
**Rubber and rubber products —
Identification of antidegradants —
Thin layer chromatographic methods**

*Caoutchouc et produits à base de caoutchouc — Identification des
agents de protection — Méthodes par chromatographie en couche
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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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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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. (standards.iteh.ai)

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

ISO 4645:2022

This second edition cancels and replaces the first edition (ISO 4645:1984), which has been technically revised. It also incorporates the Technical Corrigendum ISO 4645:1984/Cor.1:1991.

The main changes are as follows:

- the description of the principle has been improved;
- method A has been modified.

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.

Rubber and rubber products — Identification of antidegradants — Thin layer chromatographic methods

1 Scope

This document describes two methods for identification of antidegradants (antioxidants, antiozonants and stabilizers) which can be present in raw rubber, unvulcanized compounded rubber, or rubber products, by thin layer chromatography.

Method A is a simplified method that provides for the identification of known materials and can be used to check the presence or absence of a particular antidegradant which is expected to be present.

Method B is a more detailed method that enables a greater degree of separation of the spots to be obtained and therefore can be used to detect and identify an unknown antidegradant.

Antidegradants to which these methods are applicable include phosphited polyalkyl phenols, substituted bisphenols, secondary amines, substituted cresols and substituted p-phenylenediamines. Examination for other types of antidegradants is possible under the same condition when there is a standard chromatogram.

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 1407, *Rubber — Determination of solvent extract*

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

Due to the different chemical structures of antioxidants and their transformations, the partition coefficients in the liquid-solid phase are different. Antidegradants are extracted from the rubber with a solvent. The extraction solution is deposited in the form of spots on a thin layer silica gel chromatographic plate or a glass plate coated with silica gel.

If extender oil is present, the oil is removed either by column chromatography of the extract prior to the completion of the evaporation of the original solvent or by the development of the plate in light petroleum prior to the normal development in an appropriate solvent.

The colour and the shape are reported. The ratio shift value R_f of the spots of the corresponding antioxidant in the colour map is calculated

Identification of the unknown antidegradant is carried out by comparing its chromatogram with its standard chromatograms.

5 Reagents

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

Whenever there appears turbidity, precipitation, or colour change in the solution (in 5.5 and 5.6), it shall be prepared again.

WARNING — Use of fume hoods when handling volatile and toxic solvents is mandatory. Approved health and safety precautions shall be observed when using any solvent or chemical mentioned in this document.

5.1 Plate adsorbent: silica gel, particle size 2 μm to 50 μm , with or without calcium sulfate binder.

Silica gel containing a fluorescent indicator is useful in many cases to observe spots under ultraviolet radiation before spraying.

It is also possible to use commercial silica gel, Silica-G^{®1)} or Silica-HF^{®1)} for example.

5.2 Column adsorbent: silica gel, to pass a sieve of aperture 200 μm to 600 μm activated by drying, either:

- for at least 2 h at 110 °C, if the product is dry after that period; or
- overnight (approximately 16 h at 110 °C) for convenience.

5.3 Silica gel plate adhesive. A solution of sodium carboxymethyl cellulose with a mass fraction of 0,1 % to 0,2 %, prepared the day before the test. Use the clear solution (with self-made thin layer chromatography silica gel plates).

5.4 Solvents

5.4.1 Propanol.

5.4.2 Light petroleum, boiling range 35 °C to 60 °C.

5.4.3 Dichloromethane or **trichloromethane.**

5.4.4 Toluene.

5.4.5 Ethyl acetate.

5.4.6 *n*-Hexane.

5.4.7 *n*-Heptane.

5.4.8 Cyclohexane.

5.4.9 Diethylamine.

1) Silica-G and Silica-HF are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

5.4.10 Ammonium hydroxide, 25 % to 30 % (mass fraction) of NH_3 , solution ($\rho = 0,9 \text{ Mg/m}^3$).

5.4.11 Ethanol.

5.4.12 Acetone.

5.5 Developing solvents:

5.5.1 For method A:

5.5.1.1 *n*-Heptane-ethyl acetate solution: add 90 parts by volume of heptane (5.4.7) to 10 parts by volume of ethyl acetate (5.4.5).

5.5.1.2 Ethanol-ammonium hydroxide-toluene solution: add 0,5 parts by volume of ethanol (5.4.11) and 0,05 parts by volume of ammonium hydroxide (5.4.10) to 100 parts by volume of toluene (5.4.4).

5.5.1.3 Acetone-ammonium hydroxide-toluene solution: add 10 parts by volume of acetone (5.4.12) and 0,2 parts by volume of ammonium hydroxide (5.4.10) to 100 parts by volume of toluene (5.4.4).

