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Rubber and rubber products – Guide to the identification of antidegradants – Thin layer chromatographic methods

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Gummi och gummiprodukter – Vägledning för identifiering av skyddsmedel – Tunnskiktskromatografiska metoder

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Rubber and rubber products — Guide to the identification of antidegradants — Thin layer chromatographic methods

1 Scope and field of application

1.1 This International Standard describes two methods for the detection and identification, by thin layer chromatography, of antidegradants (antioxidants, antiozonants and stabilizers), which may be present in raw rubber, unvulcanized compounded rubber, or rubber products.

Method A is a simplified method, based on a single solvent system, which provides for the identification of known materials and may be used to check the presence or absence of a particular antidegradant which should be present.

Method B is a more detailed method, using additional solvents and sprays, which enables a greater degree of separation of the spots to be obtained and therefore may be used to detect and identify an unknown antidegradant.

1.2 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, provided that the requirement of 11.1 is met.

2 Reference

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

3 Principle

Extraction of antidegradants from the rubber by means of a solvent. Evaporation of the original solvent, application of a solution of the dried extract as a spot on a thin layer chromatographic plate, evaporation of the second solvent and development of the plate in an appropriate solvent. If extender oil is present, removal of the oil either by column chromatography of the extract prior to the completion of the evaporation of the original solvent or by development of the plate in light petroleum prior to the normal development in an appropriate solvent. Identification of the unknown antidegradant by comparison of its chromatogram with standard chromatograms.

4 Reagents

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

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 International Standard.

4.1 Plate adsorbent: silica gel, particle size 2 to 50 µm, with or without calcium sulphate binder.[1)]

Silica gel containing a fluorescent indicator is useful in many cases to allow observation of spots, under ultra-violet radiation, before spraying.

4.2 Column adsorbent: silica gel, to pass a sieve of aperture 200 to 600 µm[1)] 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.

4.3 Solvents:

4.3.1 Methanol.

4.3.2 Acetone.

4.3.3 Ethanol, anhydrous.

4.3.4 2-Propanol.

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

4.3.6 Dichloromethane.

4.3.7 Toluene.

1) Suitable material is available commercially. Details may be obtained from the Secretariat of ISO/TC 45 (BSI) or ISO Central Secretariat.

4.3.8 Ethyl acetate.

4.3.9 *n*-**Hexane.**

4.3.10 *n*-**Heptane.**

4.3.11 Cyclohexane.

4.3.12 Diethylamine.

4.3.13 Ammonium hydroxide, 28 to 30 % (*m*/*m*) of NH_3, solution (ϱ = 0,9 Mg/m^3).

4.3.14 Acetonitrile.

4.4 Developing solvents:

4.4.1 For method A: 90 parts by volume of the *n*-heptane (4.3.10) and 10 parts by volume of the ethyl acetate (4.3.8).

4.4.2 For method B:

4.4.2.1 Toluene.

4.4.2.2 95 parts by volume of the toluene (4.3.7) and 5 parts by volume of the ethyl acetate (4.3.8).

4.4.2.3 75 parts by volume of the cyclohexane (4.3.11) and 25 parts by volume of the diethylamine (4.3.12).

4.4.2.4 50 parts by volume of the toluene (4.3.7) and 50 parts by volume of the *n*-heptane (4.3.10).

4.4.3 Additional developing solvents which may prove useful for special problems:

4.4.3.1 100 parts by volume of the toluene (4.3.7), 10 parts by volume of the acetone (4.3.2) and 0,2 parts by volume of the ammonium hydroxide solution (4.3.13).

4.5 Spray reagents:

Most of the spray reagents below are equally suitable for colour development of both amines and phenols. The following suggestions give a base from which analytical expertise may be developed:

4.5.1 For colour development of amines:

4.5.1.1 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/dm^3 [1]. Make fresh daily.

4.5.1.2 Benzoyl peroxide, 40 g in 1 dm^3 of toluene.

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

4.5.1.3 Bismuth nitrate, solution.

Dissolve 7,5 g of anhydrous bismuth nitrate in a mixture of 1 cm^3 of concentrated nitric acid and 150 cm^3 of water.

4.5.1.4 Tetracyanoethylene (ethenetetracarbonitrile), saturated solution in dichloromethane.

4.5.2 For colour development of phenols:

4.5.2.1 Overspray, after the use of the reagent specified in 4.5.1.1: with **sodium hydroxide,** c(NaOH) = 1 mol/dm^3.

4.5.2.2 *p*-**nitrophenyldiazonium fluoborate,** 1 % (*m*/*m*) solution in methanol containing 0,5 % (*m*/*m*) of hydrochloric acid.

