Science For A Better Life

Relevant Impurity of Prothioconazole

CIPAC Meeting, June 2020

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Background and Objective

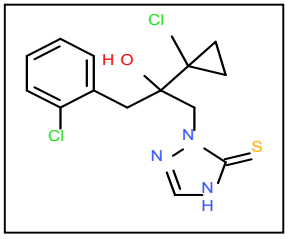
Background:

- Bayer AG, Crop Science Division wants to apply for a new FAO specification
- Peer validation for the relevant impurity **prothioconazole-desthio** of the active prothioconazole in formulations was initiated

Objective:

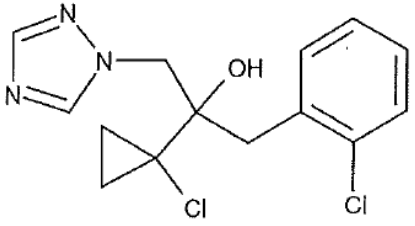
- Presentation of the outcome of the peer validation that CIPAC method (CIPAC Doc No.: CIPAC/5251/m) is suitable for the determination of **prothioconazole-desthio** in prothioconazole TC and prothioconazole EC, SC, FS formulations

Prothioconazole

Common name:	Prothioconazole	
Chemical name:	IUPAC	(RS)-2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-2,4-dihydro-1,2,4-triazole-3-thione
	CAS	3H-1,2,4-Triazole-3-thione, 2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2- hydroxypropyl]-2,4-dihydro-
CAS No.:	178928-70-6	
Trade names:	Proline,Prosaro, others	
Structure:		
Molecular Formula:	C14 H15 Cl2 N3 O S	
Molar Mass:	344.26 g/mol	
Activity:	Fungicide (class: triazolinthione)	
Relevante impurity:	prothioconazole-desthio, maximal accepted limit (MAL): 0.05% in TC	

Active substance

prothioconazole-desthio

Substance name:	prothioconazole-desthio
Chemical name:	2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1H-1,2,4-triazol-1-yl) propan-2-ol
Synonyms:	PTZ-desthio, SXX 0665
CAS No.:	120983-64-4
Structure:	
Molecular Formula:	C14 H15 Cl2 N3 O
Molar Mass:	312.2 g/mol

relevant impurity of prothioconazole

Outline of the Method and Samples

The homogenized sample containing prothioconazole plus stabilizer is dissolved in solvent mixture acetonitrile / purified water followed by prothioconazole-desthio determination using gradient reversed phase high performance liquid chromatography with ESI+ MS/MS detector and external standard calibration.

Samples:

Prothioconazole, technical, batch purity >=970 g/kg

Prothioconazole, Emulsion Concentrate (EC 250 g/L; 250 g/L Prothioconazole)

Prothioconazole, Flowable concentrate for seed treatment (FS 100 g/L; 100 g/L Prothioconazole)

Prothioconazole, Suspension Concentrate (SC 480 g/L; 480 g/L Prothioconazole)

Outline of the Method

Method Parameters:

Column: X-Terra RP 18 (Waters), 3.5 µm, 50 x 4.6 mm

Column temperature: 40 °C

Eluent & rinsing gradient:

Time [min]	1L water + 0.1 ml formic acid [%v/v]	1L ACN + 0.1 ml formic acid [%v/v/V]	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	3.0
16	50	50	2.0

Injection volume: 5 µL

Retention time: approx. 3.7 min

Detector (MS/MS):

ESI^+ (electrospray ionization in positive mode)

m/z 312 → m/z 70 amu (quantifier)

m/z 312 → m/z 125 amu (qualifier)

List of Participants

Dr. Christoph Czerwenka	AGES - Austrian Agency for Health and Food Safety Spargelfeldstraße 191 1220 Vienna Austria
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Developing Lab Schulz, Friedhelm	Bayer AG, Crop Science Division Alfred-Nobel-Strasse 50 40789 Monheim am Rhein Germany

