CIPAC

COLLABORATIVE INTERNATIONAL PESTICIDES ANALYTICAL COUNCIL LIMITED

Commission Internationale des Méthodes d'Analyse des Pesticides (CIMAP)

CIPAC Free relevant impurities methods:

Methods for relevant impurities becoming more and more important in the quality control of TK/TC and FAO-specifications. In order to meet an urgent need for methods to characterize TK/TC in a.i. and formulations, CIPAC provides selected methods as download. By downloading these methods, you accept the following conditions of use.

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PROTHIOCONAZOLE

745

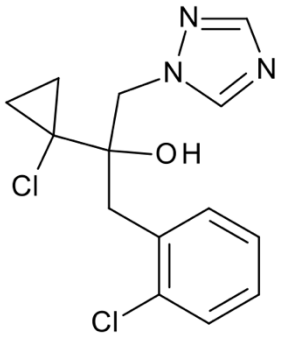
relevant impurity PROTHIOCONAZOLE-DESTHIO

ISO common name: Prothioconazole-desthio

Chemical name: 2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol

CAS No. 120983-64-4

Structure:



Empirical formula: $C_{14}H_{15}Cl_2N_3O$

Molecular mass: 312.2 g/mol

PROTHIOCONAZOLE

745

See CIPAC P page XXX

PROTHIOCONAZOLE TECHNICAL
*745/TC/M/-

1 Sampling. Ensure that all samples taken for analysis are stored closed, with minimal headspace volume and minimal exposure to light until analysis. Take at least 100 g.

2 Identity tests

2.1 HPLC-MS/MS. Use the HPLC-MS/MS method described below. The relative retention time of prothioconazole-desthio in the sample solution should not deviate by more than 5% from that of the calibration solution. Two MRM transition (quantifier and qualifiers ion) in calibration solutions and in sample solutions will be measured.

4 Prothioconazole-desthio

OUTLINE OF METHOD

The content of prothioconazole-desthio (% w/w) is determined by reversed phase high performance liquid chromatography with ESI+ MS/MS detector and external standard calibration.

* CIPAC method 2020. Based on a method supplied by Bayer Crop Science, Germany

REAGENTS

Prothioconazole-desthio, reference standard of known content

Acetonitrile, (HPLC grade or higher)

Formic acid, conc., for analysis

A.R. Purified water, (HPLC grade or higher)

L-Cysteine hydrochloride monohydrate

Eluent A: 1 L purified water + 0.1 mL formic acid

Eluent B: 1 L acetonitrile + 0.1 mL formic acid

Dilution solution: 5 mg L-Cysteine hydrochloride monohydrate in 100 mL of purified water/ acetonitrile 50/50 (v/ v)

APPARATUS

High performance liquid chromatograph equipped with an injection system capable to inject 5 µL and an ESI+ MS/MS detector in MRM mode.

Chromatographic column, stainless steel, 50 x 4.6 (i.d.) mm, packed with X-Terra RP 18; 3.5 µm or equivalent with the same selectivity.

Data system

Ultrasonic bath

Centrifuge

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Temperature 40 °C

Injection volume 5 µL

Mobile phase and Flow rate

Time [min]	1L purified water + 0.1 mL formic acid	1L acetonitrile + 0.1 mL formic acid	Flow rate [mL/min]
0.0	50	50	0.5
5.0	50	50	0.5
6.0	05	95	0.5
11.0	05	95	0.5
11.5	50	50	0.5
16.0	50	50	0.5

Retention time: approximately 3.7 minutes

MS/MS conditions

Ionization: ESI+ (electrospray ionization in positive detection mode)
Ion source: Turbo spray
Detection: MRM (multiple reaction monitoring)
MRM transition (quantifier): m/z 312→ 70 Da Dwell time:245 ms
MRM transition (qualifier): m/z 312→125 Da Dwell time:245 ms

Ion source parameters:

GS1	*50 psi*
GS2	*60 psi*
TEM	*400 °C*
IS	*5500 V*
CUR	*30 psi*
CAD	*8 psi*
DP	*60 V*
CE	*50 V*
CXP	*12 V*
EP	*10 V*

These parameters have been optimized for Sciex API 4000 triple-quad instrument. MS conditions may vary depending on the instrument and can be adapted if necessary.
In order to reduce contamination of the MS system when analysing formulations, a switching valve can be used to redirect the eluent flow to pass through the MS detector only before and after prothioconazole-desthio elution.

