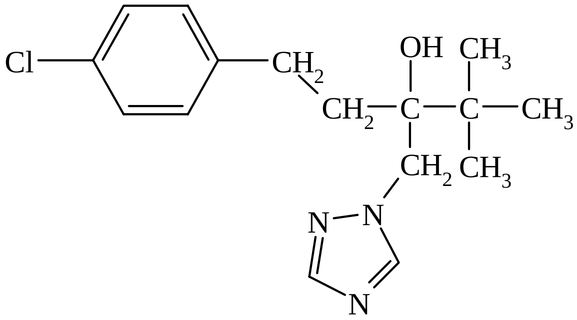
TEBUCONAZOLE
494



ISO common name	Tebuconazole
Chemical name	(*R,S*)-1-(4-Chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazole-ylmethyl)pentan-1-ol (IUPAC); α-[2-(4-chlorophenyl)ethyl]-α-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol (CA; *107534-96-3*)
Empirical formula	$C_{16}H_{24}ClN_3O$
RMM	307.4
m.p.	102.4°C
v.p.	3.1×10^{-8} Pa at 25 °C
Solubility	In water: 32 mg/l at 20°C; dichloromethane: more than 200 g/l; propan-2-ol: 50-100 g/l; toluene: 50-100 g/l; *n*-hexane: less than 0.1 g/l
Description	Colourless crystals
Formulations	Wettable powders, water dispersible granules, suspension concentrates, oil in water emulsions, suspension concentrates for seed treatment and emulsions for seed treatment

TEBUCONAZOLE TECHNICAL
[*]**494**/TC/(m)/-

1 Sampling. Take at least 100 g.

2 Identity tests As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole As for tebuconazole wettable powders **494**/WP/(M)/3.

TEBUCONAZOLE WETTABLE POWDERS
[**]**494**/TC/(m)/-

1 Sampling. Take at least 500 g.

2 Identity tests

2.1 GLC. Use the GLC method below. The relative retention time obtained from the sample should not deviate by more than 1 % from that of the standard obtained under the same conditions.

2.2 Infrared. Prepare potassium bromide discs from the sample and from a pure standard, and scan from 4000 to 400 cm^{-1}. The two spectra should not be significantly different.

2.3 Thin layer chromatography

OUTLINE OF METHOD. Tebuconazole is separated from manufacturing impurities and added modifying agents by TLC. The area containing the active ingredient is removed and the active ingredient is eluted from the removed silica gel with methanol. After evaporating the solvent the residual is used for IR.

SCOPE The method is suitable for technical solutions and formulations containing more than 1·0 % of active ingredient.

REAGENTS

TLC glass plates (20 × 20 cm) coated with silica gel 60 containing fluorescence indicator (e.g. Merck No. 5715)
Methanol
Toluene
Cyclohexane
Ethyl acetate
Cotton wool chemical pure
Potassium bromide for spectroscopy

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[*] Tentative AOAC-CIPAC method.
[**] Provisional AOAC-CIPAC method 1997.

APPARATUS

Equipment for thin layer chromatography using 20 × 20 cm TLC plates
Pasteur pipette
Micropipettes 50 µl
Applicator for 50 µl pipettes
Disposable syringe 5.0 ml, equipped with a 0.45µm filter unit (e.g. Milex-HV, Millipore)
UV lamp 254 nm
Spatula
Autosampler vial

PROCEDURE

Mark a line on a TLC plate (20 × 20 cm) for sample application at a distance of 1·0 cm from the lower edge and 2 cm from both the left and right sides.

Weigh (to the nearest 0·01 g) into a volumetric flask (5 ml) enough sample to contain 0.1 g tebuconazole. Make up to the mark with methanol and mix thoroughly. Filter about 2 ml of this suspension through a disposable syringe with a 0.45µm filter.

Apply 200 µl of the solution on the plate using four times a 50 µl pipette and a suitable holder. Fill the pipette by capillary action through one opening and empty it by bringing the opposite opening of the pipette in contact with the coating of the TLC plate at the marked line. Repeat this procedure until 200 µl of the solution is applied on the plate at the marked line.