General Safety Remark

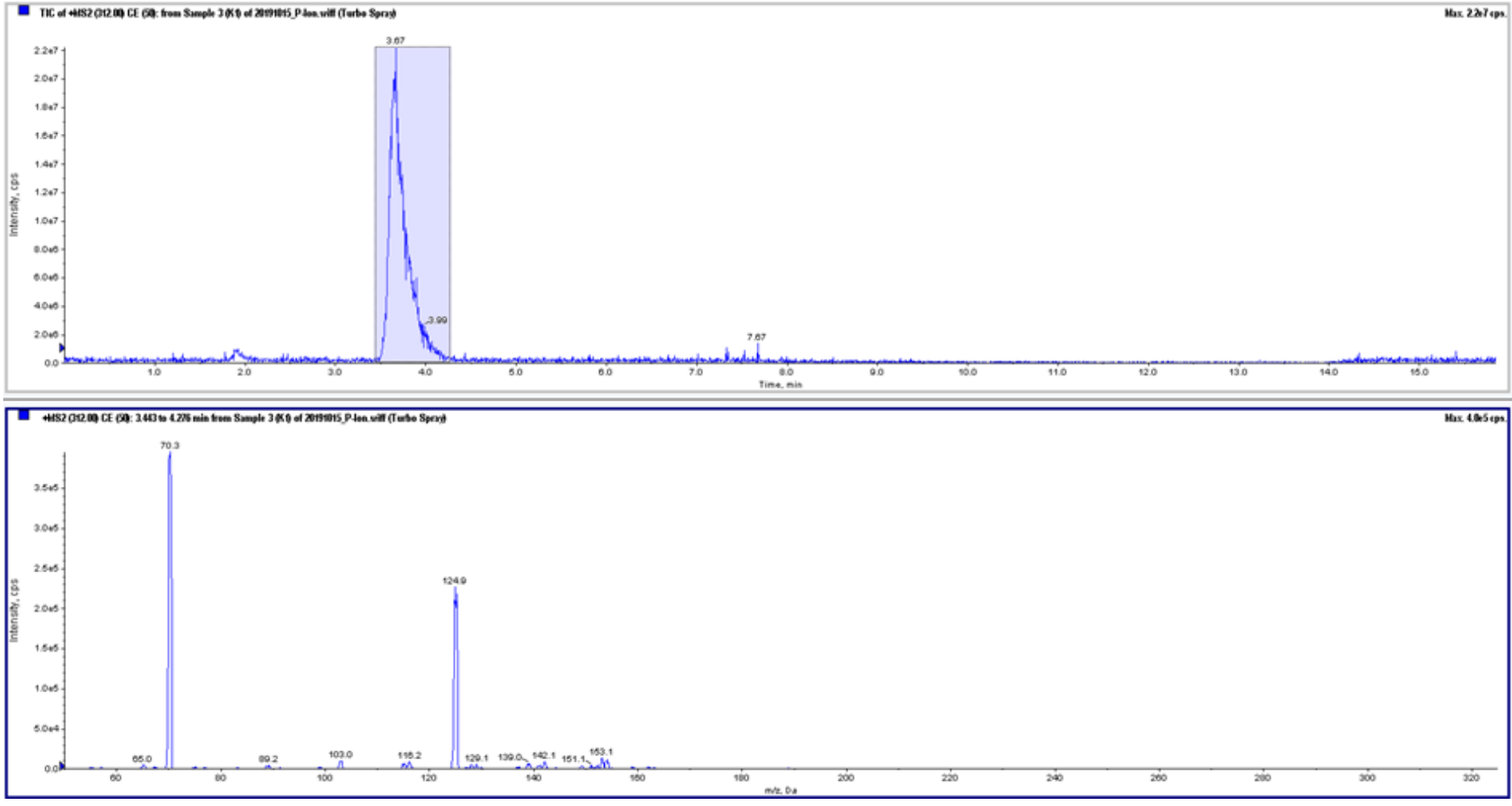
Prothioconazole (PTZ): Guideline for best practice handling of samples and sub-samples in laboratories

This best practice guideline for technical handling of the active substance PTZ and PTZ-containing formulations aims at ensuring chemical stability. It focuses on laboratory procedures. For information on safe handling of the products please refer to the Material Safety Data Sheets that are attached to each sample / product.
According to BCS experience, PTZ is sufficiently stable as active substance as well as in formulations. However, the concentration of the relevant impurity PTZ-desthio may increase if samples, and especially sub-samples, are not handled correctly.
During storage and analysis of samples and sub-samples the following must be ensured:

- Storage in closed containers
- Storage with minimum headspace volume
- Protection from light
- Storage in the refrigerator (~ 5 to 7 °C)

