Rel. Impurity of trifloxystrobin

CIPAC Peer Validation

CIPAC peer validation of an analytical method
for the determination of CGA 344605
in trifloxistrobin technical, SC, EC and WG formulations
by High Performance Liquid Chromatography

Report to CIPAC
by
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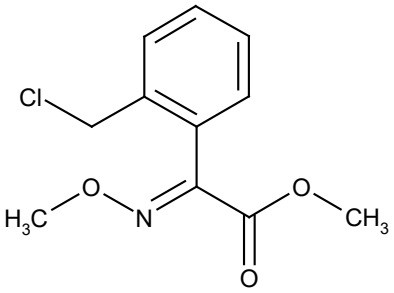
1 Introduction

1.1 Scope

The results of the peer validation of an analytical method for the determination of CGA 344605 content in trifloxystrobin in technical grade active ingredient (TGAI), trifloxystrobin suspension concentrate (SC), trifloxystrobin emulsifiable concentrate (EC) and trifloxystrobin water dispersible granule (WG) 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	CGA 344605
Synonyms	AE 1344136
Chemicla name (IUPAC)	methyl (E)-(2-chloromethylphenyl)-methoxy-imino-acetate
Structural Formula	
Empirical Formula	$C_{11}H_{12}Cl_1N_1O_3$
Molecular Weight	241.7 g/mol
CAS No	189813-45-4

1.3 Samples

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

1. Prothioconazole technical (TC)
   1.1 trifloxystrobin TC
   Batch ID: EUTE002446
   Content: 96.0 % (w/w)

2. trifloxystrobin suspension concentrate (SC)
   2.1 trifloxystrobin SC 500 (500 g/L)
   Batch ID: EM4L026661
   Declared content: 500 g/L, 43.9 % (w/w)
   2.2 trifloxystrobin SC 0 (0 g/L) (blank formulation)
   Batch ID: 2020-008522
   Declared content: 0 g/L, 0 % (w/w)

3. trifloxystrobin emulsifiable concentrate (EC)
   3.1 fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L)
   Batch ID: EM4L028674
   Declared content: 75 g/L, 6.47 % (w/w)
   3.2 fluoxastrobin+prothioconazole+trifloxystrobin EC 0 (0+0+0 g/L) (blank formulation)
   Batch ID: 2020-009165
   Declared content: 0 g/L, 0 % (w/w)

4. trifloxystrobin water dispersible granule (WG)
   4.1 trifloxystrobin WG 50 (50 g/kg)
   Batch ID: EM20001194
   Declared content: 500 g/kg, 50 % (w/w)
   4.2 trifloxystrobin WG 0 (0 g/kg) (blank formulation)
   Batch ID: 2020-008527
   Declared content: 0 g/kg, 0 % (w/w)

CGA 344605 reference standard (purity 98.4% w/w)

1.4 Participants

Dr. Trevor Bowen	Bayer AG, Crop Science Division Product Chemistry Analytics 5 65926 Frankfurt am Main Germany
Dr. Rolf Foerster	BASF SE Carl-Bosch-Strasse 38 67056 Ludwigshafen Germany
Dr. Michael Haustein	Currenta GmbH & Co. OHG CUR-ANT-PDA 41538 Dormagen Germany
Dr. Christian Mink	Syngenta Crop Protection AG Breitenloh 5 CH-4333 Münchwilen Switzerland
Developing Lab Schulz, Friedhelm	Bayer AG, Crop Science Division Formulation Analytics 1 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 an UV detector was used for quantitation of CGA 344605**.**

The CGA 344605 is separated from formulation components and active substances using a reversed phase column and gradient elution. The quantitative evaluation is carried out by comparing the peak areas with those of reference standards, 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 UV spectra of standard and sample solutions were measured under the operating conditions of the 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 standard CGA 344605 and the test solutions of technical material, formulations and blank formulations.

