Designation: D 5297 – 95 (Reapproved 2000)

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

This standard is issued under the fixed designation D 5297; 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 test method determines the purity of present commercially available rubber chemical accelerators in the range from 80 to 100 % by high performance liquid chromatography using ultraviolet detection and external standard calculations.

1.2 Expertise in high performance liquid chromatography (HPLC) is necessary to the successful application of this test method.

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 Documents

2.1 *ASTM Standards:*

D 3853 Terminology Relating to Rubber and Rubber Latices—Abbreviations for Chemicals Used in Compounding[2]

D 4483 Practice for Determining Precision for Test Method Standards in the Rubber and Carbon Black Industries[2]

D 4571 Test Methods for Rubber Chemicals—Determination of Volatile Material[2]

D 4936 Test Method for Mercaptobenzothiazole Sulfenamide Assay by Reduction/Titration[2]

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:Abbreviations*—The following abbreviations are in accordance with Terminology D 3853 and ISO 6472:

3.2.1 *MBTS*—Benzothiazyl disulfide.

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

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

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

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

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

3.2.7 *DPG*—diphenylguanidine.

3.2.8 *DOTG*—di-o-tolylguanidine.

4. Summary of Test Method

4.1 A specimen is dissolved in the appropriate solvent and a fixed loop volume is analyzed by isocratic HPLC using a thermostated C18 reversed phase column for materials 3.2.1 through 3.2.6 and a silica normal phase column for materials 3.2.7 and 3.2.8, 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 This test method is designed to determine the purity of rubber chemical accelerators.

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

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[1] This test method 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] *Annual Book of ASTM Standards*, Vol 09.01.

[3] Available from American National Standards Institute, 1l W. 42nd St., 13th Fl., New York, NY 10036.

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 3.2.7 and 3.2.8, 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:

$$k' = (t_A - t_o)/t_o \tag{1}$$

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

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

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 D 4571 and D 4936, 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:

$$k' = (t_A - t_o) / t_o \tag{3}$$

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

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

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.

NOTE 5—Different liquid chromatography columns may exhibit different elution characteristics. Suggested chromatographic starting parameters for analysis are as follows:

	Percent *m*-Hexane	Percent Ethanol^A	Percent Methanol^A	Flow rate (cm^3/min)
DPG	91	4	5	2.0
DOTC	91	4	5	2.0

^{A}Containing 0.01 *M* ethanolamine.

12.1.3 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:

$$k' = (t_A - t_o) / t_o \tag{5}$$

12.2 *Detector*—Monitor the absorbance of all components at 240 nm. The detector sensitivity should be set to 1 absorbance unit full scale (AUFS).

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

12.4 *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 45/55 (v/v) ethanol/methanol. Adjust the standard concentration if necessary by serial dilution with *m*-hexane 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 6—*Preparation of Standards*—The analytical standards are prepared by multiple recrystallizations of the guanidines. 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.

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

12.6 *Analysis*:

12.6.1 Weigh at least 50 mg to the nearest 0.01 mg of the specimen into a 50-cm^3 volumetric flask. Dissolve in 45/55 (v/v) ethanol/methanol (a sonic bath is recommended) and dilute to volume with the same solvent mixture. Adjust the concentration, if necessary, by serial dilution with *n*-hexane 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.

13. Calculation

13.1 *Response Factor*—Calculate the response factor for the standard by dividing the concentration of the standard by the measured area count and multiplying this by the purity of the standard:

$$\text{RF} = (\text{concentration/area count}) \times \%\ \text{purity} \tag{6}$$

NOTE 7—Throughout the calculation the units of concentration must be consistent (that is, mg/cm^3).

13.2 *Product Purity*—To determine the purity of the product, multiply the response factor by the measured area count of the analyte and divide by the sample concentration:

$$\%\ \text{purity} = \text{RF} \times \text{area count/sample concentration} \tag{7}$$

14. Report

14.1 Report percent accelerator to the nearest 0.1 %.

15. Precision and Bias [4]

15.1 This precision and bias section has been prepared in accordance with Practice D 4483. Refer to that practice for terminology and other statistical details.

15.2 The precision results in this precision and bias section give an estimate of the precision of this test method with the materials (accelerators) used in the particular interlaboratory program described below. The precision parameters should not be used for acceptance or rejection testing of any group of materials without documentation that they are applicable to those particular materials and the specific testing protocols that include this test method.

15.3 A Type 1 interlaboratory precision program was conducted. Both repeatability and reproducibility are short term. A period of a few days separates replicate test results. Eight laboratories participated in the sulfenamide precision program and four laboratories participated in the benzo-thiazole and guanidine precision programs. Four materials were used in the sulfenamide program. Therefore, p = 8, q = 4, and n = 2. Two materials were used in each of the benzothiazole and guanidine programs. Therefore, p = 4, q = 4, and n = 2 for the benzothiazole and guanidine programs, respectively. A test result is the value obtained from the average of two single determinations. Each material was analyzed twice on each of two separate days using the provided standards.

15.4 *Outliers*—Two cell results were determined to be cell variance outliers based on Cochran's Maximum Variance Test (see Practice D 4483, Annex 2). These results, for CBS and MBS from one laboratory, were eliminated from the calculations in Table 1.

15.5 *Precision Parameters*—Refer to Table 1 and Table 2.

15.6 *Repeatability*—The difference between two single test results (or determinations) found on identical test material under the repeatability conditions prescribed for a particular test will exceed the repeatability (*r*), as given in Table 1 and Table 2, on an average of not more than once in 20 cases in the normal and correct operation of the test method.

15.7 *Reproducibility*—The difference between two single and independent test results found by two operators working under prescribed reproducibility conditions in different laboratories on identical test material will exceed the reproducibility (*R*), as given in Table 1 and Table 2, on an average of not more than once in twenty cases in the normal and correct operation of the test method.