(b) Equilibration of the system. Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µL portions of the calibration solution C4 (see below) and repeat the injections until retention times and peak areas deviate by less than ± 5 % from the mean for three successive injections.

(c) Preparation of calibration curve. Weigh in (to the nearest 0.01 mg) approximately 20 mg (s in mg) of the prothioconazole-desthio reference standard into separate volumetric flasks (20 mL). Suspend in 10 mL acetonitrile and place the flasks in an ultrasonic bath for 5 min. Make up the flasks with purified water to just below the calibration mark and allow to cool to ambient temperature. Fill up to the mark with purified water and mix thoroughly (stock solution).
Dilute the stock solution with dilution solution (see reagents) to the final concentration of the calibration solutions used for the calibration curve. The calibration curve will contain at least six calibration points, covering a concentration range of 0.020 – 0.30 mg/L for example. See the table below:

Designation	Concentration of calibration solution [mg/l]
C1	0.0200
C2	0.0250
C3	0.0800
C4	0.1500
C5	0.2500
C6	0.3000

(d) Preparation of sample. The amount of the prothioconazole-desthio can vary in dependency of the prothioconazole content in the formulation. An amount of formulation which contains at least 50 mg of the active ingredient prothioconazole should be used. To ensure the stability of prothioconazole, keep the samples away from light, when the samples are not used.
Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (w in mg) to contain about 50 mg of prothioconazole into separate volumetric flasks (100 mL). Add approximately 5 mg of L- cysteine hydrochloride monohydrate to each flask, suspend in 50 mL acetonitrile and 10 mL purified water. Place the flasks in an ultrasonic bath for 15 min. 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 and S2).

(e) Determination. Inject in duplicate each sample solution and bracket a series of sample solution by injections of the calibration curve and blank solution (e.g. dilution solution) as follows:

- blank B,
- calibration curve C1 – C6,
- blank B,
- sample solution S1,
- sample solution S2,
- blank B,
- calibration curve C1 – C6

... (B, C1 – C1, B, S1, S1, S2, S2, B, C1 – C6, …)

Determine the peak area of prothioconazole-desthio.

(f) Calculation. For each sample solution, calculate the content of prothioconazole-desthio using the MRM transition (quantifier): m/z 312→ 70 Da.
The calibration function is established 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.

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

Where:

H_s	= area of prothioconazole-desthio in calibration solution
m	= slope of calibration function
x	= C_s = concentration of prothioconazole-desthio in calibration solution, e.g. [mg/L]
b	= intercept of calibration function

The analyte content in the sample solution is calculated using the calibration function and the determined peak area of the analyte in sample solution.

$$prothioconazole - desthio\ [mg/L] = \frac{(Hw - b)}{m}$$

Where:

Prothioconazole-desthio [mg/L]	= concentration of prothioconazole-desthio in the sample solution e.g. [mg/L]
Hw	= area of prothioconazole-desthio in sample solution
b	= intercept of calibration function
m	= slope of calibration function

The analyte content in the sample, expressed in weight percent [% (w/w)], can be calculated as follows, considering the total sample weight:

$$prothioconazole - desthio\ content\ [\%\ w/w] = \frac{prothioconazole - desthio\ [mg/L]}{c_w\ [mg/L]} \times 100\%\ (w/w)$$

Where:

Prothioconazole-desthio [% (w/w)] = concentration of prothioconazole-desthio in the sample e.g. [% (w/w)]
Prothioconazole-desthio [mg/L] = concentration of prothioconazole-desthio in the sample solution e.g. [mg/L]
Cw = concentration of sample in sample solution e.g. [mg/L]

