



Designation: D8411 – 21

Standard Test Method for Rubber Compounding Materials—Determination of 2-Mercaptobenzothiazole (MBT) in Rubber¹

This standard is issued under the fixed designation D8411; 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 standard test method covers a procedure for the determination of extractable 2-Mercaptobenzothiazole (MBT) in rubber by high performance liquid chromatography (HPLC) to assess its bioavailability.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *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

D1193 Specification for Reagent Water

D1991 Test Method for Rubber Chemicals—2-Mercaptobenzothiazole (MBT)—Assay

D5044 Test Method for Rubber Compounding Materials—Free 2-Mercaptobenzothiazole (MBT) in Benzothiazyl Disulfide (MBTS)

3. Summary of Test Method

3.1 This test method allows for the determination of 2-Mercaptobenzothiazole in cured rubber by high performance liquid chromatography apparatus equipped with a diode-array

detector (HPLC-DAD). The test is conducted after ultrasound extraction of the test sample with a chloroform-methanol mixture and shaking extraction with a simulated acid sweat solution.

4. Significance and Use

4.1 MBT is commonly used in rubber compounding as a curing accelerator for rubber and latex products. The determination of MBT in cured rubber is important to assess the risk of a release of this hazardous substance when rubber is in prolonged contact with human skin or mucous membranes.

4.2 Neo-formed MBT from MBTS might be created during the process of vulcanization when benzothiazolesulfenamides are used in the compound as accelerators, and therefore, it is part of the total MBT content in the cured rubber.

4.3 The purity of MBT as raw material can be evaluated by following Test Method D1991 based on a potentiometric titration of MBT with sodium hydroxide.

4.4 MBT is also a major impurity in MBTS. While MBTS is sparingly soluble in any organic solvent, MBT is very soluble. The amount of MBT contained in MBTS as raw material can be determined according to Test Method D5044.

4.5 This test method may be used as a quality control tool as well as for research and development work.

5. Apparatus

5.1 *High performance liquid chromatography apparatus* equipped with a diode-array detector (HPLC-DAD).

5.2 *Analytical Balance*, accuracy ± 0.1 mg.

5.3 *Ultrasonic Extraction Apparatus*, with a frequency of 40 ± 5 kHz.

5.4 *Shaking Table*, with water bath and variable frequency.

5.5 *pH Meter*, (digital is recommended), having an accuracy of ± 0.05 pH.

5.6 *Volumetric Flask*, 100 cm³.

5.7 *Conical flask with stopper*, 250 cm³.

5.8 *Volumetric Flask*, 1 dm³.

5.9 *Filter Paper*, quantitative.

¹ This test method is under the jurisdiction of ASTM Committee D11 on Rubber and Rubber-like Materials and is the direct responsibility of Subcommittee D11.20 on Compounding Materials and Procedures.

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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.

5.10 *Filtration Membrane*, organic type with a pore size of 0.45 μm .

5.11 *Flask with stopper*, 20 cm^3 .

5.12 *Pipette*, 10 cm^3 .

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade or analytical grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.³ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Types I, II, or III of Specification **D1193**.

6.3 *Methanol*, of analytical grade.

6.4 *Chloroform*, of analytical grade.

6.5 *Acetonitrile*, of chromatographic grade.

6.6 *2-Mercaptobenzothiazole reference material*, of purity $\geq 99.1\%$.

6.7 *L-histidine monohydrochloride monohydrate*, of analytical grade.

6.8 *Sodium dihydrogen phosphate dihydrate*, of analytical grade.

6.9 *Sodium chloride*, of analytical grade.

6.10 *Sodium hydroxide*, of analytical grade.

7. Sample Preparation

7.1 Sample preparation for determination of the total MBT content.

7.1.1 Weigh 0.4 g to the nearest 0.1 mg of a homogeneous test specimen and transfer it into a 20 cm^3 flask with a stopper.