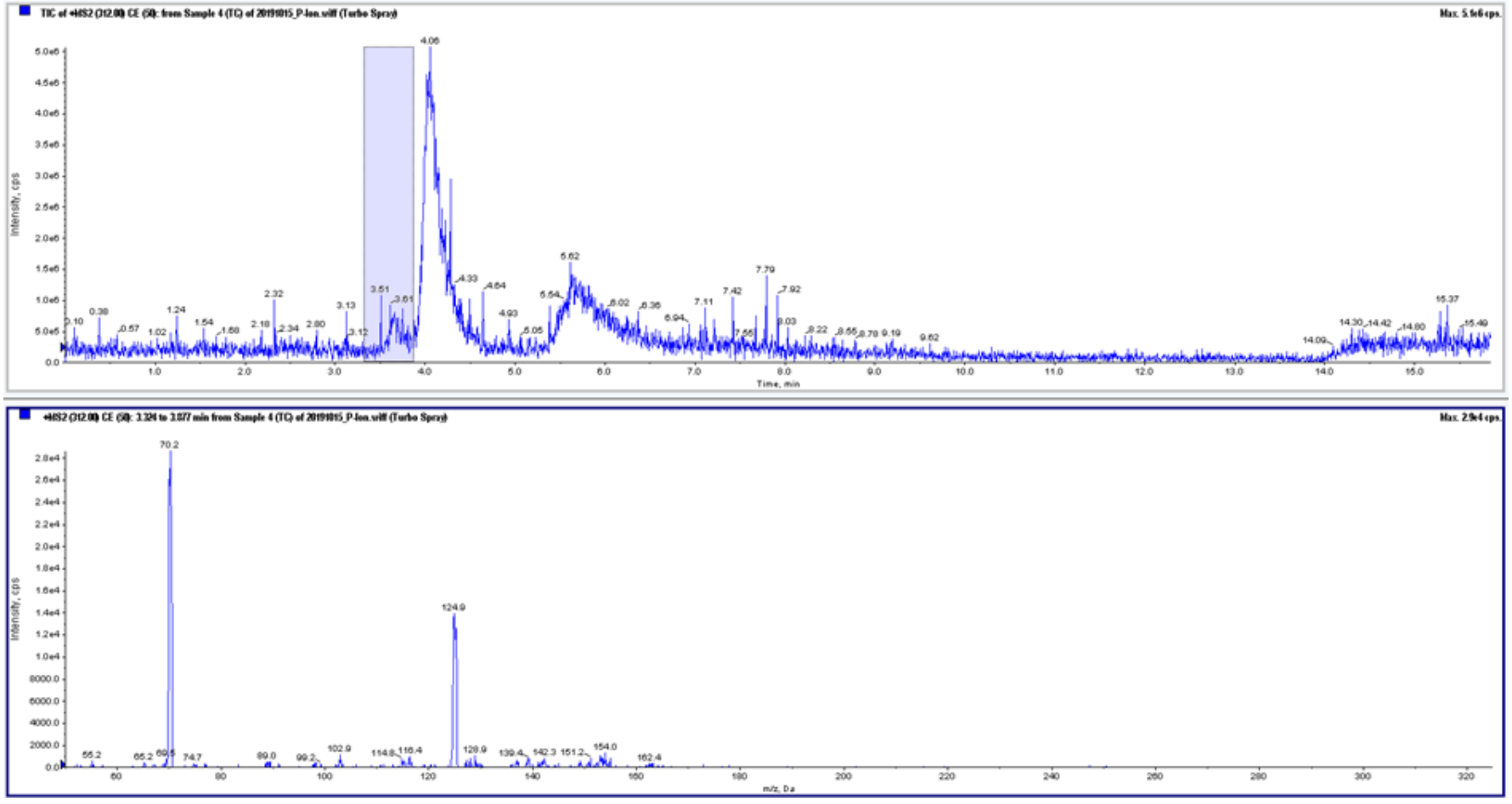
Chromatogram / Spectra

Reference item – Product Ion Scan



Chromatogram / Spectra

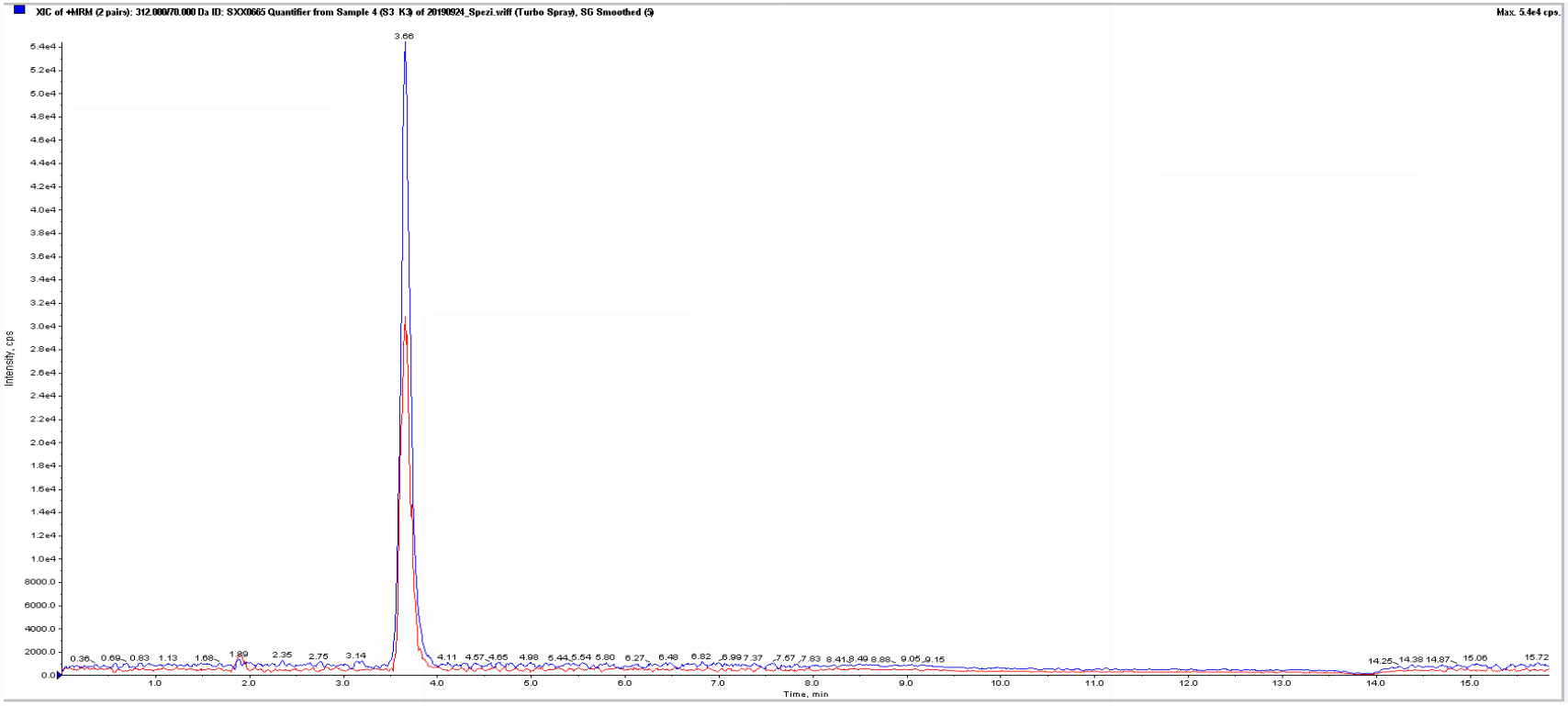
prothioconazole-desthio TC – Product Ion Scan