PROTHIOCONAZOLE EMULSIFIABLE CONCENTRATE
[*]**745**/EC/M/-

1 Sampling. As for prothioconazole technical concentrate **745**/TC/M/-

2 Identity tests. As for prothioconazole technical concentrate **745**/TC/M/-

4 Prothioconazole-desthio. As for prothioconazole technical concentrate **745**/TC/M/-

PROTHIOCONAZOLE FLOWABLE CONCENTRATE FOR SEED TREATMENT
745/FS/M/-

1 Sampling. As for prothioconazole technical concentrate **745**/TC/M/-

2 Identity tests. As for prothioconazole technical concentrate **745**/TC/M/-

[*] CIPAC method 2020. Based on a method supplied by Bayer Crop Science, Germany

4 Prothioconazole-desthio. As for prothioconazole technical concentrate **745**/TC/M/- except

substitute the point **(d) preparation of sample** for the following paragraph:

(d) Preparation of sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* in mg) to contain about 50 mg of prothioconazole into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate to each flask and 10 mL purified water to suspend the sample. Add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. 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. Clarify a part of the solution by centrifugation or filtration prior to analysis.

PROTHIOCONAZOLE SUSPENSION CONCENTRATE
[*]**745**/SC/M/-

1 Sampling. As for prothioconazole technical concentrate **745**/TC/M/-

2 Identity tests. As for prothioconazole technical concentrate **745**/TC/M/-

4 Prothioconazole-desthio. As for prothioconazole technical concentrate **745**/TC/M/- except

substitute the point **(d) preparation of sample** for the following paragraph:

(d) Preparation of sample. Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (w in mg) to contain about 50 mg of prothioconazole into separate volumetric flasks (100 mL). Add approximately 5 mg of L-cysteine hydrochloride monohydrate to each flask and 10 mL purified water to suspend the sample. Add 50 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. 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. Clarify a part of the solution by centrifugation or filtration prior to analysis.

[*] CIPAC method 2020. Based on a method supplied by Bayer Crop Science, Germany

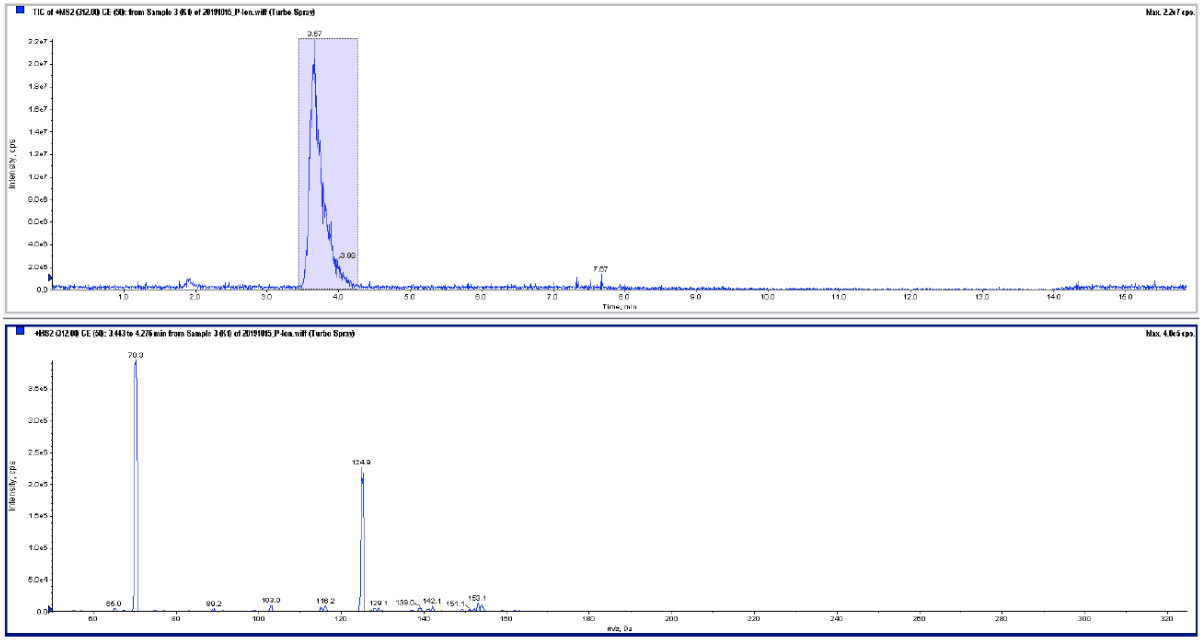


Fig. 1 Product ion Spectrum of prothioconazole-desthio
(MS/MS of m/z 312 Da)

