7.3.13A

AOAC Official Method 997.01
Tebuconazole in Fungicide and Technical Formulations

Capillary Gas Chromatographic Method
First Action 1997
Modified 1999

(Applicable to the determination of tebuconazole in technical material and in solid and liquid formulations containing tebuconazole as the only active ingredient.)

See Table **997.01** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

Test portion is dissolved in acetone containing dicyclohexyl phthalate as internal standard. Tebuconazole is separated by gas chromatography with flame ionization detection, and quantitated using peak area ratios.

B. Apparatus

(**a**) *Gas chromatograph (GC).*—With flame ionization detector and peak area integrator. Operating conditions: column temperature, ca 240°C; injector temperature, 300°C; detector temperature, 300°C; column flow (He), ca 7 mL/min; recorder speed, 0.5 cm/min; recorder range, 1 mV; injection volume, ca 1 μL; split ratio, 10:1 (minimum); detector range, 2^4; attenuation, 2^5. Detector flows: H, 30 mL/min; make-up (He), 25 mL/min; air, 240 mL/min. Retention times: tebuconazole, ca 2.5 min; dicyclohexyl phthalate (internal standard), ca 3.5 min.

(**b**) *GC column.*—Approximately 5 m × 0.53 mm id fused silica open tubular capillary column coated with methyl silicone (5 μm film thickness). (*Note:* Other lengths and film thicknesses are acceptable, but will require flow and/or temperature adjustments to obtain specified retention times. Longer retention times are acceptable.)

(**c**) *Filters.*—0.45 μm porosity; Teflon®.

(**d**) *Ultrasonic bath.*

C. Reagents

(**a**) *Acetone.*—Reagent grade.

(**b**) *Dicyclohexyl phthalate internal standard solution.*—0.50% (w/v) Dicyclohexyl phthalate (reagent grade) in acetone. (*Note:* Before use, confirm that no interfering impurities are present near retention time of tebuconazole.) Dicyclohexyl phthalate internal standard solution is stable up to 1 month at room temperature.

(**c**) *Tebuconazole reference standard.*—Approximately 95% purity (available from Bayer Corp., Agriculture Division, PO Box 4913, Hawthorne Rd, Kansas City, MO 64120-0013, USA). Store reference standard at 4–8°C. Equilibrate to room temperature before opening.

(**d**) *Tebuconazole standard solution.*—Rotate container with tebuconazole reference standard to ensure homogeneity. Accurately weigh to nearest 0.1 mg amount of tebuconazole standard containing 90–110 mg tebuconazole into 50 mL bottle with polyethylene-lined cap. Add 20.0 mL dicyclohexyl phthalate internal standard solution, cap, and mix well. Tebuconazole standard solution is stable up to 48 h at ca 20°C.

D. Preparation of Test Solution

Accurately weigh to the nearest mg test portion containing ca 100 mg tebuconazole into 50 mL bottle with polyethylene-lined cap. Pipet 20.0 mL dicyclohexyl phthalate internal standard solution, **C**(**b**), into bottle and cap. Sonicate each bottle 60 s, mix well, and let settle. Transfer aliquot of clear, supernatant liquid to autosampler vial. (*Note:* If clear supernatant liquid cannot be obtained after settling, filter portion of test suspension through 0.45 μm filter into autosampler vial.)

E. GC Determination

Adjust column temperature and/or carrier flow rate so that retention time of tebuconazole peak falls in range of ca 2–4 min. Dicyclohexyl phthalate peak should elute a minimum of 0.5 min after tebuconazole peak, with baseline separation. Unacceptable resolution between tebuconazole and dicyclohexyl phthalate internal standard or from liquid formulation components is most likely caused by excessive carrier gas flow rate. Replace GC column if problems persist.

Make repetitive injections of tebuconazole standard solution, **C**(**d**), until response ratios of standard injections, R_{std}, agree within 1% for 2 consecutive injections.

