

Rel. Impurities of prothioconazole

CIPAC Peer Validation

CIPAC peer validation of an analytical method
for the determination of prothioconazole-desthio
in prothioconazole technical, SC, EC and FS formulations
by High Performance Liquid Chromatography

Report to CIPAC
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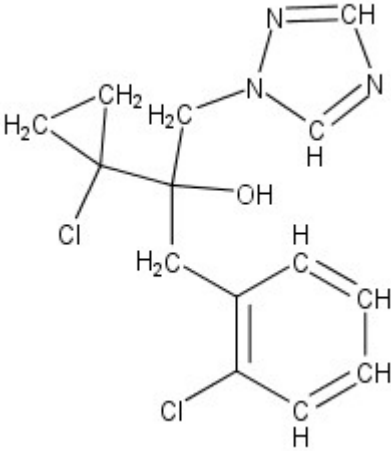
1 Introduction

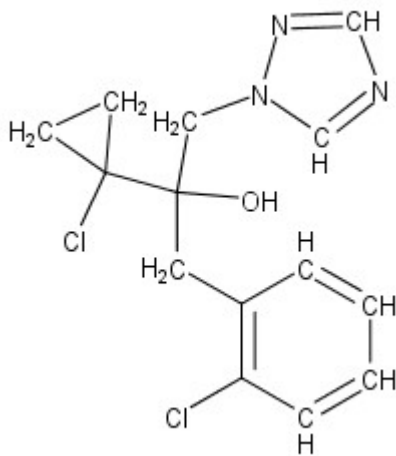
1.1 Scope

The results of the peer validation of an analytical method for the determination of prothioconazole-desthio content in prothioconazole technical grade active ingredient (TGAI), prothioconazole suspension concentrate (SC), prothioconazole emulsifiable concentrate (EC) and prothioconazole flowable conc. seed treatment (FS) formulations are reported.

The peer validation was performed according to CIPAC guideline for analytical methods for the determination of relevant impurities referred to in FAO and/or WHO specification for pesticide technical grade active ingredients and formulations.

1.2 Analyte to be determined

Analyte Name	prothioconazole-desthio
Synonyms	PTZ-desthio, SXX 0665, AE 1194888, BCS-AA53879
Three Letter Code	n/a
Structural Formula	



Empirical Formula	C ₁₄ H ₁₅ Cl ₂ N ₃ O
Molecular Weight	312.2 g/mol
CAS No	120983-64-4

1.3 Samples

Four test samples, three blank formulations and one analytical standard were sent to the participants:

1. Prothioconazole technical (TC)
1.1 prothioconazole TC
Batch ID: EDFL033963
Content: 98.9 % (w/w)

2. prothioconazole suspension concentrate (SC)
2.1 prothioconazole SC 480 (480 g/L)
Batch ID: EM4L01983
Declared content: 480 g/L, 40.3 % (w/w)
2.2 prothioconazole SC 0(0 g/L) (blank formulation)
Batch ID: 218-011680
Declared content: 0 g/L, 0 % (w/w)

3. prothioconazole emulsifiable concentrate (EC)
3.1 prothioconazole EC 250 (250 g/L)
Batch ID: EM4L021491
Declared content: 250 g/L, 25.0 % (w/w)
3.2 prothioconazole EC 0 (0 g/L) (blank formulation)
Batch ID: 2018-011806
Declared content: 0 g/L, 0 % (w/w)

4. prothioconazole flowable conc. seed treatment (FS)
prothioconazole FS 100 (100 g/L)
Batch ID: 2015-001031
Declared content: 100 g/L, 8.7 % (w/w)
prothioconazole FS 0 (0 g/L) (blank formulation)
Batch ID: 2018-011758
Declared content: 0 g/L, 0 % (w/w)

Prothioconazole-desthio, reference standard (purity 98.1% w/w)

1.4 Participants

Dr. Christoph Czerwenka	AGES - Austrian Agency for Health and Food Safety Spargelfeldstraße 191 1220 Vienna Austria
Dr. Rolf Foerster	BASF SE Carl-Bosch-Strasse 38 67056 Ludwigshafen Germany
Dr. Alexander von Tesmar	Currenta GmbH & Co. OHG CUR-ANT-PEA Chromatographie 51368 Leverkusen Germany
Dr. Christian Mink	Syngenta Crop Protection AG Breitenloh 5 CH-4333 Münchwilen Switzerland
Developing Lab Schulz, Friedhelm	Bayer AG, Crop Science Division Alfred-Nobel-Strasse 50 40789 Monheim am Rhein Germany

2 Analytical Method

2.1 Outline of the Method

Reversed Phase High Performance Liquid Chromatography (RP-HPLC) hyphenated to electrospray ionization mass spectrometry in multiple reaction monitoring mode (ESI-MS/MS in MRM mode) was used for quantitation of prothioconazole-desthio.

The prothioconazole-desthio is separated from formulation components and active substances using a reversed phase column and isocratic elution. The quantitative evaluation is carried out by comparing the peak areas with those of reference items, using an external standard method.

2.2 Method Development Prior to Peer Validation

The analytical method was developed by Bayer AG, Crop Science Division and accordingly the following procedure

1) Confirmation of Analyte identification

The ion mass spectra of standard and sample solutions were measured.

In addition, two selective m/z transitions in multiple reaction monitoring (MRM) mode were measured in standard solutions, sample, spiked sample solutions and spiked blank formulations solutions under the operation conditions described in the analytical method.

2) 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.

3) Calibration

Calibration solutions were prepared using prothioconazole-desthio standard. The solutions were analyzed and the peak areas of prothioconazole-desthio were plotted against the concentration of the calibration solutions to establish a calibration line.

4) Repeatability

Six independently prepared sample solutions for each technical material and formulation were analyzed according to the method.

In case of the technical material and the formulations SC and EC, not enough PTZ-desthio was found to evaluate the repeatability. In these cases, the test solutions were spiked with the analyte prothioconazole-desthio at a concentration of 0.01 mg/100 mL to determine the repeatability. Mean and relative standard deviations (RSD) were calculated.

5) Recovery

The technical material or the blank formulations were fortified with the analyte prothioconazole-dethio at two levels, fortification level I 0.0025 mg/100 mL and fortification level II 0.020 mg/100 mL (technical material) or 0.025 mg/100 mL (blank formulations). These solutions were analyzed, and the recoveries were calculated.

6) Limit of Quantification (LOQ)

The concentration of the lowest tested fortification level was defined as LOQ, as acceptable recovery and precision under repeatability conditions were successfully demonstrated.

2.3 Peer Validation

The peer validation was conducted in four independent laboratories. The participants are shown in 1.4. We requested the collaborators to conduct the peer validation according to the prescribed protocol, describe the operating conditions in detail and attach the calibration curve and the chromatograms for each sample.

The investigations included calibration, repeatability, recovery and LOQ. The details of each procedure are the same as those described in 2.2.

For the confirmation of the analyte identification two selective m/z transitions in multiple reaction monitoring (MRM) mode were measured¹ in standard solutions, sample and or spiked sample solutions and spiked blank formulations solutions under the operation conditions described in analytical method.

¹ Labor 4 measured only the quantifier m/z transition. This laboratory use high mass accuracy MS, which is considered adequate for confirmation of analyte identification.

3 Remarks of the participants

Laboratory	HPLC-MS/MS-System	Remarks
Developing Lab	Agilent 1260 Sciex API 4000 triple-quad-systems	n.a.
Lab 1	Agilent 1200 Sciex 4000 Qtrap	An increase in the baseline by PTZ-containing formulations was observed. Proposal to use a switching valve and to switch the eluent flow into the waste after the PTZ-desthio passed the detector, so that contamination of the instrument is reduced.
Lab 2	Agilent 1200 Sciex API 4000 triple-quad-systems	Carry-over detected after some sample injections. It cannot be eliminated. Detector signal stabilizes after several sequences/days.
Lab 3	Agilent 1290 Infinity 1 Sciex 5500 Qtrap detector.	n.a.
Lab 4	Dionex Ultimate 3000 Thermo QExactive	PRM instead of MRM used PRM: Precursor 312.06649, isolation 1 m/z Frag: HCD NCE 25 Data evaluation: m/z 70.03997 ± 5 mmu

4 Results and Discussion

4.1 Peer Validation

1) Confirmation of Analyte identification

Identity of the analyte prothioconazole-desthio was successfully demonstrated by measuring two selective m/z transitions in multiple reaction monitoring (MRM) (see Figure 6).

The ion mass spectra obtained by the developing laboratory during the method development prior to peer validation are shown in Figure 1 to Figure 4

2) Specificity

The specificity of the analytical method for prothioconazole-desthio is assessed sufficient as no significant interfering compounds were detected in the chromatograms at the retention time of the analyte.

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 Lab 4, 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. It is assumed, that the injection system was probably contaminated at the end of the sequence.

3) Calibration

The calibration lines are shown in Figure 31 to Figure 35. The calibration equation and the correlation factor are reported in addition. The calibration function of the analytical method is assessed as linear over a concentration range of 0.02 to 0.30 mg/L, as the correlation factor was satisfactory > 0.99 (in accordance with SANCO/3030/99).

4) Repeatability

The precision of the method was satisfactory with RSD values of 1.1 – 7.4 % as shown in Table 1 to Table 4.

In all cases, the Horrat value (Horwitz ratio, Hr) was ≤ 1 .

5) Recovery

The recoveries were satisfactory as shown in Table 5 to Table 12

The technical material or the blank formulations were fortified with the analyte prothioconazole-desthio at two levels, fortification level I (LOQ) 0.0025 mg/100 mL and fortification level II (close to the max. accepted level (MAL) in the formulation, see Appendix 1) 0.20 mg/100 mL (technical material) or 0.025 mg/100 mL (blank formulations). These concentrations are equal to:

	LOQ Level I	MAL Level II
prothioconazole TC	0.005 % (w/w) ²	0.04 % (w/w) ²
prothioconazole SC 480 (480 g/L)	0.002 % (w/w) ³	0.02 % (w/w) ³
prothioconazole EC 250 (250 g/L)	0.001 % (w/w) ⁴	0.01 % (w/w) ⁴
prothioconazole FS 100 (100 g/L)	0.0004% (w/w) ⁵	0.004% (w/w) ⁵

6) 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)

5 Conclusion

For all samples, the analytical method was peer-validated in terms of specificity, calibration, repeatability, recovery and limit of quantification. The RSDs of repeatability for technical material and formulations were found acceptable according to the Horrat criteria ≤ 1 .

In conclusion, the proposed method was successfully peer-validated and was considered appropriate for the determination of prothioconazole-desthio in technical material prothioconazole and SC, EC and FS formulations.

² Referred to a nominal sample concentration of 50 mg/100 mL

³ Referred to a nominal sample concentration of 125 mg/100 mL

⁴ Referred to a nominal sample concentration of 200 mg/100 mL

⁵ Referred to a nominal sample concentration of 575 mg/100 mL

6 Determination of relevant impurity prothioconazole-desthio

6.1 Tables of results

Table 1: Results prothioconazole TC (Repeatability)

	prothioconazole-desthio				
	Developing Lab	[% w/w]			
		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

Table 2: Results prothioconazole SC 480 (480 g/L) (Repeatability)

	prothioconazole-desthio				
	Developing Lab	[% w/w]			
		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

Table 3: Results prothioconazole EC 250 (250 g/L) (Repeatability)

	prothioconazole-desthio				
	Developing Lab	[% w/w]			
		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

Remark: 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 PTZ-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.

Table 4: Results prothioconazole FS 100 (100 g/L) (Repeatability)

	prothioconazole-desthio				
	Developing Lab	[% w/w]			
		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

Table 5: Results prothioconazole TC (Recovery Level I - LOQ)

	prothioconazole-desthio				
	Developing Lab	Recovery [%]			
		Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	100.8	81.74	89.9	100.6	112.8
Weighing no. 2	106.0	82.69	90.7	117.7	99.2
Weighing no. 3	96.4	81.09	93.9	124.6	102.1
Weighing no. 4	100.8	75.91	96.4	114.8	110.7
Weighing no. 5	99.2	69.43	100.0	112.0	102.3
Weighing no. 6	122.0	90.04	100.4	115.2	95.9
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

Single and mean recovery rates within the requested range of 70-130 % according to SANCO/3030/99 for a nominal analyte content < 0.01% (w/w)

Remark: The initial concentration of the analyte found in the unfortified sample was subtracted from the measured concentration of the fortified sample to calculate the recovery [%].

Table 6: Results prothioconazole TC (Recovery Level II - MAL)

	prothioconazole-desthio				
	Developing Lab	Recovery [%]			
		Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	98.1	97.1	82.6	100.4	90.3
Weighing no. 2	97.2	100.9	83.6	99.3	91.1
Weighing no. 3	94.8	98.7	84.2	99.7	92.8
Weighing no. 4	94.7	99.2	83.8	104.0	90.1
Weighing no. 5	98.1	99.3	85.0	98.7	90.6
Weighing no. 6	97.1	96.5	85.5	101.5	92.0
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

Single and mean recovery rates within the requested range of 75-125 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.01 to $< 0.1\%$ (w/w)

Remark: The initial concentration of the analyte found in the unfortified sample was subtracted from the measured concentration of the fortified sample to calculate the recovery [%].

Table 7: Results prothioconazole SC 480 (480 g/L) (Recovery Level I - LOQ)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	97.6	96.0	97.9	94.5	94.3
Weighing no. 2	99.2	98.2	106.4	94.9	96.3
Weighing no. 3	96.8	93.1	96.3	95.3	95.8
Weighing no. 4	98.4	89.4	95.9	94.5	93.8
Weighing no. 5	98.0	99.8	106.4	91.2	99.2
Weighing no. 6	98.0	97.5	100.7	90.0	93.5
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

Single and mean recovery rates within the requested range of 70-130 % according to SANCO/3030/99 for a nominal analyte content < 0.01% (w/w)

Table 8: Results prothioconazole SC 480 (480 g/L) (Recovery Level II - MAL)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	99.8	99.6	97.7	83.5	94.8
Weighing no. 2	100.1	103.1	97.5	81.6	94.1
Weighing no. 3	98.1	101.1	98.6	81.5	95.7
Weighing no. 4	99.8	100.1	98.3	80.7	95.3
Weighing no. 5	98.0	99.8	98.8	82.7	96.2
Weighing no. 6	97.2	100.5	100.0	82.7	96.6
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

Single and mean recovery rates within the requested range of 75-125 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.01 to $< 0.1\%$ (w/w)

Table 9: Results prothioconazole EC 250 (250 g/L) (Recovery Level I - LOQ)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	95.6	96.3	83.4	96.1	95.7
Weighing no. 2	94.4	95.0	85.8	94.1	101.1
Weighing no. 3	94.0	92.3	85.4	94.9	101.2
Weighing no. 4	94.4	100.6	84.2	92.9	102.0
Weighing no. 5	95.2	94.6	84.2	94.5	105.3
Weighing no. 6	93.6	98.0	85.0	94.9	101.4
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

Single and mean recovery rates within the requested range of 70-130 % according to SANCO/3030/99 for a nominal analyte content < 0.01% (w/w)

Table 10: Results prothioconazole EC 250 (250 g/L) (Recovery Level II)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	98.0	101.5	88.4	85.6	101.8
Weighing no. 2	96.0	99.5	88.3	83.7	99.0
Weighing no. 3	95.4	98.9	88.8	83.4	99.4
Weighing no. 4	95.2	98.2	88.6	81.8	97.5
Weighing no. 5	95.8	97.6	88.5	81.7	101.6
Weighing no. 6	96.0	97.1	88.6	83.3	100.6
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

Single and mean recovery rates within the requested range of 75-125 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.01 to $< 0.1\%$ (w/w)

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

Table 11: Results prothioconazole FS 100 (100 g/L) (Recovery Level I - LOQ)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	92.0	91.9	97.9	90.0	108.6
Weighing no. 2	83.1	99.3	92.3	90.0	108.0
Weighing no. 3	100.0	92.5	92.3	89.6	107.6
Weighing no. 4	86.8	97.3	86.6	88.8	105.1
Weighing no. 5	80.3	92.2	87.8	85.6	108.3
Weighing no. 6	79.1	84.9	87.0	85.6	108.3
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*

Single and mean recovery rates within the requested range of 70-130 % according to SANCO/3030/99 for a nominal analyte content < 0.01% (w/w)

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

Table 12: Results prothioconazole FS 100 (100 g/L) (Recovery Level II)

	prothioconazole-desthio				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	75.4	100.5	101.5	73.7	105.2
Weighing no. 2	91.9	101.7	101.9	73.6	105.6
Weighing no. 3	83.0	98.8	99.5	71.5	105.1
Weighing no. 4	84.4	97.7	94.7	73.0	106.0
Weighing no. 5	88.0	99.9	94.3	73.6	106.2
Weighing no. 6	88.0	102.1	95.1	71.3	107.0
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

Single and mean recovery rates within the requested range of 70-130 % according to SANCO/3030/99 for a nominal analyte content < 0.01% (w/w)

6.2 MS-Spectra

Figure 1: Product Ion Scan of reference item prothioconazole-desthio (MS/MS of m/z 312 Da)

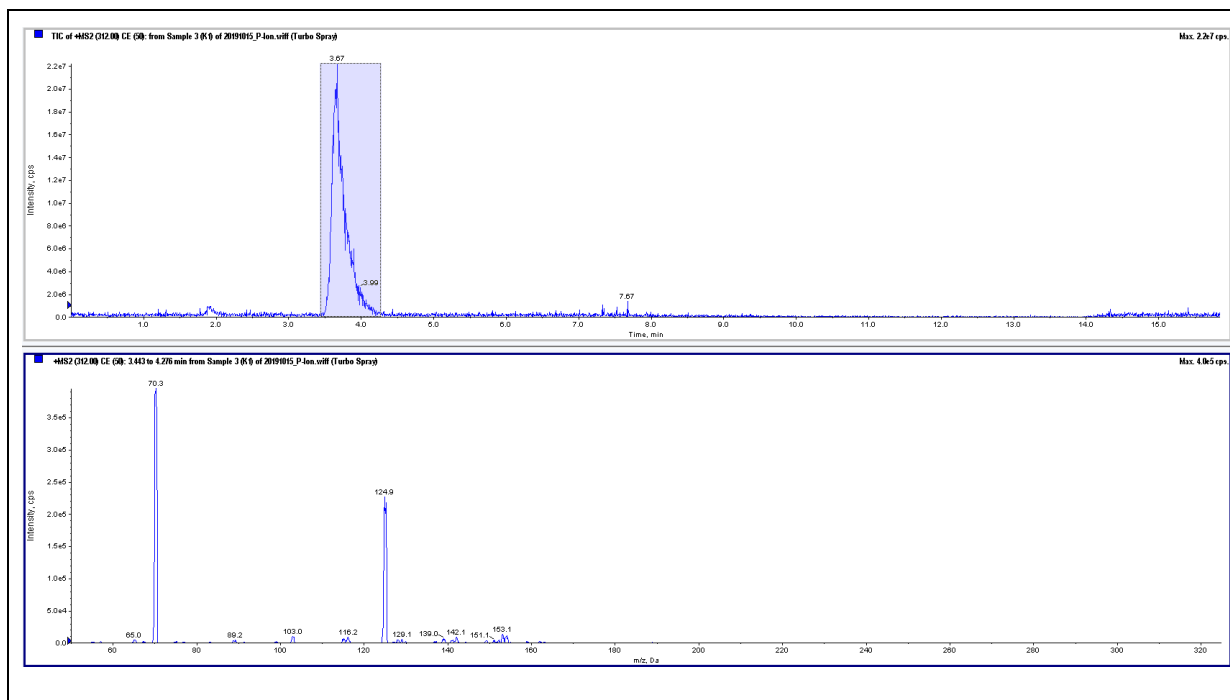


Figure 2: Product Ion Scan of prothioconazole-desthio in the technical material solution of prothioconazole TC (MS/MS of m/z 312 Da)

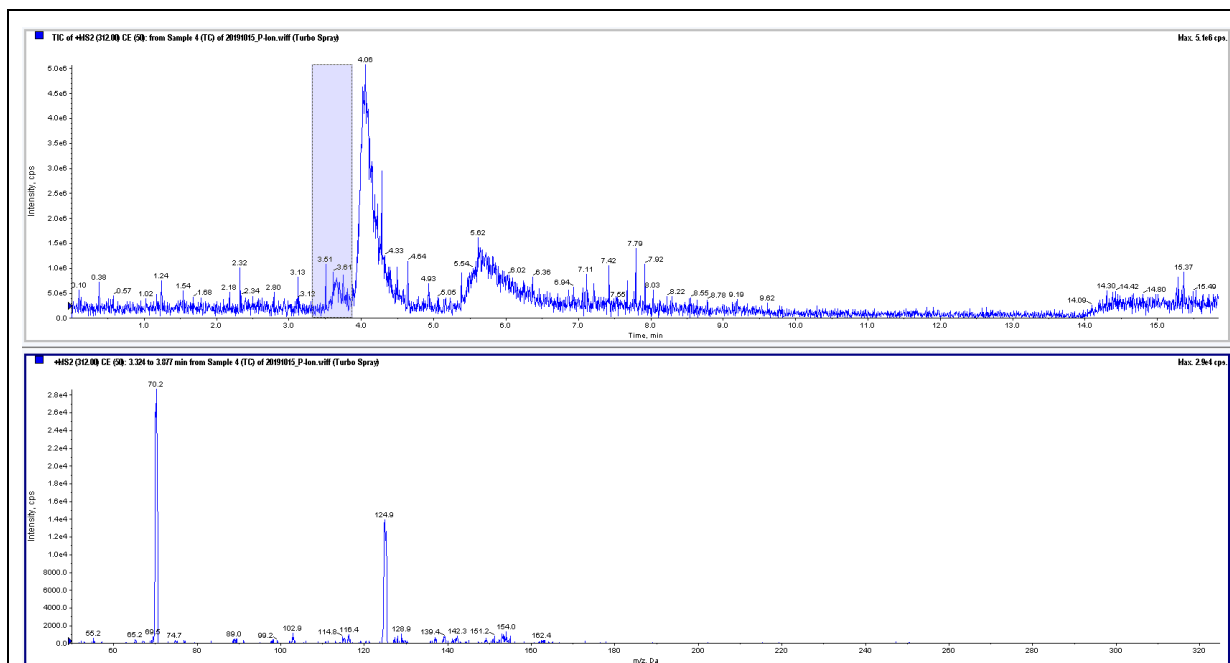


Figure 3: Product Ion Scan of prothioconazole-desthio in the Formulation solution of prothioconazole SC 480 (480 g/L) (MS/MS of m/z 312 Da)

