Designation: D5297 – 95 (Reapproved 2019)

Standard Test Methods for Rubber Chemical Accelerator—Purity by High Performance Liquid Chromatography[1]

This standard is issued under the fixed designation D5297; 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 These test methods cover the determination of the purity of present commercially available rubber chemical accelerators in the range from 80 to 100 % by high performance liquid chromatography (HPLC) using ultraviolet detection and external standard calculations.

1.2 Expertise in HPLC is necessary to the successful application of these test methods.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D3853 Terminology Relating to Rubber and Rubber Latices—Abbreviations for Chemicals Used in Compounding

D4483 Practice for Evaluating Precision for Test Method Standards in the Rubber and Carbon Black Manufacturing Industries

D4571 Test Methods for Rubber Compounding Materials—Determination of Volatile Material

D4936 Test Method for Mercaptobenzothiazole Sulfenamide Assay by Reduction/Titration

2.2 *ISO Standard:*[3]

ISO 6472 Rubber Compounding Ingredients—Abbreviations

3. Terminology

3.1 *Definitions:*

3.1.1 *external standard calculation*—a method of calculating the percent composition by measuring the area of the analyte peak, multiplying by a response factor, and dividing by the sample concentration. All components are assumed to be resolved from the component of interest.

3.1.2 *lot sample*—a production sample representative of a standard production unit, normally referred to as the sample.

3.1.3 *specimen*—also known as the test portion, it is the actual material used in the analysis. It must be representative of the lot sample.

3.2 *Abbreviations:*

3.2.1 The following abbreviations are in accordance with Terminology D3853 and ISO 6472:

3.2.2 *MBTS*—Benzothiazyl disulfide.

3.2.3 *MBS*—2-(morpholinothio)benzothiazole.

3.2.4 *CBS*—*N*-cyclohexyl-2-benzothiazolesulfenamide.

3.2.5 *TBBS*—*N*-t-butyl-2-benzothiazolesulfenamide.

3.2.6 *DIBS*—*N,N′*- diisopropyl - 2 - benzothiazolesulfenamide.

3.2.7 *DCBS*—*N,N′* - dicyclohexyl - 2 - benzothiazolesulfenamide.

3.2.8 *DPG*—diphenylguanidine.

3.2.9 *DOTG*—di-o-tolylguanidine.

4. Summary of Test Methods

4.1 A specimen is dissolved in the appropriate solvent and a fixed loop volume is analyzed by isocratic HPLC using a

[1] These test methods are under the jurisdiction of ASTM Committee D11 on Rubber and Rubber-like Materials and are 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.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036.

thermostated C18 reversed phase column for materials 3.2.2 – 3.2.7 and a silica normal phase column for materials 3.2.8 and 3.2.9, and an ultraviolet (UV) detector. Peak areas are determined using a chromatographic integrator or laboratory data system with the amount of analyte being determined by external calibration.

5. Significance and Use

5.1 These test methods are designed to determine the purity of rubber chemical accelerators.

5.2 Since the results of these test methods are based on an integrated peak area, it is assumed that all analytes of interest are resolved from interfering peaks.

6. Interferences

6.1 Components co-eluting with the analyte of interest will cause erroneous results; thus it is required that the system be capable of providing a minimum of 10 000 theoretical plates.

7. Apparatus

7.1 *Liquid Chromatograph,* consisting of the following:

7.1.1 Precision chromatographic pump,

7.1.2 Variable wavelength UV detector,

7.1.3 A method for thermostating the column at 35 ± 1°C, for example, a column oven or water jacket, and

7.1.4 A fixed loop injector with a nominal volume of 10 mm^3 (µL) or less.

7.2 *HPLC Column:*

7.2.1 A C18 (ODS) reversed phase column packed with spherical, totally porous monomolecular 5-µm particles capable of providing 40 000 theoretical plates per metre. (A minimum of 10 000 plates is required for this analysis.) This column should be reserved for this analysis.

7.2.2 For materials 3.2.8 and 3.2.9, use a silica normal phase column packed with spherical, totally porous 5-µm particles capable of providing 40 000 theoretical plates per metre. (A minimum of 10 000 plates is required for this analysis.) This column should be reserved for this analysis.

7.3 *Integrator/Data System,* capable of determining absolute amounts of analyte of interest by means of integration of detector output versus time.

7.4 *Analytical Balance,* capable of measuring within ±0.01 mg.

8. Reagents and Materials

8.1 *Acetic Acid,* glacial.

