Designation: D3156 – 96 (Reapproved 2016)

Standard Practice for Rubber—Chromatographic Analysis of Antidegradants (Antioxidants, Antiozonants and Stabilizers)[1]

This standard is issued under the fixed designation D3156; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the detection and identification by thin-layer chromatography of antidegradants (antioxidants, antiozonants, and stabilizers) that may be present in raw rubber or rubber products. Analysis for other types of antidegradants is possible as long as the requirements of the practice are met.

1.2 The values stated in SI units are to be regarded as the standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Document

2.1 *ASTM Standards:*[2]

D297 Test Methods for Rubber Products—Chemical Analysis

3. Summary of Practice

3.1 A simplified procedure (Method A, Section 13.1) based on a single-solvent system is presented, which provides for the identification of known materials. This may be used to check the presence or absence in a rubber vulcanizate or raw rubber, of an antidegradant which should be present. However, since the chromatograms obtained may not be absolutely specific for any given antidegradant, a more detailed scheme is given in 13.2.

3.2 Method B, Section 13.2, using additional solvents and sprays is included, which enables a greater degree of separation of the spots to be made and therefore may enable detection and identification of an unknown antidegradant.

3.3 Other techniques, for example: gas chromatographic (with or without treatment to obtain derivatives), spectroscopic (ultraviolet and infrared), more sophisticated thin-layer variations (two dimensional techniques), may be applied to identification of spots for improved detection and identification of an unknown antidegradant.

3.4 It is possible that the chromatographic pattern of one antidegradant may overlap that of another antidegradant; therefore each laboratory must prepare its own reference standards, based on the technique chosen from this practice.

4. Significance and Use

4.1 This practice is useful for the examination of rubber compounds or products for the presence of chemicals that prevent or greatly reduce degradation due to oxygen, ozone, or other agents.

4.2 This practice is suitable for quality assurance, factory control, and research and development applications.

5. Interferences

5.1 In the absence of extender oils, antidegradants are extracted from the rubber by a solvent and the evaporated extract is applied directly to a thin-layer chromatographic plate.

5.2 In the presence of extender oils, the oils are removed by either a pre-treatment of the plate, with light petroleum ether or by a column chromatographic technique.

5.3 Identification of the antidegradant is made by the standard technique of thin-layer chromatography, herein described.

6. Apparatus

6.1 *Spreading Device,* for making thin layer chromatographic plates with a coating 250 to 300 µm thick.

6.2 *Glass Plates,* 200 by 200-mm, or suitable for the selected tank. As an alternative to preparing plates, the use of precoated plates with a coating of 250 to 300 µm is permitted. Precoated film-backed plates are not recommended.

6.3 *Drying Oven,* 105°C minimum.

[1] This practice is under the jurisdiction of ASTM Committee D11 on Rubber and is the direct responsibility of Subcommittee D11.11 on Chemical Analysis.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

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

6.5 *Micro-pipets,* 5 and 10-mm^3 (μL).

6.6 *Chromatographic Developing Tank,* of a size suitable to hold plates, approximately 250 by 250 by 70 mm to 330 by 240 by 110 mm. Small "sandwich-type" tanks are not recommended. Add about 200 cm^3 of developing solvent (7.4) to the tank, swirl, cover, and allow to stand 15 min before using. Tank may be reused by repeating the swirling and standing step, as long as the solvent ratio remains constant.

6.7 *Extraction Apparatus,* in accordance with Section 18 or 25 of Test Methods D297.

6.8 *Chromatographic Columns,* short, liquid-solid. Those found satisfactory are as follows:

6.8.1 *Hypodermic Syringe Barrel,* 5-cm^3, fitted with a needle about 35 mm in length and 1.27 mm outside diameter (No. 18 BWG).

6.8.2 *Glass Tubes,* 120 mm in length and 10 to 12 mm in diameter, holding about 5 cm^3 of silica gel.

7. Reagents

7.1 *Plate Adsorbent*—Silica gel containing calcium sulfate.[3] Silica gel containing a fluorescent indicator may be useful in some cases to allow visualization of spots (before spraying) with ultraviolet light.