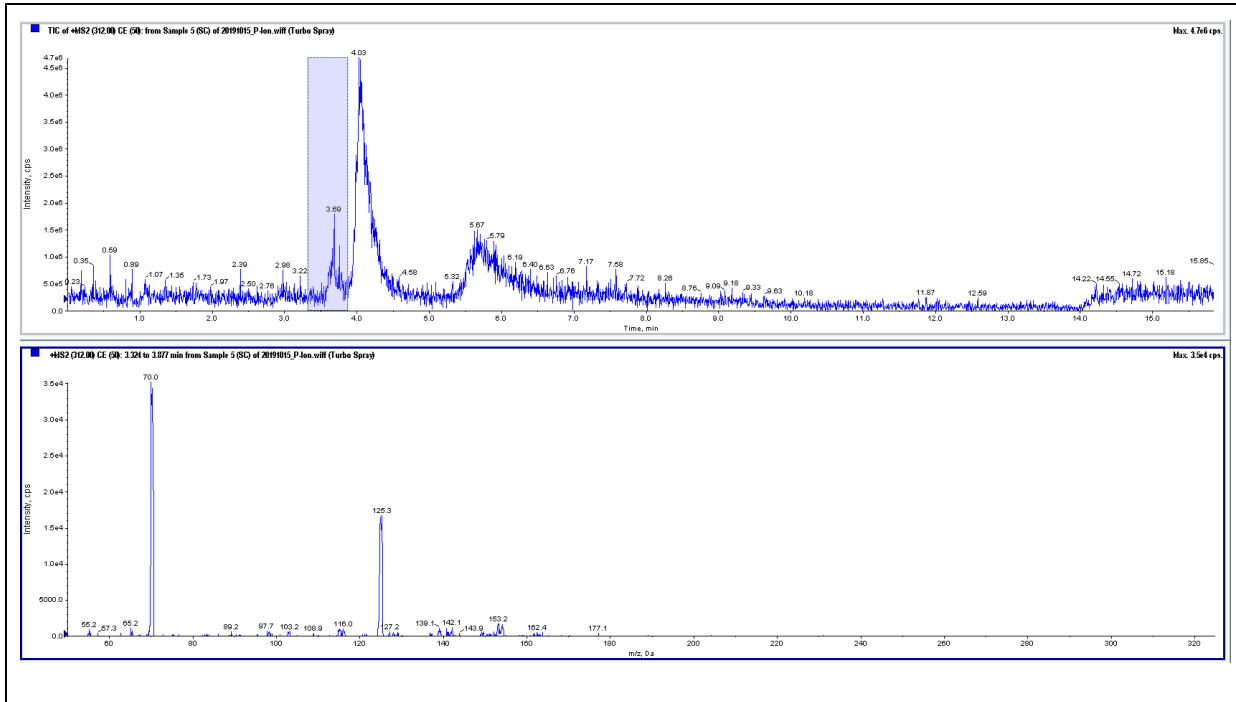


Figure 4: Product Ion Scan of prothioconazole-desthio in the Formulation solution of prothioconazole EC 250 (250 g/L) (MS/MS of m/z 312 Da)

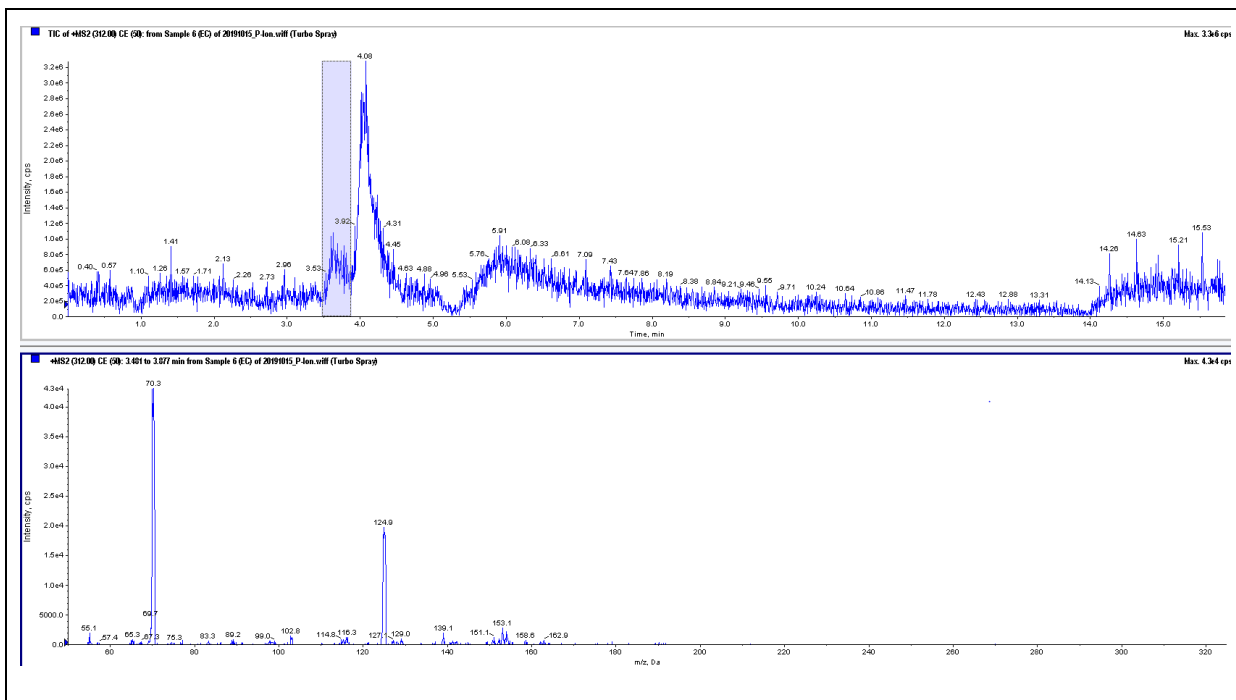
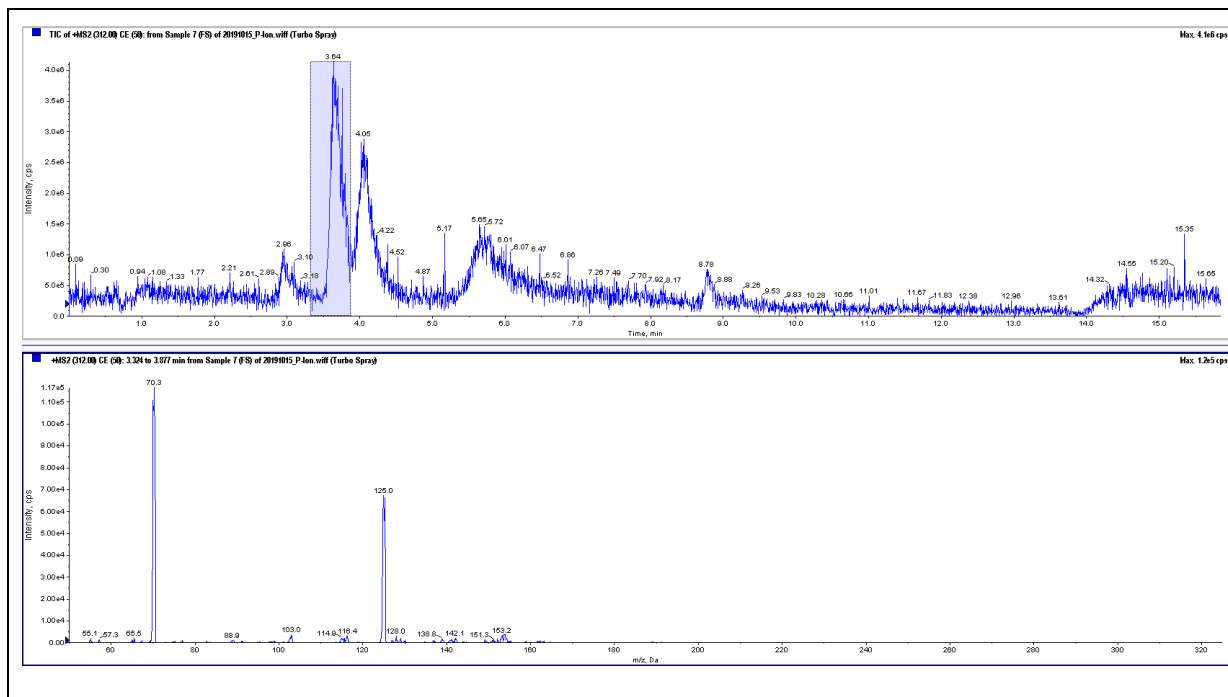


Figure 5: Product Ion Scan of prothioconazole-desthio in the Formulation solution of prothioconazole FS 100 (100 g/L) (MS/MS of m/z 312 Da)



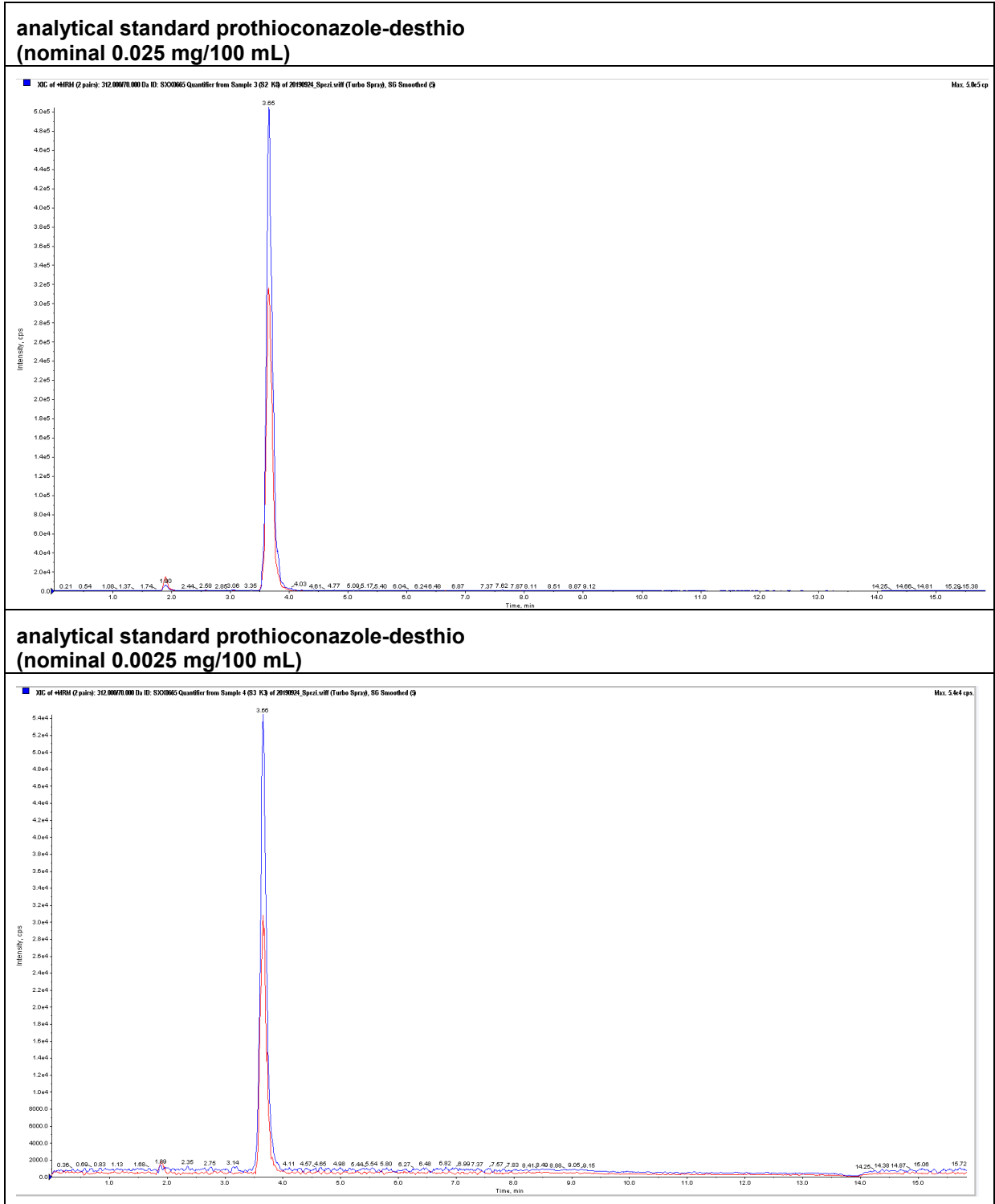
6.3 Chromatograms

An interference peak at the retention time of PTZ-desthio was detected in the chromatograms of Lab 2 and Lab 3 of the blank formulation. This peak was found to be due to carry-over from previous injections. This Peak contributes less than 1 % to the determination of PTZ-desthio at its max. accepted level and therefore it has been considered no significant.

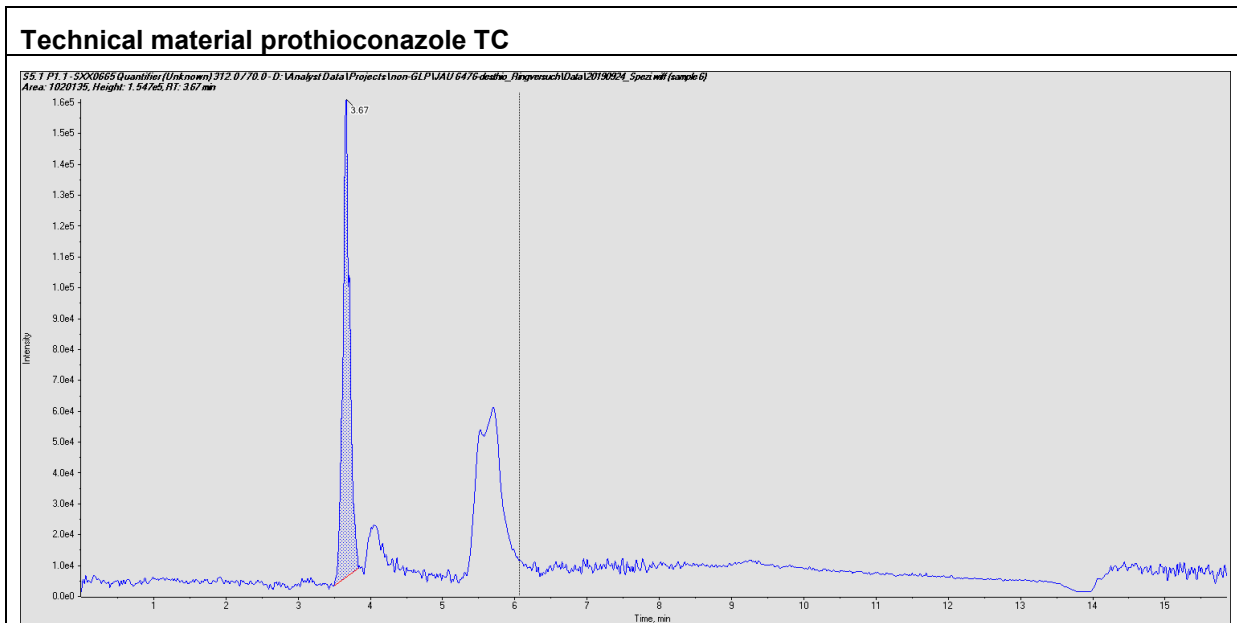
The specificity of Lab 4 could not be properly evaluated, since these samples were injected at the end of a long sequence and the carry-over effect affected the injections of both the sample solvent and the following blank formulations. The injection system was probably contaminated after more than 200 injections of the validation sequence.

Figure 6: Chromatograms of analytical standard prothioconazole-desthio Developing Lab

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



**Figure 7: Chromatogram of prothioconazole TC
Developing Lab**



**Figure 8: Chromatograms of prothioconazole SC 480 (480 g/L)
Developing Lab.**

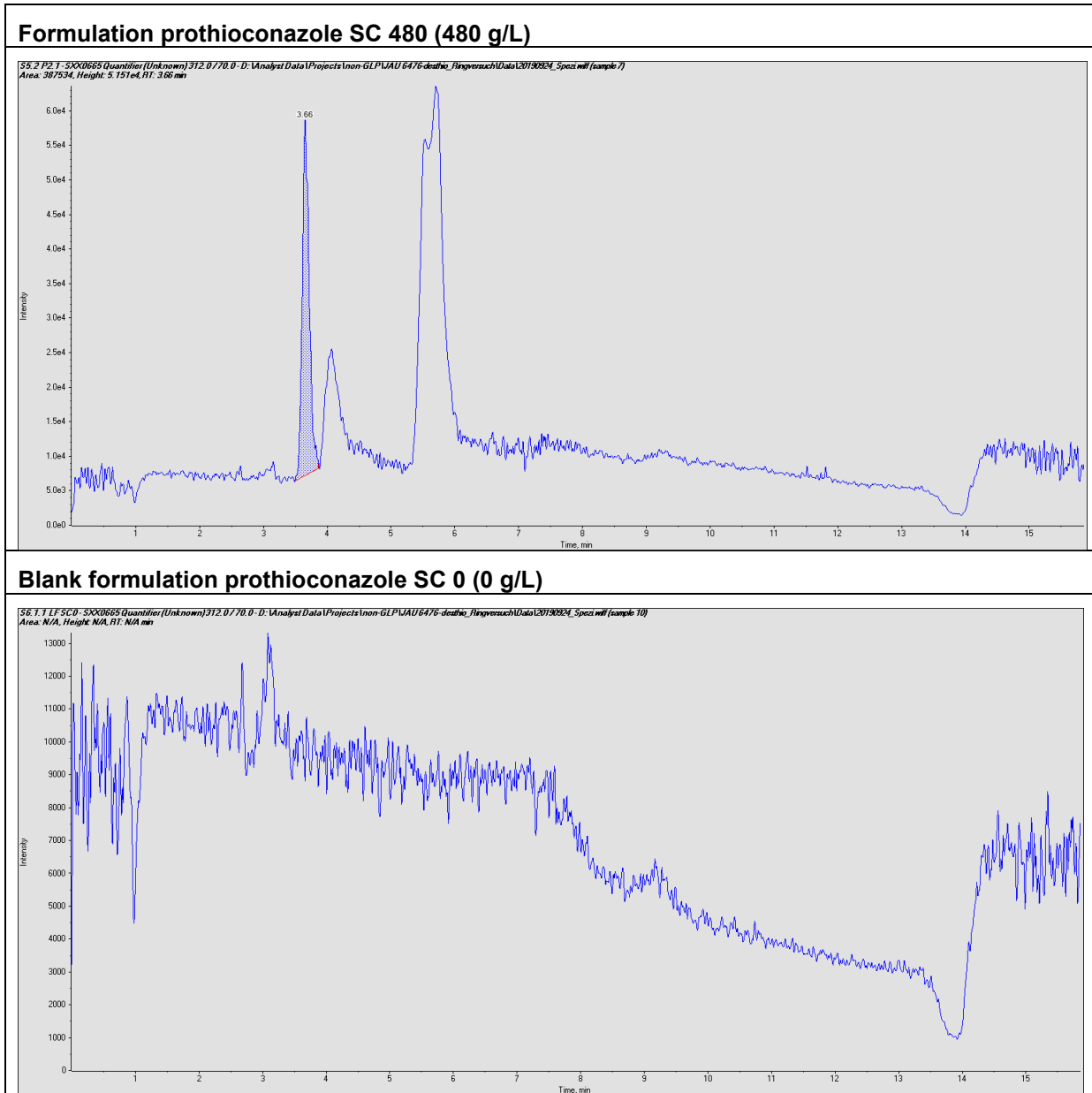
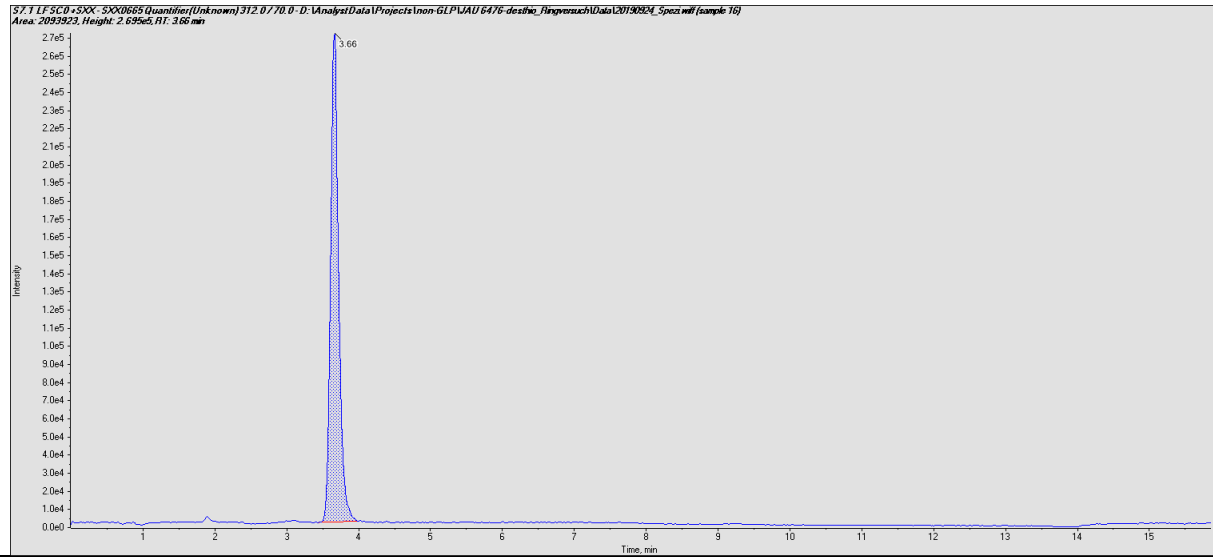
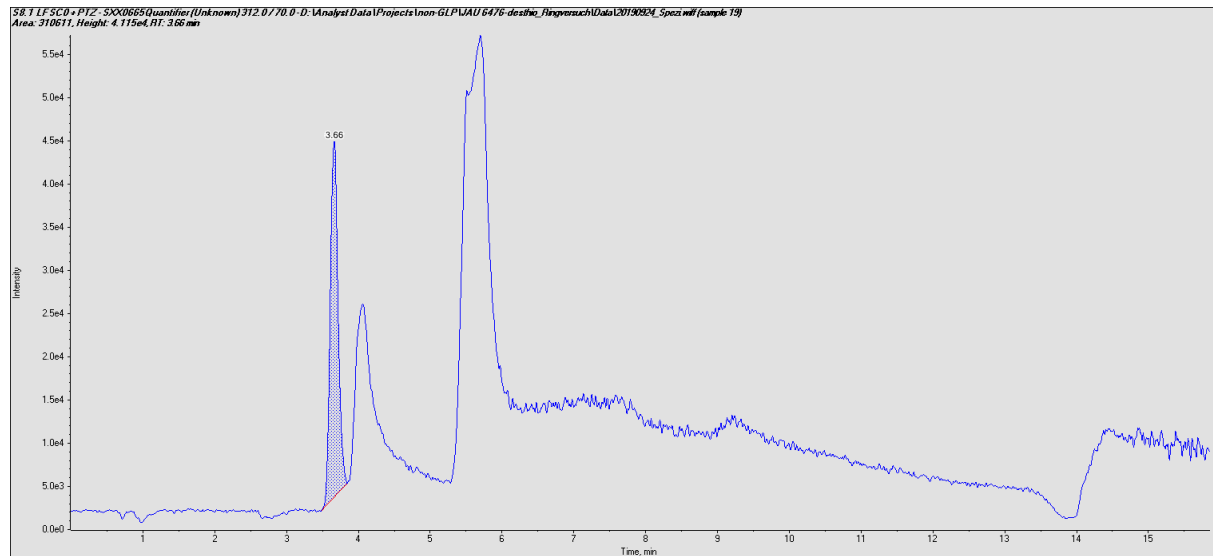