3) Calibration
   Calibration solutions were prepared using CGA 344605 standard. The solutions were analyzed and the peak areas of CGA 344605 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.
   The content of CGA 344605 found in the technical material and in the formulations SC and EC and WG was not enough to evaluate the repeatability. Therefore, the test solutions were spiked with the analyte CGA 344605 at a concentration of 0.1 mg/50 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 CGA 344605 at two levels, fortification level I 0.02 mg/50 mL and fortification level II 0.22 mg/50 mL. 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 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 specificity, calibration, repeatability, recovery and LOQ. The details of each procedure are the same as those described in 2.2.

3 Remarks of the participants

Laboratory	Remarks
Developing Lab	n.a.
Lab 1	n.a.
Lab 2	n.a.
Lab 3	n.a.
Lab 4	n.a.

4 Results and Discussion

4.1 Peer Validation

1) Confirmation of Analyte identification
   The DAD spectra obtained by the developing laboratory during the method development prior to peer validation are shown in Figure 1 to Figure 5.

2) Specificity
   The specificity of the analytical method for CGA 344605 is assessed sufficient as no significant interfering compounds were detected in the chromatograms at the retention time of the analyte.

3) Calibration
   The calibration lines are shown in Figure 48 to Figure 57. 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 8% of the max. accepted level (MAL) in the formualion (0.016 mg/50 mL) to 120% MAL (0.24 mg/50 mL), 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 0.1 – 2. % 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 CGA 344605 at two levels, fortification level I (LOQ) 0.02 mg/50 mL and fortification level II (close to the max. accepted level (MAL) in the formulation, see Appendix 1) 0.22 mg/50 mL. These concentrations are equal to:

	LOQ Level I	Level II
trifloxystrobin TC	0.040 % (w/w)[1]	0.44 % (w/w)[1]
trifloxystrobin SC 500 (500 g/L)	0.017 % (w/w)[2]	0.19 % (w/w)[2]
Fluoxastrobin+Prothioconazole+trifloxystrobin EC 300 (75+150+75 g/L)	0.0026 % (w/w)[3]	0.028 % (w/w)[3]
trifloxystrobin WG 50 (500 g/kg)	0.020% (w/w)[4]	0.22% (w/w)[4]

6) Limit of Quantification (LOQ)
A concentration of 0.02 mg/50 mL CGA 344605 was the lowest tested fortification level for which acceptable recovery and precision under repeatability conditions were successfully demonstrated. This concentration is equal to:

trifloxystrobin TC	0.040 % (w/w)
trifloxystrobin SC 500 (500 g/L)	0.017 % (w/w)
fluoxastrobin+prothioconazole+trifloxystrobin EC 300 (75+150+75 g/L)	0.0026 % (w/w)
trifloxystrobin WG 50 (500 g/kg)	0.020% (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 CGA 344605 in technical material trifloxystrobin and SC, EC and WG formulations.

[1] Referred to a nominal sample concentration of 50 mg/50 mL
[2] Referred to a nominal sample concentration of 115 mg/50 mL
[3] Referred to a nominal sample concentration of 770 mg/50 mL
[4] Referred to a nominal sample concentration of 100 mg/50 mL

6 Determination of relevant impurity CGA 344605

6.1 Tables of results

Table 1: Results trifloxystrobin TC (Repeatability)

	CGA 344605 [mg/50 mL][1]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.1107	0.1093	0.1026	0.1020	0.1102
Weighing no. 2	0.1085	0.1087	0.1031	0.1019	0.1098
Weighing no. 3	0.1010	0.1097	0.1026	0.1021	0.1101
Weighing no. 4	0.1104	0.1081	0.1026	0.1022	0.1101
Weighing no. 5	0.1078	0.1090	0.1040[3]	0.1027	0.1104
Weighing no. 6	0.1131	0.1089	0.1031	0.1021	0.1104
Mean value [mg/50 mL]	**0.110**	**0.109**	**0.103**	**0.102**	**0.110**
Mean value [% w/w] [2]	**0.220**	**0.218**	**0.206**	**0.204**	**0.220**
SD	0.00186	0.000543	0.000548	0.00028	0.000225
RSD [%]	1.69	0.50	0.54	0.27	0.20
Horwitz-Value RSD $(r)_{max}$	3.37	3.37	3.40	3.40	3.37
Horrat value H_r	**0.50**	**0.15**	**0.16**	**0.08**	**0.06**
Outliers	no	no	yes[3]	no	no