5.5.2 For method B:

5.5.2.1 Ethyl acetate-toluene solution: add 5 parts by volume of ethyl acetate (5.4.5) to 95 parts by volume of toluene (5.4.4).

5.5.2.2 Diethylamine-cyclohexane solution: add 25 parts by volume of diethylamine to 75 parts by volume of cyclohexane.

5.5.2.3 Toluene-*n*-heptane solution: add 50 parts by volume of toluene to 50 parts by volume of heptane.

5.6 Colour development and spray reagents

Most of the spray reagents are suitable for colour development of both amines and phenols. The following suggestion gives an example from which analytical expertise may be developed.

5.6.1 For colour development of most antidegradants

5.6.1.1 2,6-Dichloroquinone chlorimine ethanol solution (0,2 %)

Dissolve 0,2 g of 2,6-dichloroquinone chlorimide in 100 g of anhydrous ethanol (it is recommended to be used fresh and stored in the refrigerator after preparation).

5.6.1.2 Buffer spray reagent for use with 5.6.1.1

Dissolve 3,5 g of sodium hydroxide and 23,4 g of sodium tetraborate decahydrate in water, dilute to 1 l.

5.6.2 Special for colour development of amines (optional)

5.6.2.1 Diazotised sulphanilic acid.

Dissolve 1 g of sulphanilic acid and 1 g of potassium nitrite in 200 cm^3 of hydrochloric acid solution, $c(\text{HCl}) = 1 \text{ mol/l}$. Make fresh daily.

5.6.2.2 Benzoyl peroxide, 40 g in 1 l of toluene.

WARNING — Benzoyl peroxide is a powerful oxidizer which can explode spontaneously.

5.6.2.3 Bismuth nitrate, solution.

Dissolve 7,5 g of anhydrous bismuth nitrate in a mixture of 1 ml of concentrated nitric acid and 150 ml of water.

5.6.2.4 Tetracyanoethylene (ethenetetracarbonitrile), saturated solution in dichloromethane.

5.6.3 Special for colour development of phenols(optional)

5.6.3.1 Overspray, after the use of the reagent specified in [5.6.2.1](#): with sodium hydroxide, $c(\text{NaOH}) = 1 \text{ mol/l}$.

5.6.3.2 *p*-Nitrophenyldiazonium fiuoborate, 1 % (mass fraction) solution in methanol containing 0,5 % (mass fraction) of hydrochloric acid.

5.6.3.3 Dichloroquinonechlorimide (Gibb's Reagent) or 2,6-dibromoquinonechlorimide, 0,1 % solution in methanol.

5.6.3.4 Buffer spray, for use with reagent [5.6.3.3](#), dissolve 23,4 g of sodium tetraborate decahydrate, and 3,3 g of sodium hydroxide in 1 l of water.

5.6.3.5 Tollen's reagent.

Mix 0,5 cm³ of 5 % silver nitrate solution and 2 drops of sodium hydroxide, $c(\text{NaOH}) = 2 \text{ mol/l}$. Dissolve the precipitate in as a mass fraction of 2 % ammonium hydroxide solution as possible and add an equal volume of a volume fraction of 96 % ethanol solution.

WARNING — Prepare this reagent immediately before use and dispose of within 12 h.

6 Apparatus

Ordinary laboratory apparatus and the following.

6.1 Glass plates, of any convenient and adequate size, for example 200 mm x 200 mm, 200 mm x 50 mm, 200 mm x 70 mm, 3 mm to 5 mm thin.

6.2 Device for spreading a coating, 0,2 mm to 0,3 mm thick on the glass plates ([6.1](#)).

6.3 Pre-coated plates, covered with a layer of silica gel, 0,25 mm to 0,30 mm thick.

These may be used as an alternative to preparing plates (see [7.2](#)). Pre-coated film-backed plates with thinner coatings may be used, provided that they give good separation of the mixtures listed in [13.3](#).

6.4 Oven, capable of being controlled at $110 \text{ }^\circ\text{C} \pm 5 \text{ }^\circ\text{C}$ with air circulation.

6.5 Desiccator or drying box, for storing plates at fixed humidity.

6.6 Micro-pipettes of capacities 2 μl , 5 μl and 10 μl .

6.7 Chromatographic developing tanks with seal, large enough to hold the plates (6.1), for example of dimensions 220 mm × 125 mm × 70 mm or 250 mm × 250 mm × 70 mm or 320 mm × 240 mm × 110 mm.

Small “sandwich type” tanks are not recommended, because they do not allow adequate solvent vapour circulation between the tank wall and the sample plate.

