Trifloxystrobin

Rel. Impurity
CGA 344605

HPLC-UV Method 5289/m

CIPAC Peer Validation

by

Friedhelm Schulz
Bayer AG
Research & Development, Crop Science
Formulation Analytics 1
Building 6820, 112
40789 Monheim,
Germany

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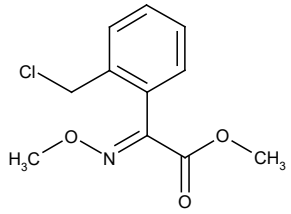
TRIFLOXYSTROBIN, relevant impurity

CGA 344605

ISO common name:	CGA 344605
Chemical name:	methyl (E)-(2-chloromethylphenyl)-methoxy-imino-acetate
CAS-No.:	189813-45-4
Structure:	



Empirical formula:	$C_{11}H_{12}Cl_1N_1O_3$
Molecular mass:	241.68 g/mol

TRIFLOXYSTROBIN TECHNICAL
617/TC/M/-

1 Sampling. Take at least 100 g.

2 Identity tests

2.1 HPLC-UV. Use the HPLC method described below. The relative retention time of CGA 344605 in the sample solution should not deviate by more than 5% from that of the calibration solution. The UV-Spectrum obtained from the sample and reference item should not differ significantly.

3 CGA 344605

OUTLINE OF METHOD

The content of CGA 344605 (% w/w) is determined by reversed phase high performance liquid chromatography using UV detection and external standard calibration.

REAGENTS

CGA 344605 reference standard of known content
Acetonitrile (HPLC grade or higher)
Purified water (HPLC grade or higher)
Phosphoric acid, 85%
Eluent A: purified water + 0.1% (v/v) phosphoric acid
Eluent B: acetonitrile+ 0.1% (v/v) phosphoric acid

APPARATUS

High performance liquid chromatograph equipped with an injection system capable to inject 5 µL and an UV/VIS or DAD detector.
Chromatographic column, stainless steel, 100 x 4.6 (i.d.) mm, packed with Ascentis Express C18; 2.7 µm or equivalent with the same selectivity.
Data system
Ultrasonic bath
Centrifuge

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Temperature 35 °C
Injection volume 5 µL

Mobile phase and Flow rate

Time [min]	purified water + 0.1% (*v/v*) phosphoric acid	acetonitrile+ 0.1% (*v/v*) phosphoric acid	Flow rate [mL/min]
0.0	54	46	1.8
6.0	40	60	1.8
6.2	5	95	1.8
8.0	5	95	1.8
8.2	54	46	1.8
13.0	54	46	1.8

Retention time: approximately 1.7 minutes
Measurement wavelength 210 nm

(b) Equilibration of the system.

Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µL portions of the calibration solution Cx (see below) and repeat the injections until retention times and peak areas deviate by less than ± 1 % from the mean for three successive injections.

(c) Preparation of calibration solutions

To prepare a stock solution, weigh (to the nearest 0.01 mg) 20 – 30 mg of the reference item CGA 344605 (*s* in mg) into a volumetric flask (50.0 mL) and add acetonitrile (approx. 40 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the reference item. Following, fill up the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mix thoroughly.

Prepare the calibration solutions either as described above or by successive dilution of the respective stock or calibration solutions with acetonitrile.

Prepare at least two independent stock solutions for preparation of the calibration solutions.

A single-point calibration can be used, if the CGA 344605 content in the sample is known (calibration solution C1 and calibration check solution C2).

For samples with varying or unknown CGA 344605 content, a multi-point calibration curve should be used, covering a suitable concentration range of the maximum expected CGA 344605 content in the sample solution, e.g. 10, 50, 100 and 120% of the max. expected CGA 344605 content (calibration solutions solutions C1, C2, C3,…, Cn).

(d) Preparation of sample
Weigh (at least to the nearest 0.1 mg) an amount (*w* in mg) of homogeneous sample containing approx. 50 mg of the active substance trifloxystrobin into a volumetric flask (50.0 mL) and add acetonitrile (approx. 40 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the sample. Following, fill up the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mix thoroughly. If necessary, clarify a part of the solution by centrifugation or filtration prior to analysis (sample solutions S1 and S2)

(e) Determination
Single-point calibration: Inject a blank solution (e.g. sample solvent) and inject 5 times the calibration solution before bracketing a series of sample solution by injections of a calibration check as follows:
- blank B,
- calibration solution C1 x 5
- calibration check C2
- sample solution S1,
- sample solution S2,
- calibration check C1 or C2
... (B, C1-C1, C2, S1, S2, C2, …)

Multi-point calibration curve: Inject a blank solution (e.g. sample solvent), the calibration curve solutions and each sample solution (single injections or in duplicate) and bracket a series of sample solutions by injecting a calibration check as follows:
- blank B,
- calibration curve C1 – Cn,
- calibration check Cx
- sample solution S1,
- sample solution S2,
- calibration check Cx
... (B, C1 – Cn, Cx, S1, S2, Cx, …)

Determine the peak area of CGA 344605.