Table 997.01 Interlaboratory study results for determination of tebuconazole in fungicide formulations by capillary gas chromatography[a]

Tebuconazole formulation	Mean concentration, %	No. of labs	s_r	s_R	RSD_r, %	RSD_R, %	r[b]	R[c]
Aqueous flowable	40.0	20	0.29	0.49	0.72	1.22	0.80	1.37
Aqueous emulsifiable concentrate	26.2	20	0.14	0.30	0.52	1.13	0.38	0.83
Flowable seed treatment	1.55	19	0.03	0.06	1.17	2.65	0.08	0.17
Emulsifiable seed treatment	2.36	17	0.02	0.04	1.50	2.40	0.06	0.10
Dry flowable	45.7	20	0.22	0.33	0.48	0.72	0.62	0.92
	26.3	20	0.14	0.19	0.54	0.72	0.40	0.53
Wettable powder	21.6	19	0.15	0.21	0.69	0.96	0.41	0.58

[a] Determination of technical grade tebuconazole was added to method in 1999 based on 2 laboratories. No collaborative study performance data is available for technical grade material.

[b] $r = 2.8 \times s_r$.

[c] $R = 2.8 \times s_R$.

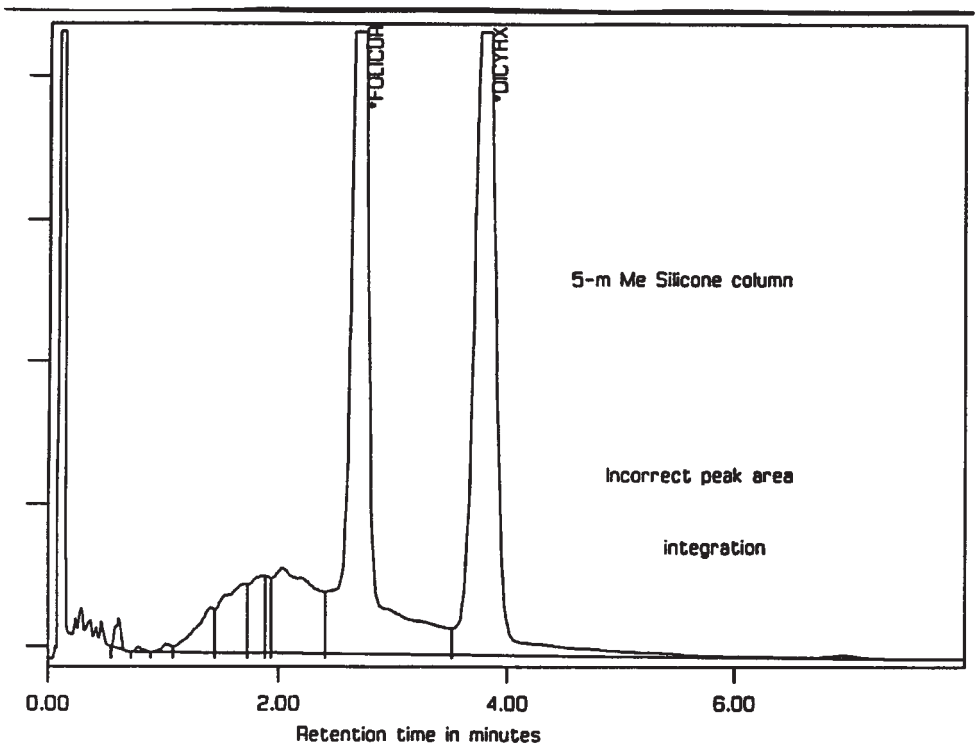

Figure 997.01A—Chromatogram of tebuconazole flowable seed treatment formulation (25 g/L) before correction.

