



Standard Test Method for Colorimetric/Spectrophotometric Procedure to Quantify Extractable Chemical Dialkyldithiocarbamate, Thiuram, and Mercaptobenzothiazole Accelerators in Natural Rubber Latex and Nitrile Gloves¹

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1. Scope

1.1 This test method is designed to quantify the amount of total extractable accelerators in natural rubber latex (NRL) and nitrile gloves. Three common classes of rubber accelerators, the mercaptobenzothiazole (MBT), thiuram, and thiocarbamate type compounds can be detected and quantified by this method. If the specific rubber accelerator(s) present in the glove material is not known, quantification is based on zinc dibutyl-dithiocarbamate (ZDBC) equivalents. This method will not detect all potential rubber accelerators, including mercaptobenzothiazole disulfide, dimorpholine, thioureas and diphenyl diamine.

1.2 For the purpose of this test method, the range of chemical accelerator measurement is based on the limit of detection (LOD) established in the performing laboratory.

1.3 This test method should be performed by experienced analysts or under the supervision of those experienced in the use of spectroscopy and working with organic solvents.

1.4 This test method has not been validated for measurement of long chain dithiocarbamates or accelerators from other rubber products, such as lubricated condoms (1).² Although this assay has been reported in the literature for the evaluation of accelerator levels in condoms, further validation for accelerator measurement from other rubber products is required by the testing laboratory prior to use.

1.5 This test method is not designed to evaluate the potential of rubber materials to induce or elicit Type IV skin sensitization reactions (for Type IV skin sensitization reactions see Test Method D6355). Total extractable accelerator content does not reflect bioavailability of individual accelerators that are de-

tected and measured by this method. This test method should be used to test and measure the total residual chemical accelerator level in NRL and nitrile gloves under controlled laboratory conditions, and should not be used to describe, appraise, or assess the hazard or risk of these materials or products under actual in-use conditions.

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

1.7 *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:³

D3577 Specification for Rubber Surgical Gloves

D3578 Specification for Rubber Examination Gloves

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

D6355 Test Method for Human Repeat Insult Patch Testing of Medical Gloves

3. Terminology

3.1 Total thiol vulcanization accelerator includes MBT, zinc dithiocarbamates (ZDTCs) and thiurams.

3.2 Definitions:

3.2.1 *limit of detection (LOD), n*—the lowest accelerator concentration that can be measured and be statistically different from the blank.

3.2.1.1 *Discussion*—The LOD is expressed as $3.3 \times$ standard error of the y-intercept of the standard plot regression line divided by the slope of the calibration line.

¹ This test method is under the jurisdiction of ASTM Committee D11 on Rubber and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products.

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² The boldface numbers in parentheses refer to a list of references at the end of this standard.

³ 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.

3.2.2 *limit of quantitation (LOQ), n*—the lowest accelerator concentration that can be measured to produce quantitatively meaningful results with acceptable precision and accuracy.

3.2.2.1 *Discussion*—The LOQ is expressed as $10 \times$ standard error of the y -intercept of the standard plot regression line divided by the slope of the calibration line.

3.2.3 *linear range, n*—area of a graph of absorbance versus concentration that approximates a straight line.

3.2.4 *spectrophotometric measurement, n*—the unit of measure of the instrument that is proportional to absorbance.

3.2.5 *standard solution, n*—the standard analyte to which the test (unknown) sample being measured is compared.

4. Summary of Test Method

4.1 The rubber material is cut into small pieces and approximately 1 g is placed into the extraction vessel. Acetonitrile is added to give a final volume/weight of 10 mL acetonitrile per gram of rubber. The extraction vessel is securely capped, placed onto a rotator and extracted at approximately 200 rpm for a minimum of 2 h at room temperature ($25 \pm 5^\circ\text{C}$). The acetonitrile extract is recovered and centrifuged in a sealed centrifuge tube at $500 \times g$ for 20 min at room temperature to remove any residual particulate matter. The acetonitrile extract supernatant fluid is transferred to a clean container and capped. Zinc dibutyldithiocarbamate (ZDBC) standards at 500 to $31.25 \mu\text{g/mL}$ in acetonitrile and a blank are prepared. Cobalt chloride ($10 \mu\text{L}$, 420 mmol/L) aqueous solution is added to 1 mL aliquots of each sample extract and standard. Each individual solution is thoroughly mixed and then incubated for 120 min at $50 \pm 5^\circ\text{C}$. The extracts and standards are cooled to room temperature for approximately 15 min after the 50°C incubation. A $100 \mu\text{L}$ aliquot of each is diluted with 1.9 mL of acetonitrile. All are mixed thoroughly and absorbance of each sample, blank and standard is measured at 320 nm on a UV spectrophotometer. Concentration of residual accelerator is obtained by extrapolation from the standard plot. Depending upon the number of samples tested, this test method takes about 5 h to complete.