7.2 *Column Adsorbent*—Silica gel 200 to 600 μm (30 to 70-mesh),[3] activated by drying at 110°C for at least 2 h, if dry in that period, or overnight (±16 h) for convenience.

7.3 *Solvents*—Use of fume hoods with volatile and toxic solvents is *mandatory*. Approved health and safety precautions should be observed for the use of any solvent or chemical mentioned in this procedure. ACS grade or equivalent should be used.

7.3.1 *Methanol.*
7.3.2 *Acetone.*
7.3.3 *Isopropanol.*
7.3.4 *Light Petroleum Ether.*
7.3.5 *Chloroform.*
7.3.6 *Toluene.*
7.3.7 *Ethyl Acetate.*
7.3.8 *n-Hexane.*
7.3.9 *n-Heptane.*
7.3.10 *Cyclohexane.*
7.3.11 *Diethylamine.*
7.3.12 *Ammonium Hydroxide* (NH_4OH, 28–30 % ammonia NH_3).
7.3.13 *Water*—References to water shall be understood to mean distilled water or water of equal purity.

7.4 *Developing Solvents:*

7.4.1 *Test Method A*—90 parts *n*-heptane and 10 parts ethyl acetate by volume.

7.4.2 *Test Method B,* used in the following order:

7.4.2.1 Toluene.

7.4.2.2 95 parts toluene and 5 parts ethyl acetate by volume.

7.4.2.3 75 parts cyclohexane and 25 parts diethylamine by volume.

7.4.2.4 50 parts toluene and 50 parts *n*-heptane by volume.

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

7.4.3.1 100 parts toluene, 5 parts acetone and 0.1 part ammonium hydroxide (NH_4OH).

7.4.3.2 100 parts toluene, 5 to 10 parts acetone and 0.1 to 0.2 parts NH_4OH.

7.5 *Spray Reagents for Color Development:*

7.5.1 *Amines:*

7.5.1.1 *Diazotized Sulfanilic Acid*—0.5 g of sulfanilic acid and 0.5 g of potassium nitrite (KNO_2) dissolved in 100 cm^3 of 1 *M* hydrochloric acid (HCl). Make fresh daily.

7.5.1.2 *Benzoyl Peroxide* (4 % solution in toluene).

7.5.1.3 *Tollen's Reagent* (0.5 cm^3 of 5 % silver nitrate ($AgNO_3$) solution + 2 drops of 2 *M* sodium hydroxide (NaOH). Dissolve the precipitate in as little 2 % ammonium hydroxide (NH_4OH) as possible, and add an equal volume of 96 % alcohol.

7.5.1.4 *Bismuth Nitrate* ($Bi(NO_3)_3$) (7.5 g) dissolved in a mixture of 1 $cm^3$of concentrated nitric acid (HNO_3, density 1.42 Mg/m^3) in 150 cm^3 of distilled water.

7.5.1.5 *Tetracyanoethylene (ethenetetracarbonitrile)*—Saturated solution in methylene chloride.

7.5.2 *Phenols:*

7.5.2.1 *Overspray,* for use with solution 7.5.1.1 1-*M* sodium hydroxide (NaOH) solution.

7.5.2.2 *p-Nitrophenyldiazonium Fluoroborate* (1 % solution in methanol containing 0.5 % hydrochloric acid (HCl, density 1.16 Mg/m^3)).

7.5.2.3 *2,6 Dichloroquinonechlorimide (0.1 % solution in methanol or toluene)*—Used with 7.5.2.4.

7.5.2.4 *Buffer Spray*, for use with 7.5.2.3—Dissolve 23.4 g of sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) and 3.3 g of sodium hydroxide (NaOH) in 1 dm^3 of water.

7.6 Reagents for preliminary "screening" tests are as follows:

7.6.1 *Ferric Chloride Solution*—Mix 0.5 g of anhydrous ferric chloride ($FeCl_3$) with 100 cm^3 of ethanol or 3A alcohol.