Typical chromatograms -
analytical standard prothioconazole-desthio

analytical standard prothioconazole-desthio

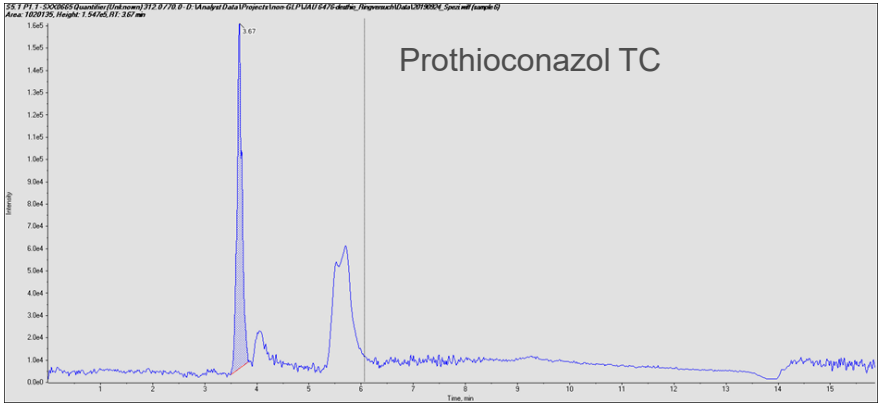
(nominal 0.0025 mg/100 mL)

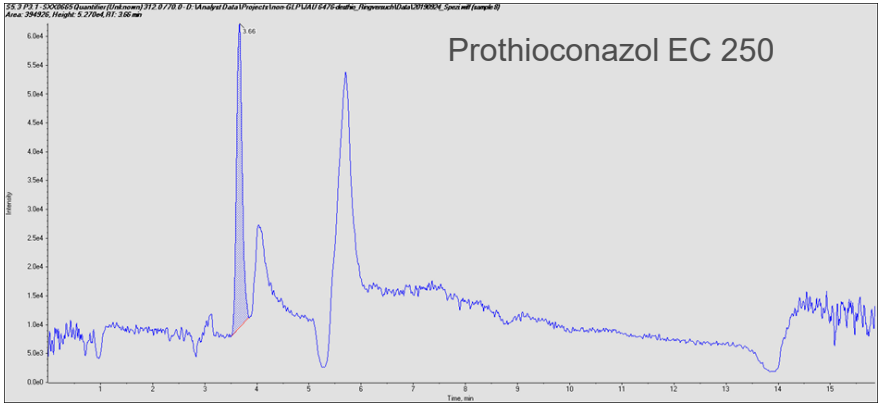


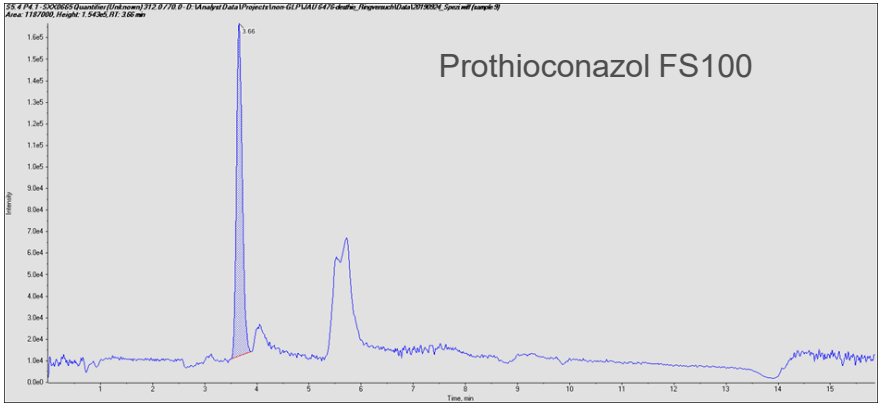
Blue: Quantifier m/z 312→70 Da; Red: Qualifier m/z 312→125 Da