8.2 *Acetonitrile,* HPLC grade.

8.3 *Chloroform,* AR grade.

8.4 *Ethanol,* HPLC grade.

8.5 *Ethanolamine.*

8.6 *n-Hexane,* HPLC grade.

8.7 *Methanol,* HPLC grade.

8.8 *Water,* HPLC grade.

9. Calibration and Standardization

9.1 A primary standard of known purity is used to determine the response factor for each analyte.

TEST METHOD A—SULFENAMIDE ACCELERATOR—PURITY

10. Procedure

10.1 *Chromatographic Conditions:*

10.1.1 Determine the mobile phase composition and the flow rate by adjusting the chromatographic parameters for the particular column chosen. The mobile phase consists of the appropriate mixture of HPLC grade acetonitrile and HPLC grade or equivalent water, both containing 0.001 *M* glacial acetic acid or less depending on the particular column chosen. (HPLC grade methanol may be added to the acetonitrile/water eluent to achieve the necessary separation for DIBS and MBTS.)

10.1.2 For the analysis of the sulfenamides, adjust the flow rate and mobile phase composition to provide a capacity factor, k', in the range from 4 to 6 for the analyte of interest, and a minimum resolution, R_s, of 2 between the MBTS impurity and the analyte of interest.

NOTE 1—Different liquid chromatography columns may exhibit different elution characteristics. Suggested chromatographic starting parameters for analysis are as follows:

	Percent H_2O[A]	Percent Acetonitrile[A]	Percent Methanol[A]	Flow rate (cm^3/min)
DCBS	5	95	0	2.5
CBS	20	80	0	2.0
TBBS	30	70	0	1.7
MBS	45	55	0	1.4
DIBS	15	0	85	1.0

[A] Containing 0.001 *M* glacial acetic acid.

10.1.3 The capacity factor, k', is defined as the retention time of the analyte, t_A, minus the retention time of an unretained solute (solvent peak), t_o , divided by t_o:

$$k' = (t_A - t_o)/t_o \quad (1)$$

10.1.4 The resolution, R_s, is a function of the capacity factor, selectivity, and the theoretical plates of the column:

$$R_s = \frac{(t_2 - t_1)}{1/2\,(tw_1 + tw_2)} \quad (2)$$

where:

t_1, t_2 = retention times of the analyte and MBTS, and
tw_1, tw_2 = peak widths at 10 % of the peak height.

10.2 *Detector*—Monitor the absorbance of all components at 275 nm. The detector sensitivity should be set to 1 absorbance unit full scale (AUFS).

10.3 *Integrator/Data System*—The integrator settings should be adjusted to give a full-scale response to 1 absorbance unit (AU).

10.4 *Standard Preparation*—Weigh at least 50 mg to the nearest 0.01 mg of the standard in a 50-cm^3 volumetric flask and dilute to volume with acetonitrile. Adjust the standard concentration if necessary by serial dilution with acetonitrile to give a maximum absorbance (peak height) between 0.4 and 0.8

AU (the linear range of the chromatographic system). The standard must be analyzed within 4 h of being diluted.

NOTE 2—*Preparation of Standards*—The analytical standards are prepared by multiple recrystallizations of the sulfenamides. Dissolve 100 g of the sulfenamide in 200 cm^3 of analytical reagent (AR) grade toluene with slight warming. Add 2 g of activated carbon and stir for 30 min. Filter the hot solution by gravity and cool in an ice/acetone bath. Filter the crystals with suction. Repeat this crystallization. Dissolve the analyte crystals from the second toluene crystallization in hot methanol, cool in an ice/acetone bath, and filter with suction. Repeat the alcohol recrystallization and dry at low pressure at 50°C overnight. The procedure can be repeated until the desired purity is obtained. The purity of the standard is estimated by gradient HPLC analysis of the impurities and differential thermal analysis (DTA). The purity of the standard should be reestimated by HPLC of the impurities every 90 days. The standard should be stored at 5°C or lower. Volatile matter and free amine content can be measured using Test Methods D4571 and Test Method D4936, respectively.

10.5 *Test Preparation*—To ensure specimen homogeneity, 5 g of the lot sample should be ground with a mortar and pestle.

