INTERNATIONAL STANDARD

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# 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 mince*



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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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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 2, *Testing and analysis.*

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 carboy-methyl 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 **trichloromathane.**

**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 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*(HCl) = 1 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, $c$(NaOH) = 1 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 $cm^3$ of 5 % silver nitrate solution and 2 drops of sodium hydroxide, $c$(NaOH) = 2 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 × 50 mm, 200 mm × 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 °C ± 5 °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 $cm^3$ 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 $cm^3$ 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 scriber, 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).

**9.3.2** Before spotting, make a baseline about 25 mm from one edge of the plate.

Use a capillary or micro-syringe to spot the sample on the baseline. Make each spot at least 2 cm apart. Allow the solvent to evaporate. The plate is then ready for development of the chromatogram. The spots can be spread out after the spotted spots are slightly dry.

# 10 Plate development

## 10.1 Method A

Using only one plate per tank, place each plate in a developing tank prepared as described in Clause 7, containing the solvent mixture (5.5.1). Do not place the plate too close to the wall of the tank and keep the liquid level below the line of the spots.

In order to ensure that the vapour of the developing agent is saturated in the chromatographic development cylinder, a piece of filter paper can be pasted on the inner wall of the chromatographic development cylinder.

Make sure the tank is covered and the solvent goes from the baseline to the top line. Remove the plate, mark the position of the solvent front and allow to dry in air for a few minutes.

The temperature during developing should not be lower than 18 °C, otherwise the result will be affected. The distance from the colour spot to the baseline shall be controlled to 50 % and 80 % of the distance from the baseline to the upper limit line, otherwise change the developing agent with appropriate polarity.

Gentle heating of the plate (maximum 50 °C) may also be used to drive off the last traces of solvent.

## 10.2 Method B

In cases where method A (see 10.1) does not resolve the spots to the satisfaction of the analyst, the developing solvents (5.5.2, 5.4.4 and 5.5.1.3) may be tried in that order. Each solvent system requires the use of an additional prepared and spotted plate.

# 11 Colour development on the plate

Spray the expanded thin layer chromatography silica gel plate with 2,6-dichloroquinone chlorimide ethanol solution (5.6.1), and then spray it with buffer spray (5.6.2) after drying. Wait until colours become visible. After the colour is stable (about 30 min), the identification is performed.

# 12 Expression of results

## 12.1 Method A

### 12.1.1 For amine type antidegradants

Spray the plate or desired portion of the plate (see 9.3.1) with a fine spray of colour developing and spray reagents acid (5.6.2) until colours become visible.

Calculate the ratio shift values $R_f$ from Formula (1):

$$R_f = \frac{a}{A} \tag{1}$$

where

*a* is the distance travelled by the leading edge of the spot from the starting line;

*A* is the distance travelled by the solvent front from the starting line.

Compare the $R_f$ values and colours obtained with those from standard chromatograms prepared in each laboratory (see Clause 13).

All amine types, including some mixtures, can be identified by this method.

NOTE Phenolic type antidegradants also produce colours with this spray.

#### 12.1.2 For phenolic type antidegradants

In a few cases, identification of phenolic antidegradants can be made more certain by using chlorimide spray (5.6.3). It can prove advantageous to follow the chlorimide spray with the buffer spray (5.6.3). Calculate the $R_f$ values and observe the colours. Heat the plate at 105 °C for a few minutes and observe the colours again. Known antidegradants shall be treated in the same manner as unknown substances for valid comparisons.

NOTE Amine type antidegradants also produce strong colours (usually blues and greens) with this spray.

### 12.2 Method B

Plates developed in accordance with 10.2 may be sprayed with any of the reagents mentioned in 5.6. This can result in additional information useful for differentiation in some difficult separations. The sequence of plate development and spray reagents should be the same for known substances as for unknown substances.

### 12.3 Confirmation tests

For confirmation of identity, prepare a plate with the unknown antidegradant and the antidegradant it has been tentatively identified as, treated in the same manner, in adjacent lanes.

Another technique which is sometimes useful is to add a known antidegradant to the test solution. This ensures that the known antidegradant has the same "background" interference as the unknown antidegradant.