(f) Calculation.
For each sample solution, calculate the content of CGA 344605.

Single-point calibration curve:
Calculate the response factors of the calibration solutions. Average the response factors of the calibration solutions. These must agree within ± 2 % of the average, otherwise repeat the determination.
Calculate the content of the sample solutions

$$fi = \frac{Hs \cdot 100}{s \cdot P}$$

$$\text{CGA 344605 [\% } (w/w)] = \frac{Hw \cdot 100}{w \cdot f}$$

Where

fi	= single response factor
Hs	= area of CGA 344605 in calibration solution
s	= mass of reference item CGA 344605 in the calibration solution (mg)
P	= purity of CGA 344605 reference item [% (w/w)]
CGA 344605 [% (w/w)]	= concentration of CGA 344605in the sample, e.g. *[% (w/w)]*
H*w*	= area of CGA 344605 in sample solution
w=	= mass of sample taken (mg)
f =	= average response factor

Multi-point calibration curve:
Calculate the calibration function by plotting the resulting peak area of the analyte versus the nominal concentration of the analyte in calibration solution. The calibration function is obtained using preferably linear regression (1st order). If necessary, quadratic regression functions (2nd order) can also be used.
Calculate the analyte content in the sample solution using the calibration function and the determined peak area of the analyte in sample solution.
Calculate the analyte content in the sample, expressed in weight percent [% (*w/w*)], considering the total sample weight:

$$Hs = m \cdot x + b$$

$$\text{CGA 344605 } [mg/l] = \frac{(Hw - b)}{m}$$

$$\text{CGA 344605 } [\%\ (w/w)] = \frac{\text{CGA 344605 } [mg/l]}{c_w [mg/l]} \cdot 100\%\ (w/w)$$

Where

Hs	= area of CGA 344605 in calibration solution
m	= slope of calibration function
x	= C_S = concentration of CGA 344605 in calibration solution, e.g. [mg/l]
b	= intercept of calibration function
CGA 344605 *[mg/l]*	= concentration of CGA 344605in the sample solution, e.g. [mg/l]
Hw	= area of CGA 344605 in sample solution
CGA 344605 [% (w/w)]	= concentration of CGA 344605in the sample, e.g. *[% (w/w)]*
Cw [mg/l]	= concentration of sample in sample solution e.g. [mg/l]

TRIFLOXYSTROBIN EMULSIFIABLE CONCENTRATE 617/EC/M/-

1 Sampling

As for trifloxystrobin technical concentrate 617/TC/M/-

2 Identity tests

As for trifloxystrobin technical concentrate 617/TC/M/-

3 CGA 344605

As for trifloxystrobin technical concentrate 617/TC/M/-

TRIFLOXYSTROBIN WATER DISPERSIBLE GRANULES 617/WG/M/-

1 Sampling

As for trifloxystrobin technical concentrate 617/TC/M/-

2 Identity tests

As for trifloxystrobin technical concentrate 617/TC/M/-

3 CGA 344605

As for trifloxystrobin technical concentrate 617/TC/M/- except

substitute the points *(c) Preparation of calibration solutions and (d) Preparation of sample* for the following paragraphs:

(c) Preparation of calibration solutions
To prepare a stock solution, weigh (to the nearest 0.01 mg) 20 – 30 mg of the reference item CGA 344605 (*s* in mg) into a volumetric flask (50.0 mL) and add water (10 mL) and acetonitrile (approx. 30 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the reference item. Following, fill up the flask with the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mixed thoroughly.

Prepare the calibration solutions either as described above or by successive dilution of the respective stock or calibration solutions with 80% acetonitrile and 20% water (*v/v*).

Prepare at least two independent stock solutions for preparation of the calibration solutions.

A single-point calibration can be used, if the CGA 344605 content in the sample is known (calibration solution C1 and calibration check solution C2).
For samples with varying or unknown CGA 344605 content a multi-point calibration curve should be used, covering a suitable concentration range of the maximum expected CGA 344605 content in the sample solution, e.g. 10, 50, 100 and 120% of the max. expected CGA 344605 content (calibration solutions C1, C2, C3,…, Cn).