[1] The amount of CGA 344605 determined in the test item was < LOQ. Therefore , the precision was determined using test item solutions spiked with reference item CGA 344605. For the calculation of the RSD, the results in mg/50 mL were used to eliminate the error of the variability of the test item weight in the test solutions (error propagation)

[2] Calculated from mean value in [mg/50 mL] for a a nominal sample concentration of 50 mg/50 mL

[3] The results of laboratory 2 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 2: Results trifloxystrobin SC 500 (500 g/L) (Repeatability)

	CGA 344605 [mg/50 mL][1]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.1060	0.1062	0.1003	0.0995	0.1068
Weighing no. 2	0.1071	0.1066	0.0996	0.0996	0.1070
Weighing no. 3	0.1053	0.106	0.0981	0.0993	0.1076
Weighing no. 4	0.1044	0.1065	0.1029	0.0994	0.1069
Weighing no. 5	0.1055	0.1059	0.0995	0.0995	0.1073
Weighing no. 6	0.1054	0.1059	0.0989	0.0994	0.1055
Mean value [mg/50 mL]	**0.106**	**0.106**	**0.0999**	**0.0995**	**0.107**
Mean value [% w/w] [2]	**0.0918**	**0.0918**	**0.0869**	**0.0865**	**0.0929**
SD	0.000906	0.000306	0.00165	0.000105	0.000723
RSD [%]	0.86	0.29	1.65	0.11	0.68
Horwitz-Value RSD $(r)_{max}$	3.84	3.84	3.87	3.87	3.83
Horrat value H_r	**0.22**	**0.08**	**0.43**	**0.03**	**0.18**
Outliers	no	no	no	no	no

[1] The amount of CGA 344605 determined in the test item was < LOQ. Therefore , the precision was determined using test item solutions spiked with reference item CGA 344605. For the calculation of the RSD, the results in mg/50 mL were used to eliminate the error of the variability of the test item weight in the test solutions (error propagation)

[2] Calculated from mean value in [mg/50 mL] for a nominal sample concentration of 115 mg/50 mL

Table 3: Results fluoxastrobin+prothioconazole+trifloxystrobin EC 300 (75+150+75 g/L) (Repeatability)

	CGA 344605 [mg/50 mL][1]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.1124[3]	0.1004	0.0934	0.0932	0.1098[3]
Weighing no. 2	0.1001	0.1018	0.0908	0.0934	0.1027
Weighing no. 3	0.1036	0.0991	0.0903	0.0941	0.1034
Weighing no. 4	0.1025	0.0999	0.0908	0.0936	0.1026
Weighing no. 5	0.1026	0.1043	0.0932	0.0936	0.1029
Weighing no. 6	0.1034	0.0984	0.0907	0.0935	0.1026
Mean value [mg/50 mL]	**0.102**	**0.101**	**0.0915**	**0.0936**	**0.103**
Mean value [% w/w] [2]	**0.0133**	**0.0131**	**0.0119**	**0.0122**	**0.0134**
SD	0.00137	0.00213	0.00138	0.000301	0.000336
RSD [%]	1.34	2.12	1.51	0.32	0.33
Horwitz-Value RSD $(r)_{max}$	5.13	5.15	5.22	5.21	5.13
Horrat value H_r	**0.26**	**0.41**	**0.29**	**0.06**	**0.06**
Outliers	yes[3]	no	no	no	yes[3]

[1] The amount of CGA 344605 determined in the test item was < LOQ. Therefore , the precision was determined using test item solutions spiked with reference item CGA 344605. For the calculation of the RSD, the results in mg/50 mL were used to eliminate the error of the variability of the test item weight in the test solutions (error propagation)

[2] Calculated from mean value in [mg/50 mL] for a nominal sample concentration of 770 mg/50 mL

[3] The results of the developing laboratory and Lab 4 show an upper outlier at 95% confidence according to Dixon Test. This value was disregarded for the calculation of the mean value and the standard deviation

Table 4: Results trifloxystrobin WG 50 (500 g/kg) (Repeatability)