Blue: Quantifier (m/z 312→ 70 Da; Red: Qualifier(m/z 312→125 Da

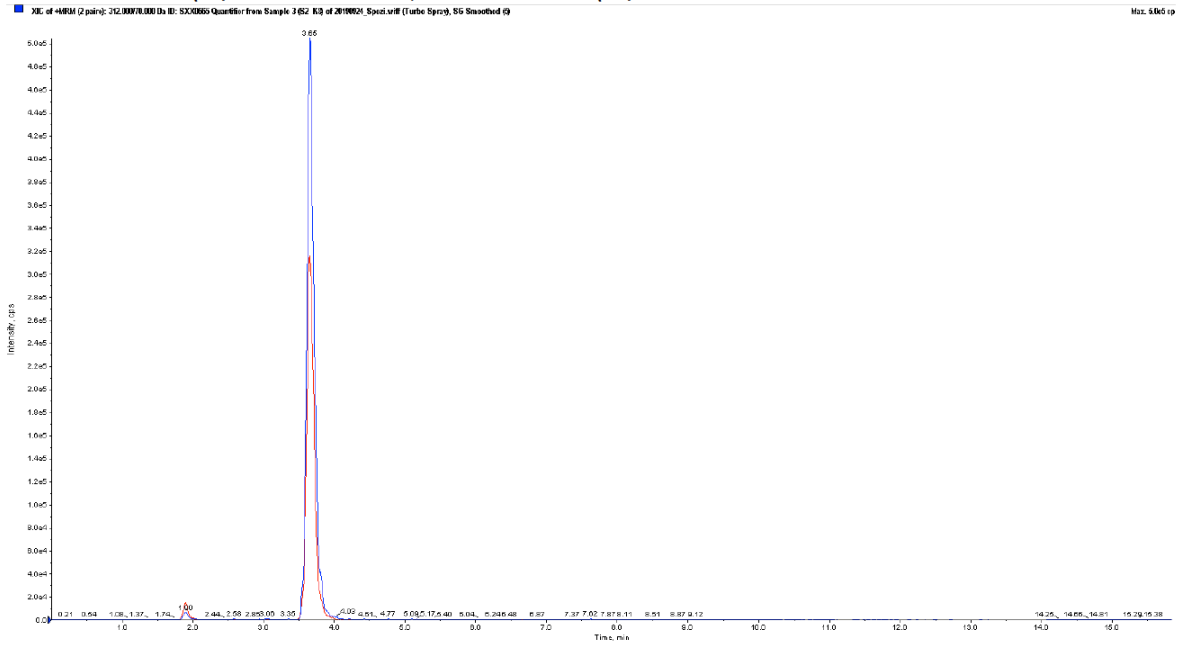


Fig. 2 Chromatogram of Analytical Standard prothioconazole-desthio