Figure 8. continuation

**Blank formulation prothioconazole SC 0 (0 g/L)
spiked with reference item prothioconazole-desthio****Blank formulation prothioconazole SC 0 (0 g/L)
spiked with reference item prothioconazole**

**Figure 9: Chromatograms of prothioconazole EC 250 (250 g/L)
Developing Lab**

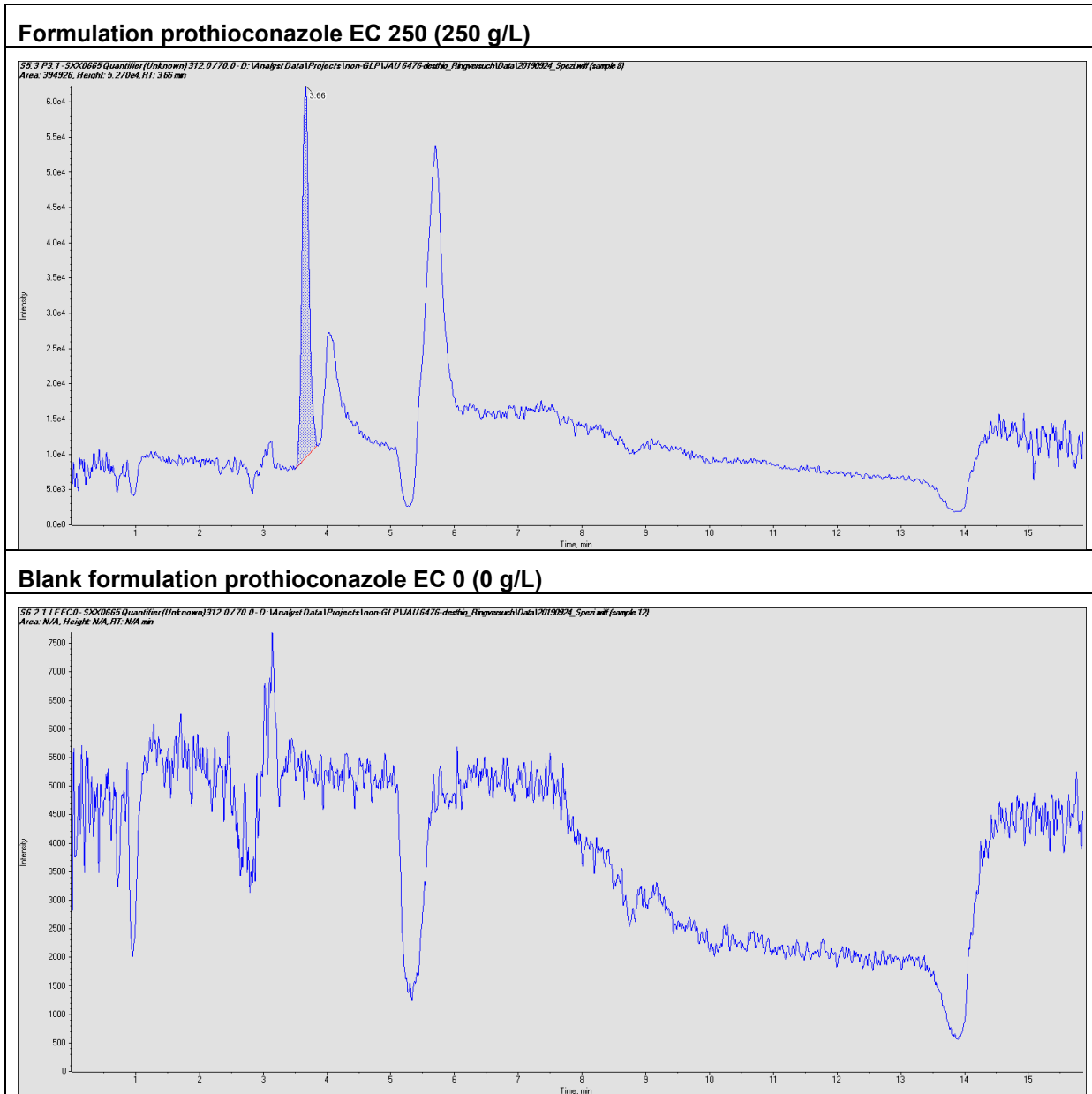
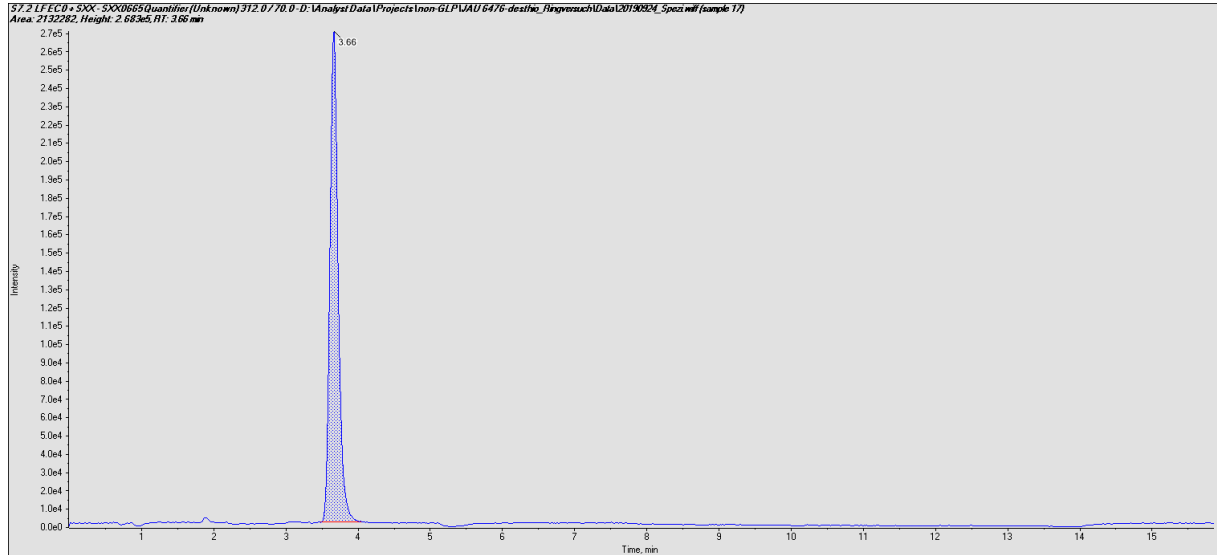
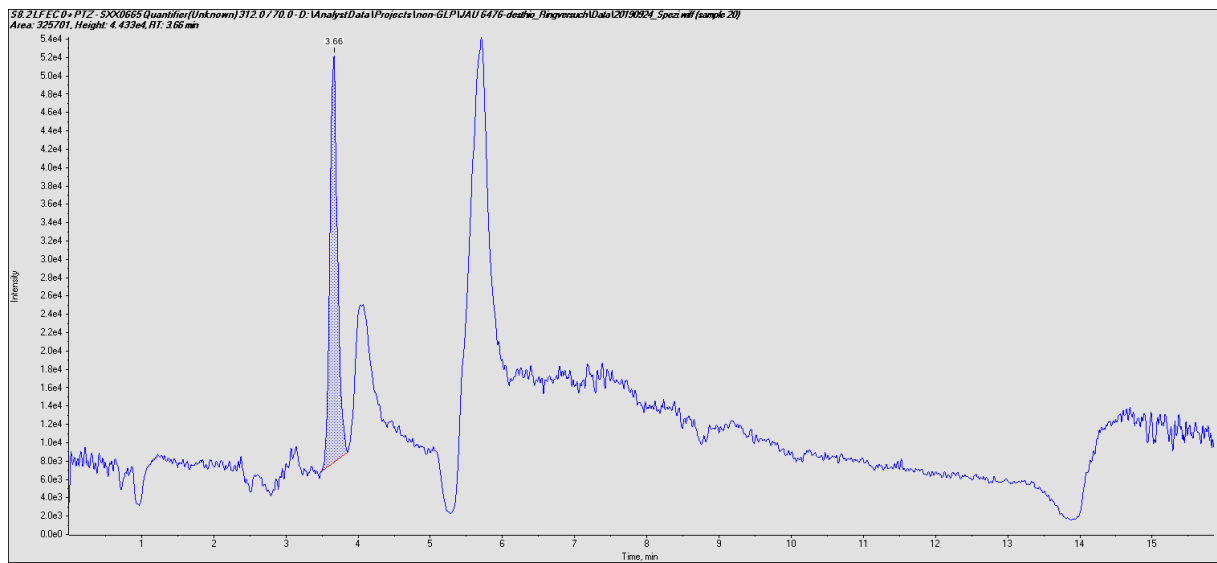


Figure 9. continuation

**Blank formulation prothioconazole EC 0 (0 g/L)
spiked with reference item prothioconazole-desthio****Blank formulation prothioconazole EC 0 (0 g/L)
spiked with reference item prothioconazole**

**Figure 10: Chromatogram of prothioconazole FS 100 (100 g/L)
Developing Lab**

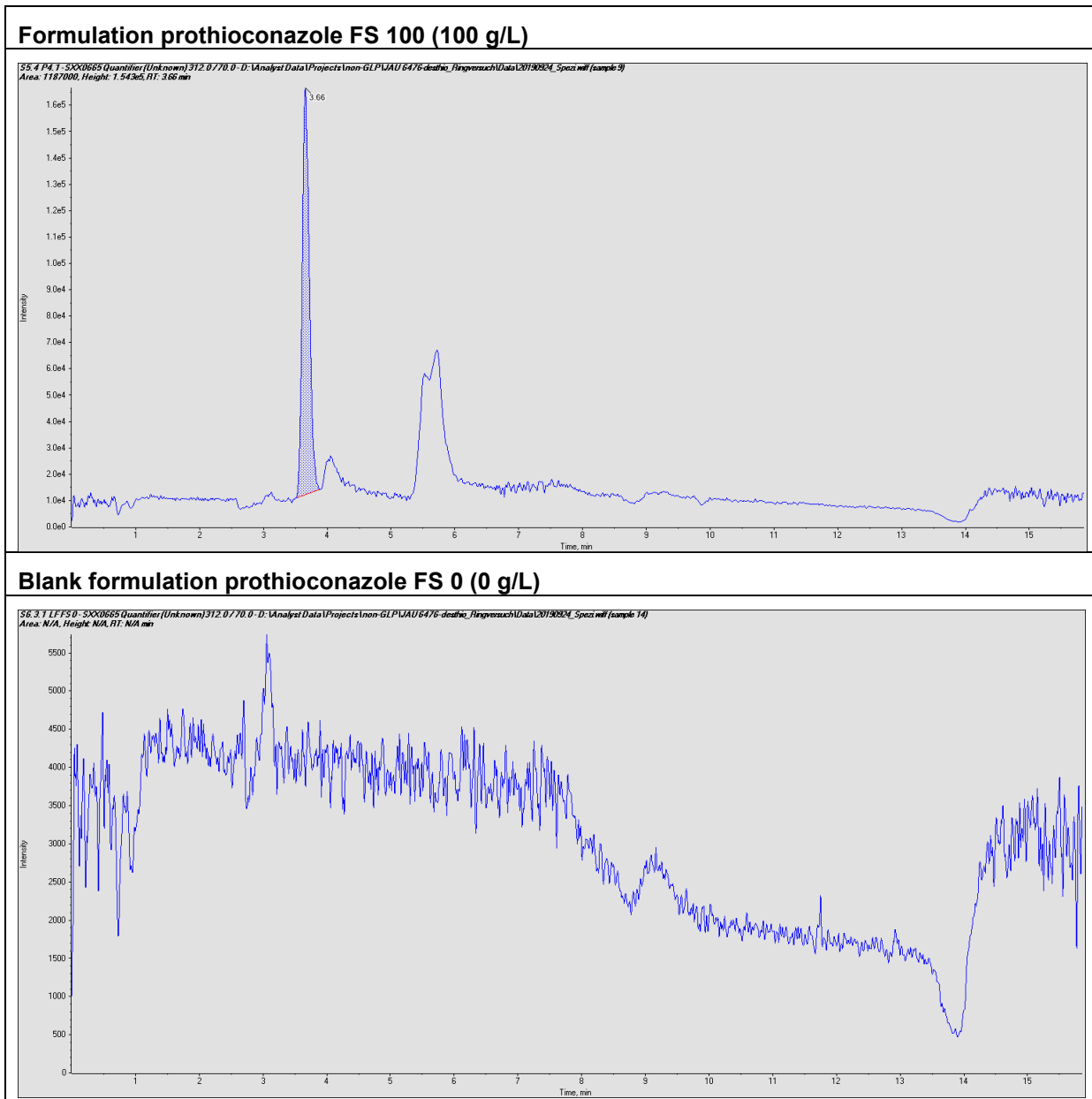
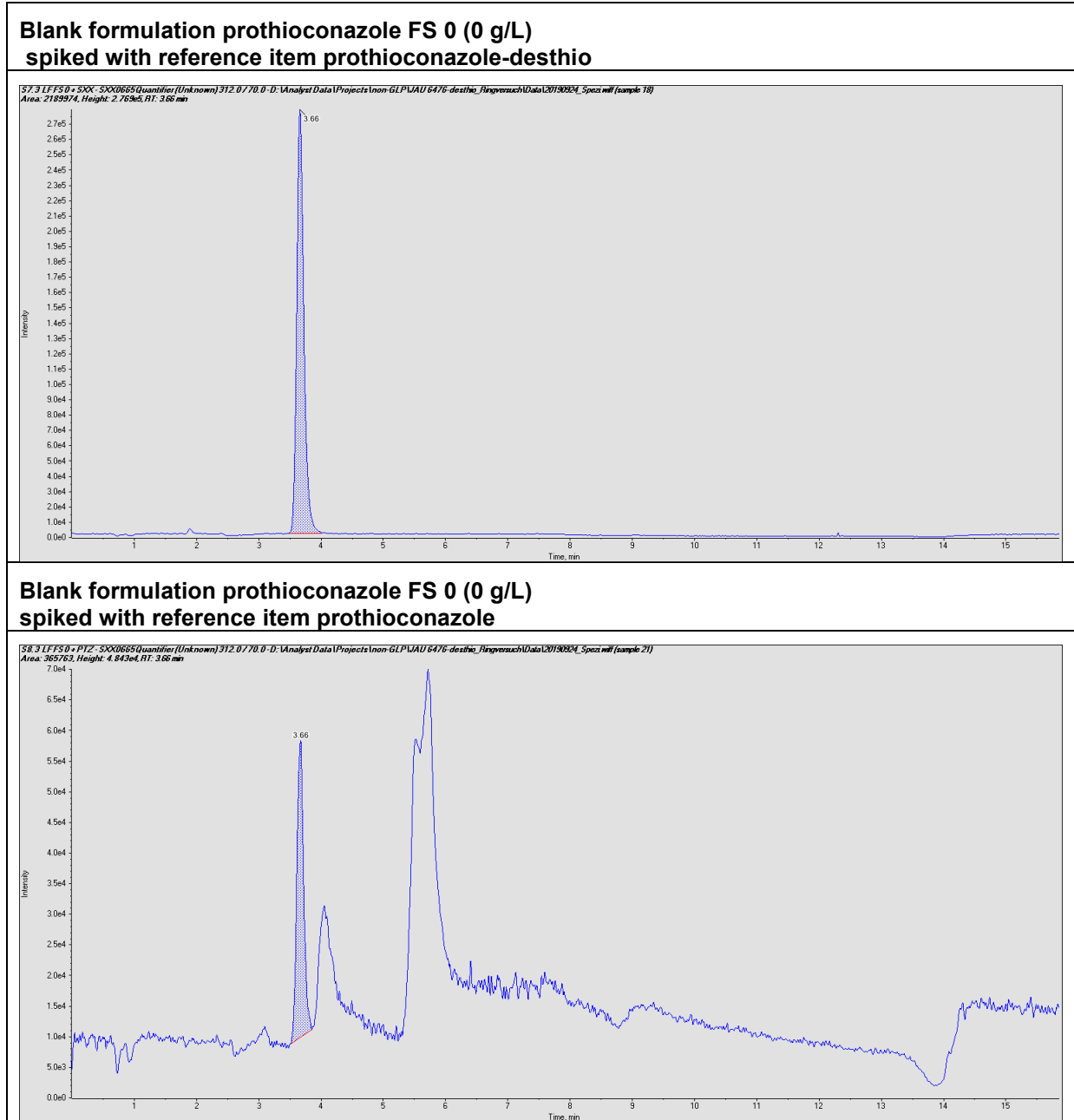
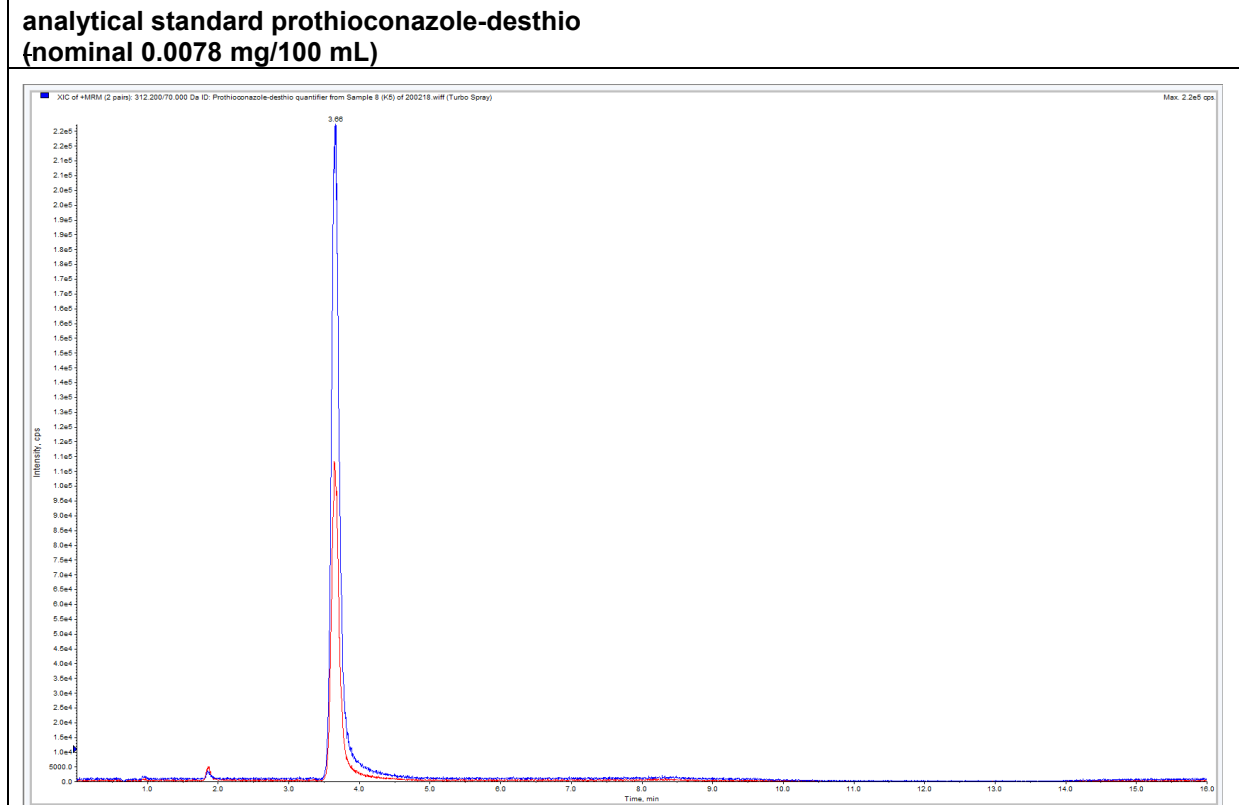