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

4.5.2.4 Buffer spray for use with reagent 4.5.2.3: dissolve 23,4 g of **sodium tetraborate decahydrate,** and 3,3 g of **sodium hydroxide** in 1 dm^3 of water.

4.5.2.5 Tollen's reagent.

Mix 0,5 cm^3 of 5 % silver nitrate solution and 2 drops of sodium hydroxide, c(NaOH) = 2 mol/dm^3. Dissolve the precipitate in as little 2 % (*m*/*m*) ammonium hydroxide solution as possible, and add an equal volume of 96 % (*V*/*V*) ethanol solution.

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

5 Apparatus

Ordinary laboratory apparatus and the following

5.1 Glass plates, of any convenient and adequate size, for example 200 mm × 200 mm.

5.2 Device for spreading a coating 250 to 300 µm thick on the glass plates (5.1).

5.3 Pre-coated plates, covered with a layer of silica gel, 250 to 300 µm thick.

These may be used as an alternative to preparing plates (see 6.2). Pre-coated film-backed plates with thinner coatings may be used, provided that they give good separation of the mixtures listed in 11.3.

1) Hitherto expressed as "1 M or 1 N solution".

5.4 Oven, capable of being controlled at 100 ± 5 °C.

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

5.6 Micro-pipettes, of capacities 2, 5 and 10 mm^3.[1)]

5.7 Chromatographic developing tanks, large enough to hold the plates (5.1), for example of dimensions 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.

5.8 Extraction apparatus, as described in ISO 1407.

5.9 Rotary vacuum evaporator (optional, see 7.3).

5.10 Short liquid-solid chromatographic column.

Those found to be satisfactory comprise:

5.10.1 5 cm^3 hypodermic syringe barrel, fitted with a needle about 35 mm in length and 1,25 mm in diameter.

5.10.2 Glass tubes, 120 mm in length and 10 to 12 mm in diameter, holding about 5 cm^3 silica gel.

5.11 Spray apparatus.

5.12 Mask for spraying portions of plates (optional, see 8.5.1).

6 Preparation of developing tank and plates

6.1 Preparation of developing tank

Add about 200 cm^3 of the developing solvent (4.4.1 or 4.4.2) to a tank (5.7), swirl, cover and allow to stand for about 15 min before using.

The tank may be re-used by repeating swirling and allowing to stand, provided that the composition of the solvent remains constant.

6.2 Preparation of plates

6.2.1 Prepare plates by making a slurry of 2 parts of water and 1 part of the silica gel (4.1). Allow to stand, with occasional gentle stirring, taking care to avoid the formation of air bubbles, until the mixture has thickened slightly. Spread the slurry evenly over the glass plates (5.1) using the spreading device (5.2). The thickness of the layer should be 250 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 at least 2 h in the oven (5.4), controlled at 100 ± 5 °C, or if more convenient, overnight (approximately 16 h).

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

6.2.3 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.

6.2.4 Plates may be 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 result when plates are spotted at room temperature.

6.3 Preparation of pre-coated plates

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

7 Preparation of test portion

7.1 Sheet the test portion thinly using a laboratory mill with a tight nip and running at even speed or cut it into very small pieces (length of edges < 2 mm) and place 2 to 5 g between two filter papers. Transfer to the extraction apparatus (5.8) and extract with an appropriate solvent as specified in ISO 1407 for 4 h with the test portion in the extraction cup, or for 1 to 2 h with the rubber immersed in the solvent.

Alternative extraction procedures, such as shaking with dichloromethane (vulcanizates only) at room temperature for a short time, or standing overnight in 2-propanol (4.3.4) or acetonitrile (4.3.14) are also satisfactory.

7.2 Simultaneously with the extraction, carry out a preliminary screening, if necessary, as described in the annex.

7.3 Evaporate the extract (7.1) in a beaker on a hot plate, at not more than 50 °C, using a stream of nitrogen to aid evaporation in the final stages. The use of a vacuum rotary evaporator, if available, is helpful. When about 1 cm^3 of solution remains, examine visually for the presence of extender oil. If extender oil is present, proceed as described in 7.4 to 7.7. If extender oil is absent, evaporate the extract to dryness using gentle heating (at a maximum of 50 °C) under a stream of nitrogen. Dissolve the dried extract in 0,5 to 1,0 cm^3 of dichloromethane with gentle heating to obtain a clear solution and then proceed directly with spotting of the thin layer plate as described in clause 8.