(d) Preparation of sample.
Weigh (at least to the nearest 0.1 mg) an amount (*w* in mg) of homogeneous sample containing approx. 50 mg of the active substance trifloxystrobin into a volumetric flask (50.0 mL) and add water to suspend the sample (10 mL) and acetonitrile (approx. 30 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the sample. Following, fill up the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mix thoroughly. If necessary, clarify a part of the solution by centrifugation or filtration prior to analysis (sample solutions S1 and S2).

TRIFLOXYSTROBIN SUSPENSION CONCENTRATE 617/SC/M/-

1 Sampling

As for trifloxystrobin technical concentrate 617/TC/M/-

2 Identity tests

As for trifloxystrobin technical concentrate 617/TC/M/-

3 CGA 344605

As for trifloxystrobin technical concentrate 617/TC/M/-

substitute the points *(c) Preparation of calibration solutions and (d) Preparation of sample* for the following paragraphs:

(c) Preparation of calibration solutions
To prepare a stock solution, weigh (to the nearest 0.01 mg) 20 – 30 mg of the reference item CGA 344605 (*s* in mg) into a volumetric flask (50.0 mL) and add water (10 mL) and acetonitrile (approx. 30 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the reference item. Following, fill up the flask with the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mixed thoroughly.

Prepare the calibration solutions either as described above or by successive dilution of the respective stock or calibration solutions with 80% acetonitrile and 20% water (*v/v*).

Prepare at least two independent stock solutions for preparation of the calibration solutions.

A single-point calibration can be used, if the CGA 344605 content in the sample is known (calibration solution C1 and calibration check solution C2).
For samples with varying or unknown CGA 344605 content a multi-point calibration curve should be used, covering a suitable concentration range of the maximum expected CGA 344605 content in the sample solution, e.g. 10, 50, 100 and 120% of the max. expected CGA 344605 content (calibration solutions C1, C2, C3,…, Cn).

(d) Preparation of sample.
Weigh (at least to the nearest 0.1 mg) an amount (*w* in mg) of homogeneous sample containing approx. 50 mg of the active substance trifloxystrobin into a volumetric flask (50.0 mL) and add water to suspend the sample (10 mL) and acetonitrile (approx. 30 mL). Place the flask in an ultrasonic bath for about 10 minutes to dissolve the sample. Following, fill up the flask with acetonitrile to just below the calibration mark. After the temperature is equilibrated, fill up the flask to the calibration mark with acetonitrile and mix thoroughly. If necessary, clarify a part of the solution by centrifugation or filtration prior to analysis (sample solutions S1 and S2).

