

Method 641
***The Determination of
Thiabendazole in Municipal
and Industrial Wastewater***

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1. SCOPE AND APPLICATION

1.1 This method covers the determination of thiabendazole in municipal and industrial wastewater.

<i>Parameter</i>	<i>CAS No.</i>
Thiabendazole	148-79-8

1.2 The estimated detection limit (EDL) for thiabendazole is listed in Table 1. The EDL was calculated from the minimum detectable response being equal to 5 times the background noise using a 100- μ L injection. The EDL for a specific wastewater may be different depending on the nature of interferences in the sample matrix.

1.3 This is a liquid chromatographic method applicable to the determination of thiabendazole in municipal and industrial discharges. When this method is used to analyze unfamiliar samples for thiabendazole, compound identification should be supported by at least one additional qualitative technique.

1.4 This method is restricted to use by or under the supervision of analysts experienced in the operation of liquid chromatographs and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Sections 9.2 and 9.3.

2. SUMMARY OF METHOD

2.1 Thiabendazole is analyzed in the sample matrix after solubilization with acid and filtration to remove particulate matter. Chromatographic conditions are described which permit the separation and accurate measurement of thiabendazole by direct aqueous injection and HPLC with fluorescence detection.

3. INTERFERENCES

3.1 Solvent, reagents, glassware, and other sample-processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 9.1.

3.1.1 The use of high-purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

- 3.1.2** Glassware must be scrupulously cleaned.¹ Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water and reagent water. It should then be drained dry and heated in a muffle furnace at 400°C for 15 to 30 minutes. Solvent rinses with acetone and pesticide-quality hexane may be substituted for the heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store the glassware inverted or capped with aluminum foil.
- 3.2** Matrix interferences may be caused by fluorescing contaminants that coelute with thiabendazole. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. Matrix interferences caused by the presence of particulate matter are removed by filtration. Unique samples may require additional cleanup approaches to achieve the detection limit listed in Table 1.

4. SAFETY

- 4.1** The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified²⁻⁴ for the information of the analyst.

5. APPARATUS AND EQUIPMENT

- 5.1** Sampling equipment for discrete sampling.
- 5.1.1** Vial: 25-mL capacity or larger, equipped with a screw-cap with hole in center (Pierce 13074 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105°C before use.
- 5.1.2** Vial: 3.5-mL, equipped with a screw-cap with hole in center (Pierce 13019 or equivalent). Wash vial and cap as in Section 5.1.1.
- 5.1.3** Septum: PTFE-faced silicone (Pierce 12722 or equivalent). Detergent wash and dry at 105°C for 1 hour before use.
- 5.1.4** Septum: PTFE-faced silicone (Pierce 12712 or equivalent). Detergent wash and dry at 105°C for 1 hour before use.
- 5.2** Syringe: Glass, 5-mL with Leur tip.

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- 5.3 Syringe-filter holder: Stainless steel with Leur connection (Rainin 38 to 101 or equivalent).
- 5.4 Filters: 13 mm, Nylon 66, 0.45 μ pore (Rainin 38 to 112 or equivalent).
- 5.5 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.
- 5.6 High-performance liquid chromatography (HPLC) apparatus: Analytical system complete with liquid chromatograph and all required accessories including syringes, analytical columns, and mobile phases. The system must be compatible with the specified detectors and strip-chart recorder. A data system is recommended for measuring peak areas.
- 5.6.1 Isocratic pumping system, constant flow.
- 5.6.2 Injector valve (Rheodyne 7125 or equivalent) with 100- μ L loop.
- 5.6.3 Column: 250 mm long by 4.6 mm ID, stainless steel, packed with reverse-phase Ultrasphere ODS, 10 μ .
- 5.6.4 Fluorescence detector, for excitation at 300 nm and emission at 360 nm (Perkin Elmer 650 to 1S or equivalent). Fluorometer should have dispersive optics for excitation and utilize either filter or dispersive optics at the emission detector.
- 5.6.5 Strip-chart recorder compatible with detector, 250 mm. (A data system for measuring peak areas is recommended.)