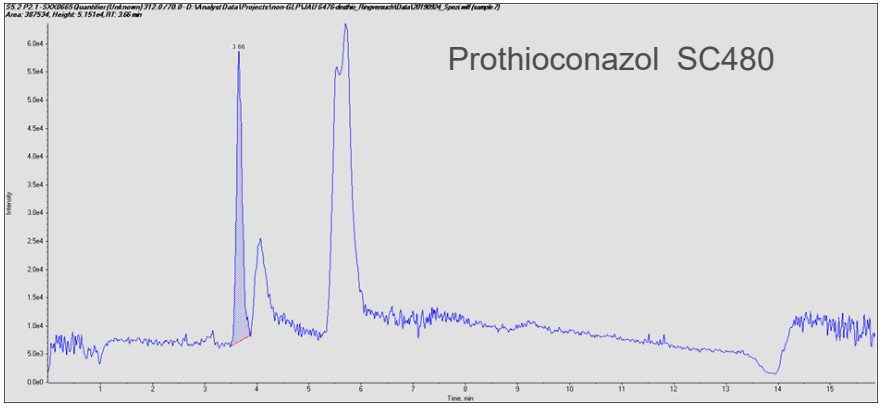
Typical chromatograms

Prothioconazol TC, EC 250, FS100, SC480









Statistical evaluation

Statistical evaluation was performed based on the data of 5 participants.

The investigated items are:

- Specificity and Interference
- Linearity
- Precision
- Accuracy (Level I & Level II)
- LOQ

Statistical evaluation

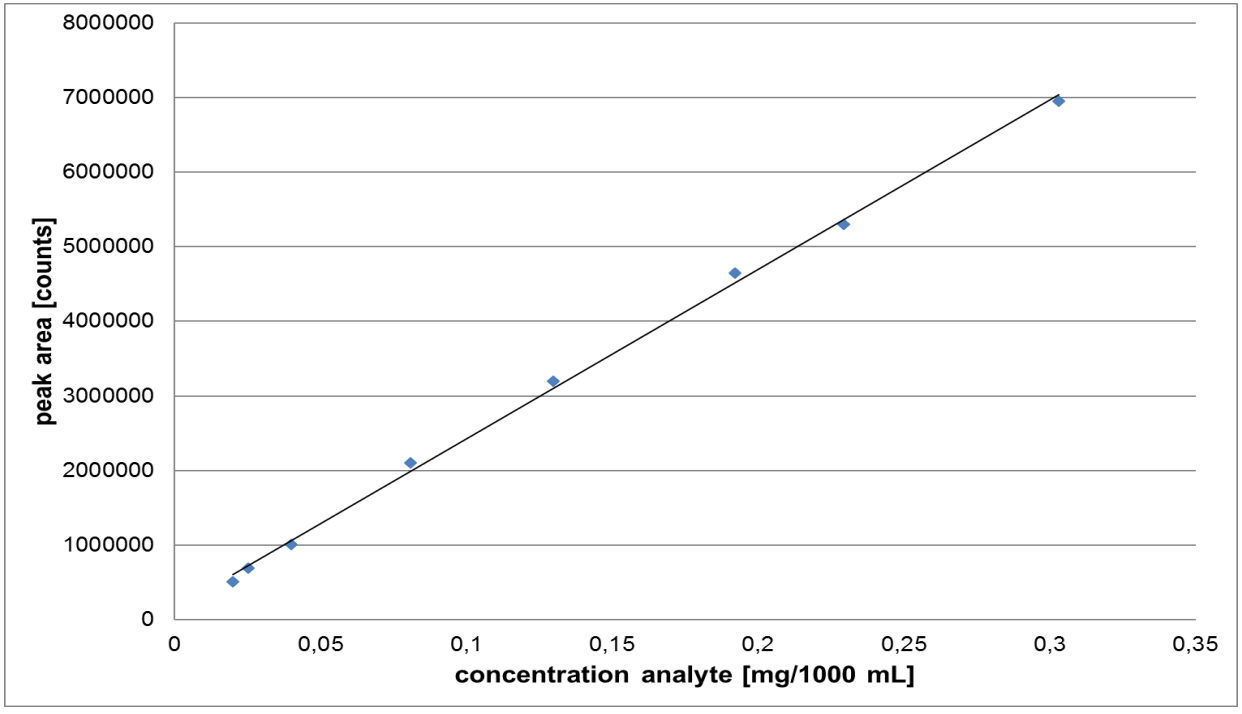
- **Identity:** The ion mass spectra of standard and sample solutions were measured. (developing lab). In addition, two selective m/z transitions in multiple reaction monitoring (MRM) mode were measured in standard solutions, sample or ~~,~~ spiked sample solutions and spiked blank formulations solutions under the operation conditions described in the analytical method (4 labs, 1 lab used a detector with accurate mass)
- **Specificity:** Interferences at the retention time of the analyte in the technical material and in the formulations were checked by comparing the chromatograms of the reference item prothioconazole-desthio and the test solutions of technical material, formulations and blank formulations.

