

5.1.55

AOAC Official Method 966.28
Thiabendazole in Feeds

Spectrophotometric Method

First Action 1966

Final Action 1967

Method I

(Applicable to all feeds.)

A. Principle

Thiabendazole is extracted from feed with 0.1M HCl. Interferences are removed by adjusting extract to pH 5–6 with sodium citrate and extracting with CHCl₃. Thiabendazole is re-extracted with 0.1M HCl and reduced with Zn slurry in 30% glycerol in presence of *p*-phenylenediamine. Oxidation with ferric iron yields blue complex which is extracted with butyl alcohol and measured at 605 nm.

B. Reagents

(a) *Zinc dust*.—Reagent grade. Crush fine lumps with spatula immediately before use.

(b) *Zinc slurry*.—Weigh 50 mg *p*-phenylenediamine 2HCl (*Caution*: *p*-Phenylenediamine may be harmful.) and 2 g Zn dust into dry 100 mL glass-stoppered graduate. Add 100 mL 30% (v/v) glycerol solution, stopper, and shake ca 30 s to suspend Zn dust uniformly. (There must be no agglomeration of Zn.) Prepare just before use and use immediately.

(c) *Ferric solution*.—Dissolve 15.0 g FeNH₄(SO₄)₂ 12H₂O in 75 mL H₂O, add 10.0 mL 0.5M H₂SO₄, dilute to 100 mL, and mix.

(d) *Thiabendazole standard solutions*.—(1) *Stock solution*.—0.5 mg/mL. Dissolve 50.0 mg Thiabendazole Reference Standard (available from Merial, Ltd., 631 Route 1 South, North Brunswick, NJ 08902, USA; www.us.merial.com) in 0.1M HCl and dilute to 100 mL. Solution is stable 1 month. (2) *Intermediate solution*.—50 g/mL. Dilute 10 mL stock solution to 100 mL with 0.1M HCl. Solution is stable 1 month. (3) *Working solution*.—5 g/mL. Dilute 20.0 mL intermediate solution to 200 mL with 0.1M HCl. (Use same 0.1M HCl as in extraction of feed.)

C. Determination

Weigh 2.000 g ground test portion into 250 mL standard-taper 24/40 flat-bottom extraction flask. (For feeds containing <0.025% (<250 mg/kg) thiabendazole, weigh 5.000 g; Table 966.28.)

Add 100.0 mL 0.1M HCl to test portion and add magnetic stirring bar. Connect flask to reflux condenser (Allihn, drip tip) and reflux gently on magnetic hot plate, while stirring, 30 min. Cool, transfer mixture to centrifuge tube, and centrifuge ca 5 min. Dilute measured aliquot of supernate to concentration of 5 g thiabendazole/mL (serial dilutions may be necessary; Table 966.28). Such dilutions determine “dilution factor,” *DF*.

Mark series of 50 mL centrifuge tubes 1, 2, 3, 4, etc. Place 20.0 mL 0.1M HCl in tube 1 and 20.0 mL (100 g) working standard solution

Table 966.28. Test portion sample weights, dilutions, and factors

Declaration, % (mg/kg)	Test portion weight, g	Dilution(s)	<i>DF</i>
0.01 (100)	5	None	1
0.025 (250)	2	None	1
0.1 (1000)	2	25–100	4
1.0 (10000)	2	10–100; 25–100	40
6.0 (60000)	2	10–100; 10–250	250

in tubes 2 and 3. Place 20.0 mL aliquots of test solutions in tubes 4, 5, etc. Add 3 g sodium citrate, 3 g NaCl, and 20.0 mL CHCl₃ to each tube, stopper tightly with polyethylene stopper, and shake mechanically 5 min. Centrifuge ca 5 min and discard top layers. With pipet, transfer 10 mL CHCl₃ extract to dry, marked, centrifuge tubes, add 25.0 mL 0.1M HCl to each, stopper, and shake 5 min. Centrifuge, and transfer, with pipet, 15 mL of top acid layer to another marked tube. (Because of timing, handle 10 tubes at one time.)

With rapid delivery pipet, add 5 mL freshly prepared Zn slurry, **B(b)**, to each tube. (5 mL pipet with tip cut off to give delivery in ca 5 s is suitable. Hold pipet directly over center of solution.) *Do not shake tube but immediately stopper tightly and let stand 4 min*. Start timing after delivery of slurry to first tube.

After 4 min, add 5.0 mL ferric solution, **B(c)**, to each tube with rapid pipet, stopper, and mix by inverting tube. Let stand 5 min; then shake vigorously and centrifuge ca 3 min. With pipet, transfer 15 mL clear, colored solution to marked, dry, centrifuge tubes. Let stand 45 min from addition of ferric solution. Then add 5.00 mL *n*-butyl alcohol and 3 g anhydrous Na₂SO₄ to each tube. Stopper, and immediately shake each tube ca 5 s to avoid caking of Na₂SO₄; then shake all tubes ca 3 min or until Na₂SO₄ is completely dissolved, and centrifuge.

Transfer clear butyl alcohol solution (top layer) to dry 1 cm cell and read *A* at 605 nm against *n*-butyl alcohol as reference

$$\text{Thiabendazole, \%} = \frac{(A - A_0)(C)(DF)}{360(A - A_0)W}$$

$$\text{Thiabendazole, mg/kg} = \frac{(A - A_0)(C)(DF)}{360(A - A_0)W} 10^4$$

where *A* refers to test portion, *A*₀ to reagent blank (tube 1), *A* to standard, *C* = g thiabendazole standard in final 15.0 mL colored solution (18 g), *DF* = dilution factor (Table 966.28), and *W* = g original test sample.

References: *JAOAC* 47, 235(1964); 49, 312(1966).

CAS-148-79-8 (thiabendazole)