6. **REAGENTS AND CONSUMABLE MATERIALS**

- 6.1 Reagent water: Reagent water is defined as a water in which an interferent is not observed at the EDL of each parameter of interest.
- 6.2 Sodium hydroxide solution (10N): Dissolve 40 grams of NaOH in reagent water and dilute to 100 mL.
- 6.3 Sodium thiosulfate: ACS, granular.
- 6.4 Sulfuric acid solution (1+1): Slowly add 50 mL of H₂SO₄ (specific gravity 1.84) to 50 mL of reagent water.
- 6.5 HPLC buffer (pH 8.2): Add 8 mL of triethanolamine (Eastman 1599) and 1 mL of glacial acetic acid (ACS) to 1 L of reagent water.
- 6.6 High-purity methanol: HPLC quality, distilled in glass.
- 6.7 Stock standard solution (1.0 μ g/ μ L): Stock standard solutions are prepared from pure standard material or purchased as a certified solution.
- 6.7.1 Prepare the stock standard solution by accurately weighing about 0.0100g of pure material. Dissolve the material in pesticide-quality methanol, dilute to volume in

a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

- 6.7.2 Transfer the stock standard to a PTFE-sealed screw-cap bottle. Store at 4°C and protect from light. The stock standard should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards.
- 6.7.3 The stock standard must be replaced after 6 months, or when comparison with quality control check samples indicates a problem.

7. *SAMPLE COLLECTION, PRESERVATION, AND STORAGE*

- 7.1 Collect all samples in duplicate. Grab samples must be collected in glass containers. Conventional sampling practices⁵ should be followed, except that the bottle must not be prewashed with sample before collection.
- 7.2 The samples must be iced or refrigerated at 4°C from the time of collection until analysis. Chemical preservatives should not be used in the field unless more than 24 hours will elapse before delivery to the laboratory. If the samples will not be analyzed within 48 hours of collection, the sample should be adjusted to a pH range of 1.0 to 3.0 with sodium hydroxide or sulfuric acid, and 35 mg of sodium thiosulfate per liter of sample for each part per million of free chlorine should be added.
- 7.3 All samples must be analyzed within 30 days of collection.⁶

8. *CALIBRATION AND STANDARDIZATION*

- 8.1 Establish liquid chromatographic operating parameters equivalent to those indicated in Table 1.
- 8.2 Prepare calibration standards at a minimum of three concentration levels of thiabendazole by adding volumes of the stock standard to a volumetric flask and diluting to volume with HPLC mobile phase. One of the standards should be at a concentration near, but greater than, the EDL, and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.
- 8.3 Using injections of 100 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results are used to prepare a calibration curve for thiabendazole. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation), linearity of the calibration curve can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

- 8.4** The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for thiabendazole varies from the predicted response by more than $\pm 10\%$, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or factor must be prepared.
- 8.5** Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

9. QUALITY CONTROL

9.1 Monitoring for interferences.

9.1.1 Analyze a laboratory reagent blank as described in Section 10 each time a set of samples is extracted. A laboratory reagent blank is an aliquot of reagent water. If the reagent blank contains a reportable level of thiabendazole, immediately check the entire analytical system to locate and correct for possible interferences and repeat the test.

9.2 Assessing accuracy.

9.2.1 After every 10 samples, and preferably in the middle of each day, analyze a laboratory control standard. Calibration standards may not be used for accuracy assessments and the laboratory control standard may not be used for calibration of the analytical system.

9.2.1.1 Laboratory control standard concentrate: From the stock standard prepared as described in Section 6.7, prepare a laboratory control standard concentrate that contains thiabendazole at a concentration of $2 \mu\text{g}/\text{mL}$ in methanol or other suitable solvent.⁷

9.2.1.2 Laboratory control standard: using a pipette or microliter syringe, add $50.0 \mu\text{L}$ of the laboratory control standard concentrate to a 10-mL aliquot of reagent water contained in a 10-mL volumetric flask.

9.2.1.3 Analyze the laboratory control standard as described in Section 10. Calculate the percent recovery (P_i) with the equation:

Equation 1

$$P_i = \frac{100S_i}{T_i}$$

where

S_i = Analytical results from the laboratory control standard, in $\mu\text{g/L}$

T_i = Known concentration of the spike, in $\mu\text{g/L}$

9.2.2 At least annually, the laboratory should participate in formal performance evaluation studies, where solutions of unknown concentrations are analyzed and the performance of all participants is compared.

9.3 Assessing precision.

9.3.1 Precision assessments for this method are based upon the analysis of field duplicates (Section 7.1). Analyze both sample vials for at least 10% of all samples. To the extent practical, the samples for duplication should contain reportable levels of thiabendazole.

9.3.2 Calculate the relative range (RR_i) with the equation:

Equation 2

$$RR_i = \frac{100R_i}{X_i}$$

where

R_i = Absolute difference between the duplicate measurements X_1 and X_2 , in $\mu\text{g/L}$

X_i = Average concentration found $\left(\frac{X_1 + X_2}{2} \right)$, in $\mu\text{g/L}$

9.3.3 Individual relative range measurements are pooled to determine average relative range or to develop an expression of relative range as a function of concentration.