  Carry over has been detected in the chromatograms of some laboratories. The peak areas contribute less than 1 % to the determination of PTZ-desthio at its max. accepted level and therefore it has been considered no significant.

  In case of one Lab, the specificity samples were injected at the end of the validation sequence and no proper evaluation could be carried out. The carry over was seen in the chromatograms of the blank formulations as well as chromatograms of sample solvent.

Statistical evaluation

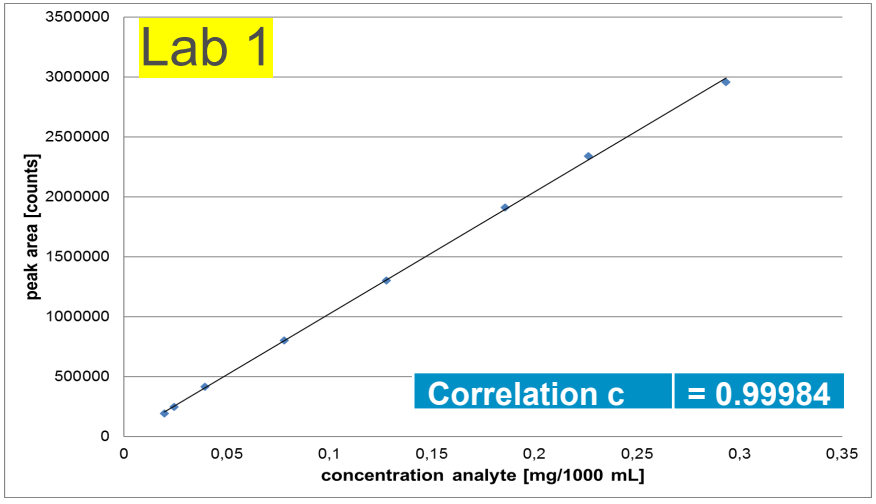
Linearity, developing lab

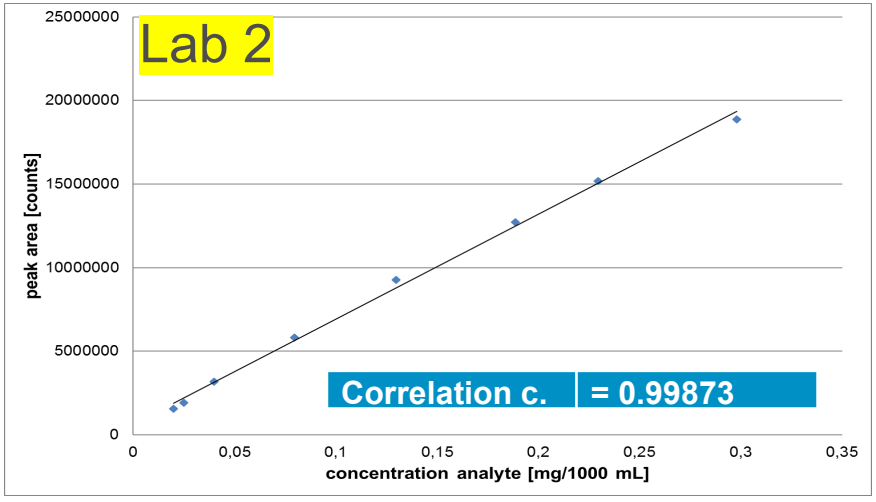


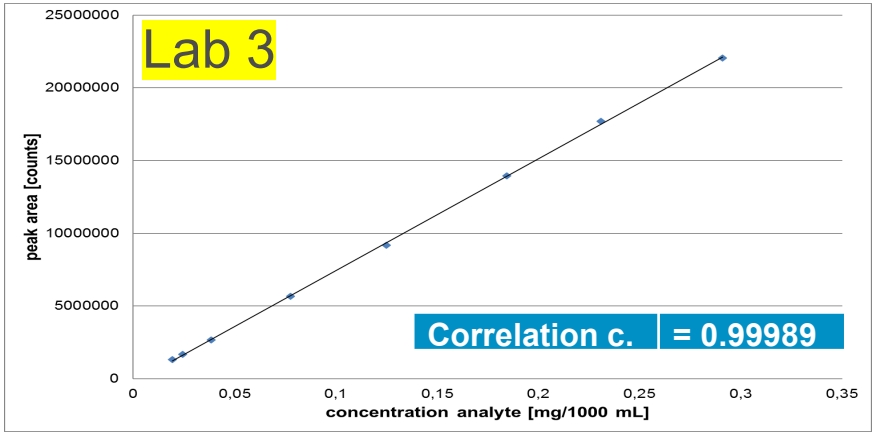
Number of values	**n = 8**
Regression equation	y = a + b x (1st order) y = 155347.6 + 22709821. x
Correlation coefficient	= 0.99916