Figure 10. continuation



**Figure 11: Chromatogram of analytical standard prothioconazole-desthio
Lab 1**

Blue: Quantifier; Red: Qualifier



**Figure 12: Chromatograms of technical material prothioconazole TC
Lab 1**

Blue: Quantifier; Red: Qualifier

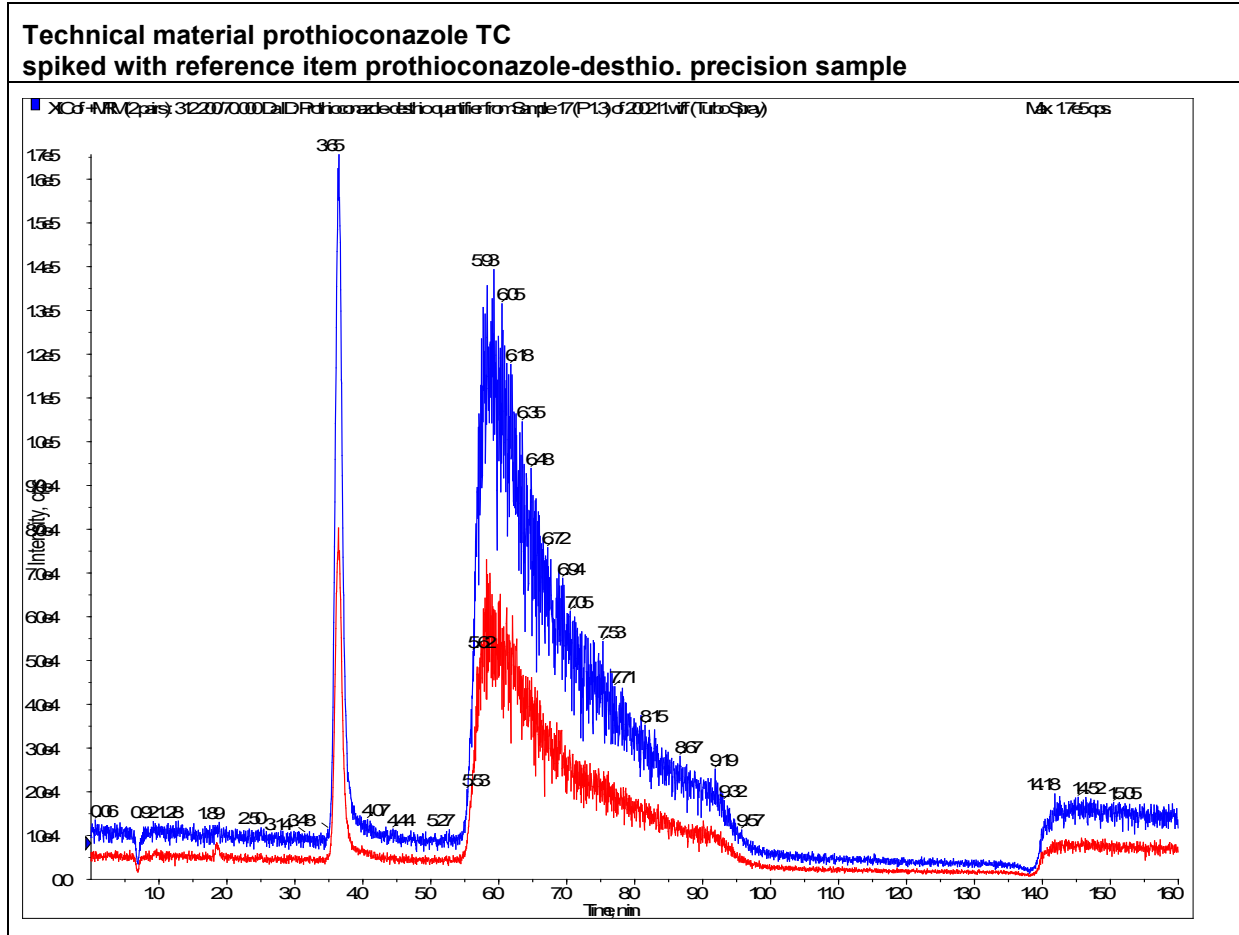


Figure 13: Chromatograms of prothioconazole SC 480 (480 g/L) Lab 1

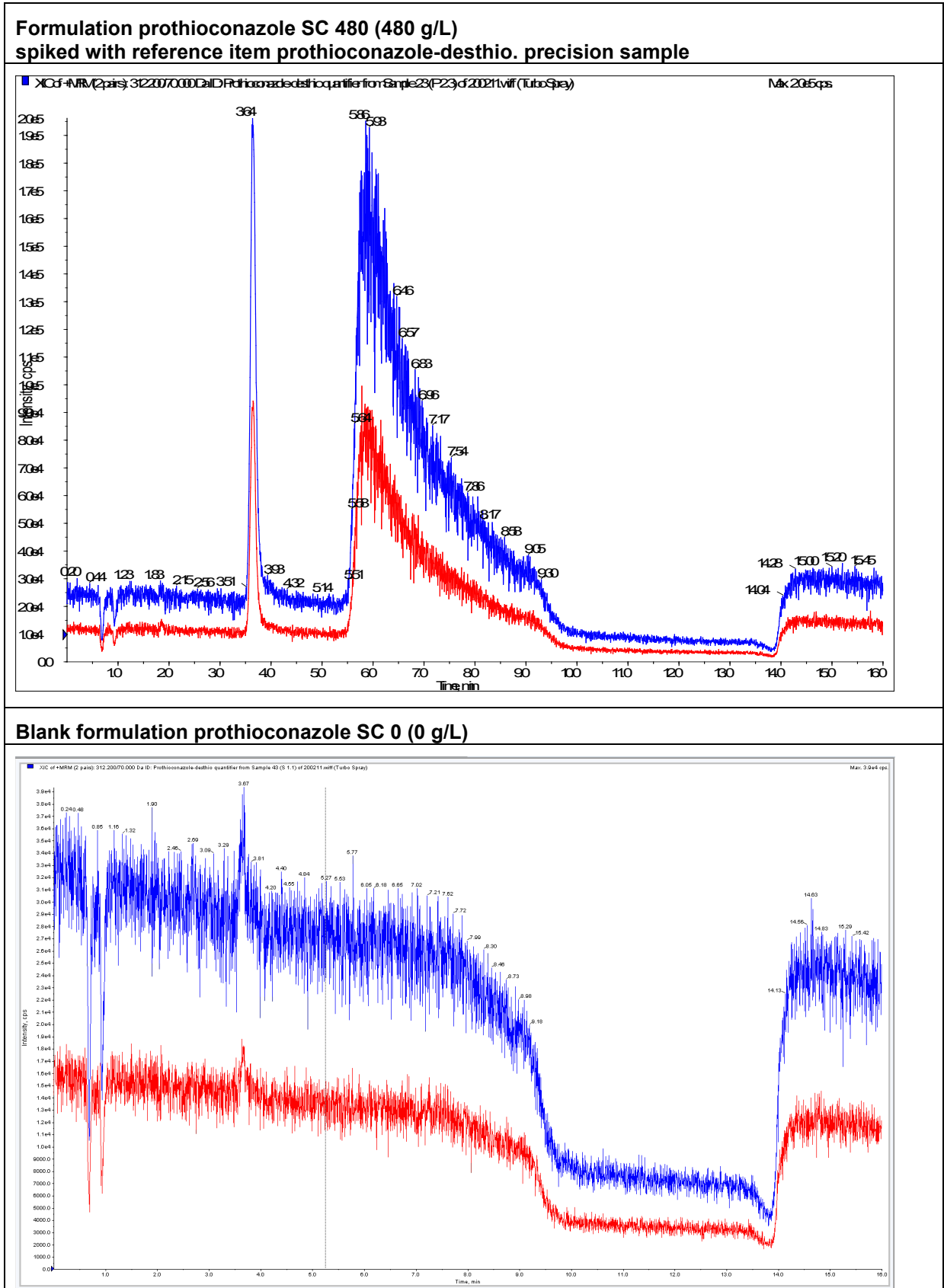


Figure 13. continuation

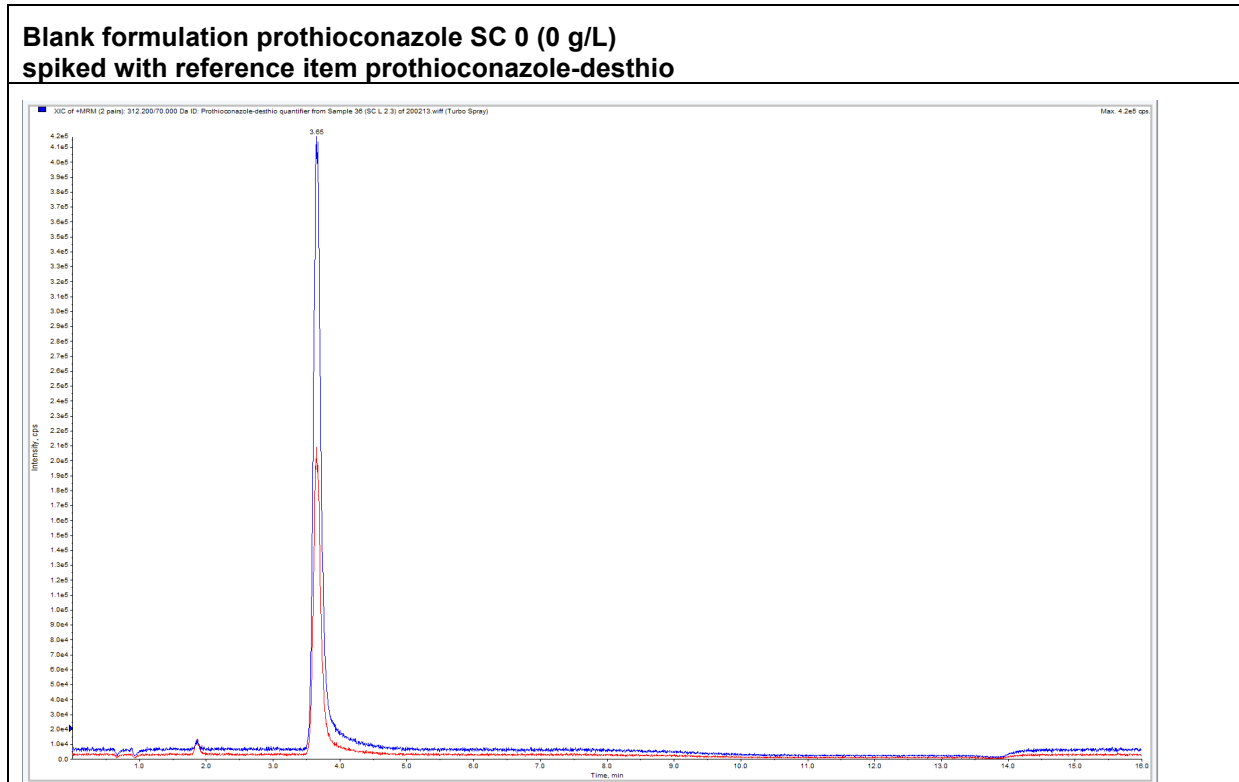


Figure 14: Chromatograms of prothioconazole EC 250 (250 g/L) Lab 1

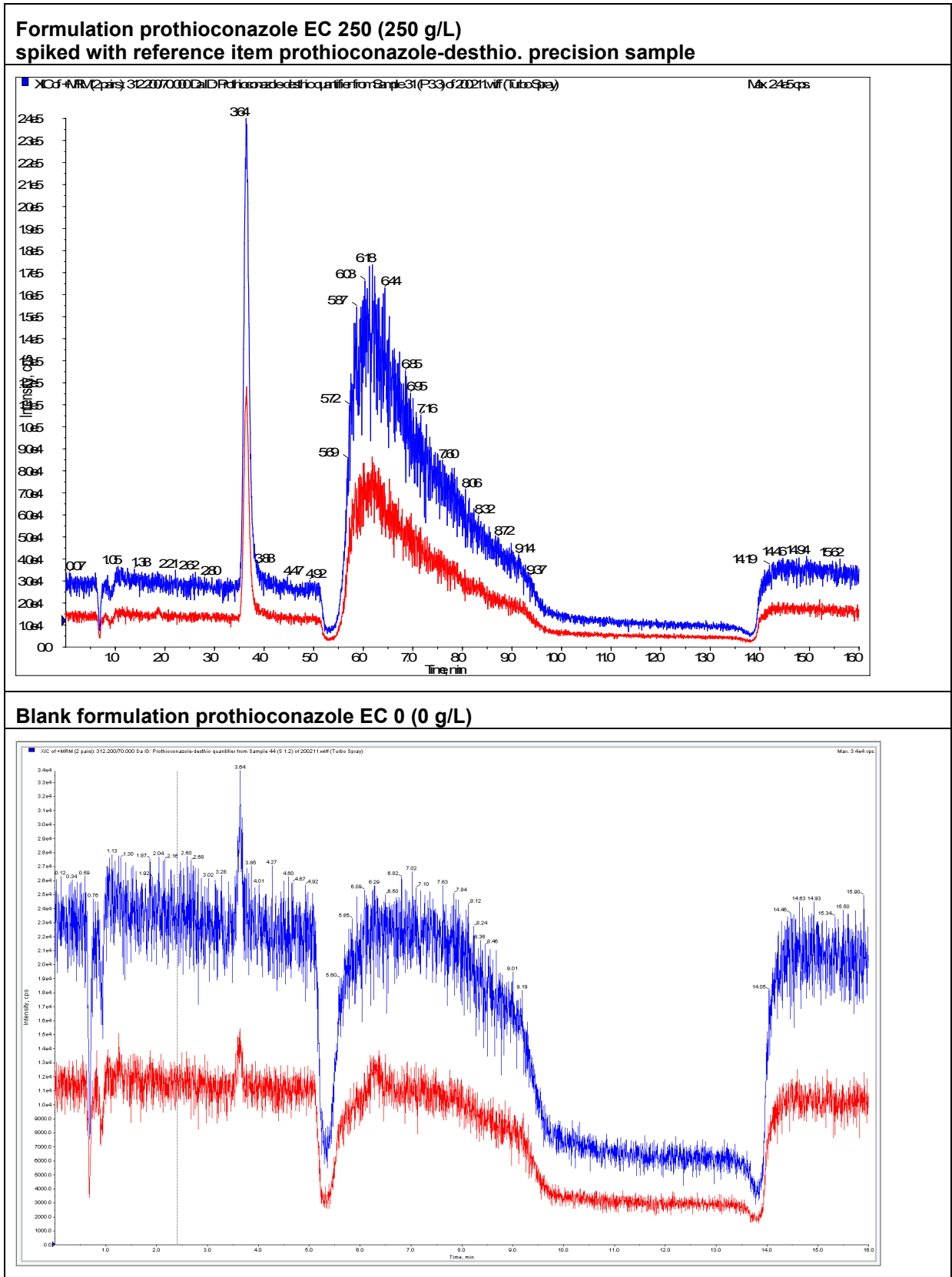
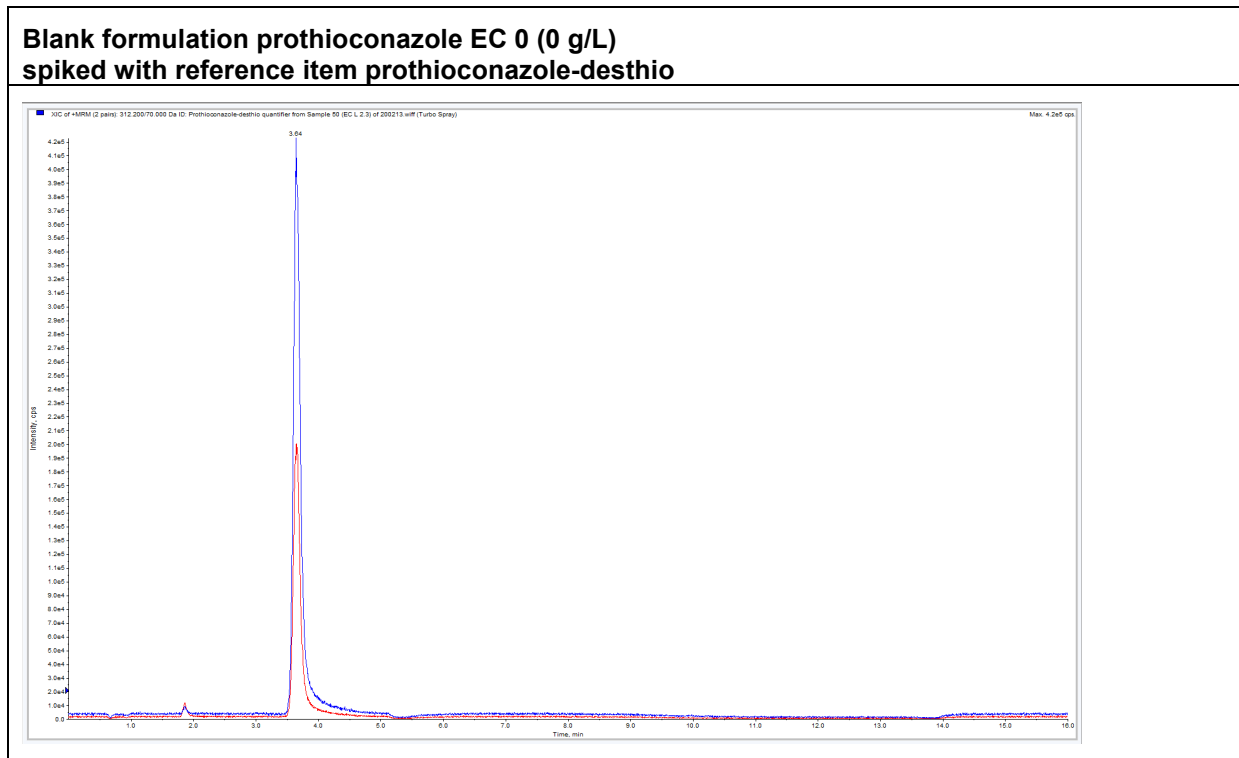


