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**Trifloxystrobin**

**617**

**TRIFLOXYSTROBIN**

**617**

**Chemical name: methyl (E)-methoxyimino-{(E)-α-[1-(α,α,α-trifluoro-m-
 tolyl)ethylideneaminooxy]-o-tolyl}acetate**

**ISO common name: Trifloxystrobin**

**CAS-No.:** 141517-21-7

**Structure:**



**Molecular mass: 408.4**

**Empirical formula: C20 H19 F3 N2 O4**

**m.p.: 72.9 °C**

**b.p.: Decomposition occurs at about 285°C before boiling point is reached**

**Solubility: acetone, dichloromethane, ethylacetate > 500 g/L;
 methanol 76 g/L (all at 25°C)**

**Description: The technical product is a gray white powder.**

**TRIFLOXYSTROBIN TECHNICAL**

**617/TC/M/-**

**1 Sampling. Take at least 100 g. Grind the sample thoroughly in a mortar.**

**2 Identity tests**

**2.1 HPLC. Use the HPLC method described below. The relative retention time of Trifloxystrobin in the sample solution should not deviate by more than 2% from that of the calibration solution.**

**2.2 UV spectrometry.** Record the UV spectrum during the HPLC determination. The UV spectrum obtained from the sample should not differ significantly from that of the standard. (Fig. 1)

**2.2 Infrared.** Prepare by direct application pure trifloxystrobin and the sample onto the diamond probe of a “Golden Gate” accessory. Scan from 4000 to 650 cm-1. The spectrum produced from the sample should not differ significantly from that of the standard. (Fig. 2)

**3 Trifloxystrobin**

**OUTLINE OF THE METHOD.**

**Trifloxystrobin content is determined (g/kg) by reversed phase high performance liquid chromatography using UV detection at 280 nm and external standard calibration.**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

**REAGENTS**

**Trifloxystrobin reference standard of known content**

**Acetonitrile (HPLC grade)**

**Phosphoric acid 85 % (puriss. p. a.)**

**Purified water (HPLC grade)**

**Eluent A: 10 mMol phosphoric acid in 1 L purified water**

**Eluent B: acetonitrile**

**APPARATUS**

**High performance liquid chromatograph equipped with an injection system capable to inject 3 µl1 and an UV spectrophotometric detector operated at 280 nm.**

**Liquid chromatography column, stainless steel, 50 x 4.6 (i.d.) mm, packed with Kinetex C 18; 2.6 µm or equivalent with the same selectivity.**

**Electronic integrator or data system**

**Ultrasonic bath**

**PROCEDURE**

**(a) *Operating conditions* (typical):**

 Column temperature: 50°C

 Injection volume: 3 µl1

 Detector wavelength: 280 nm

 **Mobile phase and Flow rate:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Time [min]** | **10 mMol phosphoric acid in 1 L purified water [%v/v]** | **Acetonitrile****[%v/v]** | **Flow rate[ml/min]** |
| **0.0** | **40** | **60** | **2** |
| **1.6** | **40** | **60** | **2** |
| **1.7** | **05** | **95** | **3** |
| **2.5** | **05** | **95** | **3** |
| **2.6** | **40** | **60** | **3** |
| **2.9** | **40** | **60** | **2** |
| **3.5** | **40** | **60** | **2** |

 Retention time: approximately 1.3 min

(b) *Equilibration of the system*. Pump sufficient mobile phase through the column to equilibrate the system. Inject 3 µl1 portions of the calibration solution C1 and repeat the injections until retention times and peak areas deviate by less than ± 1 % from the mean for three successive injections.

*(c) Calibration solution.* Weigh in duplicate (to the nearest 0.1 mg) approximately 50 mg (*s* mg) of the trifloxystrobin reference standard into separate volumetric flasks (100 ml). Add 90 ml acetonitrile and place the flasks in an ultrasonic bath for 15 min2. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly (Calibration solutions C1, C2) (Fig. 3).

*(d) Sample preparation.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 50 mg (*w* mg) of trifloxystrobin reference standard into separate volumetric flasks (100 ml). Add 90 ml acetonitrile and place the flasks in an ultrasonic bath for 15 min2. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly (Sample solutions S1, S2 for TC 1 and S3, S4 for TC 2) (Fig. 4).

*(e) Determination.* Inject in duplicate each sample solution and bracket a series of sample solution injections by injections of the calibration solutions as follows: calibration solution 1, calibration solution 2, calibration solution 1, sample solution 1, sample solution 1, sample solution 2, sample solution 2, calibration solution 1, … (C1, C2, C1, S1, S1, S2, S2, C1, …).

Determine the peak areas of trifloxystrobin.