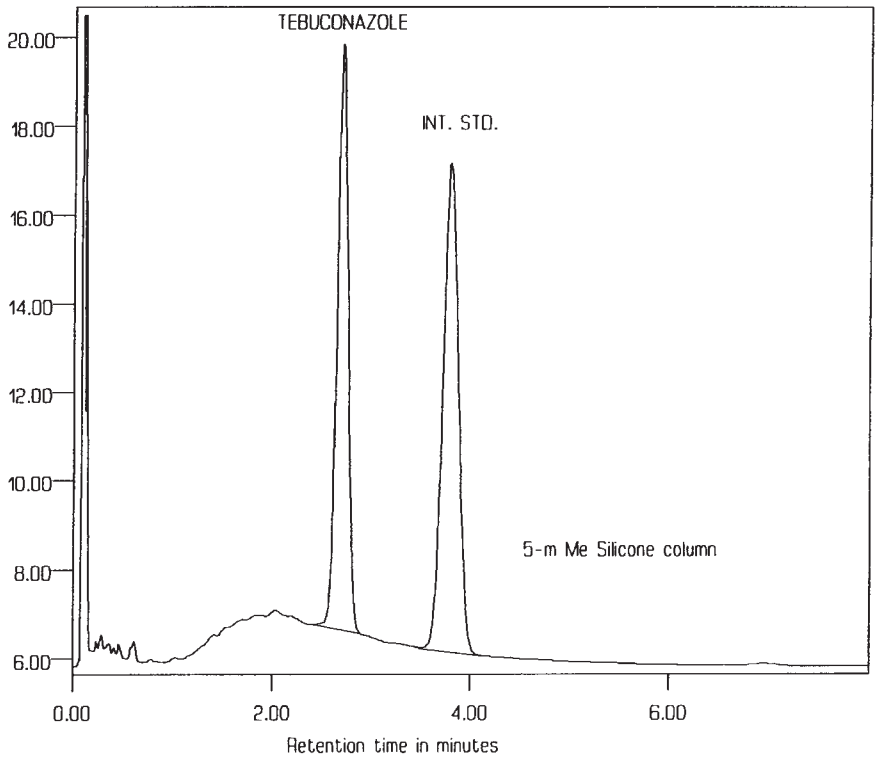

Figure 997.01B—Chromatogram of tebuconazole flowable seed treatment formulation (25 g/L) after correction.

Inject no more than 3 duplicate aliquots of test solution (6 injections) between injections of tebuconazole standard solution. Response ratios of test solution injections, *R*, must agree within 1%. Otherwise repeat analysis starting with injections of tebuconazole standard solution. [*Note:* Analyses of flowable seed treatment (FS) formulations (25 g/L) are subject to potential integration interferences. Peak areas may require manual electronic integration for this type of matrix only. *See* Figure **997.01A** for peak area integration before correction and Figure **997.01B** for correctly integrated chromatogram of FS formulation.]

Average response ratios of tebuconazole standard solution injections immediately preceding and following test solution injections. Bracketing standard injections must agree within 1%. Repeat analysis that does not meet this criterion.

F. Calculation

Calculate response ratio of tebuconazole standard solution, R_{std}, as follows:

$$R_{std} = \frac{P_{t-std}}{P_{i-std}}$$

where $P_{t\text{-}std}$ = peak area of tebuconazole in tebuconazole standard solution; $P_{i\text{-}std}$ = peak area of dicyclohexyl phthalate in tebuconazole standard solution.

Calculate response ratio of test solution, *R*, as follows:

$$R = \frac{P_t}{P_i}$$

where P_t = peak area of tebuconazole in test solution; P_i = peak area of dicyclohexyl phthalate in test solution.

Calculate percent tebuconazole in test portion as follows:

$$\text{Tebuconazole, \%} = (R_{ave}/R_{ave\text{-}std}) \times (W_{std}/W) \times P$$

where R_{ave} = average of 2 response ratios of tebuconazole in test solution; $R_{ave\text{-}std}$ = average of 2 response ratios of tebuconazole in tebuconazole standard solution (obtained immediately before and after test solution injection); W_{std} = weight of tebuconazole reference standard, mg; W = weight of test portion, mg; P = percentage purity of tebuconazole reference standard.

Reference: *J. AOAC Int.* **80,** 703(1997).

CAS-80443-41-0 (tebuconazole)

Revised: June 2000