Figure 14. continuation



**Figure 15: Chromatograms of prothioconazole FS 100 (100 g/L)
Lab 1**

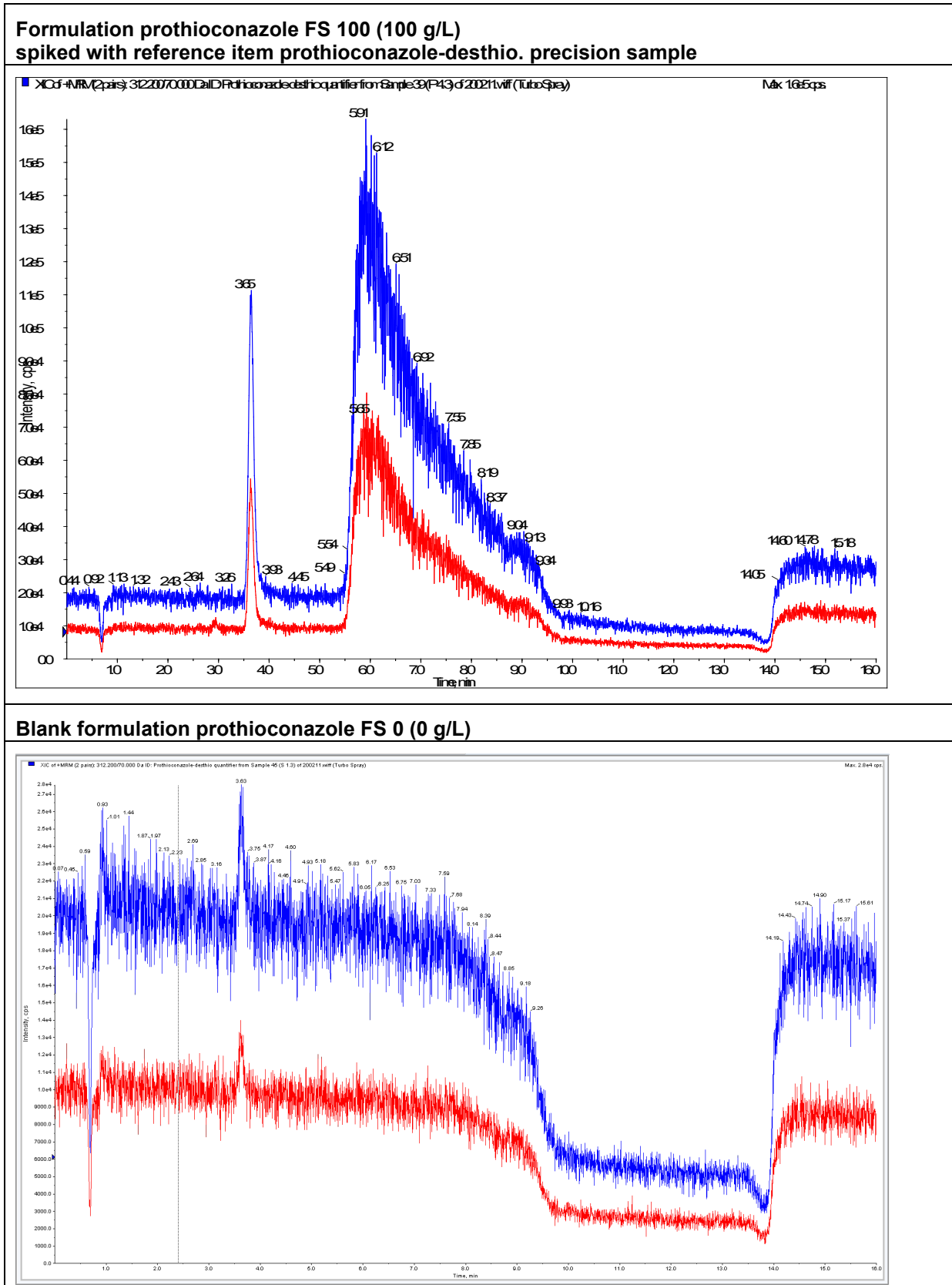
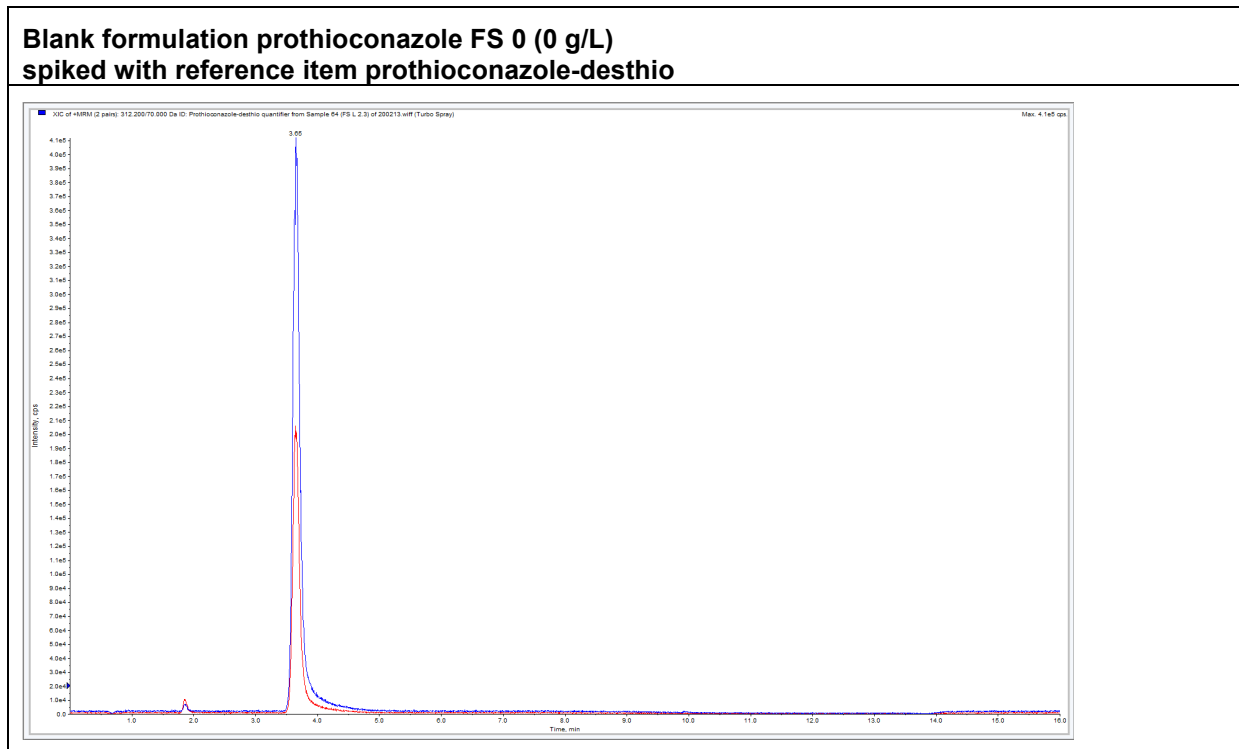
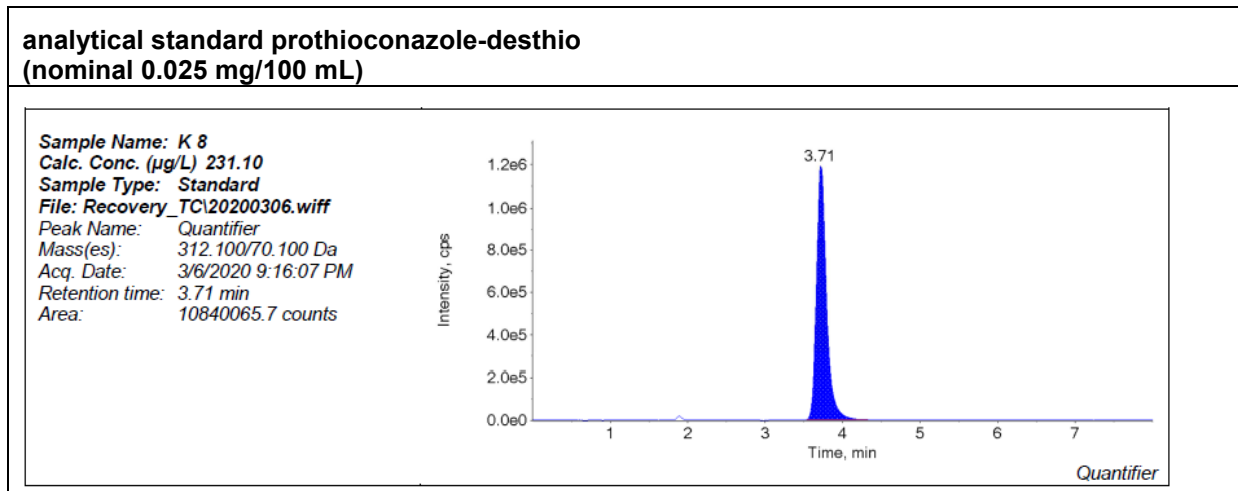


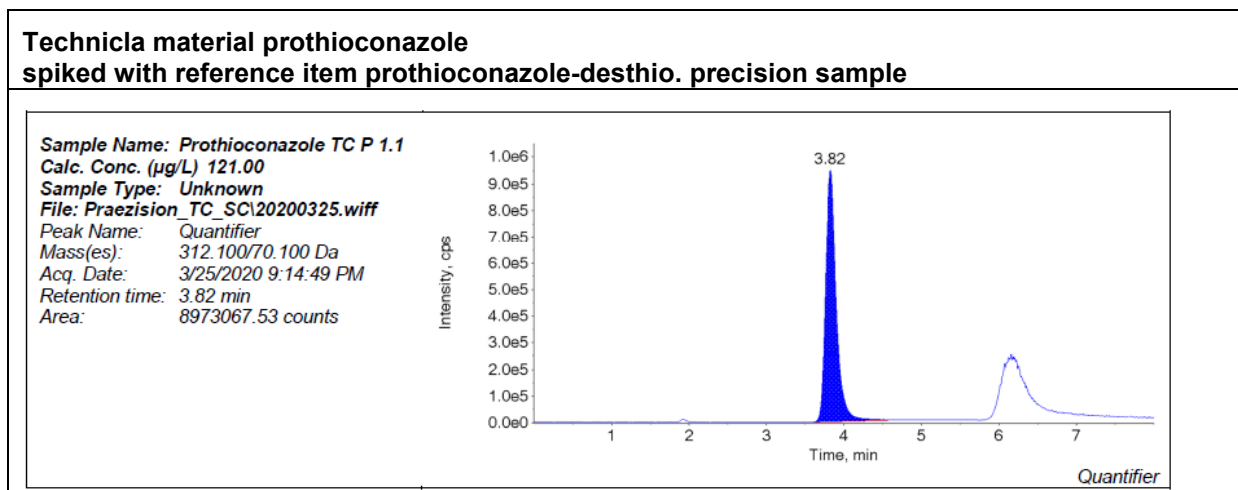
Figure 15. continuation



**Figure 16: Chromatograms of analytical standard prothioconazole-desthio
Lab 2**



**Figure 17: Chromatogram of prothioconazole TC
Lab 2**



**Figure 18: Chromatograms of prothioconazole SC 480 (480 g/L)
Lab 2**

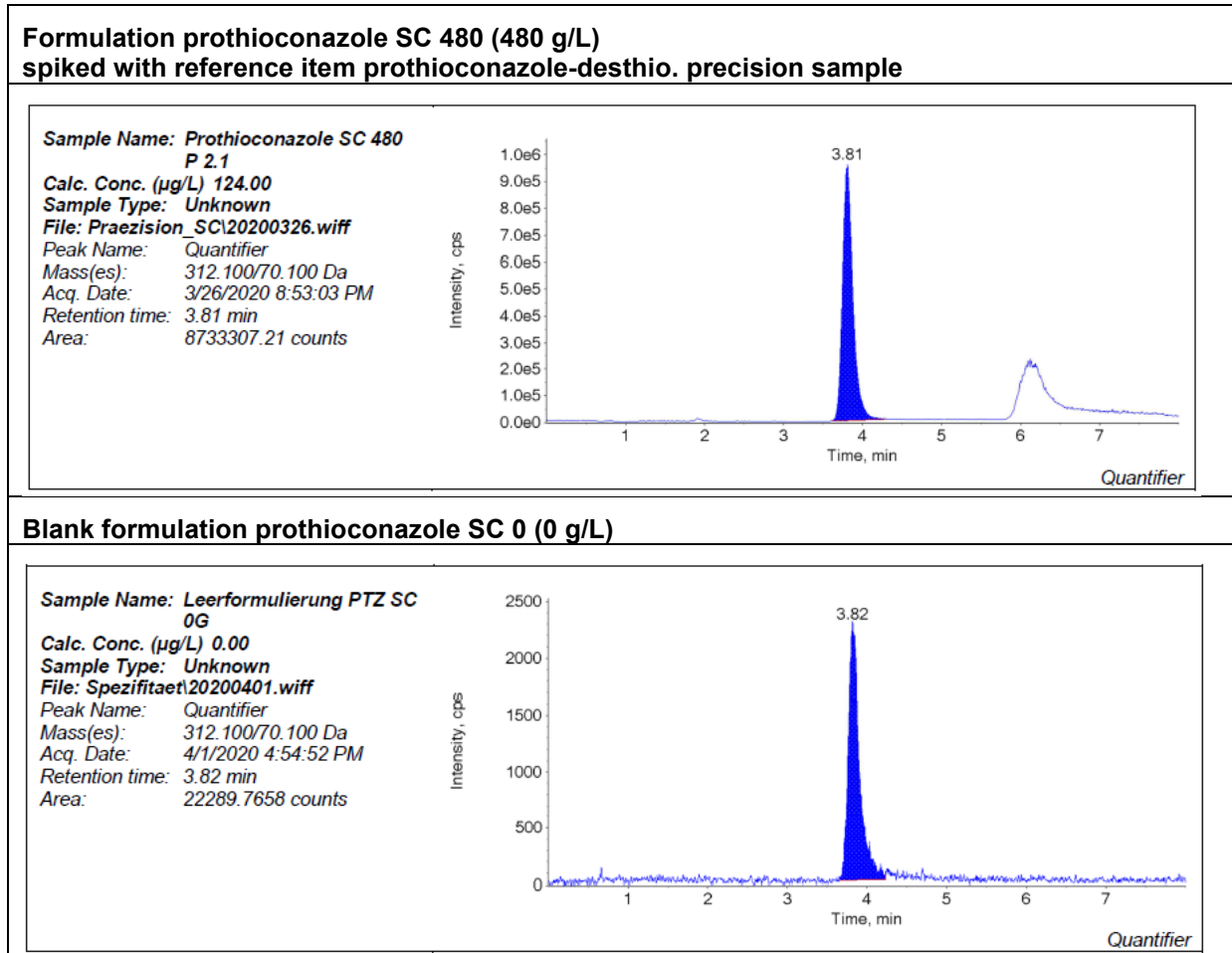
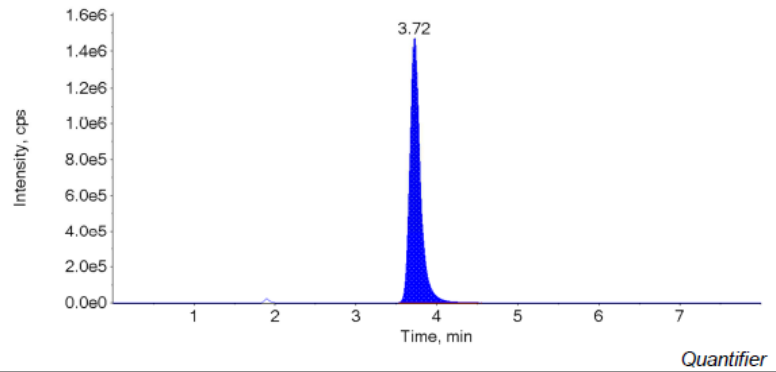


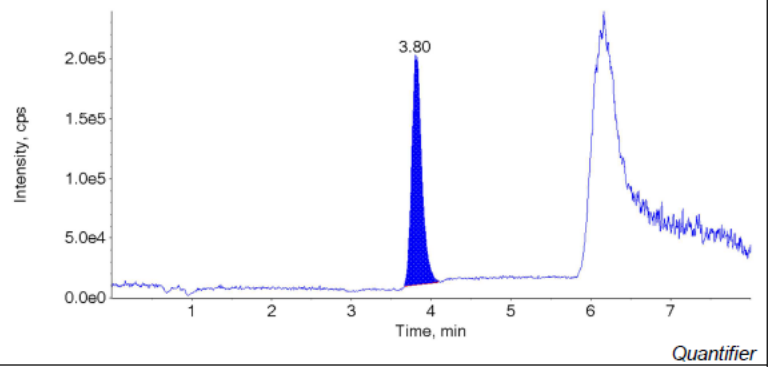
Figure 18. continuation

**Blank formulation prothioconazole SC 0 (0 g/L)
spiked with reference item prothioconazole-desthio**

Sample Name: LF PTZ SC Recovery L
2.1
Calc. Conc. ($\mu\text{g/L}$) 242.40
Sample Type: Unknown
File: Recovery_SC\20200310.wiff
Peak Name: Quantifier
Mass(es): 312.100/70.100 Da
Acq. Date: 3/11/2020 4:15:15 AM
Retention time: 3.72 min
Area: 13425427.6 counts

**Blank formulation prothioconazole SC 0 (0 g/L)
spiked with reference item prothioconazole**

Sample Name: Leerformulierung PTZ SC
0G + PTZ
Calc. Conc. ($\mu\text{g/L}$) 0.00
Sample Type: Unknown
File: Spezifitaet\20200401.wiff
Peak Name: Quantifier
Mass(es): 312.100/70.100 Da
Acq. Date: 4/1/2020 9:11:00 PM
Retention time: 3.80 min
Area: 1685195.07 counts



**Figure 19: Chromatograms of prothioconazole EC 250 (250 g/L)
Lab 2**

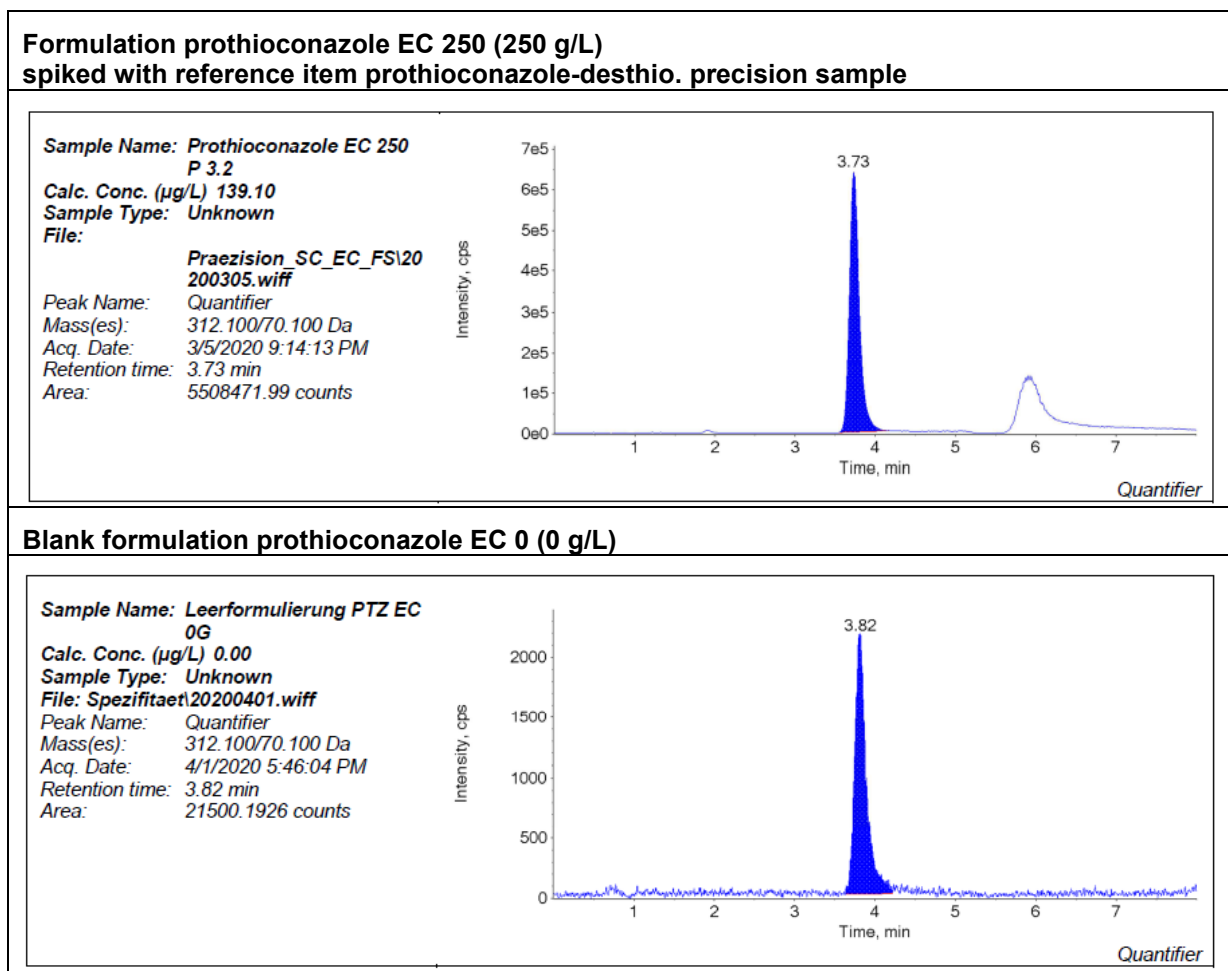
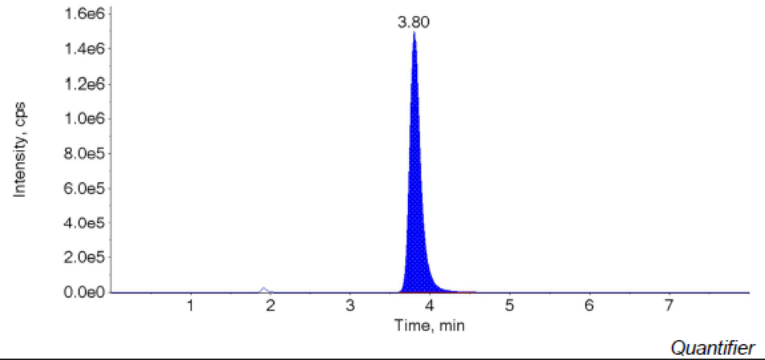


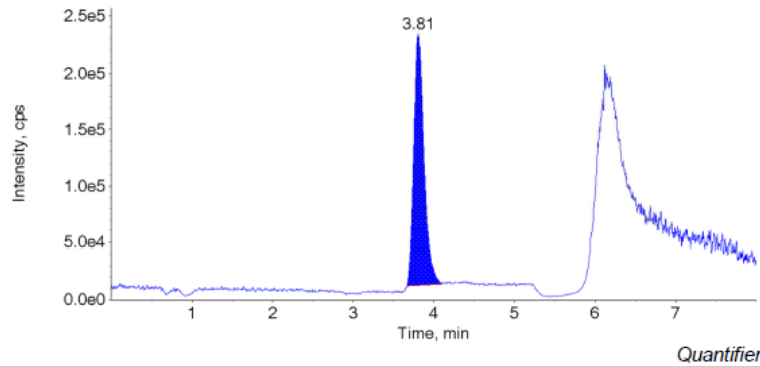
Figure 19. continuation

**Blank formulation prothioconazole EC 0 (0 g/L)
spiked with reference item prothioconazole-desthio**

Sample Name: LF PTZ EC Recovery L
2.1
Calc. Conc. ($\mu\text{g/L}$) 219.40
Sample Type: Unknown
File: Recovery_EC\20200330.wiff
Peak Name: Quantifier
Mass(es): 312.100/70.100 Da
Acq. Date: 3/31/2020 8:18:34 AM
Retention time: 3.80 min
Area: 14496091.5 counts

**Blank formulation prothioconazole EC 0 (0 g/L)
spiked with reference item prothioconazole**

Sample Name: Leerformulierung PTZ EC
0G + PTZ
Calc. Conc. ($\mu\text{g/L}$) 0.00
Sample Type: Unknown
File: Spezifitaet\20200401.wiff
Peak Name: Quantifier
Mass(es): 312.100/70.100 Da
Acq. Date: 4/1/2020 8:02:42 PM
Retention time: 3.81 min
Area: 1953352.64 counts