7.6.2 *Ferric Sulfate Solution*—Mix 1 g of ferric sulfate $(FeSO_4)_3$ with 100 cm^3 of water.

7.6.3 *Hydroxylamine Hydrochloride Solution*—Mix 1.0 g of hydroxylamine hydrochloride with 100 cm^3 of water.

7.6.4 *p-Nitroaniline Solution*—Mix 2.8 g of *p*-nitroaniline with 32 cm^3 of hydrochloric acid (density 1.16 Mg/m^3). Dilute to 250 cm^3 with water.

7.6.5 *Sodium Nitrite Solution*—Mix 1.44 g of sodium nitrite ($NaNO_2$) with 250 cm^3 of water.

7.6.6 *Glacial Acetic Acid* (99.7 % w/w).

7.6.7 *Titanium Tetrachloride Solution*—Mix the contents of one 5-cm^3 ampoule of titanium tetrachloride ($TiCl_4$) with 1 dm^3 of acetic acid (7.6.6).

7.6.8 *Anhydrous Alcohols*—Ethanol, 3A alcohol, or isopropanol.

[3] The sole source of supply of silica gel known to the committee at this time is E. Merck A.G., Frankfurter Str. 250, Darmstadt, Germany. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

8. Plate Preparation

8.1 Make a slurry of 2 parts of water to 1 part of silica gel (7.1) by mass. Immediately, spread the slurry evenly over the glass plate with the spreading device. The layer thickness should be 250 to 300 µm. Allow the plates to stand at room temperature to set the binder. Dry for 45 min at 105°C.

8.2 Plates may be spotted while warm, if it has been proven that no decomposition of the antidegradant sought, takes place. Spotting, while warm, sometimes results in more compact spots.

8.3 Plates may be stored in a desiccator over silica gel. Unused plates should be reactivated after 4 days standing.

8.4 Before using, make "lanes" on the plate, about 20 mm in width, by scoring with a knife or scriber. If this step leads to uneven development from lane to lane, eliminate.

8.5 When precoated plates are used, follow the manufacturer's directions for plate conditioning.

9. Sampling

9.1 Due to the nature of this practice and the wide variety of compounds, or products that may be examined by this practice, no directions are given for sampling other than that the selected sample shall be at the discretion of the analyst.

10. Preliminary "Screening" Tests

10.1 Some preliminary spot tests that have been useful for "screening" possible antidegradant types prior to thin-layer analysis are tabulated below. These tests are performed on a few cubic centimetres of an "extract" of about 1 g of milled rubber, warmed in 10 cm^3 of anhydrous alcohol (7.6.8).

10.1.1 Add dropwise 0.5 % ferric chloride (7.6.1) until color appears; with excess reagent, the color changes to an oxidized form. Dialkyl phenylenediamines give a pink color, alkyl aryl phenylenediamines give a blue color, while diaryl phenylenediamines give a green color.

10.1.2 If no color was shown with the above, test for quinolines by adding 1 % aqueous ferric sulfate (7.6.2) plus 1 % aqueous hydroxylamine hydrochloride (7.6.3). If quinolines are present, a red color is observed. Phenylenediamines interfere.

10.1.3 If no color was observed in 10.1.1, mix 10 cm^3 of a solution of *p*-nitroaniline (7.6.4) with 10 cm^3 of sodium nitrite solution (7.6.5). Cool mixture in an ice bath. Add this solution dropwise to the sample solution with glacial acetic acid (7.6.6). Amine antidegradants (for example, phenyl-beta-naphthylamine and the condensation product of acetone-diphenylamine) give purple to red colors. Phenylenediamines interfere.

10.2 Add 5 cm^3 of acetic acid (7.6.6) and 5 cm^3 of titanium tetrachloride (7.6.7) to 3 cm^3 of "extract". Most phenolics give a red color, except hindered phenols. Phenolic resins respond with a red color also.

11. Preparation of Test Specimen

11.1 Sheet the test specimen thinly or cut into fine pieces (2 by 2 by 2 mm) and place 2 to 5 g between the filter paper sheets. Place in the extraction apparatus (6.7) and extract with methanol for 4 h, or 1 to 2 h for rapid reflux extraction. An alternative extraction procedure is to allow the specimen to stand overnight in isopropanol.