5. Significance and Use

5.1 Dialkyldithiocarbamates (DTCs), benzothiazoles, and thiurams are often used as vulcanization accelerators in NRL products. Zinc DTC accelerators are added either directly or are formed in situ during the vulcanization process via reaction between a thiuram(s) and zinc oxide. DTCs, benzothiazoles, and thiurams have been detected in leachates from medical devices made of rubber such as gloves. Studies have shown these chemicals can cause allergic contact dermatitis. A simple selective method to monitor rubber accelerator levels in rubber extracts would be useful for quality control, product screening and research.

5.2 This colorimetric assay measures dialkyldithiocarbamates, including zinc dialkyldithiocarbamates (ZDTC), mercaptobenzothiazole (MBT) and thiurams as a total thiol vulcanization accelerator level in rubber products. A UV spectrophotometer with detection at 320 nm is used to measure the ZDTC, mercaptobenzothiazole and thiurams.

Sample extracts diluted at 1:20 prior to measurement on the spectrophotometer is usually sufficient to quantify the residual accelerator level from most commercially available rubber gloves; however, sample dilution can be adjusted (from neat extract to $> 1:20$ dilution) based on analytical needs. Thiurams and ZDTCs complex with cobalt turning the extract to a concentration-dependent shade of green. ZDTCs reacts quickly while thiurams react very slowly (requiring a heat catalyst). Mercaptobenzothiazole does not complex to Co(III), however, it absorbs strongly at 320 nm. It can be distinguished from both ZDTCs and thiurams by its strong absorbance at 320 nm without the cobalt dependent visible green color. Cobalt complexed thiurams and ZDTCs, but not MBT, also have and absorbance at 370 nm (2).

6. Interferences

6.1 Suspended solids such as powder or cotton flock can interfere with spectrophotometric measurements and care must be taken not to resuspend particulate following centrifugation. Some extracts may require additional steps to remove particulate such as higher speed centrifugation or possibly filtration, dependent on the physical nature of the particulate material. The rubber accelerators, mercaptobenzothiazole disulfide, dimorpholine, thioureas, diphenyl diamine, and diphenylguanidine are not detected by this assay and do not interfere with measurement of MBT or ZDTCs. Potential exists for interference from leached dyes or other additives that absorb at 320 nm; however, this has not been reported.

7. Apparatus

7.1 *Polypropylene or Glass Extraction Tubes*, with screw top lids (50 mL, conical bottom).

7.2 *Polypropylene Cryotubes*, (3.6 mL), with screw tops for cobalt reaction of extracts and standards and for the final 1/20 dilution prior to UV analyses.

7.3 *Parafilm*.

7.4 *Adjustable Positive Displacement Pipettes*, (5 to 10 mL, 1 mL and 250 μL).

7.5 *Laboratory Shaker*, (200 rpm).

7.6 *Laboratory Vortex Mixer*.

7.7 *Water or Dry Bath*, capable of maintaining the temperature at 50°C .

7.8 *Centrifuge*, (capable up to $500 \times g$).

7.9 *UV Spectrophotometer*.

8. Materials

8.1 *Chemical Accelerator Standard*—Use specific thiuram, zinc dithiocarbamate or mercaptobenzothiazole if specific species in the specimen is known.⁴

⁴ If species is not known use zinc dibutyldithiocarbamate (ZDBC), the sole source of supply of which known to the committee at this time is ChemService, Inc., 660 Tower Lane, PO Box 599, West Chester, PA 19381 (cat. No. Ou-76 (M.W. = 474.2)). 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.

NOTE 1—Storage problems for zinc diethyldithiocarbamate have been reported and care needs to be taken if this is to be used as the reference standard.

8.2 CoCl_2 —Cobalt (II) Chloride hexahydrate.

8.3 Water, (distilled/deionized (dH_2O)).

8.4 Acetonitrile, (HPLC grade).

9. Standards and Reagent Preparation

9.1 *Stock ZDBC*—Weigh 10 mg ZDBC (or appropriate accelerator standard) and add 10 mL acetonitrile (final concentration of 1000 $\mu\text{g/mL}$). Mix until completely dissolved. (Prepare a fresh mixture immediately before use.)

NOTE 2—(1) To improve precision in weighing milligram quantities of chemical compounds, a larger mass may be used and dissolved in greater volumes of solvent to yield a final concentration of 1 $\mu\text{g/mL}$. In addition, it is easier and more accurate to adjust the volume of acetonitrile added to the ZDBC vs. weighing exactly 10 or 100 mg to achieve the stock solution. (2) It is advisable to use positive displacement pipettes for accurate addition of acetonitrile due to its low surface tension. (3) Seal all vials with Parafilm around the screw caps to prevent leakage and evaporation.