	CGA 344605 [mg/50 mL][1]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	0.1115	0.1077	0.1040	0.1031	0.1105
Weighing no. 2	0.1095	0.1059	0.1039	0.1033	0.1111
Weighing no. 3	0.1099	0.1057	0.1036	0.1034	0.1102
Weighing no. 4	0.1096	0.1049	0.1022	0.1037	0.1112
Weighing no. 5	0.1095	0.1051	0.1022	0.1053	0.1090
Weighing no. 6	0.1088	0.1041	0.1020	0.1040	0.1107
Mean value [mg/50 mL]	**0.110**	**0.106**	**0.103**	**0.104**	**0.110**
Mean value [% w/w] [2]	**0.110**	**0.106**	**0.103**	**0.104**	**0.110**
SD	0.000919	0.00122	0.000943	0.0008	0.000802
RSD [%]	0.84	1.16	0.92	0.77	0.73
Horwitz-Value RSD $(r)_{max}$	3.74	3.76	3.77	3.77	3.73
Horrat value H_r	**0.22**	**0.31**	**0.24**	**0.20**	**0.20**
Outliers	no	no	no	no	no

[1] The amount of CGA 344605 determined in the test item was < LOQ. Therefore , the precision was determined using test item solutions spiked with reference item CGA 344605. For the calculation of the RSD, the results in mg/50 mL were used to eliminate the error of the variability of the test item weight in the test solutions (error propagation)

[2] Calculated from mean value in [mg/50 mL] for a nominal sample concentration of 100 mg/50 mL

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

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	101.8	103.3	95.64	98.70	99.95
Weighing no. 2	99.17	105.5	96.13	98.70	98.08[1]
Weighing no. 3	98.86	106.1	96.13	98.27	99.48
Weighing no. 4	98.95	105.6	96.13	100.0	99.48
Weighing no. 5	102.2	103.0	95.64	99.57	99.95
Weighing no. 6	101.3	105.3	99.60[1]	98.27	99.95
Mean value	**100.4**	**104.8**	**96.6**	**98.9**	**99.5**
SD	1.55	1.31	1.52	0.71	0.72
RSD [%]	1.54	0.96	1.57	0.72	0.73
Outliers	no	no	yes[1]	no	yes[1]

[1] The results of laboratory 2 show an upper outlier and the results of laboratory 4 show a lower outlier at 95% confidence according to Dixon Test. However, these values were considered valid and therefore they were not disregarded to calculate the mean value and the RSD.

Mean recovery rates are within the requested range of 75-125 % according to SANCO/3030/99 for a nominal analyte content ≥0.01 - < 0.1% (*w/w*)

Remark: The initial concentration of the analyte found in the unfortified sample, which was significant at the concentration of recovery level I, was substracted from the measured concentration of the fortified sample to calcultate the recovery [%].

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

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	101.9	103.3	100.5	100.8	100.5
Weighing no. 2	101.4	103.0	101.2	100.8	100.5
Weighing no. 3	100.8	103.2	100.5	101	100.3
Weighing no. 4	101.0	103.2	100.1	100.7	100.3
Weighing no. 5	102.2	103.7	100.8	101.1	100.4
Weighing no. 6	100.9	103.2	100.6	100.9	100.4
Mean value	**101.4**	**103.3**	**100.6**	**100.9**	**100.4**
SD	0.58	0.23	0.37	0.15	0.09
RSD [%]	0.57	0.23	0.36	0.15	0.09
Outliers	no	no	no	no	no

Mean recovery rates are within the requested range of 80-120 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.1 to < 1% (*w/w*)

Table 7: Results trifloxystrobin SC 500 (500 g/L) (Recovery Level I - LOQ)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	98.34	96.73	94.15	99.13	97.14
Weighing no. 2	104.2	99.21	94.15	100.0	96.20
Weighing no. 3	98.56	98.42	94.65	100.0	97.61
Weighing no. 4	102.1	98.66	93.16	101.3	97.14
Weighing no. 5	99.34	97.48	95.14	100.9	103.2[1]
Weighing no. 6	101.7	98.42	94.15	100.4	95.26
Mean value	**100.7**	**98.2**	**94.2**	**100.3**	**97.8**
SD	2.33	0.894	0.659	0.764	2.80
RSD [%]	2.31	0.91	0.70	0.76	2.86
Outliers	no	no	no	no	yes[1]

[1] The results of laboratory 4 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.