11.2 Evaporate the extract from 11.1 in a beaker, using a low temperature hot plate (not more than 50°C) and a stream of nitrogen, to aid in evaporation near the end of the evaporation step. A rotary evaporator is helpful, if available. When about 1 cm^3 of solution remains, examine for the presence of extender oil. If present, proceed with 11.3, if absent, proceed directly with the spotting of the thin-layer plate (Section 12).

11.3 If visual examination indicates the presence of oil, dissolve the residue in about 2 cm^3 of chloroform ($CHCl_3$) and proceed with 11.4.

11.4 Prepare a silica gel column from activated silica gel (7.2) by placing a glass wool plug at the end of the column (6.8.1 or 6.8.2) and fill immediately. Store in a desiccator no longer than 1 to 2 h before using. Preferably, use a freshly prepared column.

11.5 Pour the $CHCl_3$ onto the *dry* silica gel column (11.4). Wash with *n*-hexane until the glass wool plug becomes colorless. Use a maximum of 25 cm^3 of *n*-hexane. The oils are largely removed at this point.

NOTE 1—An alternative method for removing oil is to develop the prepared plate with light petroleum ether, until the oils have moved to the top of the plate, carefully dry to remove the ether, then proceed with plate development Section 13.

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

11.7 Evaporate the eluant to dryness with gentle heating (maximum of 50°C) and a stream of nitrogen. Dissolve in 0.5 to 1.0 cm^3 of acetone or chloroform with gentle heating to obtain a clear solution and proceed to Section 12 for plate spotting.

12. Plate Spotting

12.1 The technique of spotting thin-layer plates cannot be described exactly. In the case of the antidegradants dealt with in this method, a few general rules, or guidelines, are given here. Each operator must, however, develop his own technique by practice.

12.1.1 *Amount of Sample*—In general, 50 to 100 µg is the desired amount of sample. Less can sometimes be used.

12.1.2 *Quantity of Solution*—The best chromatograms are obtained when the sample is applied in a volume of 5 mm^3 (µL) or less. 10 mm^3 is permissible, but larger volumes spread the spot and reduce separation efficiency.

12.1.3 *Concentration of Sample*—It follows from 12.1.1 and 12.1.2 that the ideal sampling technique would be to spot within the range from a 1 to 2 % solution. 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.

12.2 *Spotting Technique:*

12.2.1 A plate, scored, with lanes (if desired) and ready for spotting should contain at least eight usable lanes. Several samples, or alternating samples and knowns, may be spotted on one plate. One may also use four lanes for color development with one spray and four lanes for use with another spray.

12.2.2 Apply the spots along a line about 25 mm from one edge of the plate, one spot in each lane. The plate is now ready for development of the chromatogram.

13. Plate Development (Elution)

13.1 *Test Method A*—Using only one plate per tank, place the plate in the prepared tank (6.6), containing the solvent mixture of 7.4.1.

13.1.1 Do not place the plate too close to the wall surface and keep the solvent from below the line of spots.

13.1.2 Replace the cover and allow the solvent front to move about 150 mm from the line of spots.

13.1.3 Remove the plate, mark the position of the solvent front, and allow to air dry for a few minutes. Low temperature heating of the plate (maximum 50°C) may also be used to drive off the last traces of solvent.

13.2 *Test Method B*—In cases where Test Method A (13.1) did not resolve the spots to the satisfaction of the analysis, the developing solvents of 7.4.2 and 7.4.3 may be used in the order listed. Each solvent system requires the use of an additional prepared-and-spotted plate.

13.3 As an aid to development of a particular technique, the analyst should get good separation of increasingly polar mixtures by using the developing solvents listed in 7.4, before attempting to analyze unknowns. It is understood that knowns have been treated in the same manner as unknowns before comparisons are valid.