**Figure 20: Chromatograms of prothioconazole FS 100 (100 g/L)
Lab 2**

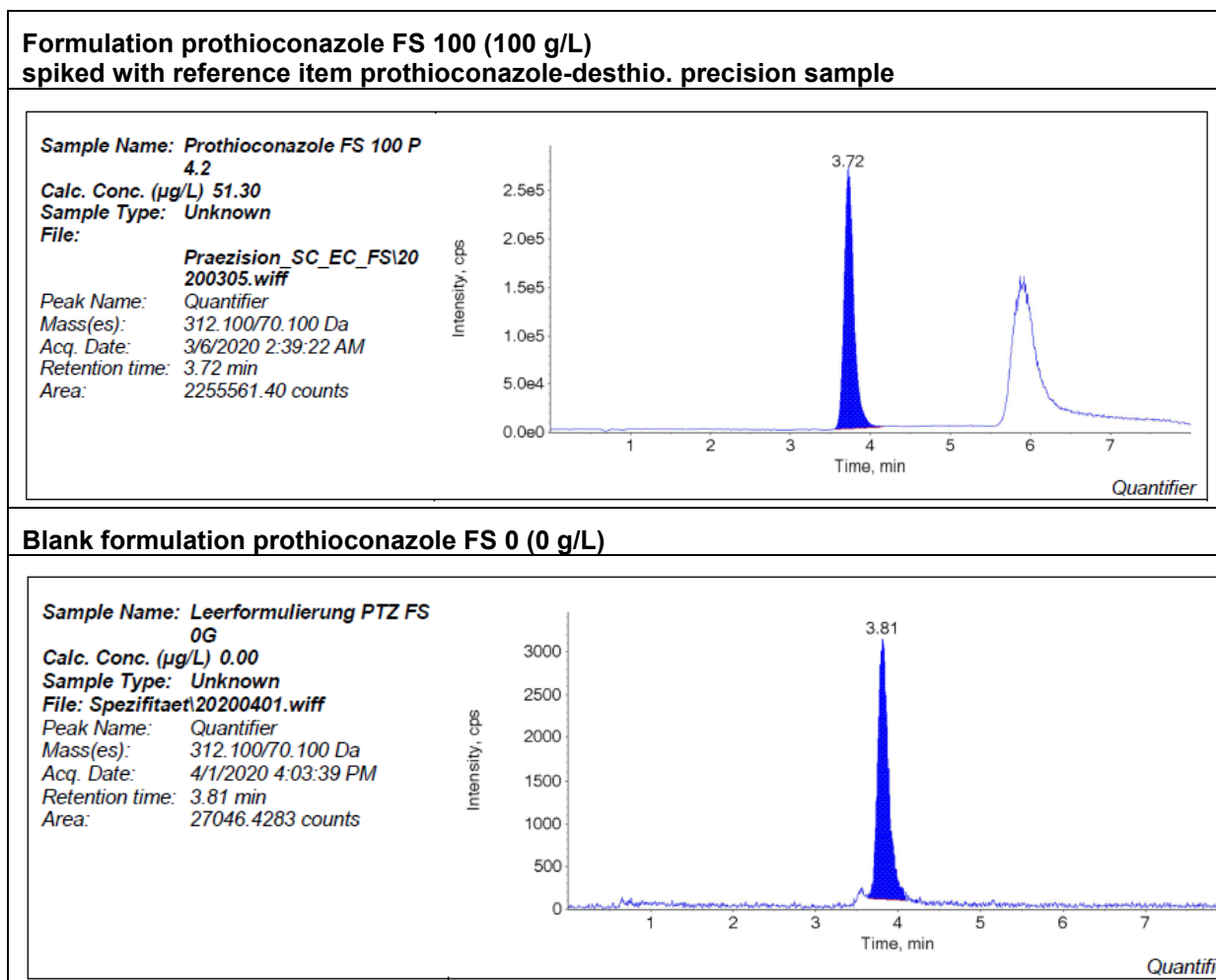


Figure 20. continuation

**Blank formulation prothioconazole FS 0 (0 g/L)
spiked with reference item prothioconazole-desthio**

Sample Name: LF PTZ FS Recovery L
2.1

Calc. Conc. ($\mu\text{g/L}$) 252.00

Sample Type: Unknown

File: Recovery_FS\20200331.wiff

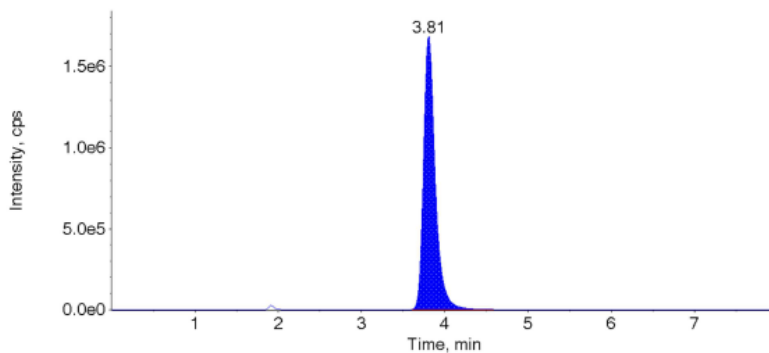
Peak Name: Quantifier

Mass(es): 312.100/70.100 Da

Acq. Date: 4/1/2020 5:08:37 AM

Retention time: 3.81 min

Area: 16582731.7 counts



Quantifie

**Blank formulation prothioconazole FS 0 (0 g/L)
spiked with reference item prothioconazole**

Sample Name: Leerformulierung PTZ FS
0G + PTZ

Calc. Conc. ($\mu\text{g/L}$) 0.00

Sample Type: Unknown

File: Spezifitaet\20200401.wiff

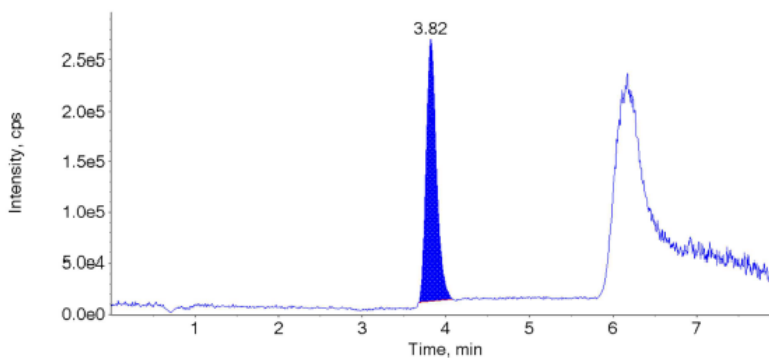
Peak Name: Quantifier

Mass(es): 312.100/70.100 Da

Acq. Date: 4/1/2020 7:11:28 PM

Retention time: 3.82 min

Area: 2176725.31 counts



Quantifie

Figure 21: Chromatograms of analytical standard prothioconazole-desthio Lab 3

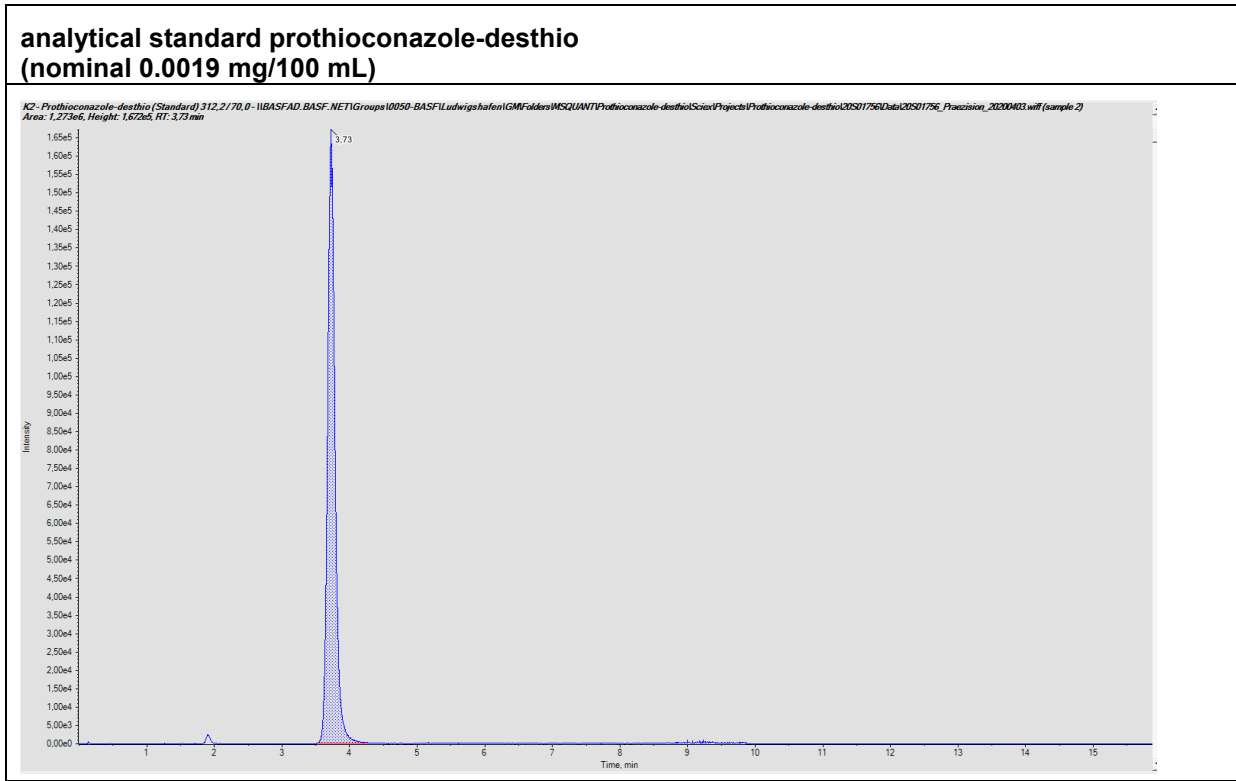
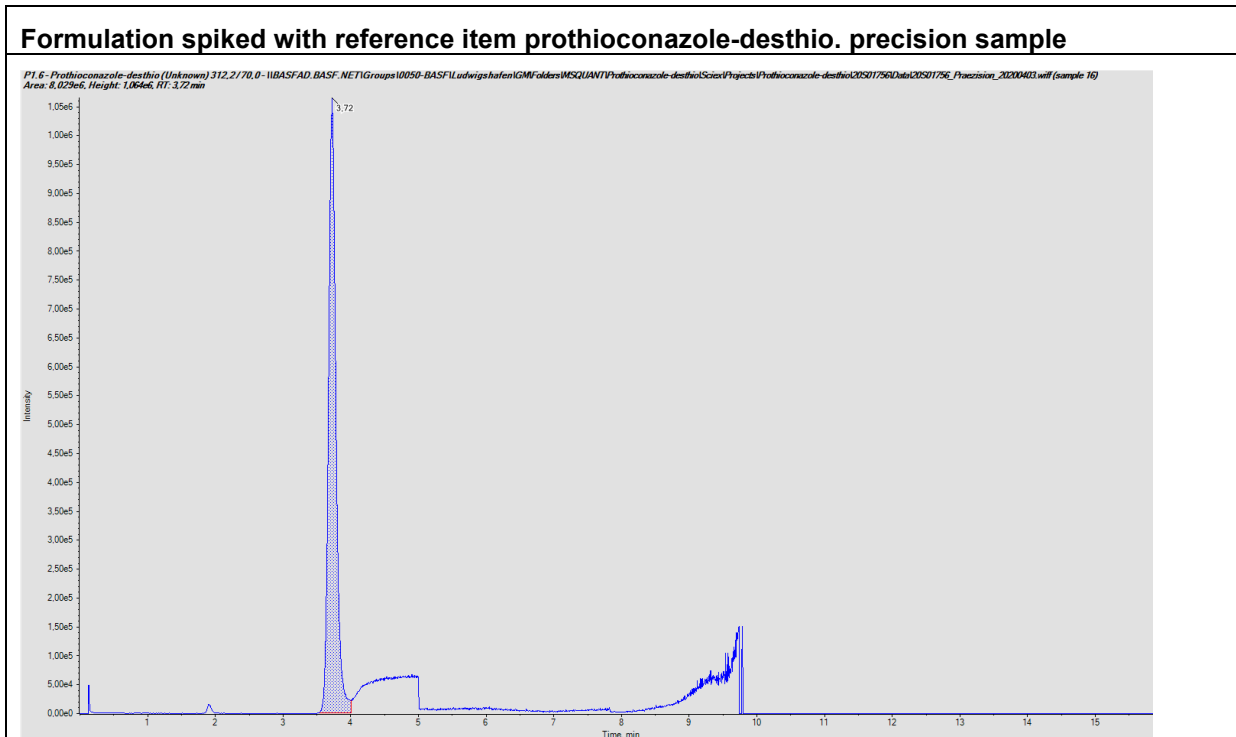


Figure 22: Chromatograms of prothioconazole TC Lab 3



**Figure 23: Chromatograms of prothioconazole SC 480 (480 g/L)
Lab 3**

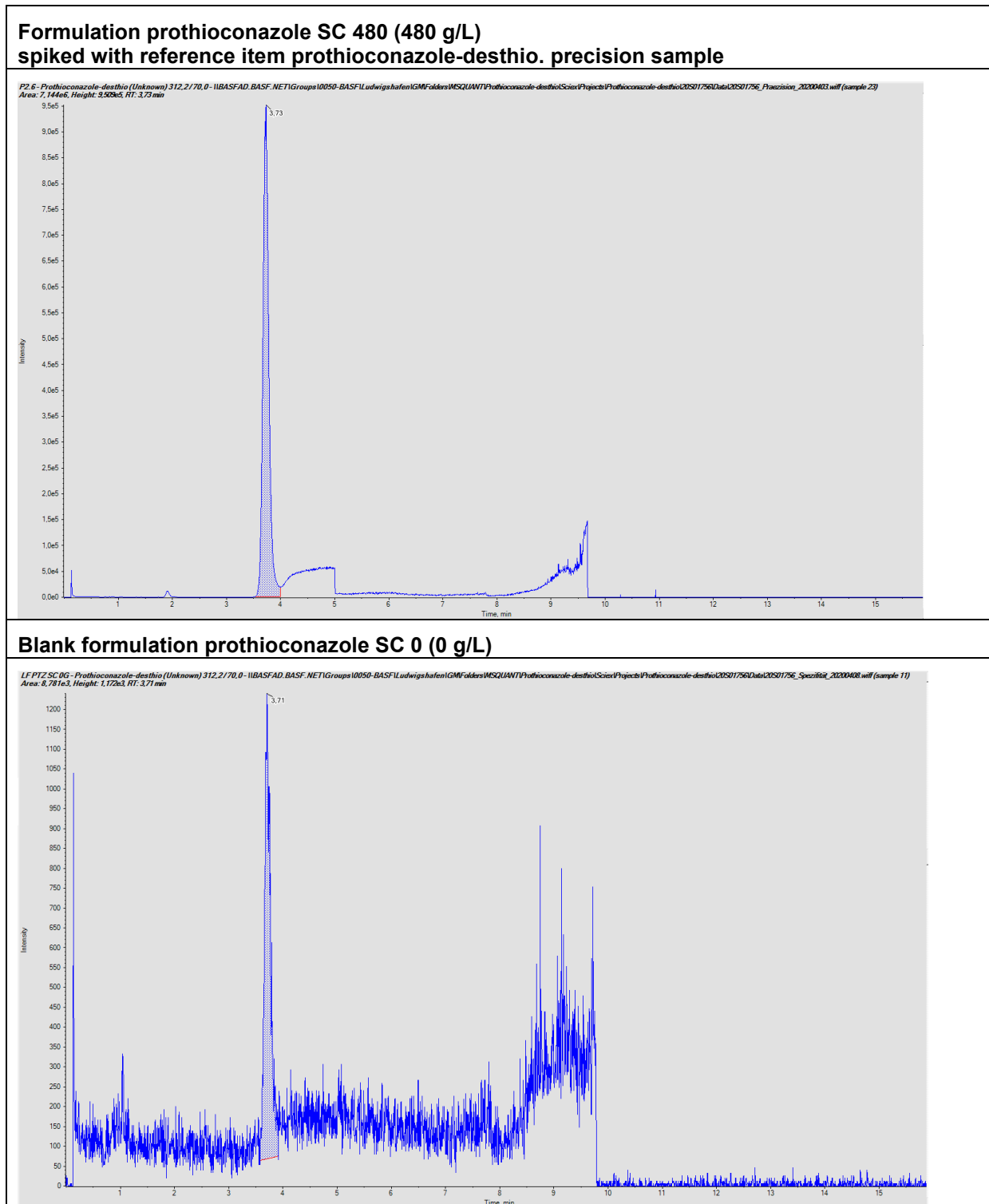
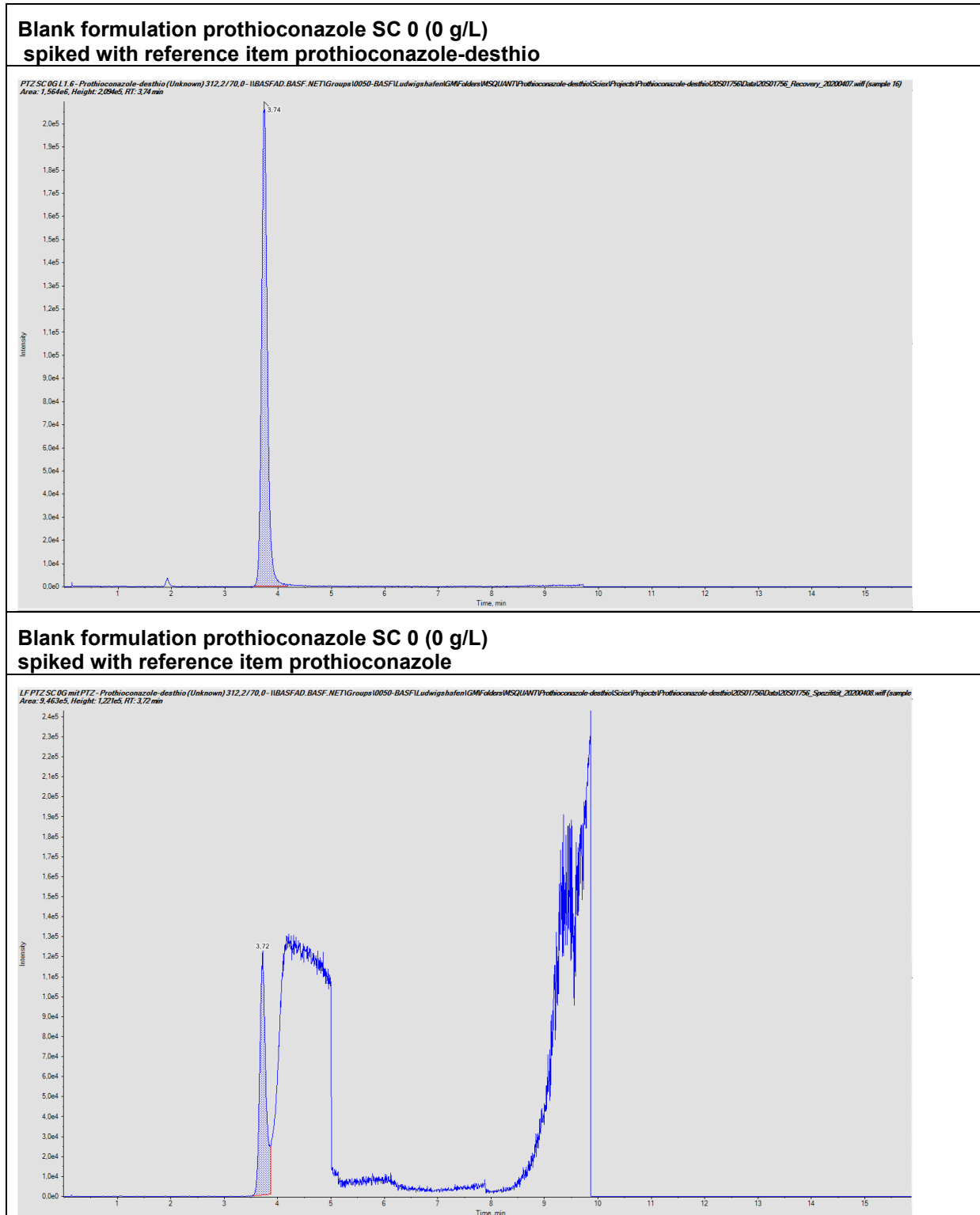