Mean recovery rates are within the requested range of 75-125 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.01 - < 0.1% (*w/w*)

Table 8: Results trifloxystrobin SC 500 (500 g/L) (Recovery Level II - MAL)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	100.7	99.24	100.3	98.79	96.31[1]
Weighing no. 2	99.50	99.34	98.97	98.53	97.72
Weighing no. 3	99.82	99.33	99.78	98.57	97.63
Weighing no. 4	100.7	99.11	99.33	98.62	97.72
Weighing no. 5	101.1	98.97	99.92	98.57	97.29
Weighing no. 6	100.4	99.25	99.42	98.66	97.50
Mean value	**100.4**	**99.2**	**99.6**	**98.6**	**97.4**
SD	0.60	0.14	0.47	0.09	0.54
RSD [%]	0.60	0.14	0.48	0.09	0.55
Outliers	no	no	no	no	yes[1]

[1] 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.

Mean recovery rates are within the requested range of 80-120 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.1 to < 1% (*w/w*)

Table 9: Results fluoxastrobin+prothioconazole+trifloxystrobin EC 300 (75+150+75 g/L) (Recovery Level I - LOQ)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	178.3[1]	108.5	87.22	93.07	100.4
Weighing no. 2	94.41	105.3	85.73	93.51	93.85
Weighing no. 3	99.65	115.3	87.22	93.94	92.91
Weighing no. 4	99.00	106.4	83.75	92.64	95.73
Weighing no. 5	98.56	107.0	85.73	93.51	94.32
Weighing no. 6	95.68	110.6	84.24	93.51	95.26
Mean value	**97.5**	**108.9**	**85.7**	**93.4**	**95.4**
SD	2.28	3.66	1.45	0.45	2.64
RSD [%]	2.34	3.58	1.70	0.48	2.77
Outliers	yes[1]	no	no	no	no

[1] The results of the developing laboratory show an upper outlier at 95% confidence according to Dixon Test. This value was disregarded for the calculation of the mean value and the standard deviation

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

Table 10: Results fluoxastrobin+prothioconazole+trifloxystrobin EC 300 (75+150+75 g/L) (Recovery Level II)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	98.91	101.5	98.16	97.32	95.59[1]
Weighing no. 2	99.03	101.2	98.12	97.49	96.95
Weighing no. 3	100.4	101.3	97.98	97.40	96.69
Weighing no. 4	98.82	100.8	98.34	97.23	96.91
Weighing no. 5	99.03	100.9	99.51[1]	97.10	96.82
Weighing no. 6	100.0	100.7	97.76	97.32	96.91
Mean value	**99.4**	**101.1**	**98.3**	**97.3**	**96.7**
SD	0.66	0.31	0.62	0.14	0.53
RSD [%]	0.67	0.31	0.63	0.14	0.54
Outliers	no	no	yes[1]	no	yes[1]

[1] The results of laboratory 2 show an upper outlier and the results of laboratory 4 show an lower outlier at 95% confidence according to Dixon Test. However, these values were considered valid and therefore they were not disregarded to calculate the mean value and the RSD.

Mean recovery rates are 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 11: Results trifloxystrobin WG 50 (500 g/kg) (Recovery Level I - LOQ)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	98.43	85.54	92.67[1]	94.37	106.5
Weighing no. 2	108.2	79.75	94.65	95.67	115.4
Weighing no. 3	100.3	73.27	94.65	103.5	114.0
Weighing no. 4	103.4	74.11	94.65	94.81	114.0
Weighing no. 5	106.9	78.81	95.64	102.6	109.8
Weighing no. 6	105.3	75.59	95.64	96.54	107.0
Mean value	**100.3**	**77.9**	**94.7**	**97.9**	**111.1**
SD	3.81	4.56	1.08	4.06	3.87
RSD [%]	3.66	5.85	1.15	4.14	3.49
Outliers	no	no	yes[1]	no	no

[1] The results of laboratory 2 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.