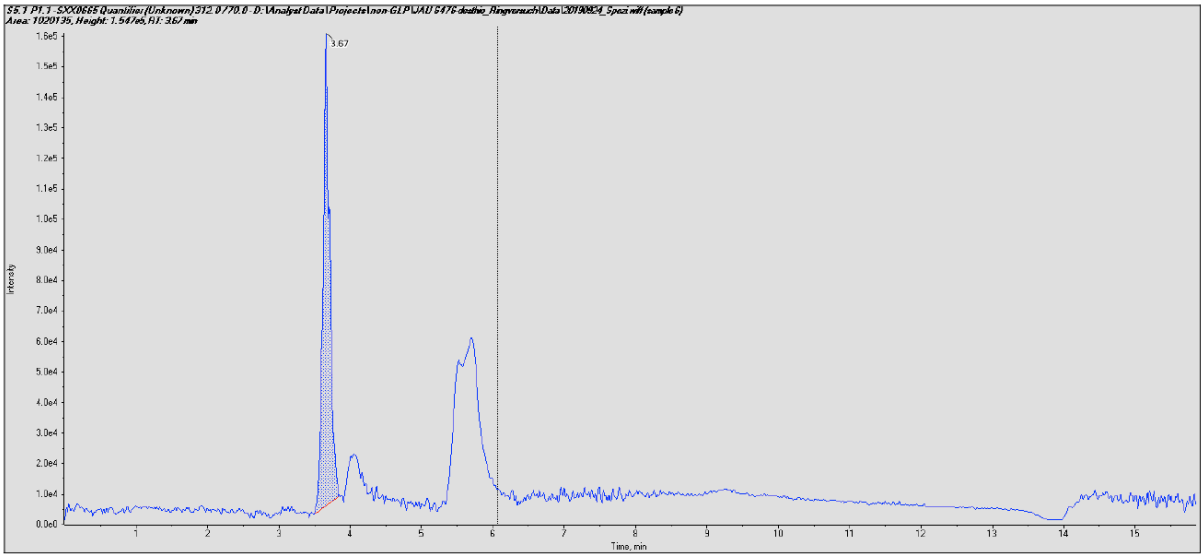


Fig. 3 Chromatogram of Prothioconazole TC

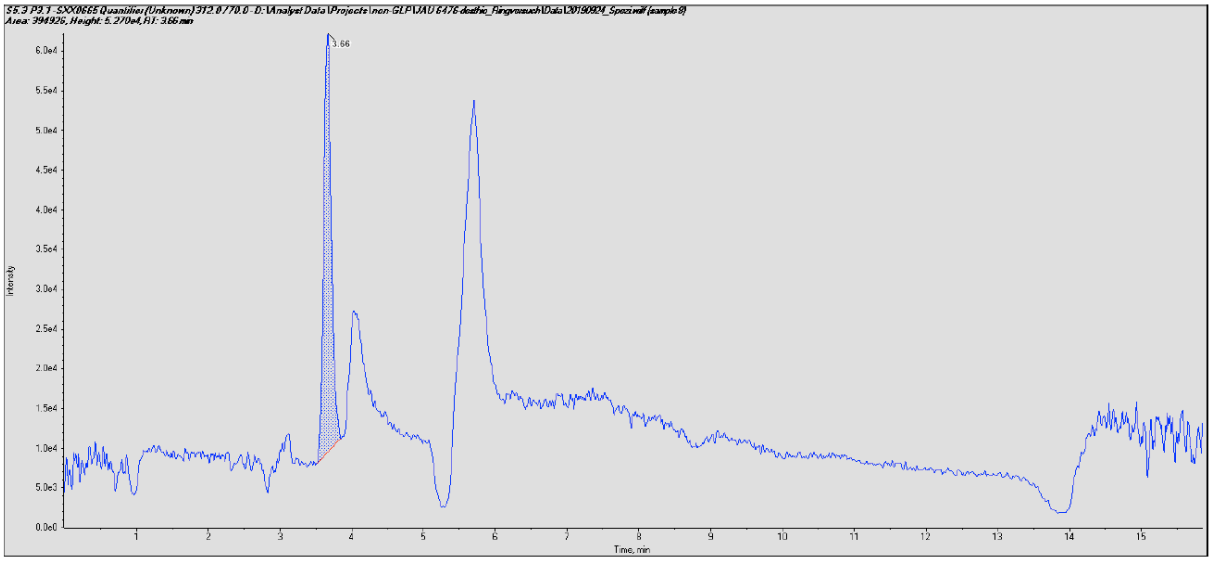


Fig. 4 Chromatogram of Prothioconazole EC