10.6 *Analysis:*

10.6.1 Weigh at least 50 mg to the nearest 0.01 mg of the specimen into a 50-cm^3 volumetric flask. Dissolve in acetonitrile (a sonic bath is recommended) and dilute to volume with acetonitrile. Adjust the concentration, if necessary, by serial dilution with acetonitrile to give a maximum absorbance within 10 % of the standard absorbance. Filter through a chemically resistant filter with a nominal pore size less than or equal to 0.5 µm. Analyze within 4 h of dilution. Chromatograph the standard and measure the area.

TEST METHOD B—BENZOTHIAZOLE ACCELERATOR—PURITY

11. Procedure

11.1 *Chromatographic Conditions:*

11.1.1 Determine the mobile phase composition and the flow rate by adjusting the chromatographic parameters for the particular column chosen. The mobile phase consists of the appropriate mixture of HPLC grade acetonitrile and HPLC grade or equivalent water, both containing 0.001 *M* glacial acetic acid or less depending on the particular column chosen.

11.1.2 For the analysis of the benzothiazoles, adjust the flow rate and mobile phase composition to provide a capacity factor, k', in the range from 4 to 6 for the analyte of interest, and a minimum resolution, R_s, of 2 between the MBTS impurity and the analyte of interest.

NOTE 3—Different liquid chromatography columns may exhibit different elution characteristics. Suggested chromatographic starting parameters for analysis are as follows:

	Percent H_2O[A]	Percent Acetonitrile[A]	Percent Methanol[A]	Flow rate (cm^3/min)
MBT	65	35	0	2.0
MBTS	20	80	0	2.0

[A] Containing 0.001 *M* glacial acetic acid.

11.1.3 The capacity factor, k', is defined as the retention time of the analyte, t_A, minus the retention time of an unretained solute (solvent peak), t_o, divided by t_o:

$$k' = (t_A - t_o)/t_o \quad (3)$$

11.1.4 The resolution, R_s, is a function of the capacity factor, selectivity, and the theoretical plates of the column:

$$R_s = \frac{(t_2 - t_1)}{1/2\,(tw_1 + tw_2)} \quad (4)$$

where:

t_1, t_2 = retention times of the analyte and MBTS, and
tw_1, tw_2 = peak widths at 10 % of the peak height.

11.2 *Detector*—Monitor the absorbance of all components at 275 nm. The detector sensitivity should be set to 1 absorbance unit full scale (AUFS).

11.3 *Integrator/Data System*—The integrator settings should be adjusted to give a full-scale response to 1 absorbance unit (AU).

11.4 *Standard Preparation*—Weigh at least 50 mg to the nearest 0.01 mg of the standard in a 50-cm^3 volumetric flask and dilute to volume with acetonitrile for MBT and chloroform for MBTS. Adjust the standard concentration if necessary by serial dilution with acetonitrile to give a maximum absorbance (peak height) between 0.4 and 0.8 AU (the linear range of the chromatographic system). The standard must be analyzed within 4 h of being diluted.

NOTE 4—*Preparation of Standards*—The analytical standards may be prepared by multiple recrystallizations of the benzothiazoles. The purity of the standard is estimated by gradient HPLC analysis of the impurities and differential thermal analysis (DTA). The purity of the standard should be reestimated by HPLC of the impurities every 90 days. The standard should be stored at 5°C or lower.

11.5 *Test Preparation*—To ensure specimen homogeneity, 5 g of the lot sample should be ground with a mortar and pestle.

11.6 *Analysis:*

11.6.1 Weigh at least 50 mg to the nearest 0.01 mg of the specimen into a 50-cm^3 volumetric flask. Dissolve MBT in acetonitrile and MBTS in chloroform (a sonic bath is recommended) and dilute to volume with acetonitrile for MBT and chloroform for MBTS. Adjust the concentration, if necessary, by serial dilution with acetonitrile to give a maximum absorbance within 10 % of the standard absorbance. Filter with a chemically resistant filter with a nominal pore size less than or equal to 0.5 µm. Analyze within 4 h of being diluted. Chromatograph the standard and measure the area.

TEST METHOD C—GUANIDINE ACCELERATOR—PURITY

12. Procedure

12.1 *Chromatographic Conditions:*

12.1.1 Determine the mobile phase composition and the flow rate by adjusting the chromatographic parameters for the particular column chosen. The mobile phase consists of the appropriate mixture of HPLC grade *n*-hexane, ethanol, and methanol containing 0.01 *M* ethanolamine or less depending on the particular column chosen.

12.1.2 For the analysis of the guanidines, adjust the flow rate and mobile phase composition to provide a capacity factor, k', in the range from 6 to 8 for the analyte of interest.