Figure 23. continuation



**Figure 24: Chromatograms of prothioconazole EC 250 (250 g/L)
Lab 3**

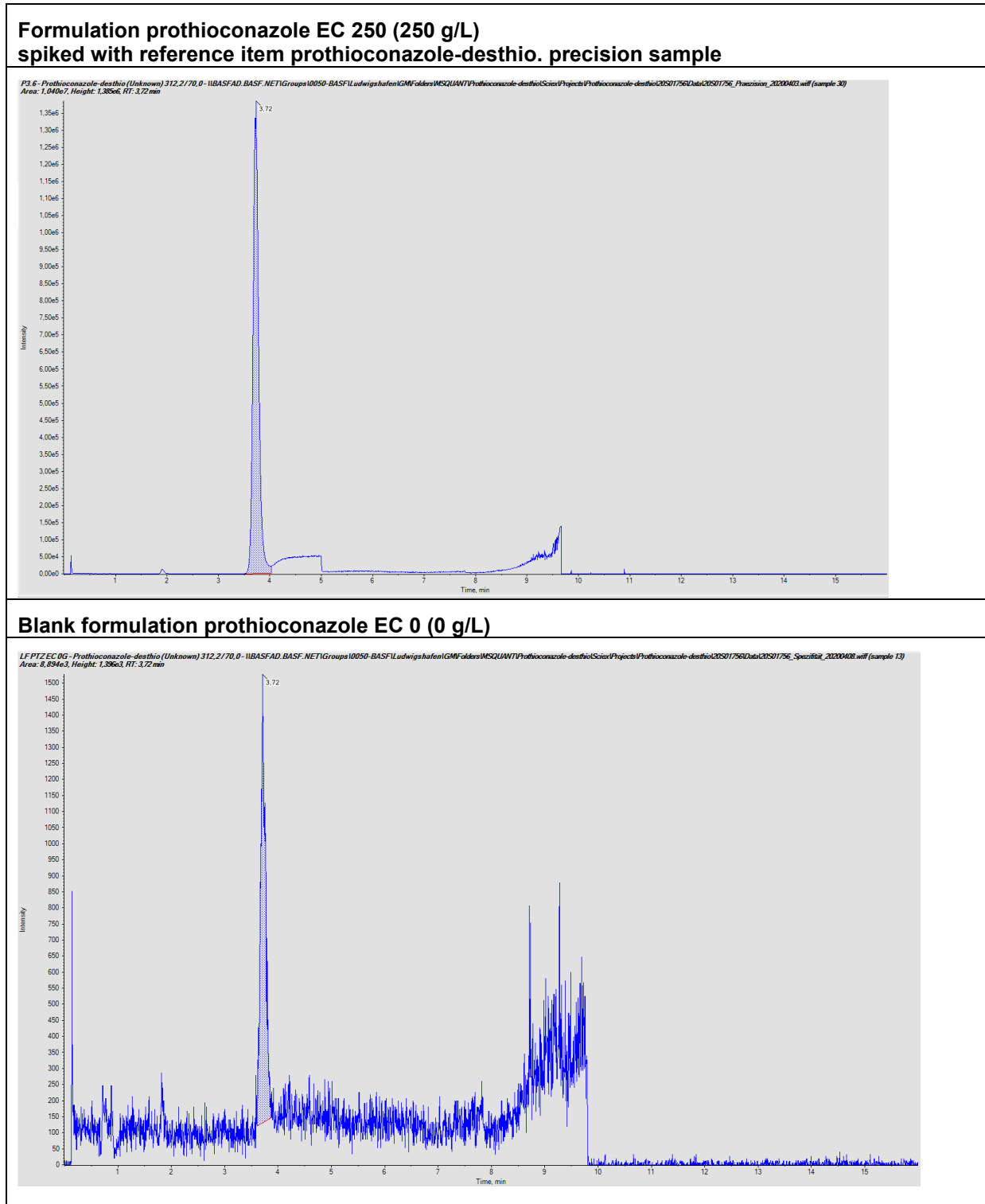
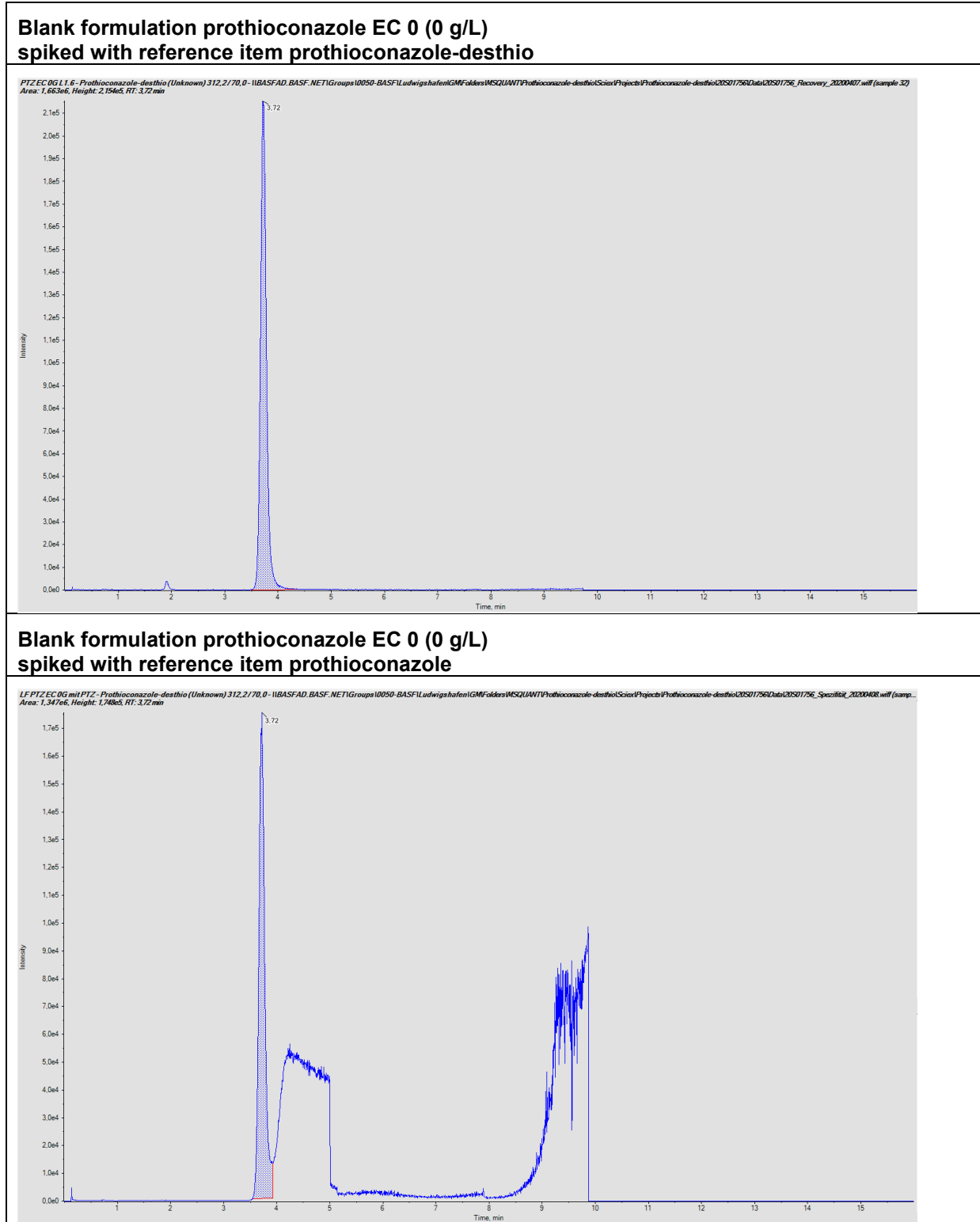


Figure 24. continuation



**Figure 25: Chromatograms of prothioconazole FS 100 (100 g/L)
Lab 3**

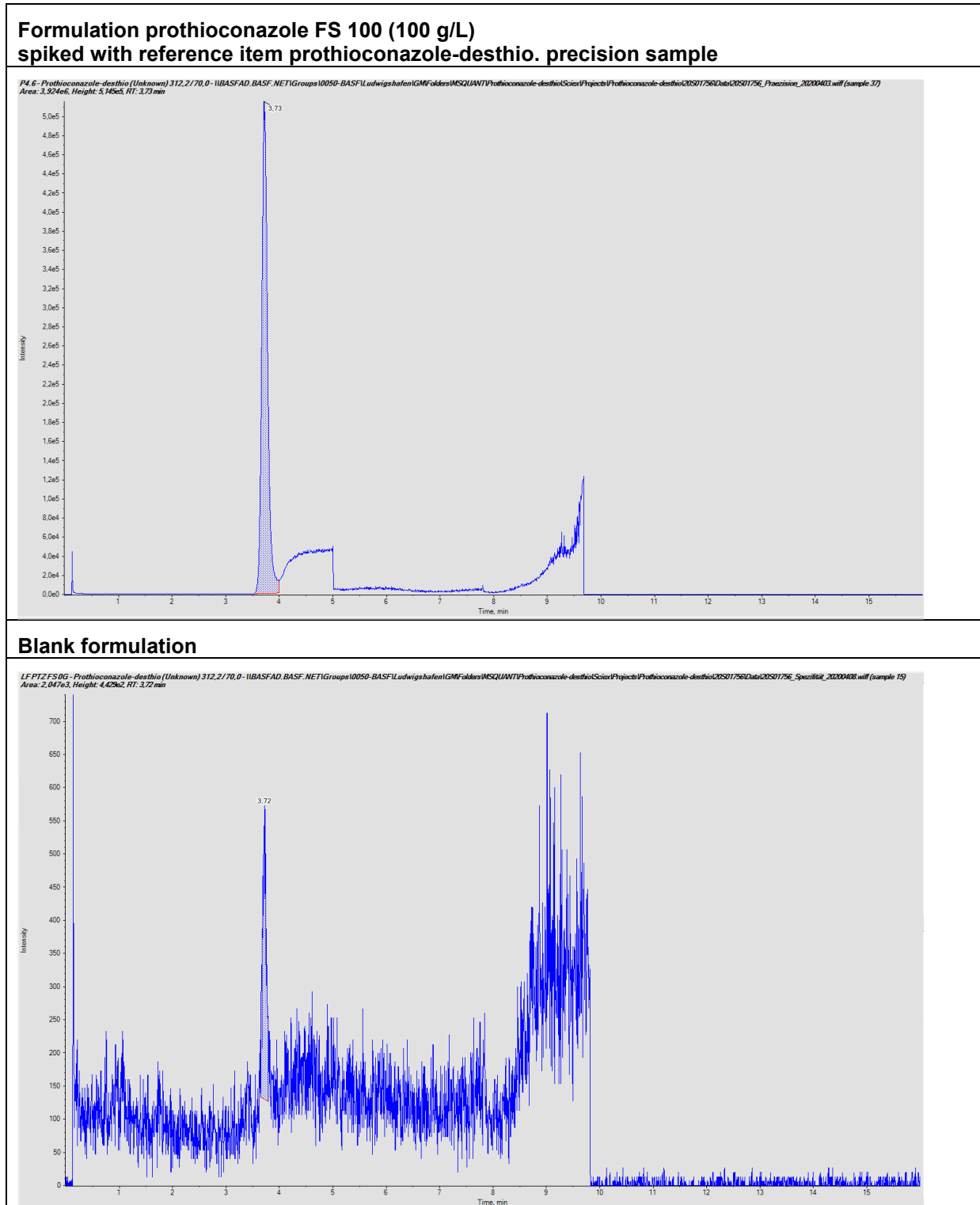


Figure 25. continuation

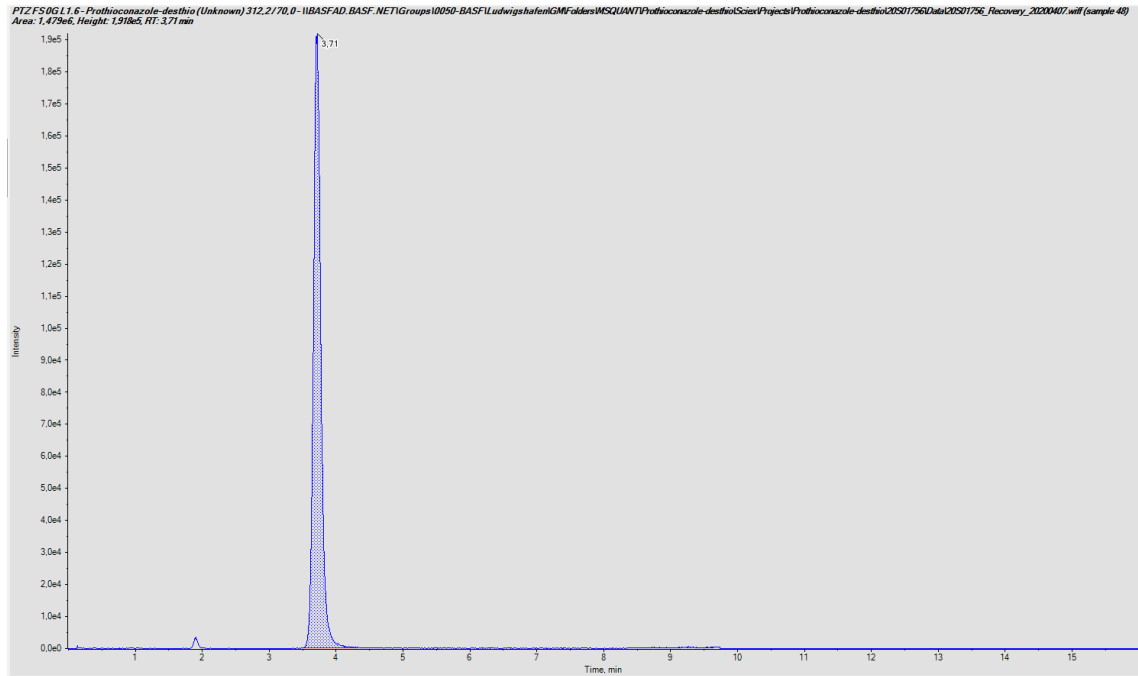
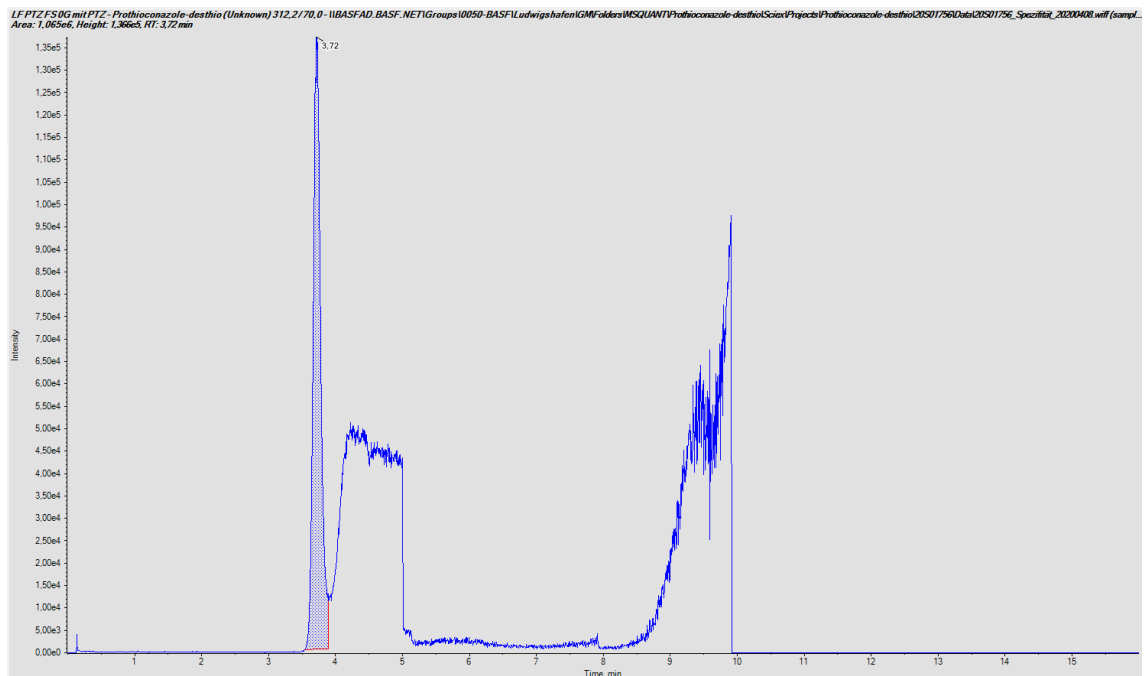
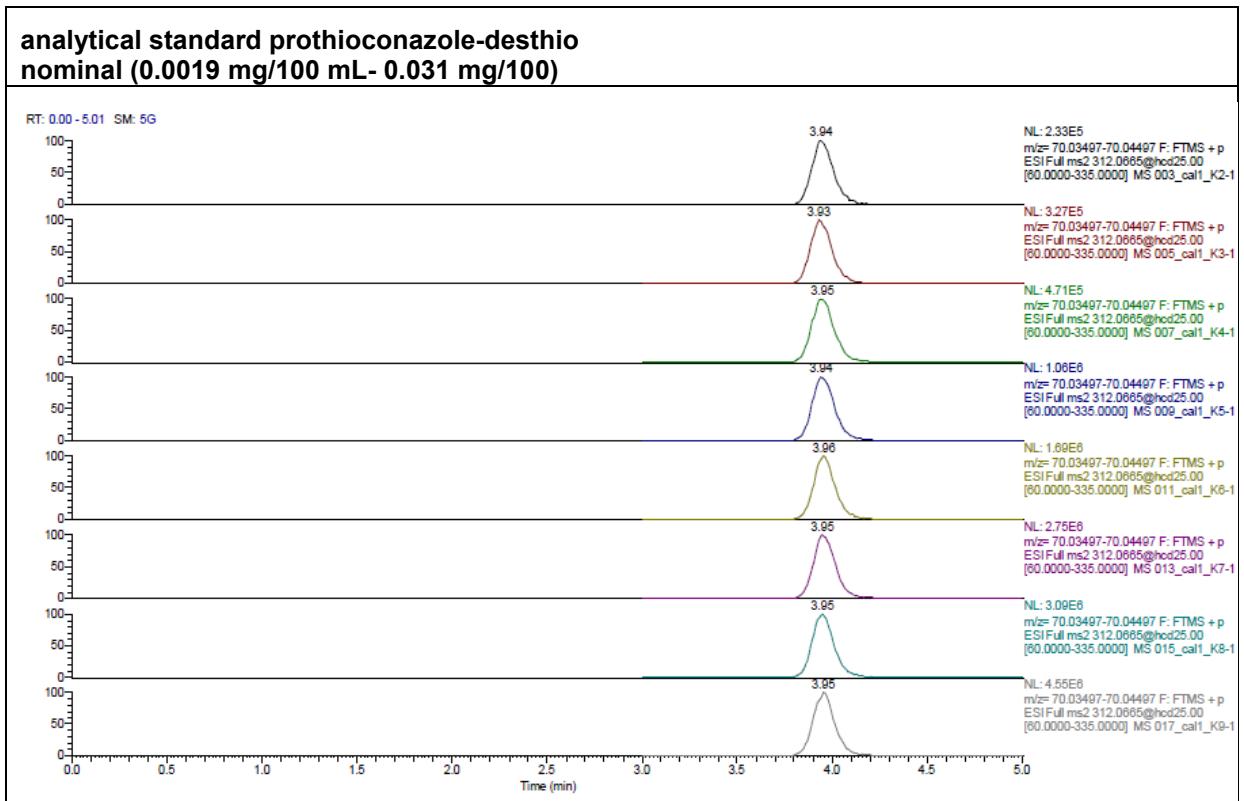
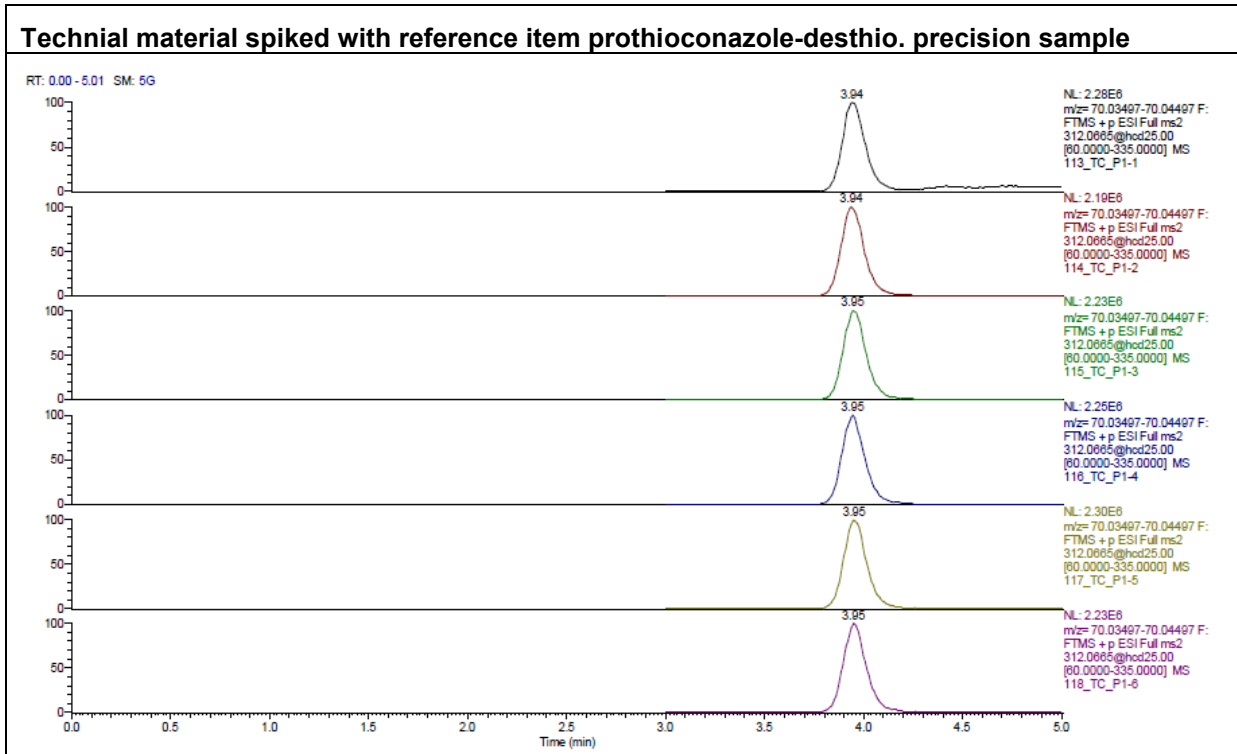
**Blank formulation prothioconazole FS 0 (0 g/L)
spiked with reference item prothioconazole-desthio****Blank formulation prothioconazole FS 0 (0 g/L)
spiked with reference item prothioconazole**

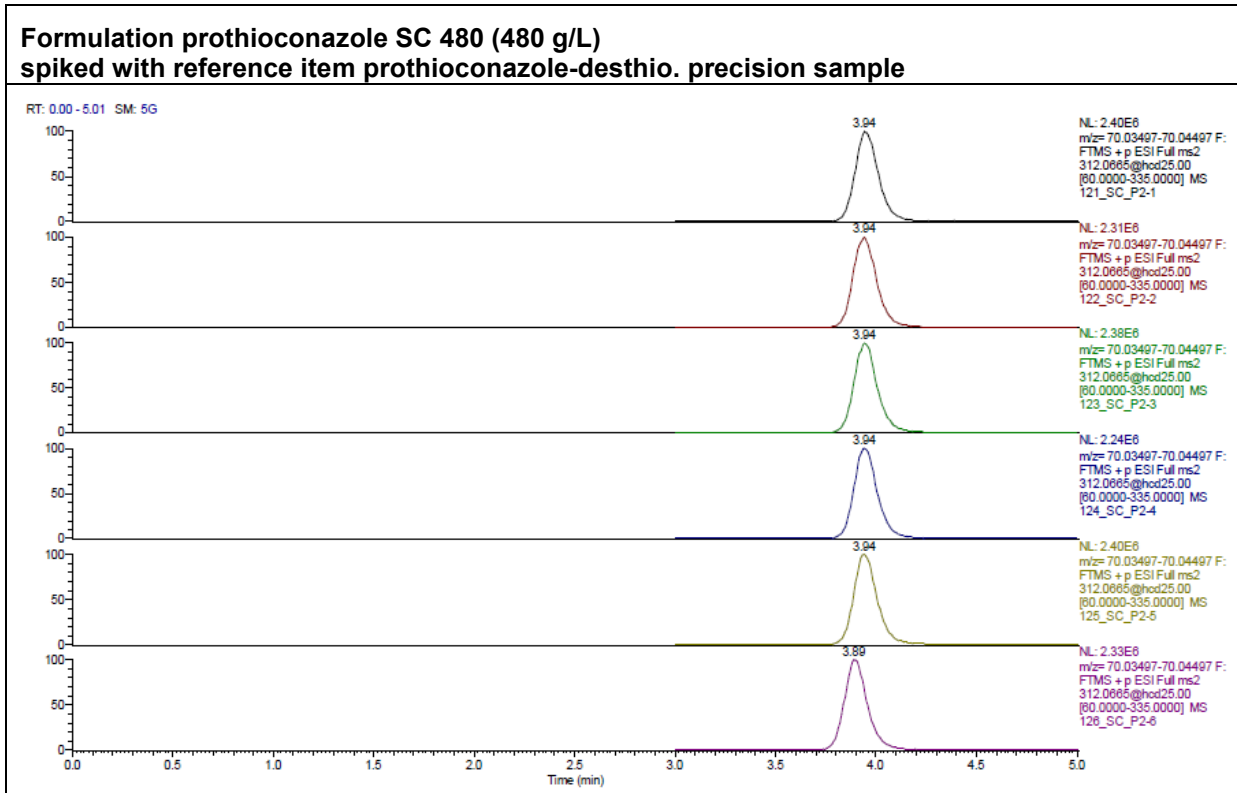
Figure 26: Chromatograms of analytical standard prothioconazole-desthio Lab 4