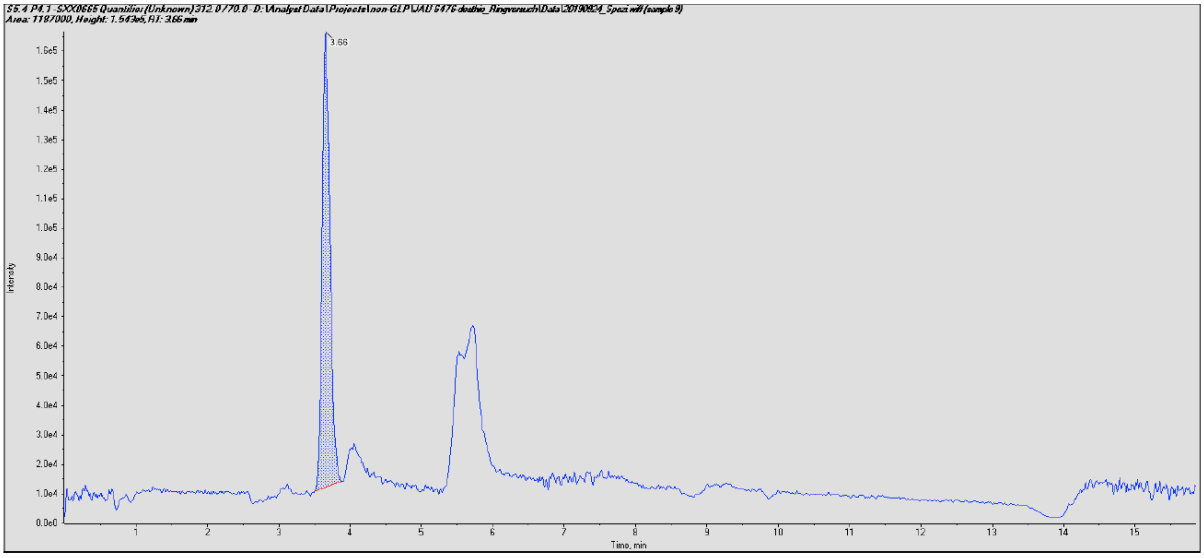


Fig. 5 Chromatogram of Prothioconazole FS

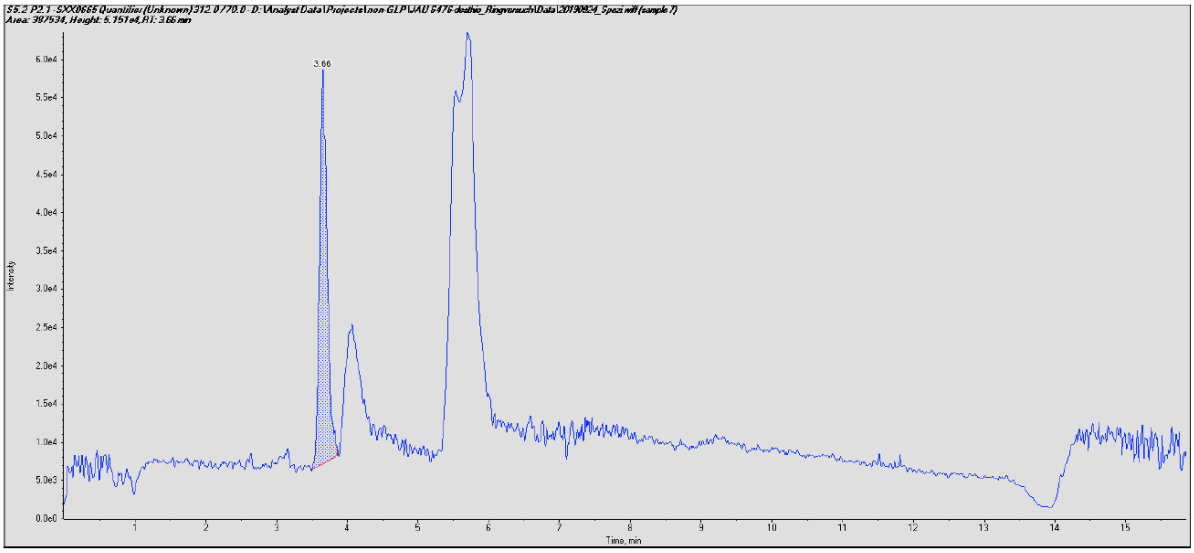


Fig. 6 Chromatogram of Prothioconazole SC