9.2 *Prepare Dilutions*—Starting with the stock solution of 1000 $\mu\text{g/mL}$ ZDBC (or appropriate accelerator standard), perform five 1:1 serial dilutions using acetonitrile as the diluent to produce the following standard concentrations 500, 250, 125, 62.5, and 31.25 $\mu\text{g/mL}$ (500 – 31.25 ppm). Ensure that each standard is dissolved by thoroughly mixing before proceeding with the next dilution.

NOTE 3—If MBT is employed as the standard, the 500 $\mu\text{g/mL}$ standard absorbance will be outside the linear range of the curve.

9.3 *Solvent Blank*—Blank spectrophotometer using reagent blank (see 12.3).

9.4 *Cobalt Chloride Reagent*—To 50 mg of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ add 500 μL dH_2O (100 mg/mL).

NOTE 4—Larger masses of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ may be used with appropriate volume of dH_2O to achieve a final concentration of 100 mg/mL (420 mmol/L). This reagent is stable up to 1 month at room temperature in a sealed container.

10. Hazards

10.1 Laboratory personnel should adhere to standard good laboratory practices. Care should be taken when working with all chemical reagents. Acetonitrile is a volatile solvent and all solvent transfer steps should be conducted in a chemical hood. Chemical resistant glove use is recommended when handling organic solvents.

11. Extraction

11.1 *Extraction Medium*—Acetonitrile.

11.2 *Test Specimen*:

11.2.1 Cut the entire test specimen into small pieces (approximately 1 cm^2). Mix the small pieces to ensure that the sample analyzed is representative of the entire specimen. A weight of at least 1 g sample should be placed in extraction solution to provide a final concentration of 1 g sample/10 mL acetonitrile. The test specimen weight used and acetonitrile volume added should be recorded. Three separate 1 g/10 mL extracts should be prepared.

11.2.2 Perform the extraction of each rubber test specimen using the extraction solution as described below:

11.2.2.1 The test specimen shall be extracted in acetonitrile for 2 h at a temperature of $25 \pm 5^\circ\text{C}$ using continuous shaking at approximately 200 rpm on laboratory shaker. Periodically check each sample visually to ensure that the specimen pieces are covered with extraction solvent (that is, not adhering to vial wall above the solvent). Fifty-millilitre polypropylene conical bottom tubes work well for this purpose. If specimen pieces are above the solvent they can easily be tapped down into the solvent without opening the vials. Wrapping the top of the tube with Parafilm after screwing on the lid ensures a good seal to prevent potential loss/leakage of solvent.

11.2.2.2 Remove the samples from the shaker after extraction. Separate the NRL/nitrile pieces from the acetonitrile extract by carefully decanting into a new 50 mL centrifuge tube. Centrifuge the acetonitrile sample extract in a sealed centrifuge tube at $500 \times g$ for 20 min at room temperature to remove any residual particulate matter. Separate the extract from residual particulate by carefully decanting or pipetting the extract to prevent re-suspension of the particulate. If extract is cloudy or visible particulate remains additional centrifugation or filtration is required to remove particulate.

12. Colorimetric/Spectrophotometric Assay

12.1 Take a 1 mL aliquot of each of the ZDBC standard dilutions and add 10 μL of the CoCl_2 reagent solution into each.

12.2 Take a 1 mL aliquot of each rubber extract test sample and add 10 μL of the CoCl_2 reagent solution into each. Repeat this to provide duplicate measures for each test sample.

12.3 Prepare a reagent blank using 1 mL the acetonitrile and 10 μL of the CoCl_2 reagent solution.

12.4 Mix all samples, standards and blanks thoroughly and then incubate for 120 min at $50 \pm 5^\circ\text{C}$. All lids should be sealed with Parafilm after they are screwed onto the tube. After incubation, allow the tubes to come to room temperature before proceeding (approximately 15 min).

12.5 Remove a 100 μL aliquot of the mixture from each sample (extracts, standards and reagent blanks) and dilute with 1.9 mL of acetonitrile. Mix thoroughly and measure the absorbance of each sample and standard at 320 nm on a UV spectrophotometer. The spectrophotometer should be blanked using this reagent blank. If the absorbance reading of a sample is greater than the 500 $\mu\text{g/mL}$ ZDBC standard, dilute the sample 1:1 with reagent blank and read the absorbance again.

13. Calibration

13.1 The spectrophotometer is calibrated using the external standard method. Equal volumes of the standard solution are used to measure the absorbance as prepared in Section 12. This allows a direct correlation of absorbance value to known standard concentrations.

13.2 The calibration standard absorbance is determined at 320 nm.

NOTE 5—Glass or quartz cuvettes may provide more accurate results than plastic.