*(f) Calculation*

Calculate the response factors from the calibration solutions bracketing the injections of the sample solutions. Average the response factors of the calibration solutions preceding and following the sample solution injections. These must agree within ±1 % of the average otherwise repeat the determination. Calculate the content of the sample solutions.

= 

Trifloxystrobin content (g/kg) = 

Where:

fi = single response factor

f = average response factor

HS = peak area of trifloxystrobin standard in the calibration solution

HW = peak area of trifloxystrobin in the sample solution

s = weight of the trifloxystrobin standard in the calibration solution (mg)

w = weight of the sample (mg)

P = purity of the trifloxystrobin standard (g/kg)

**Repeatability r** = 8.90 to 9.28 g/kg at 980 g/kg active ingredient content

**Reproducibility R** = 11.91 to 13.38 g/kg at 980 g/kg active ingredient content

**TRIFLOXYSTROBIN ANY OTHER LIQUID**

**617/AL/M/-**

**1 Sampling.** Take at least 500 ml. Shake the sample well prior to weighing.

**2 Identity tests.**

**2.1 HPLC**. As for Trifloxystrobin 617/TC/M/-

**2.2 UV spectrometry**. As for Trifloxystrobin 617/TC/M/-

**3 Trifloxystrobin.**

**Same approach as for Trifloxystrobin 617/TC/M/-**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

As for Trifloxystrobin 617/TC/M/- except

Disposable PTFE syringe filter compatible with organic solvents and a 0.45 µm pore diameter or centrifuge.

*(d) Sample preparation.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 2.5 mg (*w* mg) of trifloxystrobin reference standard into separate volumetric flasks (50 ml). Add 20 ml acetonitrile, make up the flasks with acetonitrile to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile and mix thoroughly. Filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S5, S6) (Fig. 5).

**Repeatability r** = 0.0045 g/kg at 0.125 g/kg active ingredient content

**Reproducibility R** = 0.0048 g/kg at 0.125 g/kg active ingredient content

**TRIFLOXYSTROBIN EMULSIFIABLE CONCENTRATE**

**617/EC/M/-**

**1 Sampling.** Take at least 500 ml. Shake the sample well prior to weighing.

**2 Identity tests.**

**2.1 HPLC**. As for Trifloxystrobin 617/TC/M/-

**2.2 UV spectrometry**. As for Trifloxystrobin 617/TC/M/-

**3 Trifloxystrobin.**

**Same approach as for Trifloxystrobin 617/TC/M/-**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

**As for Trifloxystrobin 617/AL/M/- except**

*(d) Sample preparation.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 50 mg (*w* mg) of trifloxystrobin reference standard into separate volumetric flasks (100 ml). Add 90 ml acetonitrile and place the flasks in an ultrasonic bath for 15 min2. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S7, S8) (Fig. 6).

**Repeatability r** = 0.96 g/kg at 137.5 g/kg active ingredient content

**Reproducibility R** = 1.67 g/kg at 137.5 g/kg active ingredient content

**TRIFLOXYSTROBIN FLOWABLE CONCENTRATE FOR SEED TREATMENT**

**617/FS/M/-**

**1 Sampling.** Take at least 500 ml. Shake the sample well prior to weighing.

**2 Identity tests.**

**2.1 HPLC**. As for Trifloxystrobin 617/TC/M/-

**2.2 UV spectrometry**. As for Trifloxystrobin 617/TC/M/-

**3 Trifloxystrobin.**

**Same approach as for Trifloxystrobin 617/TC/M/-**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

**As for Trifloxystrobin 617/AL/M/- except**

*(d) Sample preparation.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 50 mg (*w* mg) of trifloxystrobin reference standard into separate volumetric flasks (100 ml). Add 5 ml purified water and 90 ml acetonitrile. Place the flasks in an ultrasonic bath for 15 min2. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with purified water and mix thoroughly. Filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S9, S10) (Fig. 7)

**Repeatability r** = 1.91 g/kg at 261.6 g/kg active ingredient content

**Reproducibility R** = 2.77 g/kg at 261.6 g/kg active ingredient content

**4 Suspensibility**

**REAGENTS AND APPARATUS as for MT 184**

**PROCEDURE**

***(a)* *Preparation of suspension and determination of sedimatation. MT 184.***

***(b)*** Determination of trifloxystrobin in the bottom 25 ml of suspension: After removal of the top 225 ml of suspension transfer by pipette 0.5 ml\* out of the remaining and well homogenized 25 ml to a 100 ml volumetric flask and add approx. 50 ml acetonitrile. To dissolve the substance, place the flask in an ultrasonic bath for 15 min and make up the flask with acetonitrile to just below the calibration mark. Wait until the temperature has stabilized, then fill the flask up to the calibration mark with acetonitrile. Determine the mass of trifloxystrobin (Q g) by 617/TC/M/-).