Fig. 1 UV Spectrum of CGA 344605

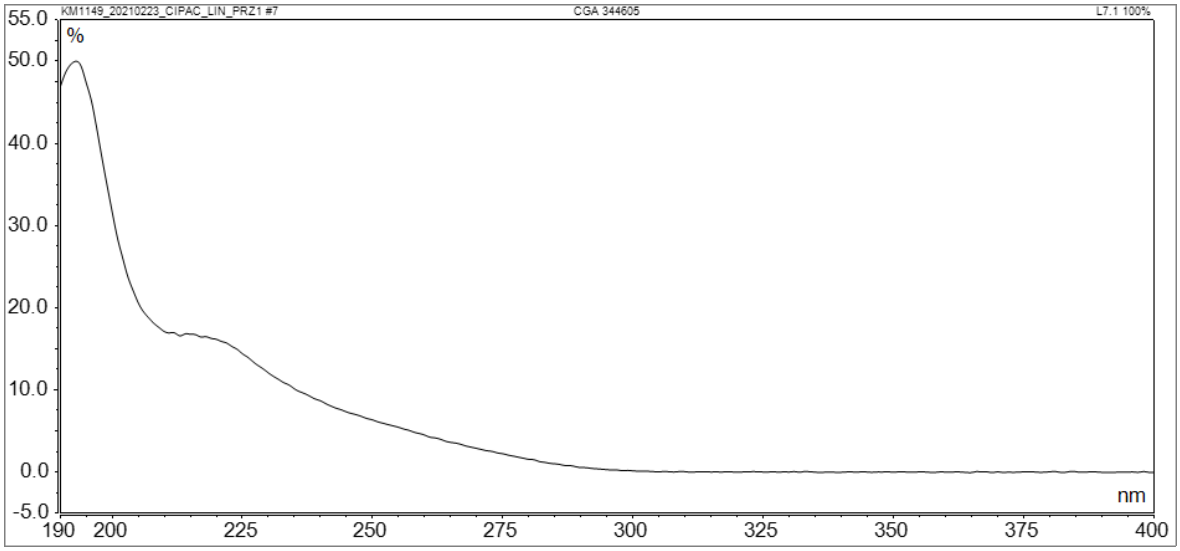


Fig. 2 Chromatogram of Analytical Standard CGA 344605

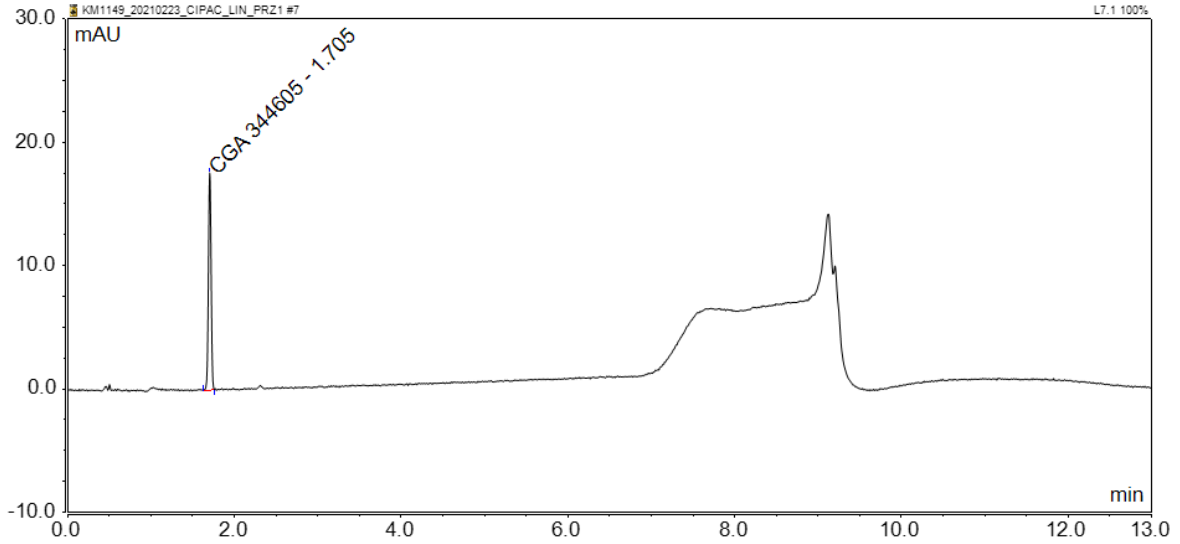


Fig. 3 Chromatogram of Trifloxistrobin TC, spiked with CGA 344605

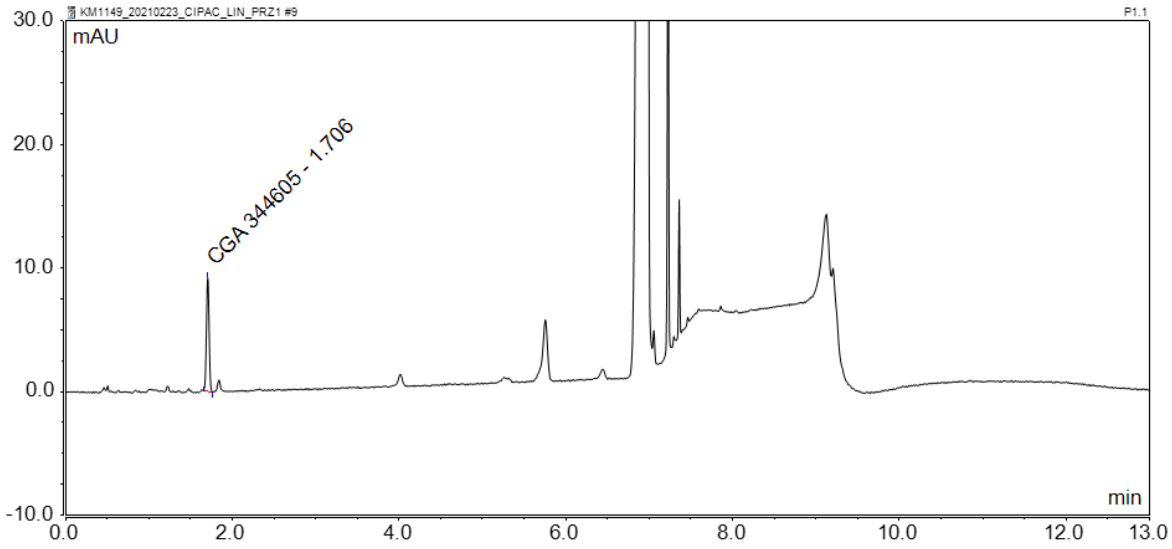