14. Calculation

14.1 Record the absorbance at 320 nm of the test samples and standards from the colorimetric/spectrophotometric assay.

14.2 Perform a linear regression using the ZDBC (or corresponding accelerator) standard concentration versus their corresponding absorbance value. Prepare a linear plot of the data as an X,Y plot of absorbance at 320 nm versus $\mu\text{g/mL}$ ZDBC.

14.3 Obtain the slope of the linear regression line and y-intercept of the calibration regression line for quantification of sample accelerator concentrations from the standard plot.

14.4 Record the $\mu\text{g/mL}$ and $\mu\text{g/g}$ (as described in Specifications [D3577](#) and [D3578](#)) of residual accelerator concentration for each test specimen extract obtained from the regression plot (adjust concentration for dilutions required for samples with concentrations outside the standard range).

15. Report

15.1 The working laboratory should maintain a record of all observations and calculations derived from the data.

15.2 The report shall include a description of the rubber device including product and lot number, when available. The accelerator concentrations should be expressed in $\mu\text{g/g}$ and $\mu\text{g/dm}^2$ (Specifications [D3577](#) and [D3578](#)).

16. Precision and Bias

16.1 An interlaboratory program for determining precision was conducted according to Practice [D4483](#). Bias data is not available due to lack of standard materials containing known leachable accelerator contents. Each individual laboratory should establish its own LOD and LOQ. The overall average LOD for ZDBC from participating laboratories was 15.8 ± 9.7 $\mu\text{g/mL}$.

16.2 [Table 1](#) lists the glove types and accelerator species content used for determination of precision. The gloves were purchased from a commercial vendor and accelerator species determined by high performance liquid chromatographic – photodiode array analyses as previously reported ([1](#), [2](#)).

TABLE 1 Glove Type and Content

| | | |
|---------|-----------------|---|
| Glove 1 | Powder-Free NRL | Zinc diethyldithiocarbamate (ZDEC), ZDBC, MBT |
| Glove 2 | Powder-Free NRL | ZDBC |
| Glove 3 | Powdered NRL | ZDEC, ZDBC |
| Glove 4 | Nitrile | ZDEC |

16.3 The precision and bias section gives an estimate of the precision of this test method with commercially available medical glove extracts used in the interlaboratory comparison program as 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 of the test method.

16.3.1 A Type 1 precision was evaluated.

16.3.2 Both repeatability and reproducibility are short term, a period of minutes to a few days separates replicate test results.

16.3.3 A test result is a mean value as specified by this test method obtained on duplicate measurements of the glove material extract.

16.3.4 Four different glove materials were used ([Table 1](#)) in this interlaboratory comparison program and were tested by six different laboratories. Multiple gloves from a single lot of each individual type of glove were cut into approximately 1 cm^2 pieces. Pieces from each glove lot type were mixed to minimize intralot variation before supplying to participating laboratories.

16.3.5 The interlaboratory comparison program was performed in 2007 to 2008.

16.3.6 The results of the precision calculations for repeatability and reproducibility are given in [Table 2](#).

16.4 Bias is the difference between an average test value and the reference (true) test property value. Reference materials do not exist for rubber with known residual accelerator levels to test this method. Bias therefore cannot be determined.

17. Keywords

17.1 colorimetric; mercaptobenzothiazole; spectrophotometric; thiol accelerator; thiuram; zinc dialkyldithiocarbamate

TABLE 2 Precision Data For Measurement of Thiol Accelerator (in ZDBC equivalences) from NRL and Nitrile Medical Exam Gloves^A

| Sample | Mean Accelerator µg/g | Within Laboratories | | | Between Laboratories | | |
|---------|--------------------------|---------------------|-----|-----|----------------------|------|-----|
| | | Sr | r | (r) | SR | R | (R) |
| Glove 1 | 5378 | 254 | 712 | 14 | 1767 | 4946 | 93 |
| Glove 2 | 2247 | 71 | 199 | 9 | 166 | 464 | 20 |
| Glove 3 | 2757 | 136 | 381 | 14 | 213 | 598 | 22 |
| Glove 4 | 3946 | 135 | 378 | 10 | 445 | 1246 | 32 |

^A Sr = within laboratory standard deviation

r = repeatability between test results of a single laboratory = 2.83 times the square root of the repeatability variance

(r) = repeatability (in percentage)

SR = between laboratory standard deviation

R = reproducibility between laboratories = 2.83 times the square root of the repeatability variance

(R) = reproducibility (in percentage)

REFERENCES

- (1) Depree, G.J., Bledsoe, T.A., and Siegel, P.D., "Determination of zinc dialkyldithiocarbamates in latex condoms," *J Chromatographic Sci.*, Vol. 42, No. 2, pp. 80-84, 2004.
- (2) Depree, G.J., Bledsoe, T.A., Siegel, P.D., "Survey of sulfur containing rubber accelerator levels in latex and nitrile exam gloves," *Contact Dermatitis*, Vol. 53, pp. 107-113, 2005.

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