7.1.2 Prepare a solvent mixture of CHCl_3 : MeOH (2:1, v/v). Add by pipette, 10 cm^3 of the chloroform-methanol mixture and close the flask with a stopper.

7.1.3 Ultrasonically extract the test specimen in the flask with the chloroform-methanol mixture for 14 h at a temperature not exceeding 40 $^{\circ}\text{C}$. Periodically add cold water or another cooling agent to the bath during the ultrasound extraction to keep the temperature below 40 $^{\circ}\text{C}$.

7.1.4 Transfer the extracted solution into a 100 cm^3 volumetric flask, pouring the solution into the flask using a funnel and filter paper, dilute it homogeneously with methanol to the mark.

³ ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

TABLE 1 HPLC mobile phase with gradient—The composition of the mobile phase is variable

Time, in min	0 – 2	2 – 17	17 – 22	22 – 25	25 – 28
Mobile phase A, in %	70	10	10	70	70
Mobile phase B, in %	30	90	90	30	30

7.2 Sample preparation for determination of the bioavailable MBT content.^{4,5}

7.2.1 Weigh to the nearest 0.1 mg: 0.5 g of L-histidine hydrochloride monohydrate ($\text{C}_6\text{H}_9\text{O}_2\text{N}_3\cdot\text{HCl}\cdot\text{H}_2\text{O}$), 5.0 g of sodium chloride (NaCl), and 2.2 g of sodium dihydrogen phosphate dihydrate ($\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$), and transfer them into a 1 dm^3 flask.

7.2.2 Prepare the acid sweat solution by adding water to the flask to a constant volume of 1 dm^3 .

7.2.3 Adjust the pH value of the acid sweat solution to 5.5 ± 0.2 by 0.1 mol/dm^3 sodium hydroxide (NaOH) solution.

7.2.4 Extract 2.0 g of the test specimen in a 250 cm^3 conical flask with a stopper with 50 cm^3 of the acid sweat solution using a shaking table with a water bath at 40 $^{\circ}\text{C}$ and 1.8 Hz (110 rpm) for 1 h.

8. Conditions of HPLC Apparatus

8.1 The following parameters are recommended for the HPLC-DAD setup. Depending on the type of HPLC equipment, other specific parameters might apply.

8.1.1 chromatographic column: adsorbent: C18 grafted silica, particle size: 5 μm , inner diameter: 4.6 mm, column length: 250 mm, or a column with similar performance

8.1.2 wavelength range: 200 nm to 400 nm

8.1.3 test wavelength: 320 nm

8.1.4 temperature of column: 35 $^{\circ}\text{C}$

8.1.5 mobile phase A: water containing 1 % acetonitrile

8.1.6 mobile phase B: acetonitrile

8.1.7 gradient program, see **Table 1**

8.1.8 flow: 1.0 cm^3/min

8.1.9 injection volume: 10 mm^3

NOTE 1—The combination of solvents will affect the retention times and peak separation efficiency. The above recommendation is one of many possibilities. The specific solvents and ratios used can be determined by the technician to fit the needs of the lab. It is important to maintain the separation of the peaks so they can be unambiguously identified and quantified.

9. Calibration

9.1 MBT reference solutions for the determination of the total MBT content are used for calibration in the range from 0.05 mg/cm^3 to 0.50 mg/cm^3 . For the bioavailable content the calibration range is from 0.001 to 0.100 mg/cm^3 .

9.2 Prepare a reference stock solution of MBT in methanol with the concentration of 1 mg/cm^3 . For this purpose, dissolve 100 mg of the MBT reference material in 100 cm^3 of methanol in a volumetric flask.

⁴ Chinese National Standard GB/T 3922-2013, Standardization Administration of the People's Republic of China (SAC), Beijing 2013.

⁵ Textilien - Farbechtheitsprüfungen - Teil E04: Farbechtheit gegen Schweiß (ISO 105-E04:2013); Deutsche Fassung EN ISO 105-E04:2013