6.8 Extraction apparatus, as described in ISO 1407.

6.9 Rotary vacuum evaporator (optional).

6.10 Short liquid-solid chromatographic column(optional).

6.10.1 5 cm³ hypodermic syringe barrel, fitted with a needle about 35 mm in length and 1,25 mm in diameter.

6.10.2 Glass tubes, 120 mm in length and 10 mm to 12 mm in diameter, holding about 5 cm³ silica gel.

6.11 Spray apparatus.

6.12 Mask for spraying portions of plates (optional, see 9.3.1).

6.13 Ultrasonic extraction apparatus.

7 Preparation of developing tank and plates

7.1 Preparation of developing tank

Add about 200 ml of the developing solvent (5.4.1 or 5.4.2) to a tank (6.7) or the developing solvent level is 15 mm to 20 mm above the bottom of the tank, before using swirl gently, cover and allow the developing solvent to stand for about 15 min until the liquid level is stable.

Since the developing agent is volatile and the volatilization rate is inconsistent, the chromatographic developing cylinder should be sealed as far as possible to ensure the consistency of the developing agent's polarity.

The tank may be re-used by repeating swirling and allowing to stand, provided that the composition of the solvent remains constant and no precipitation.

7.2 Preparation of plates

7.2.1 Prepare plates by making a slurry of 2 parts of water or silica gel plate adhesive (5.3) and 1 part of the silica gel (5.1). Allow to stand, with occasional gentle stirring until the mixture has thickened slightly, take care to avoid the formation of air bubbles. Spread the slurry evenly over the glass plates (6.1) using the spreading device (6.2). The thickness of the layer should be 200 μm to 300 μm. Allow the plates to stand at room temperature until the silica gel sets. Dry completely and activate the silica gel, by placing the plates for 1,5 h to 2 h in the oven (6.4), controlled at 110 °C ± 5 °C.

7.2.2 The plates may be stored in a desiccator over silica gel. Unused plates shall be reactivated after 4 days.

7.2.3 Before use, “lanes” may be made on the plate, about 20 mm wide by scoring with a knife or scribe, but the procedure may be omitted if edge effects spoil the chromatogram.

7.2.4 Plates may be well spotted while warm if it has been proved that no decomposition of the antidegradant takes place.

Spotting the plates while warm sometimes results in more compact spots, but it has been observed that better duplication will be obtained when plates are spotted at room temperature.

7.3 Preparation of pre-coated plates

If pre-coated plates are used, follow the manufacturer's instructions for conditioning.

8 Preparation of test portion

8.1 Take and prepare rubber sample in accordance with ISO 4661-2. Weigh 2,5 g to 3,0 g of the sample, cut the sample into very small pieces, or about 1 mm × 1 mm × 1 mm size, wrap it with filter paper and put it in the extractor (6.8). Extract with an appropriate solvent as specified in ISO 1407 for 2 h to 4 h with the test portion in the extraction cup, or for 18 h to 12 h with the rubber immersed in the solvent. If the immersion effect is not satisfied, ultrasonic vibration can be used.

Alternative extraction procedures, such as shaking with dichloromethane or chloroform (vulcanizates only) at room temperature for a short time (or no more than 30 min) or immersing overnight in 2-propanol (5.4.1) is also satisfactory.

If the oil content in the sample is too high and is likely to affect the results, it can be extracted with anhydrous ethanol. After concentrating and cooling, the oil and ethanol are separated, and the ethanol layer is taken for test.

Alternatively, the thin layer chromatography silica gel plate with the sample extract is first developed in petroleum ether to the top of the thin layer chromatography silica gel plate, and the thin layer is taken out. After the chromatographic silica gel plate is naturally dried in a ventilated place, it is expanded and developed in the required developing agent. See Annex B for the description of an alternative method.

8.2 Simultaneously with the extraction, carry out a preliminary screening, if necessary, as described in Annex A.

9 Plate spotting

9.1 General

The technique of spotting thin layer plates cannot be described exactly, although a few general rules or guidelines can be given. Each operator should, however, develop his own technique by practice.

9.2 Quantity of solution to apply

The best chromatograms are obtained when the test solution is applied in a volume of 5 µl or less; 10 µl is permissible, but larger volumes spread the spot and reduce the efficiency of separation. Spreading of the spot depends on the solvent used and is particularly bad if the solvent is acetone.

Some complex mixtures can produce streaks. If streaking occurs, it is advisable to decrease the sample amount to obtain continuous spots from the components of the mixture.

9.3 Spotting technique

9.3.1 Several samples, or alternating samples and known substances, may be spotted on one plate providing the spots are at least 2 cm apart. Four lanes may be used for colour development with one spray and four lanes with another spray, using a mask (6.12).