Then twice focus the line on the plate at about 1·5 cm with methanol. After drying develop the plate over a distance of 12 cm from the lower edge using a mixture of toluene-cyclohexane-ethyl acetate, 50+50+35 (v/v) as mobile phase in a tank saturated with mobile phase (about 45 minutes). Remove the plate from the tank and allow the solvent to evaporate. View the plate under UV light (254 nm) and identify and mark the area of the active ingredient (R_F about 0·2).

Remove the silica gel at the marked zone with a spatula. Transfer the removed silica gel to a Pasteur pipette containing a wad of chemical pure cotton wool. Cover the silica gel column with a wad of cotton wool. Put methanol (2 ml) onto the top of the column and collect the first 5 to 10 drops in a vial containing potassium bromide (about 200 mg). Evaporate the solvent, dry, and use the material for preparing a disc for IR, see 2.2 above.

3 Tebuconazole

OUTLINE OF METHOD The sample is dissolved in acetone containing dicyclohexyl phthalate as internal standard. Tebuconazole is determined with capillary gas chromatography in the split injection mode with flame ionisation detection.

REAGENTS

Acetone

Tebuconazole standard of known purity (purity at least 950 g/kg). Store at 4 - 8 °C. Equilibrate to room temperature before opening the storing vessel.

Dicyclohexyl phthalate internal standard. Before use, confirm that no interfering impurities are present near the retention time of tebuconazole.

Internal standard solution. Prepare a solution of 5g/l dicyclohexyl phthalate in acetone. The solution is stable for one month when stored at room temperature.

Calibration solution. Homogenise the tebuconazole standard. Then weigh (to the nearest 0.1 mg) into a bottle equipped with a polyethylene-lined cap (50 ml) 90 -110 mg (*s* mg) tebuconazole. Add by pipette internal standard solution (20.0 ml), cap and mix well. The solution is stable for up to 48 h when stored at about 20 °C.

APPARATUS

Gas chromatograph equipped with a flame ionisation detector

Column 5 m × 0.53 mm i.d., open fused silica column coated with methyl silicone; film thickness 5 µ. *Note:* Other lengths and film thicknesses are acceptable, but will require flow and/or temperature adjustments to obtain the specified retention times

Automatic digital integrator or *chromatography data system*

Ultrasonic bath

Filter PTFE, porosity 0.45µm

PROCEDURE

(a) Operating conditions (typical)

Column	
Material	fused silica
Dimensions	5 m × 0.53 mm i.d.
Injector system	
Injector	split mode
Injector temperature	300 °C
Split ratio	10:1 (minimum)
Injection volume	1 µl

Detector system	
Type	FID
Range	2^4
Attenuation	2^5
Oven temperature	240 °C
Gas flow rates	
Helium (carrier)	7 ml/min
Helium (make up)	25 ml/min
Hydrogen	30 ml/min
Air	240 ml/min
Retention times	tebuconazole: about 2.5 min dioctyl phthalate: about 3.5 min

(b) Preparation of the sample solution. Weigh (to the nearest 0.1 mg) into a bottle (50 ml) equipped with a polyethylene cap enough sample to contain about 100 mg (*w* mg) tebuconazole. Add by pipette internal standard solution (20.0 ml), cap, place the bottle in an ultrasonic bath for 60 s, and mix well. Allow to settle. Transfer an aliquot of the clear supernatant liquid to an autosampler vial. If a clear supernatant liquid cannot be obtained after settling, filter a portion of the test sample solution through a 0.45 µm filter into the autosampler vial.

(c) Equilibration of the system. Adjust the column temperature and/or carrier gas rate so that the retention time of the tebuconazole peak falls within 2 to 4min. The dicyclohexyl phthalate peak should elute at a minimum of 0.5 min after the tebuconazole peak, with baseline separation. Unacceptable resolution between tebuconazole and dicyclohexyl phthalate, or from liquid formulation components, is most likely caused by a high flow rate of the carrier gas. Replace the GC column if problems persist. Make several injections of the calibration solution. Response ratios (R') must agree within 1 % for two consecutive injections.