Mean recovery rates are 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 12: Results trifloxystrobin WG 50 (500 g/kg) (Recovery Level II)

	CGA 344605 Recovery [%]				
	Developing Lab	Lab 1	Lab 2	Lab 3	Lab 4
Weighing no. 1	99.59	93.99	97.35	102.3[1]	99.38
Weighing no. 2	100.7	95.33	98.39	98.05	99.42
Weighing no. 3	99.63	96.40	97.31	97.23	99.68
Weighing no. 4	101.0	95.66	97.4	97.84	99.42
Weighing no. 5	100.4	94.60	99.06	98.49	99.38
Weighing no. 6	99.49	95.22	101.7	98.62	99.72
Mean value	**100.1**	**95.2**	**98.5**	**98.8**	**99.5**
SD	0.65	0.84	1.70	1.81	0.16
RSD [%]	0.65	0.88	1.73	1.83	0.16
Outliers	no	no	no	yes[1]	no

[1] The results of laboratory 3 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.

Mean recovery rates are within the requested range of 80-120 % according to SANCO/3030/99 for a nominal analyte content ≥ 0.1 to < 1% (*w/w*)

6.2 UV-Spectra

Figure 1: UV spectra of reference standard CGA 344605
(Top: in acetonitrile; Bottom: in Acetonitrile/Water)