Fig. 4 Chromatogram of Trifloxistrobin EC, spiked with CGA 344605

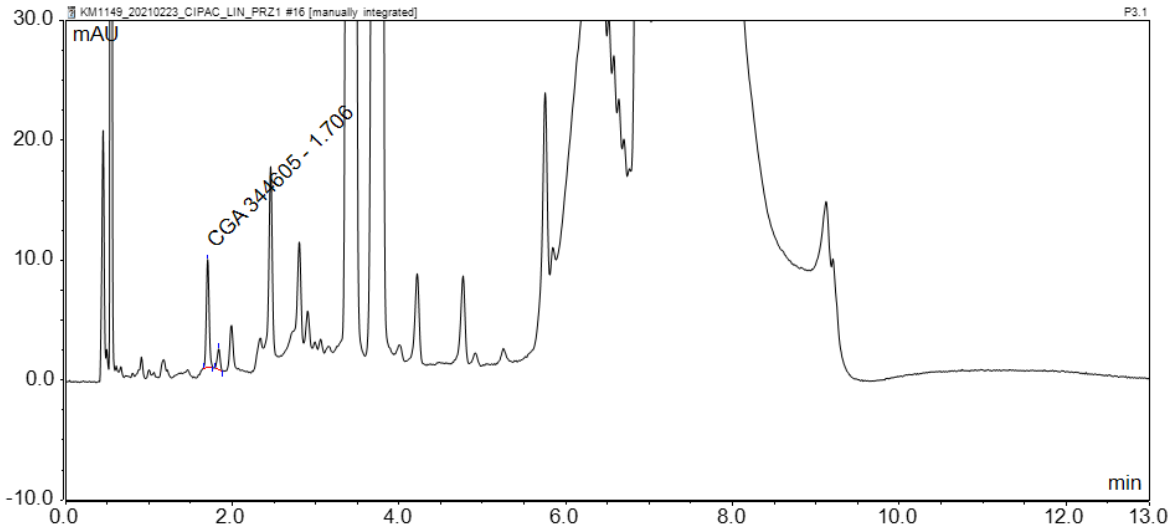


Fig. 5.Chromatogram of Trifloxistrobin WG

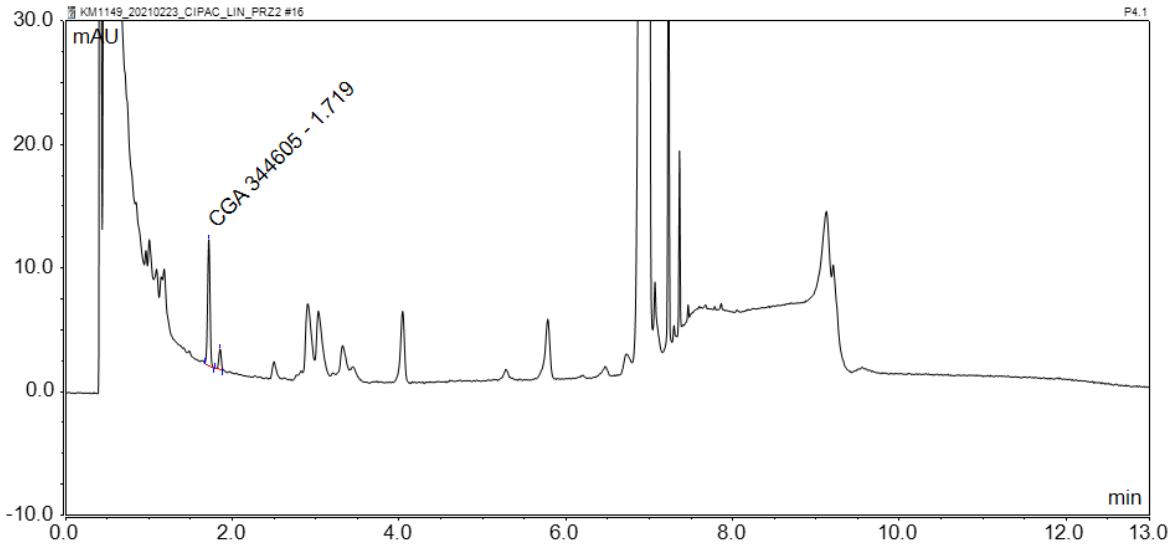


Fig. 6. Chromatogram of Trifloxistrobin SC, spiked with CGA 344605