(d)Determination. Inject duplicate aliquots of the test sample (no more than three samples i.e. 6 injections) between the injections of the calibration solution. Response ratios of the sample injections (R) must agree within 1%. Otherwise repeat the analysis, starting with injections of the calibration solution. Average the response ratios of the calibration solution injections immediately preceding and following the sample solution injections. The bracketing calibration solution injections must agree within 1 %. Repeat any part of the analysis that does not meet this criterion.

(e)Calculation. Calculate the response ratio of the calibration solution as follows:

$$R' = \frac{H_s}{I_r}$$

$$R = \frac{H_w}{I_q}$$

where:

H_s = area of the tebuconazole peak in the calibration solution
H_w = area of the tebuconazole peak in the sample solution
I_r = area of the internal standard peak in the calibration solution
I_q = area of the internal standard peak in the sample solution

$$\text{Content of tebuconazole} = \frac{R'_a \times s \times P}{R_a \times w} \text{ g/kg}$$

where:

R_a = average of the two response ratios of the calibration solution injections
R'_a = average of the two response ratios of the sample solution injections
s = mass of tebuconazole in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of the tebuconazole standard (g/kg)

Repeatability r = 4 g/kg at 216 g/kg active ingredient content
Reproducibility R = 6 g/kg at 216 g/kg active ingredient content

TEBUCONAZOLE WATER DISPERSIBLE GRANULES

[*] **494**/WG/(M)/-

1 Sampling. Take at least 500 g.

2 Identity tests. As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole. As for tebuconazole wettable powders **494**/WP/(M)/3.

Repeatability r = 6 g/kg at 457 g/kg active ingredient content
= 4 g/kg at 263 g/kg active ingredient content
Reproducibilty R = 9 g/kg at 457 g/kg active ingredient content
= 5 g/kg at 263 g/kg active ingredient content

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[*] Provisional AOAC-CIPAC method 1997.

TEBUCONAZOLE OIL IN WATER EMUSIONS

[*] **494**/EW/(M)/-

1 Sampling. Take at least 500 ml.

2 Identity tests. As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole. As for tebuconazole wettable powders **494**/WP/(M)/3.

Repeatability.r = 4 g/kg at 262 g/kg active ingredient content
Reproducibility R = 8 g/kg at 262 g/kg active ingredient content

TEBUCONAZOLE SUSPENSION CONCENTRATES

[*]**494**/SC/(M)/-

1 Sampling. Take at least 1l.

2 Identity tests. As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole. As for tebuconazole wettable powders **494**/WP/(M)/3.

Repeatability r = 8 g/kg at 400 g/kg active ingredient content
Reproducibility R = 14 g/kg at 400 g/kg active ingredient content

TEBUCONAZOLE FLOWABLE CONCENTRATES FOR SEED TREATMENT

[*]**494**/FS(M)/-

1 Sampling. Take at least 1l.
2 Identity tests. As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole. As for tebuconazole wettable powders **494**/WP/(M)/3 except:

APPARATUS

Automatic digital integrator or *chromatography data system* suitable for manual electronic integration

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[*] Provisional AOAC-CIPAC method 1997.

and add at: *(d) Determination*
Note: Analyses of flowable seed treatment formulations are subject to potential integration interferences. Peak areas may require manual electronic integration.

Repeatability r = 0.8 g/kg at 15.5 g/kg active ingredient content
Reproducibility R = 1.7 g/kg at 15.5 g/kg active ingredient content

TEBUCONAZOLE EMULSIONS FOR SEED TREATMENT

[*] **494**/ES(M)/-

1 Sampling. Take at least 1l.

2 Identity tests. As for tebuconazole wettable powders **494**/WP/(M)/2.

3 Tebuconazole. As for tebuconazole wettable powders **494**/WP/(M)/3

Reproducibility R = 1.0 g/kg at 23.6 g/kg active ingredient content
Repeatability r = 0.6 g/kg at 23.6 g/kg active ingredient content

Figure 25 to be included

Fig. 25 Infrared spectra of the different modifications of tebuconazole

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[*] Provisional AOAC-CIPAC method 1997.