## 13 Standard chromatograms

**13.1** To identify the unknown antidegradant from its thin layer chromatogram, it is necessary to obtain "standard" chromatograms for authentic samples of any antidegradants whose presence is suspected in the test portion. The standard chromatograms should be obtained using identical solvents and sprays reagents, and preferably at the same time and on the same plate, as the chromatogram of the antidegradant to be identified.

**13.2** If a record of the standard chromatograms is to be kept, the best method is to use colour photographs. However, it is possible to copy the chromatograms as simple line drawings, noting the colour, shape and pattern of the spots. The chromatographic pattern is important because many complex antidegradants contain several components. They will often give tailing spots and even streaks on the chromatogram. For this reason, an accurate drawing or picture is more useful than a table of $R_f$ values and colours alone. Some analysts have found the use of colour charts to be an aid in the description of the colour obtained, for example: munsell charts or equivalent.

**13.3** As an aid to development of a particular technique, the analyst should first try to obtain good separation of the following mixtures using the developing solvents listed in 5.5 before attempting to analyse unknown substances.

Low polarity antidegradants — typified by a mixture of the diaryl amines, phenyl-*β*-naphthylamine and phenyl-*α*-naphthylamine — should be resolved using reagent 5.5.1.

Medium polarity antidegradants — typified by a mixture of the substituted p-phenylenediamines *N*,*N*'-bisd-ethyl-3-methylpentyl)-*p*-phenylenediamine, *N*-isopropyl-*N*'-phenyl-*p*-phenylenediamine and *N*-phenyl-*N*'-cyclohexyl-*p*-phenylenediamine — should be resolved using reagent 5.5.1.3.

**WARNING — Phenyl *β*-naphthylamine and phenyl α-naphthylamine may contain low levels of the potent carcinogen *β*-naphthylamine.**

High polarity antidegradants — typified by a mixture of *N*-phenyl-*N*'-(p-toluene-sulfonyl)-*p*-phenylenediamine, *N*,*N*-disec-butyl-*p*-phenylenediamine and *N*/*N*-disopropyl-*p*-phenylenediamine — should be well resolved by reagents 5.5.1.3 and 5.5.2.1.

**13.4** The developing solvent or solvents should be selected at the discretion of the analyst for the particular problem encountered.

## 14 Test report

The test report shall contain the following information:

a) all details necessary for identification of the sample;

b) a reference to this document, i.e. ISO 4645:2022;

c) the method used, method A or B;

d) the result(s), the antidegradant(s) found using the methods described in this document;

e) any deviations from the procedure or any unusual features observed;

f) the date of the test.

# Annex A
(informative)

# Preliminary spot tests

## A.1 General

Some preliminary spot tests, which have been useful for "screening" possible antidegradant types prior to thin layer analysis are described in this annex.

## A.2 Reagents

**A.2.1 Iron(III) chloride**, 0,5 % ethanolic solution.

**A.2.2 Iron(III) sulfate**, 1 % aqueous solution.

**A.2.3 Hydroxylamine hydrochloride**, 1 % aqueous solution.

**A.2.4 *p*-Nitroaniline solution.**

Dissolve 2,8 g of p-nitroaniline in 32 $cm^3$ of warm, concentrated hydrochloric acid. Dilute to 250 $cm^3$ with water.

**A.2.5 Sodium nitrite solution.**

Dissolve 1,44 g of sodium nitrite in 250 $cm^3$ of water.

**A.2.6 Acetic acid,** glacial, 99,7 % solution, $\rho$ = 1,05 $Mg/m^3$.

**A.2.7 Titanium(IV) chloride solution.**

Dissolve 5 $cm^3$ of titanium(IV) chloride in 2 000 $cm^3$ of glacial acetic acid.

## A.3 Procedure

**A.3.1** These tests should be performed on a few cubic centimetres of an "extract" from about 1 g of milled or finely cut rubber, warmed in 10 $cm^3$ of anhydrous ethanol.

**A.3.2** Add, drop by drop, the iron(III) chloride ethanolic solution (A.2.1), until colour appears, avoiding excess reagent.

Most phenolic compounds, except hindered phenols, give a red colour. Phenolic resins give a red colour.

**A.3.3** If no colour is produced in A.3.2, test for quinolines by mixing equal amounts of the test solution, the iron(III) sulfate solution (A.2.2) and the hydroxylamine hydrochloride solution (A.2.3).

Quinolines give a red colour. Phenylenediamines interfere.