10. PROCEDURE

10.1 Sample preparation.

10.1.1 Adjust the pH of the sample to pH 1 to 3 with sulfuric acid solution.

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- 10.1.2** Assemble the syringe-filtration assembly by attaching the filter holder (with filter) to a 5-mL glass syringe equipped with a Leur tip.
- 10.1.3** Remove the barrel from the syringe and pour a 4- to 5-mL aliquot of the acidified sample into the syringe, allowing room for reinsertion of the syringe barrel.
- 10.1.4** Filter a portion of the sample through 0.45- μ filter using a syringe-filter holder. The first few milliliters should be discarded. Collect the filtrate in a 4-mL vial equipped with a PTFE-sealed screw-cap.
- 10.1.5** The syringe and filter holder should be rinsed with acetone or methanol and then HPLC-grade water between samples.
- 10.2** Cleanup and separation.
- 10.2.1** Cleanup procedures may not be necessary for a relatively clean sample matrix. Use of fluorescent detectors, however, often obviates the necessity for cleanup of relatively clean sample matrices. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that recovery is no less than 85%.
- 10.3** Liquid chromatographic analysis.
- 10.3.1** Table 1 summarizes the recommended operating conditions for the liquid chromatograph. Included in this table are the estimated retention time and estimated detection limit that can be achieved by this method. An example chromatogram achieved by this column is shown in Figure 1. Figure 2 is a chromatogram of thiabendazole in a POTW wastewater sample. Other columns, chromatographic conditions, or detectors may be used if data quality comparable to Table 2 is achieved.
- 10.3.2** Calibrate the system daily as described in Section 8.
- 10.4** Inject 100 μ L of the filtered aqueous sample. Monitor the column eluent at excitation wavelength 300 nm (5 nm slit width) and emission wavelength 360 nm (10 nm slit width). Record the resulting peak size in area or peak height units.
- 10.5** The retention-time window used to make identifications should be based upon measurements of actual retention-time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.
- 10.6** If the response for the peak exceeds the working range of the system, dilute the sample with mobile phase and reanalyze.
- 10.7** If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

11. CALCULATIONS

11.1 Determine the concentration of thiabendazole in the sample.

11.1.1 Calculate the amount of thiabendazole injected from the peak response using the calibration curve or calibration factor in Section 8.2.2. The concentration in the sample can be calculated from the following equation:

Equation 3

$$\text{Concentration, } \mu\text{g/L} = \frac{(A) (100)}{(V_i)}$$

where

A = Amount of the thiabendazole injected, in ng

V_i = Volume of sample injected, in μL

11.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

12. METHOD PERFORMANCE

12.1 The EDL and associated chromatographic conditions for thiabendazole are listed in Table 1.⁸ The EDL is defined as the minimum response being equal to five times the background noise, using a 100- μL injection.

12.2 Single-operator accuracy and precision studies were conducted by Environmental Science and Engineering, Inc.,⁶ using a spiked POTW sample. The results of these studies are presented in Table 2.

References

1. ASTM Annual Book of Standards, Part 31, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, Pennsylvania, p. 679, 1980.
2. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.
3. "OSHA Safety and Health Standards, General Industry" (29 *CFR* 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).
4. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
5. ASTM Annual Book of Standards, Vol. 11.01, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, 1986.
6. Test procedures for Pesticides in Wastewaters, EPA Contract Report 68-03-2897, unpublished report available from U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, March 1979.
8. "Evaluation of Ten Pesticides Methods," U.S. Environmental Protection Agency, Contract No. 68-03-1760, Task No. 11, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Table 1. Liquid Chromatography of Thiabendazole*

