



**International
Standard**

ISO 7836

**Natural rubber — Identification of
phytosterols, including β -sitosterol**

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

**First edition
2024-07**

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Published in Switzerland

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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 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.

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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, *R_f*, 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 \times 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 μ l to 5 μ l.

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.

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.