6) containing the developing solvent (5.3). Close the chamber to allow for solvent advancement to the top of the pencil line for at least 30 min. Withdraw the plate from the developing chamber once the solvent reaches the solvent front.

7.2.5 Identification of β-sitosterol

Spray the TLC plate with the spray reagent (5.2). Dry the plate through evaporation by blowing with a hair dryer (6.7). Identify β-sitosterol which is coloured purple.

Measure the *Rf* value.

NOTE *Rf* value is the ratio of the distance the spot has moved compared to the solvent front. The presence of β-sitosterol is confirmed by similar *Rf* value of the sample to that of standard within ±5 %.

8 Expression of results

The results obtained are expressed as presence or absence of β-sitosterol in the sample.

NOTE The minimum detection limit of natural rubber is at 3 %.

9 Test report

The test report shall include the following information:

a) a reference to this document, i.e. ISO 7836:2024, and the method used;

b) all details necessary for the complete identification of the product tested;

c) the results obtained, β-sitosterol detected in sample (expressed as presence or absence of β-sitosterol in natural rubber);

d) any unusual features noted during the determination;

e) any operations not included in this document to which reference is made, as well as any incident which can have affected the results;

f) the detection limit of this method;

g) the date of the test.