**\* This is calculated for a** 50% suspension, in case of other concentrations the volume has to be adjusted but it has to be ensured to work within the linear range.

**Alternatively a sample weight of 500 mg of the remaining suspension can be used instead of 0.5 ml.**

**TRIFLOXYSTROBIN SUSPENSION CONCENTRATE**

**617/SC/M/-**

**1 Sampling.** Take at least 500 ml. Shake the sample well prior to weighing.

**2 Identity tests.**

**2.1 HPLC**. As for Trifloxystrobin 617/TC/M/-

**2.2 UV spectrometry**. As for Trifloxystrobin 617/TC/M/-

**3 Trifloxystrobin.**

**Same approach as for Trifloxystrobin 617/TC/M/-**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

**Same approach as for Trifloxystrobin 617/FS/M/-**

(Sample solutions S11, S12) (Fig. 8)

**Repeatability r** = 2.90 g/kg at 570 g/kg active ingredient content

**Reproducibility R** = 6.97 g/kg at 570 g/kg active ingredient content

**4 Suspensibility**

**REAGENTS AND APPARATUS as for MT 184**

**PROCEDURE**

***(a)* *Preparation of suspension and determination of sedimatation. MT 184.***

***(b)*** Determination of trifloxystrobin in the bottom 25 ml of suspension: After removal of the top 225 ml of suspension transfer by pipette 0.5 ml\* out of the remaining and well homogenized 25 ml to a 100 ml volumetric flask and add approx. 50 ml acetonitrile. To dissolve the substance, place the flask in an ultrasonic bath for 15 min and make up the flask with acetonitrile to just below the calibration mark. Wait until the temperature has stabilized, then fill the flask up to the calibration mark with acetonitrile. Determine the mass of trifloxystrobin (Q g) by 617/TC/M/-).

**\* This is calculated for a** 50% suspension, in case of other concentrations the volume has to be adjusted but it has to be ensured to work within the linear range.

**Alternatively, a sample weight of 500 mg of the remaining suspension can be used instead of 0.5 ml.**

**TRIFLOXYSTROBIN WATER DISPERSABLE GRANULE**

**617/WG/M/-**

**1 Sampling.** Take at least 500 g. **Grind the sample thoroughly in a mortar.**

**2 Identity tests.**

**2.1 HPLC**. As for Trifloxystrobin 617/TC/M/-

**2.2 UV spectrometry**. As for Trifloxystrobin 617/TC/M/-

**3 Trifloxystrobin.**

**Same approach as for Trifloxystrobin 617/TC/M/-**

**3.1 Determination of Trifloxystrobin by reversed phase HPLC**

**Same approach as for Trifloxystrobin 617/FS/M/-**

(Sample solutions S13, S14) (Fig. 9)

**Repeatability r** = 3.53 g/kg at 500 g/kg active ingredient content

**Reproducibility R** = 6.50 g/kg at 500 g/kg active ingredient content

**4 Suspensibility. As for Trifloxystrobin 617/FS/M/- except**

*(b)* Determination of trifloxystrobin in the bottom 25 ml of suspension. After removal of the top 225 ml of suspension transfer by pipette 5 ml\* out of the remaining and well homogenized 25 ml to a 100 ml volumetric flask and add approx. 50 ml acetonitrile. To dissolve the substance, place the flask in an ultrasonic bath for 15 min and make up the flask with acetonitrile to just below the calibration mark. Wait until the temperature has stabilized, then fill the flask up to the calibration mark with acetonitrile. Determine the mass of trifloxystrobin (Q g) by 617/TC/M/-)

**\* This is calculated for a** 2% suspension, in case of other concentrations the volume has to be adjusted but it has to be ensured to work within the linear range.

**Alternatively, a sample weight of 5000 mg of the remaining suspension can be used instead of 5 ml.**

Note 1: Injection volume of 5 µl is also possible and is covered with the linear range.

Note 2: Ultrasonication can be reduced to 5 min or even avoided in case of complete solubility.

-10.0

0.0

12.5

25.0

37.5

50.0

60.0

200

220

240

260

280

300

320

340

360

380

400

%

nm

**Fig. 1 UV-Spectrum of Trifloxystrobin**



**Fig. 2 Infrared Spectrum of Trifloxystrobin**



**Fig. 3 Chromatogram of Trifloxystrobin Analytical Standard**



**Fig. 4 Chromatogram of Trifloxystrobin TC**



**Fig. 5 Chromatogram of Trifloxystrobin AL**



**Fig. 6 Chromatogram of Trifloxystrobin EC**



**Fig. 7 Chromatogram of Trifloxystrobin FS**



**Fig. 8 Chromatogram of Trifloxystrobin SC**



**Fig. 9 Chromatogram of Trifloxystrobin WG**