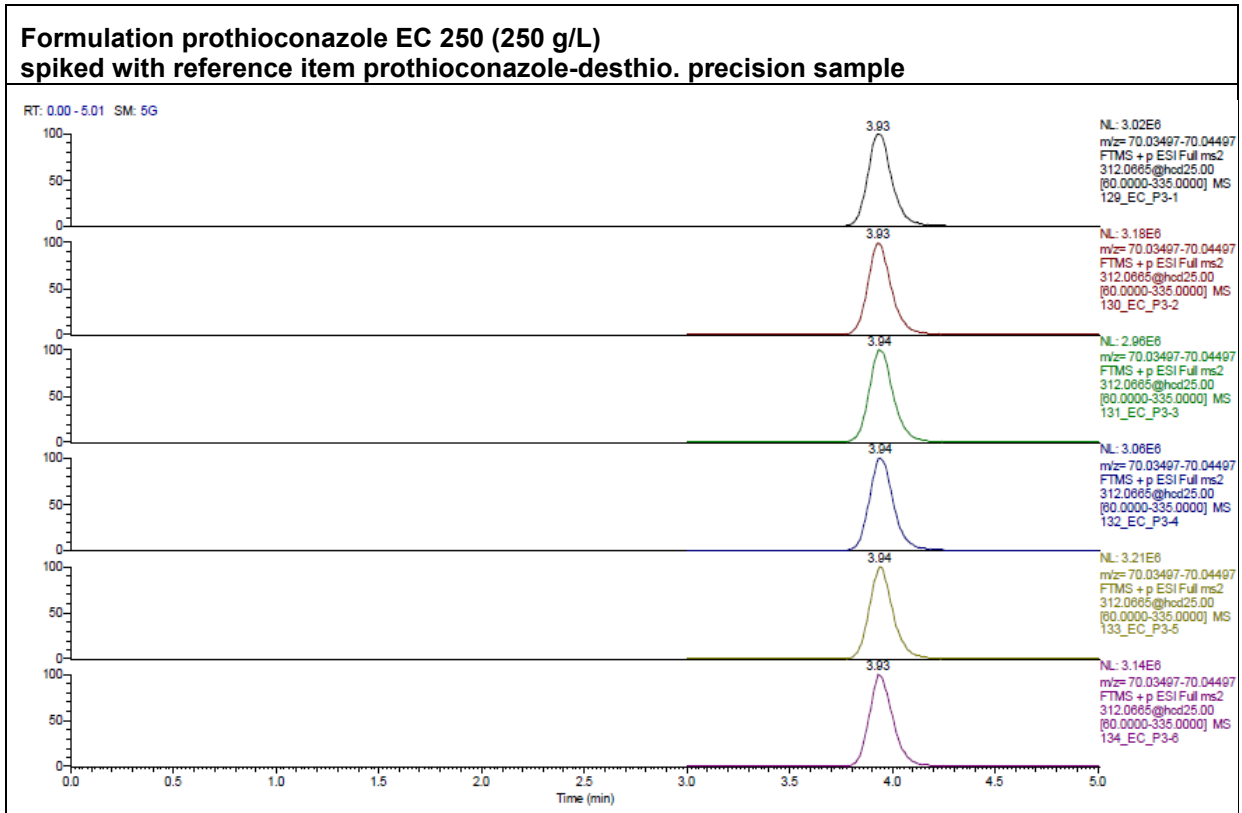
**Figure 27: Chromatograms of prothioconazole TC
Lab 4**



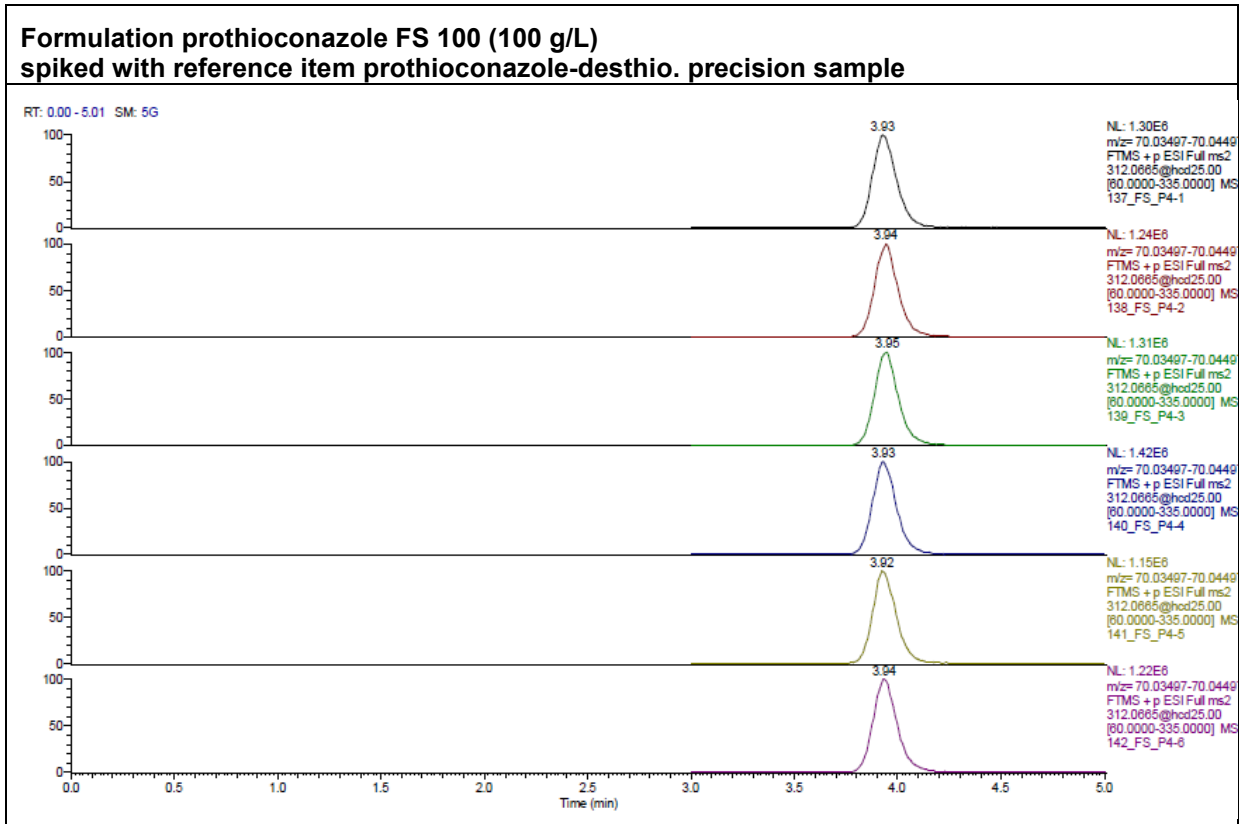
**Figure 28: Chromatograms of prothioconazole SC 480 (480 g/L)
Lab 4**



**Figure 29: Chromatogram of prothioconazole EC 250 (250 g/L)
Lab 4**

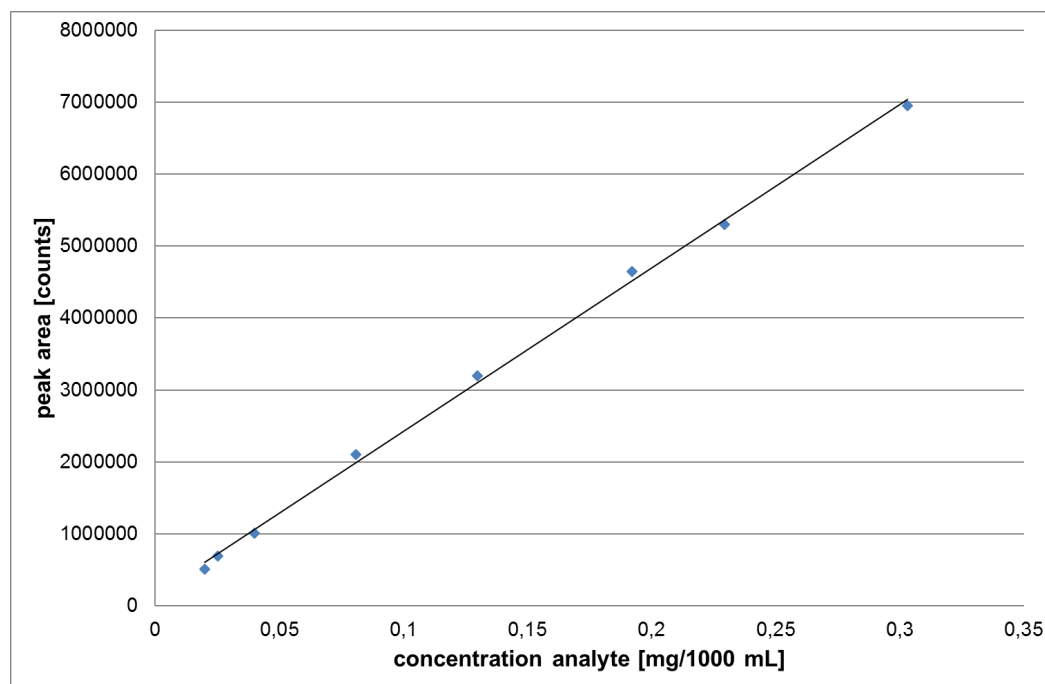


**Figure 30: Chromatogram of prothioconazole FS 100 (100 g/L)
Lab 4**



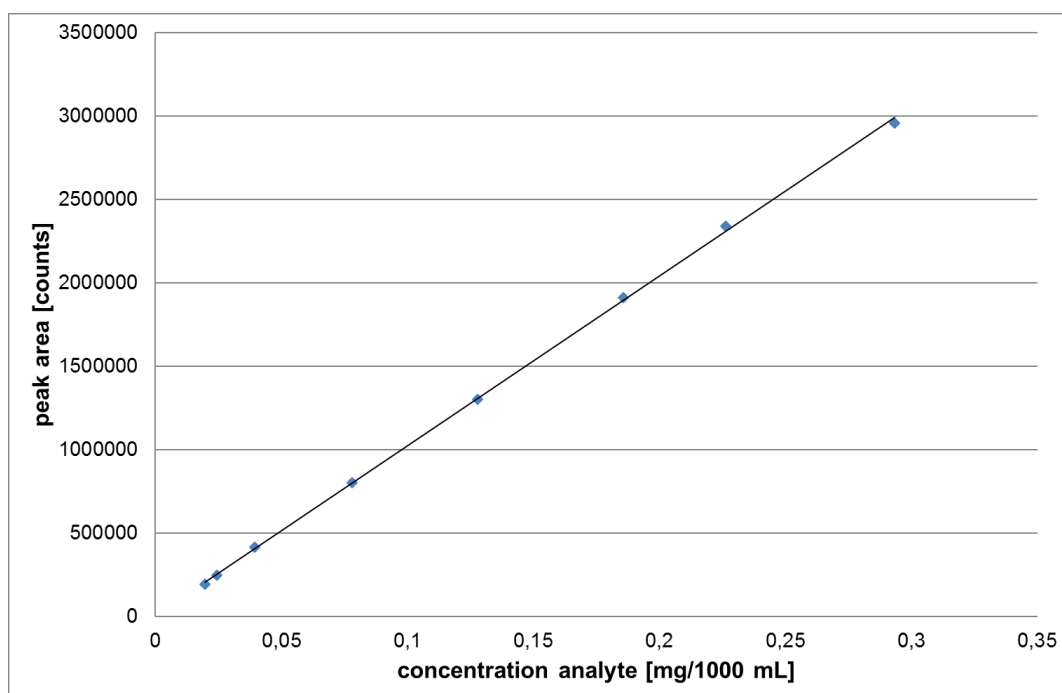
6.4 Calibration function (Linearity)

**Figure 31: Calibration function of prothioconazole-desthio
Developing Lab**



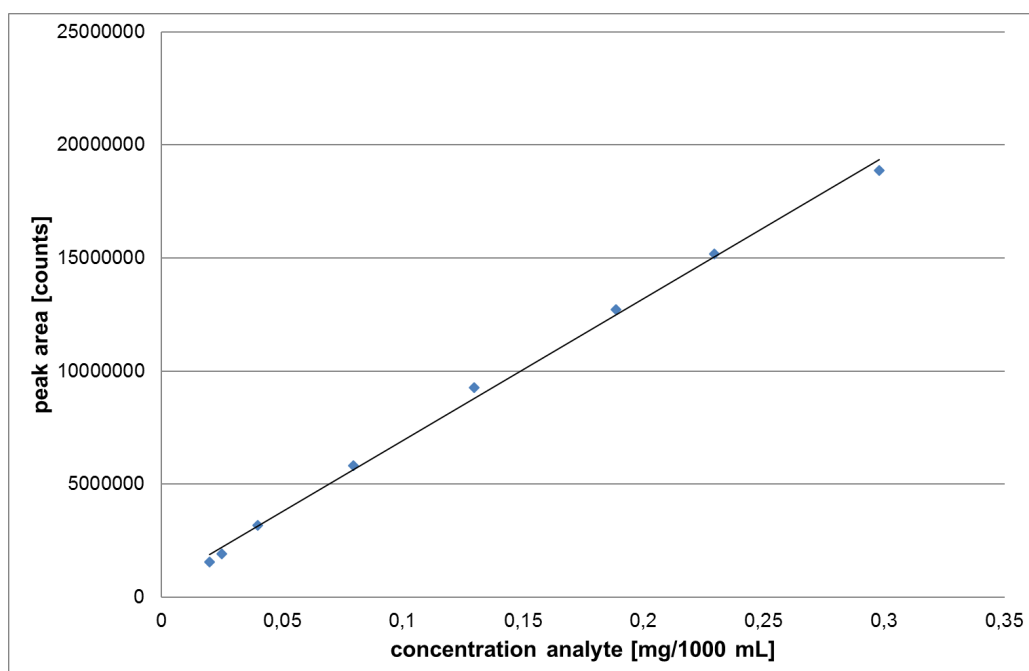
Number of values $n = 8$
Regression equation $y = a + b x$ (1st order)
 $y = 155347.6 + 22709821. x$
Correlation coefficient = 0.99916

**Figure 32: Calibration function of prothioconazole-desthio
Lab 1**



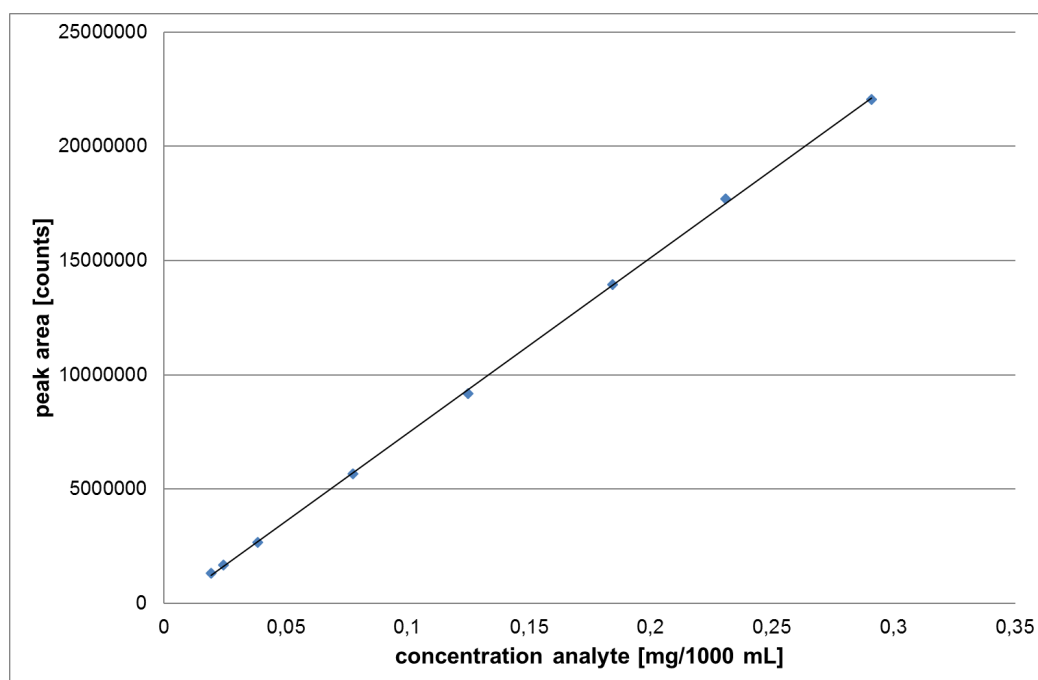
Number of values $n = 8$
Regression equation $y = a + b x$ (1st order)
 $y = 6232.7 + 10171073.9 x$
Correlation coefficient = 0.99984

**Figure 33: Calibration function of prothioconazole-desthio
Lab 2**



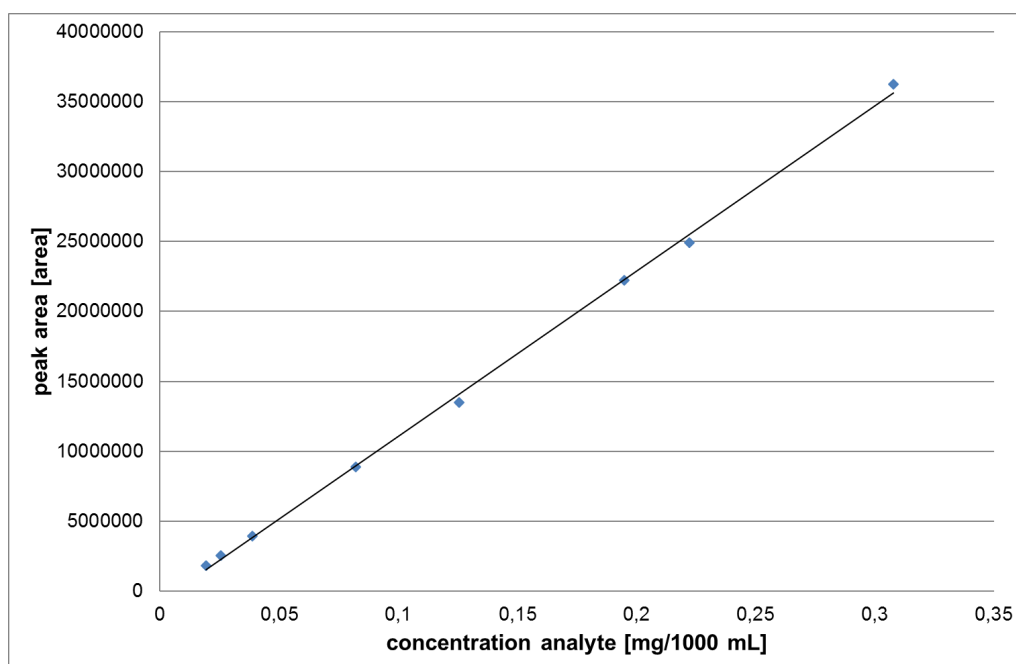
Number of values $n = 8$
Regression equation $y = a + b x$ (1st order)
 $y = 639394.7 + 62800104.6 x$
Correlation coefficient **= 0.99873**

**Figure 34: Calibration function of prothioconazole-desthio
Lab 3**



Number of values $n = 8$
Regression equation $y = a + b x$ (1st order)
 $y = -239526.48 + 76851412.3 x$
Correlation coefficient **= 0.99989**

**Figure 35: Calibration function of prothioconazole-desthio
Lab 4**



Number of values $n = 8$

Regression equation $y = a + b x$ (1st order)

$$y = -728940.05 + 118074916.62 x$$

Correlation coefficient = 0.99941

Appendix 1: Calculation of max. limit of prothioconazole-desthio in the formulations

The max. accepted level (MAL) of an analyte in formulation ($MAL_{formulation}^{analyte}$) is calculated based on its MAL in the respective technical grade active substance ($MAL_{TGAS}^{analyte}$) and the declared content of this active substance in the formulation ($DC_{formulation}^{a.s.}$) according to the following formula

$$MAL_{formulation}^{analyte} [\% (w/w)] = \frac{MAL_{TGAS}^{analyte} [\% (w/w)] \cdot DC_{formulation}^{a.s.} [\% (w/w)]}{100\% (w/w)}$$

The MAL for prothioconazole-desthio in the technical grade active substance prothioconazole is 0.5 g/kg. equal to 0.05% (w/w).

Thus the MAL of prothioconazole-desthio in the formulations is calculated as follow.

Formulation	$MAL_{TGAS}^{analyte}$	$DC_{formulation}^{a.s.(PTZ)}$	$MAL_{formulation}^{analyte}$	
	[g/kg]	[% (w/w)]	[g/kg]	[% (w/w)]
prothioconazole SC 480 (480 g/L)	0.5	40.3	0.20	0.020
prothioconazole EC 250 (250 g/L)	0.5	25.0	0.125	0.0125
prothioconazole FS 100 (100 g/L)	0.5	8.7	0.0435	0.00435