NOTE — Small amounts of residual alcohol may change mobilities of the spots (R_f values).

7.4 Prepare a silica gel column from the activated silica gel (4.2) by placing a glass wool plug at the end of the column (5.10) and filling immediately. The column shall, preferably, be used when freshly prepared, but otherwise shall be used within 2 h of preparation and shall be stored in a desiccator during this period.

1) Hitherto expressed as "µl".

7.5 Dissolve the residue obtained as described in 7.3 in about 2 cm^3 of dichloromethane and pour this solution onto the dry silica gel column. Wash with the *n*-hexane (4.3.9) until the glass wool plug becomes colourless. Use no more than 25 cm^3 of the *n*-hexane, and discard it after washing is complete.

A large part of the oils will have been removed at this stage.

NOTE — An alternative method for complete removal of oil is to develop the prepared plate with the light petroleum (4.3.5) until the oils have moved to the top of the plate, dry to remove the ether, then proceed with plate development as described in clause 9.

7.6 After the last of the *n*-hexane has drained off the column, place a clean beaker under the column. Wash the column alternately with acetone and methanol until all colour is removed, except a slight stain that normally cannot be removed even with excessive washing. Discard the silica gel.

7.7 Evaporate the eluant to dryness using gentle heating (maximum 50 °C) under a stream of nitrogen. Dissolve in 0,5 to 1,0 cm^3 of dichloromethane, with gentle heating to obtain a clear solution, and proceed as described in clause 8.

8 Plate spotting

8.1 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.

8.2 Amount of antidegradant

In general, 50 to 100 µg is the desired amount of antidegradant. Less can sometimes be detected.

8.3 Quantity of solution to apply

The best chromatograms are obtained when the test solution is applied in a volume of 5 mm^3 or less; 10 mm^3 is permissible, but larger volumes spread the spot and reduce efficiency of separation. Spreading of the spot depends on the solvent used, and is particularly bad if the solvent is acetone.

8.4 Concentration of test solution

It follows from 8.2 and 8.3 that the ideal technique would be to spot using a solution with a concentration range of 10 to 20 g/dm^3. Some complex mixtures may produce streaks at this concentration. If streaking occurs, it is advisable to decrease the amount of sample in order to obtain discrete spots from the components of the mixture.

8.5 Spotting technique

8.5.1 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 the mask of 5.12.

8.5.2 Apply the spots, by means of a micropipette (5.6), along a line about 25 mm from one edge of the plate, applying each spot at least 2 cm apart. Allow the solvent to evaporate. The plate is then ready for development of the chromatogram.

9 Plate development

9.1 Method A

Using only one plate per tank, place each plate in a developing tank prepared as described in clause 6, containing the solvent mixture (4.4.1). Do not place the plate too close to the wall of the tank, and keep the liquid level below the line of the spots. Replace the cover and allow the solvent front to advance about 150 mm beyond the line of spots. Remove the plate, mark the position of the solvent front and allow to dry in air for a few minutes; gentle heating of the plate (maximum 50 °C) may also be used to drive off the last traces of solvent.

9.2 Method B

In cases where method A (see 9.1) does not resolve the spots to the satisfaction of the analyst, the developing solvents (4.4.2.1, 4.4.2.2, 4.4.2.3, 4.4.2.4 and 4.4.3.1) may be tried in that order. Each solvent system requires the use of an additional prepared and spotted plate.

10 Colour development on the plate

10.1 Method A

10.1.1 For amine type antidegradants

Spray the plate or desired portion of the plate (see 8.5.1) with a fine spray of the diazotized sulphanilic acid (4.5.1.1) until colours become visible.

Calculate the R_f values from the formula

$$R_f = \frac{\text{distance travelled by the leading edge of the spot from the starting line (see 8.5.2)}}{\text{distance travelled by the solvent front from the starting line}}$$

Compare the R_f values and colours obtained with those from standard chromatograms prepared in each laboratory (see clause 11).

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

NOTE — Phenolic type antidegradants also produce colours with this spray.

See also sub-clause 10.3.

10.1.2 For phenolic type antidegradants

10.1.2.1 Overspray the plate, after treatment according to 10.1.1, with the sodium hydroxide solution (4.5.2.1). Phenolic antidegradants will change colour, with R_f values and colours characteristic of individual chemicals or mixtures.