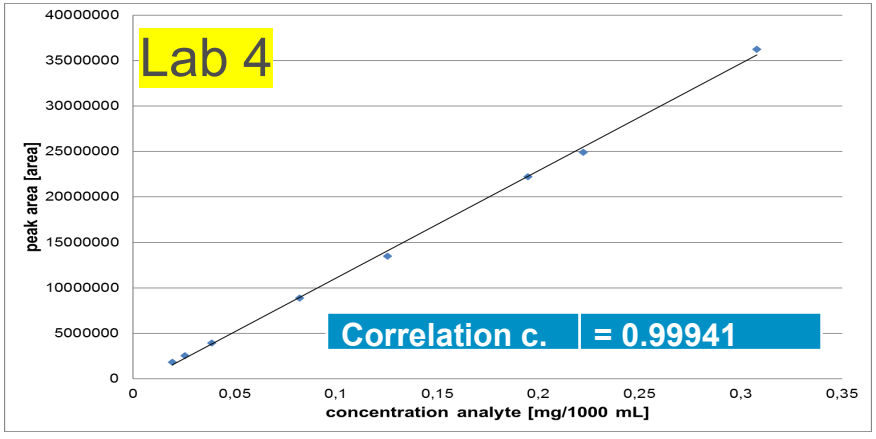
Statistical evaluation

Linearity, Lab 1-4









Statistical Summary - Repeatability

Prothioconazol TC (spiked with prothioconazole-desthio)

	prothioconazole-desthio				
	[% w/w]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.0211	0.0218	0.0233	0.0215	0.0264
Weighing no. 2	0.0226	0.0223	0.0236	0.0229	0.0256
Weighing no. 3	0.0223	0.0220	0.0245	0.0214	0.0249
Weighing no. 4	0.0222	0.0222	0.0243	0.0220	0.0258
Weighing no. 5	0.0223	0.0215	0.0240	0.0222	0.0257
Weighing no. 6	0.0233	0.0219	0.0248	0.0214	0.0256
Mean value	0.0222	0.0220	0.0241	0.0219	0.0257
SD	0.0007	0.0003	0.00056	0.00061	0.00049
RSD [%]	**3.21**	**1.30**	**2.34**	**2.78**	**1.92**
Horwitz-Value RSD $(r)_{max}$	4.75	4.76	4.70	4.76	4.65
Horrat value H_r	**0.68**	**0.27**	**0.50**	**0.58**	**0.41**
Outliers	no	no	no	no	no

Statistical Summary - Repeatability

Prothioconazol SC 480 (spiked with prothioconazole-desthio)

	prothioconazole-desthio				
	[% w/w]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.00849	0.00953	0.00997	0.00818	0.0103
Weighing no. 2	0.00892	0.00863	0.00997	0.00804	0.0108
Weighing no. 3	0.00927	0.00910	0.0100	0.00787	0.0110
Weighing no. 4	0.00823	0.00926	0.00996	0.00783	0.0102
Weighing no. 5	0.00903	0.01013	0.00943	0.00801	0.0105
Weighing no. 6	0.00835	0.00920	0.00917	0.00758	0.0100
Mean value	0.00872	0.00931	0.00975	0.00792	0.0105
SD	0.00042	0.00050	0.00036	0.00021	0.00038
RSD [%]	**4.78**	**5.38**	**3.68**	**2.65**	**3.67**
Horwitz-Value RSD $(r)_{max}$	5.47	5.42	5.38	5.55	5.32
Horrat value H_r	**0.87**	**0.99**	**0.68**	**0.48**	**0.69**
Outliers	no	no	no	no	no

Statistical Summary - Repeatability

Prothioconazol EC 250 (spiked with prothioconazole-desthio)

	prothioconazole-desthio				
	[% w/w]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.00526	0.00737	0.00683	0.00687	0.00867
Weighing no. 2	0.00518	0.00688	0.00692	0.00674	0.00889
Weighing no. 3	0.00516	0.00725	0.00673	0.00663	0.00864
Weighing no. 4	0.00511	0.00723	0.00694	0.00679	0.00871
Weighing no. 5	0.00525	0.00730	0.00690	0.00693	0.00882
Weighing no. 6	0.00536	0.00684	0.00689	0.00703	0.00875
Mean value	0.00522	0.00715	0.00687	0.00683	0.00874
SD	0.00009	0.00023	0.000077	0.00014	0.000095
RSD [%]	**1.70**	**3.16**	**1.13**	**2.03**	**1.09**
Horwitz-Value RSD $(r)_{max}$	5.91	5.64	5.67	5.68	5.47
Horrat value H_r	**0.29**	**0.56**	**0.20**	**0.36**	**0.20**
Outliers	no	no	no	no	no

Comment: The absolute mean values of the analyte are distributed in a range larger than expected. This can be justified by the fact that the formulation was spiked with prothioconazole-desthio and the amount added is not identical in all laboratories. Also, a different storage of the samples in each laboratory before the analysis is not excluded, and therefore the original concentration of the analyte in the sample may have risen differently.