13.3.1 Low-polarity antidegradants, mixture of phenyl-alpha-naphthylamine (PAN) and phenyl-beta-naphth-ylamine (PBN), should be well resolved using 7.4.1.[4,5]

NOTE 2—Use PBN that is free of beta-naphthylamine, a carcinogen.

13.3.2 Medum-polarity antidegradants—mixture of *N,N'* bis(1-ethyl-methyl-pentyl)-*p*-phenylenediamine (FLEXZONE 8L), *N* -isopropyl-*N'*-phenyl-*p*-phenylenediamine (FLEXZONE 3C), and *N*-phenyl-*N* 'cyclohexyl-*p*-phenylenediamine (FLEXZONE 6H)—should be well resolved using 7.4.3.1.[5,6]

13.3.3 High-polarity antidegradants—mixture of *p*-tolyl-sulfonylamine (ARANOX), *N,N*-disecondary butyl-*p*-phenylenediamine (NAUGALUBE 440), and *N,N*-diisopropyl-*p*-phenylene-diamine (NAUGALUBE 403)—should be well resolved using 7.4.3.1 or 7.4.2.2.[5,7]

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

14. Color Development

14.1 *Test Method A:*

14.1.1 *For Amine Type Antidegradants*—Spray the plate or desired portion of the plate with a *fine* spray of diazotized sulfanilic acid (7.5.1) until colors become visible. Identification is made by R_f numbers (ratio of the distance the spot has moved to the solvent front position) and colors compared to standard chromatograms prepared in your laboratory Section 15. Any amine type, including some mixtures, have been identified by this reagent. As further proof of identity, prepare plates with the unknown, and the suspected antidegradant in adjacent lanes.

14.1.2 *For Phenolic-Type Antidegradants:*

14.1.2.1 Overspray the plate after treatment according to 10.1.1 with NaOH solution (7.5.2.1). Phenolic antidegradants will show up with R_f numbers and colors being characteristic of the individual chemicals or mixtures. For further confirmation, prepare a plate with the unknown and the suspected antidegradant in adjacent lanes.

14.1.2.2 In a few cases, identification of phenolic antidegradants may be made more certain by using the chlorimide spray. Spray the unused portion of the plate or a second plate with the buffer spray (7.5.2.4) followed by the chlorimide spray (7.5.2.3). Similar results may be obtained with the chlorimide spray first, then buffer spray. Observe the R_f numbers and the colors. Heat the plate at 105°C for a few minutes and observe the colors again.

14.2 *Test Method B*—Plates developed in (13.2) may be sprayed with any of the reagents mentioned in (7.5) using one spray per plate. This may result in additional information, useful for differentiation of some difficult separations. The sequence of plate development and spray reagents should be the same for knowns as for unknowns. Another technique that is sometimes useful is to add known to unknown. This ensures that the known has the same "background" interference as the sample.

15. Standard Chromatograms

15.1 The use of this practice requires that the thin-layer chromatograms be prepared for all antidegradants one may expect to find in raw rubbers or rubber products.

15.2 A record of these chromatograms should be kept. The best method is by color photographs. However, it is possible to copy the chromatograms as simple line drawings, noting the color, the 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 colors alone. Color charts[8] are useful in describing the colors of the spots.

[4] PAN and PBN are trademarks of the ICI America, Stamford, CT.

[5] Rubber chemicals with other trade names may be substituted, provided they have the same chemical structure.

[6] FLEXZONE is a trademark of Uniroyal Chemical Co., Naugatuck, CT 06770.

[7] ARANOX and NAUGALUBE are trademarks of Uniroyal Chemical Co., Naugatuck, CT 06770.

[8] The sole source of supply of the apparatus known to the committee at this time is the Royal Horticultural Society, 80 Vincent Square London, United Kingdom. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

16. Precision

16.1 Repeatability and reproducibility data have not been generated according to this practice.

16.2 Antidegradants examined by this method in a cooperative testing program, included phosphited polyalkyl phenols, substituted bisphenols, secondary amines, substituted cresols, and substituted *p*-phenylenediamines.

17. Keywords

17.1 antidegradants; rubber; thin layer chromatography