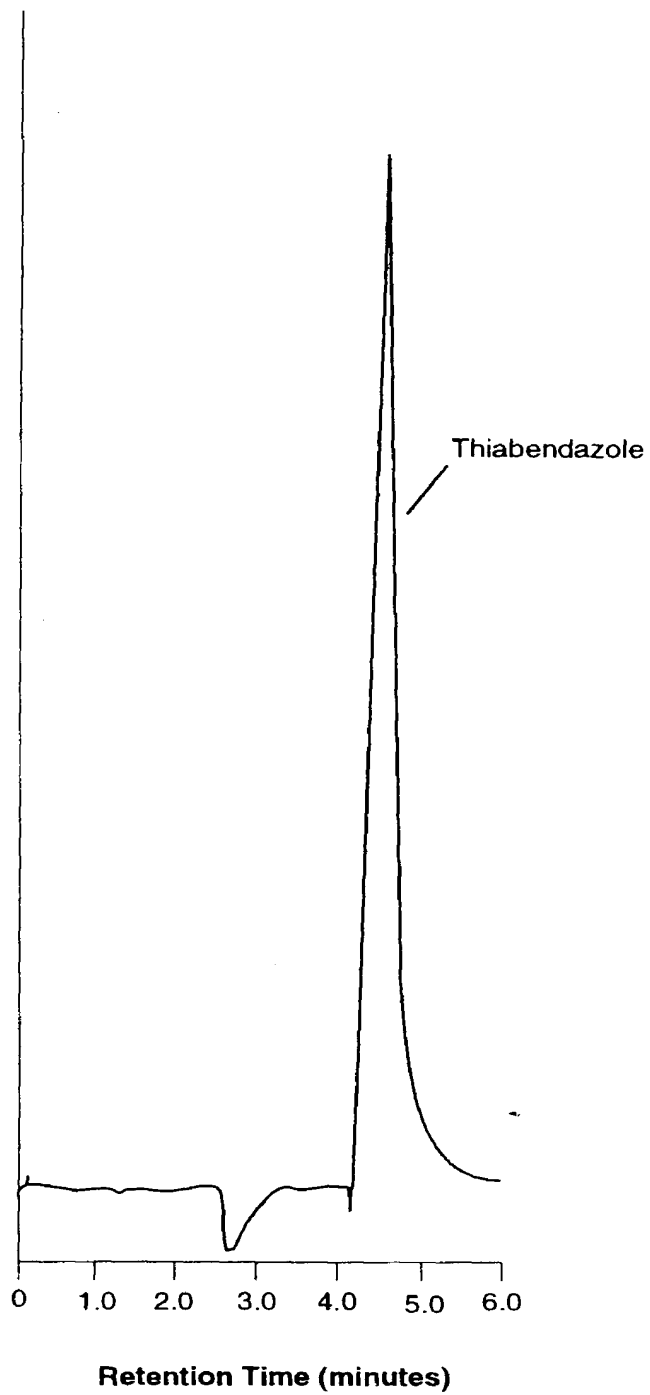
Compound	Retention Time (min)	Estimated Detection Limit (µg/L)
Thiabendazole	4.3	1.7

*HPLC conditions: 10 µ reverse-phase Ultrasphere ODS; Column, 250 mm long by 4.6 mm ID; isocratic 70% methanol/30% buffer; flow rate 1 mL/min.

Table 2. Single-Operator Accuracy and Precision*

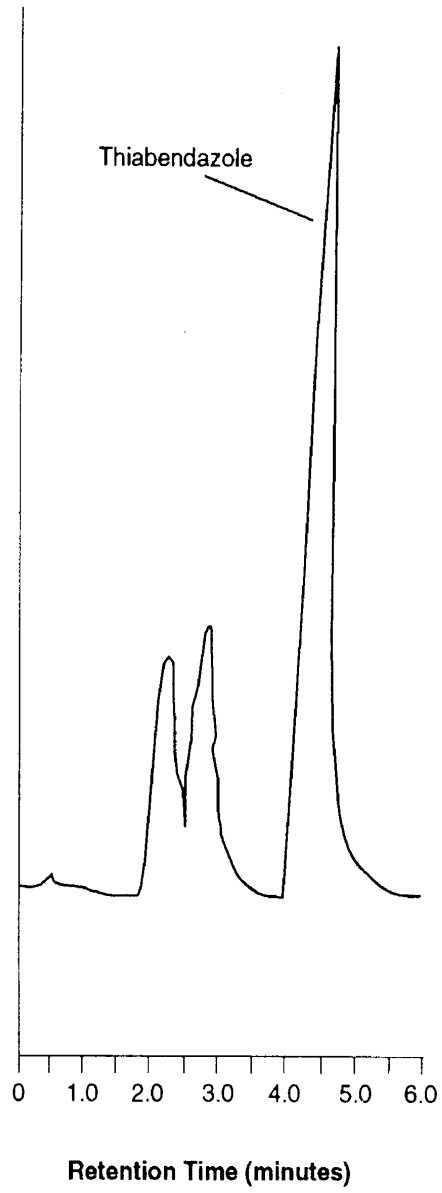
Parameter	Spike Concentration (µg/L)	Number of Replicates	Average Percent Recovery	Standard Deviation (%)
Thiabendazole	12.5	7	100	9.5
	625	7	92.8	4.5

*POTW effluent was used in this study.



A52-002-67A

Figure 1. HPLC of Thiabendazole



AS2-002-68A

Figure 2. Chromatogram of Thiabendazole in Wastewater Sample