15.8 *Bias*—Impurities that are not resolved from the analyte of interest will produce a falsely high result. There may be other sources of bias which have not been determined.

TABLE 1 Precision (Type 1)[A]—Sulfenamide Purity

Material	Mean level[B]	Within laboratories[C]			Between laboratories[C]		
		s_r	r	(r)	S_R	R	(R)
CBS	98.7	0.601	1.68	1.70	0.602	1.68	1.70
DCBS	97.0	0.426	1.19	1.23	1.166	3.26	3.36
TBBS	96.0	0.394	1.10	1.15	1.113	3.12	3.25
MBS	92.8	0.648	1.81	1.95	1.882	5.27	5.68

[A]This is short-term precision (days) with $p = 8$, $q = 4$, $n = 2$.
[B]Mean level values (in percent).
[C] Symbols are defined as follows:
s_r = within laboratory standard deviation,
r = repeatability (in measurement units),
(r) = repeatability (in percent),
S_R = between laboratory standard deviation,
R = reproducibility (in measurement units), and
(R) = reproducibility (in percent).

TABLE 2 Precision (Type 1)[A]—Benzothiazole and Guanidine Purity

Material	Mean level	Within laboratories			Between laboratories		
		s_r	r	(r)	S_R	R	(R)
MBT	94.4	0.132	0.370	0.392	1.184	3.32	3.52
MBTS	95.1	0.130	0.364	0.383	1.152	3.22	3.39
DPG	98.3	0.403	1.128	1.148	0.850	2.38	2.42
DOTG	96.9	0.251	0.702	0.724	0.409	1.144	1.18

[A]This is short-term precision (days) with $p = 8$, $q = 4$, $n = 2$.

[4] Supporting data are on file at ASTM Headquarters. Request RR: D11-1063.

16. Keywords

16.1 benzothiazole accelerator; guanidine accelerator; high-performance liquid chromatography; rubber accelerator; sulfenamide accelerator

APPENDIXES

(Nonmandatory Information)

X1. RECOMMENDATIONS

X1.1 Degas the eluents.

X1.2 Use an appropriate guard column.

X1.3 Acid clean the glassware.

X1.4 Keep the temperature of the samples and standard the same.

X2. TYPICAL CHROMATOGRAMS

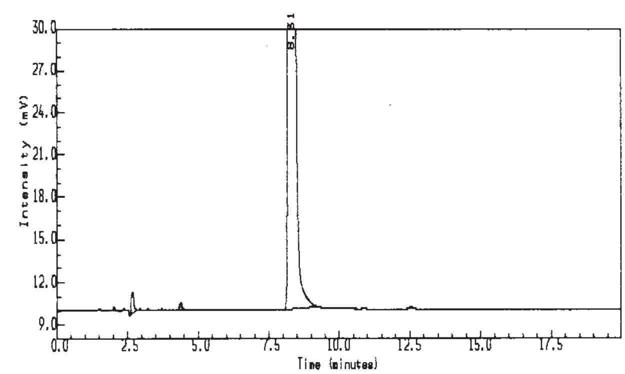
X2.1 A typical chromatogram of a CBS production sample is shown in Fig. X2.1.

X2.2 A typical chromatogram of an MBT production sample is shown in Fig. X2.2.

X2.3 A typical chromatogram of a DPG production sample is shown in Fig. X2.3.

Injection Report

Acquired on 1-MAY-1991 at 14:18



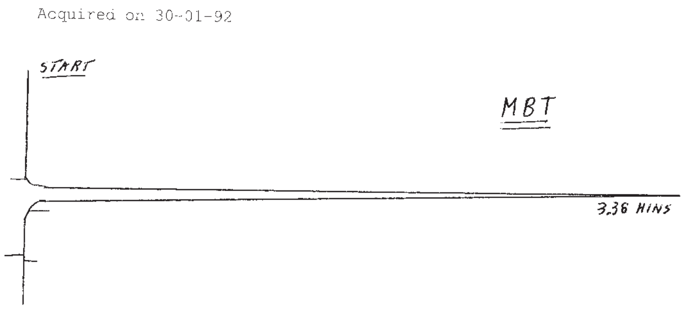
Sample Name : CBS
Sample Id :
Sample Type : Sample Amount=685.00000
Bottle No : 4

PEAK INFORMATION

Peak	RT mins	Hght uV	Area uVs	Area %	Width
1	2.018	240	701	0.03	2.9
2	2.684	1477	9100	0.39	5.9
3	4.382	572	3398	0.15	5.6
4	8.311	226520	2293662	99.43	9.6

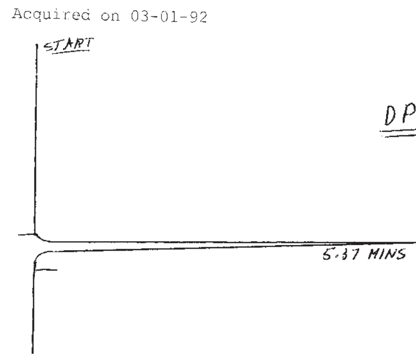
Totals	Hght uV	Area uVs	Area %
Unknowns	0	0	0.00
Quantified	228809	2306861	100.00
Grand Total	228809	2306861	100.00

FIG. X2.1 Sample Chromatogram



PEAK #	AREA %	RT MINS	AREA
1	100	3.38	1107888
TOTAL	100		1107888

FIG. X2.2 Typical Chromatogram—MBT



PEAK #	AREA %	RT MINS	AREA
1	100	5.37	1133449
TOTAL	100		1133449

FIG. X2.3 Typical Chromatogram—DPG