Statistical Summary - Repeatability

Prothioconazol FS 100

	prothioconazole-desthio				
	[% w/w]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.00112	0.00103	0.00086	0.000816	0.00134
Weighing no. 2	0.00113	0.00105	0.00090	0.000842	0.00122
Weighing no. 3	0.00097	0.00109	0.00090	0.000829	0.00132
Weighing no. 4	0.00095	0.000999	0.00084	0.000840	0.00143
Weighing no. 5	0.00103	0.00100	0.00085	0.000881	0.00117
Weighing no. 6	0.00103	0.00103	0.00088	0.000942	0.00125
Mean value	0.00104	0.00103	0.00087	0.000858	0.00129
SD	0.00007	0.00003	0.000026	0.000046	0.000095
RSD [%]	**7.17**	**3.16**	**2.94**	**5.41**	**7.40**
Horwitz-Value RSD $(r)_{max}$	7.54	7.54	7.74	7.76	7.30
Horrat value H_r	**0.95**	**0.42**	**0.38**	**0.70**	**1.01**
Outliers	no	no	no	no	no

Statistical Summary - Recovery Level I + II

prothioconazole TC

(Recovery Level I - LOQ)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**104.2**	**80.2**	**95.2**	**114.2**	**103.8**
SD	9.26	6.94	4.49	7.89	6.60
RSD [%]	8.89	8.66	4.72	6.91	6.36
Outliers	no	no	no	no	no

(Recovery Level II – MAL)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**96.6**	**98.6**	**84.1**	**101.0**	**91.2**
SD	1.52	1.60	1.03	1.93	1.06
RSD [%]	1.57	1.62	1.22	1.91	1.16
Outliers	no	no	no	no	no

Statistical Summary - Recovery Level I + II

prothioconazole SC 480

(Recovery Level I - LOQ)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**98.0**	**95.7**	**100.6**	**93.4**	**95.5**
SD	0.80	3.82	4.80	2.20	2.12
RSD [%]	0.82	3.99	4.77	2.36	2.22
Outliers	no	no	no	no	no

(Recovery Level II – MAL)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**98.8**	**101.0**	**98.5**	**82.1**	**95.4**
SD	1.19	1.29	0.902	1.03	0.894
RSD [%]	1.20	1.28	0.92	1.25	0.937
Outliers	no	no	no	no	no

Statistical Summary - Recovery Level I + II

prothioconazole EC 250

(Recovery Level I - LOQ)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**94.5**	**96.1**	**84.7**	**94.6**	**101.1**
SD	0.75	2.88	0.897	1.07	3.11
RSD [%]	0.79	3.0	1.06	1.13	3.08
Outliers	no	no	no	no	no

(Recovery Level II – MAL)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**96.1**	**98.8**	**88.5**	**83.3**	**100.0**
SD	1.02	1.58	0.187	1.45	1.67
RSD [%]	1.06	1.60	0.21	1.74	1.67
Outliers	yes*	no	no	no	no

* The results of the developing laboratory showed an upper outlier at 95% confidence according to Dixon Test. However, this value was not disregarded to calculate the mean value and the RSD, as it was considered valid.

Statistical Summary - Recovery Level I + II

prothioconazole FS 100

(Recovery Level I - LOQ)	prothioconazole-desthio				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**86.9**	**93.0**	**90.7**	**88.3**	**107.7**
SD	7.94	4.99	4.36	2.14	1.29
RSD [%]	9.14	5.36	4.81	2.42	1.20
Outliers	no	no	no	no	yes*
(Recovery Level II – MAL)	**prothioconazole-desthio**				
	Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Mean value	**85.1**	**100.0**	**97.8**	**72.8**	**106.0**
SD	5.71	1.68	3.55	1.13	0.71
RSD [%]	6.71	1.68	3.63	1.55	0.70
Outliers	no	no	no	no	no

* The results of Laboratory 4 showed a lower outlier at 95% confidence according to Dixon Test. However, this value was not disregarded to calculate the mean value and the RSD, as it was considered valid.

Statistical evaluation

Limit of Quantification - LOQ

A concentration of 0.0025 mg/100 mL prothioconazole-desthio was the lowest tested fortification level for which acceptable recovery and precision under repeatability conditions were successfully demonstrated. This concentration is equal to:

- prothioconazole TC 0.005 % (w/w)
- prothioconazole SC 480 (480 g/L) 0.002 % (w/w
- prothioconazole EC 250 (250 g/L) 0.001 % (w/w)
- prothioconazole FS 100 (100 g/L) 0.0004% (w/w)

Conclusions

- **The statistical evaluation is reported from the 5 laboratories which participated in the peer validation.**

- **The data presented in the statistical summary show that the method is suitable in terms of accuracy and reproducibility for all samples tested.**

- **The acceptance of the proposed method for the determination of the relevant impurity prothioconazole-desthio in prothioconazole TC and prothioconazole EC, SC, FS formulations tested is recommended.**

Science For A Better Life

Many thanks to the participants of the small collaboration trial and the support of from
Maria Teresa Garcia-Sanchez (Currenta)
and Mario Müller (Bayer)

Forward-Looking Statements

This website/release/presentation may contain forward-looking statements based on current assumptions and forecasts made by Bayer management.

Various known and unknown risks, uncertainties and other factors could lead to material differences between the actual future results, financial situation, development or performance of the company and the estimates given here. These factors include those discussed in Bayer's public reports which are available on the Bayer website at http://www.bayer.com/.

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