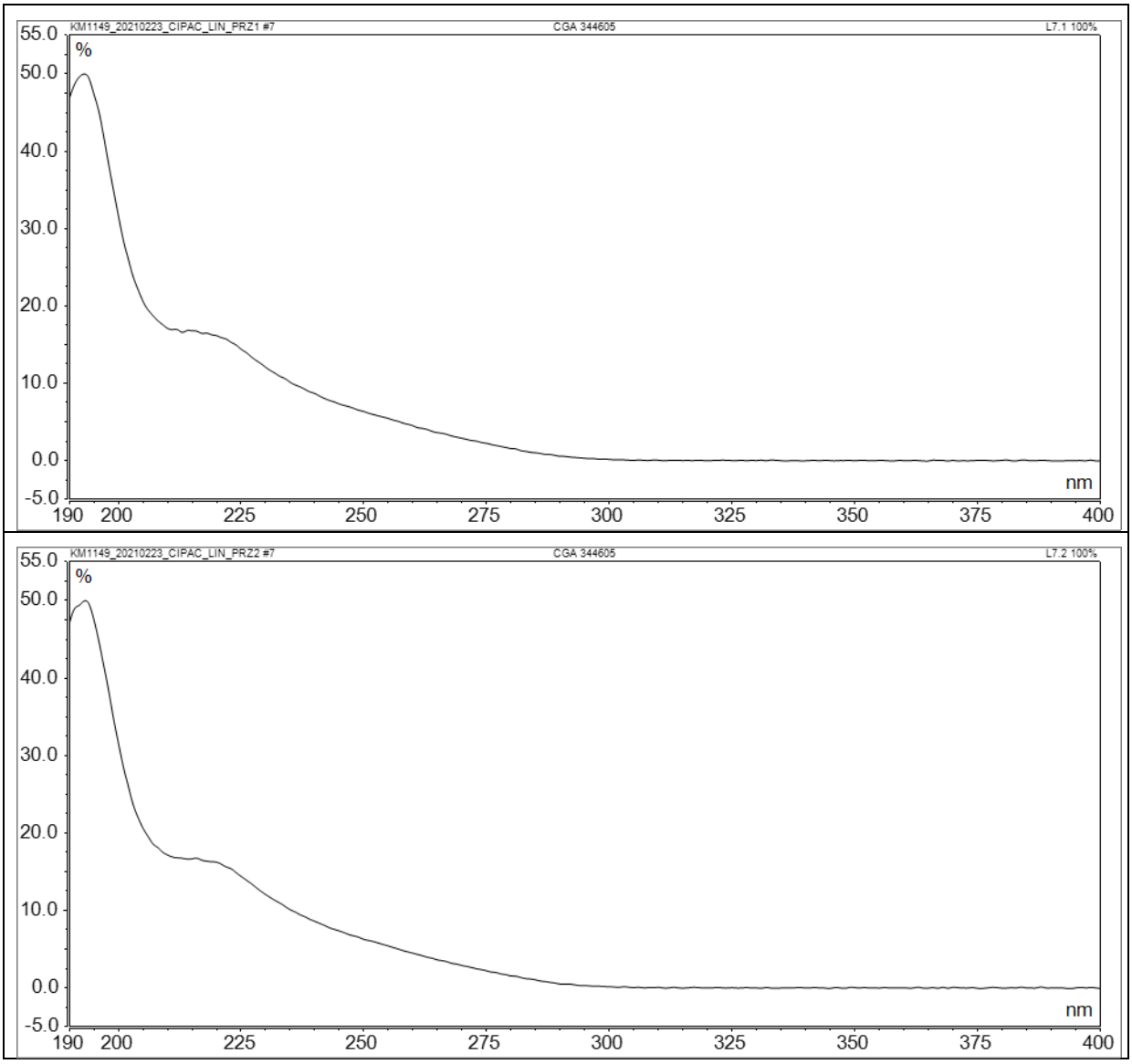


Figure 2: UV spectra of CGA 344605 in spiked test item trifloxystrobin TC

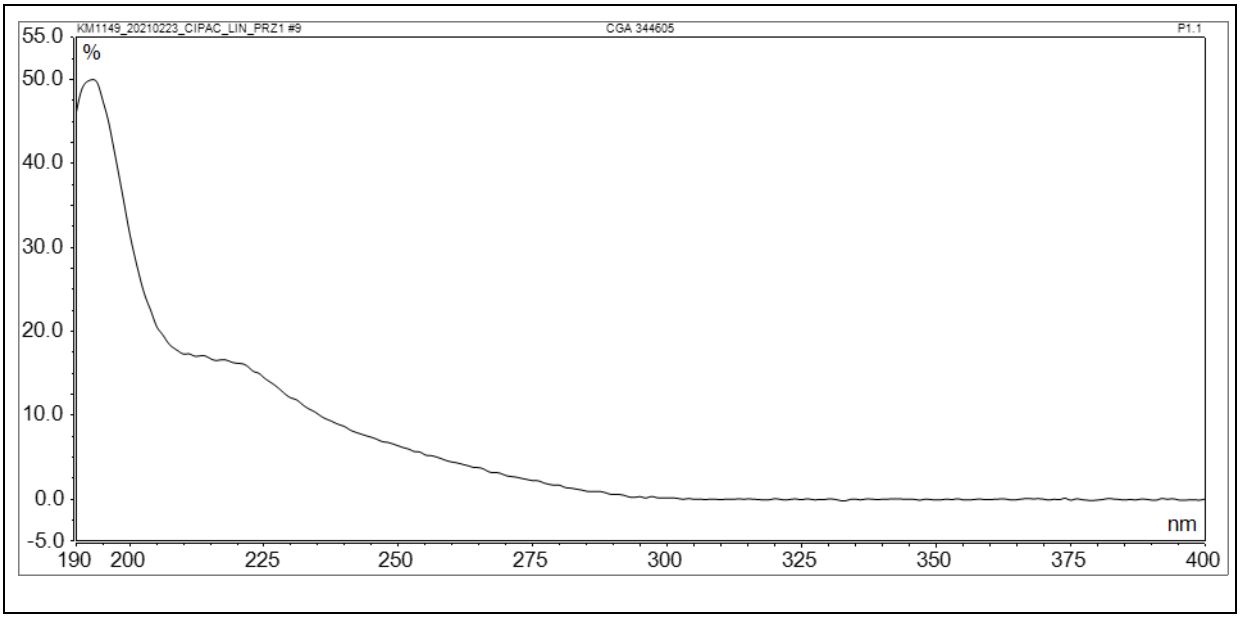


Figure 3: UV spectra of CGA 344605 in spiked test item trifloxystrobin SC 500 (500 g/L)

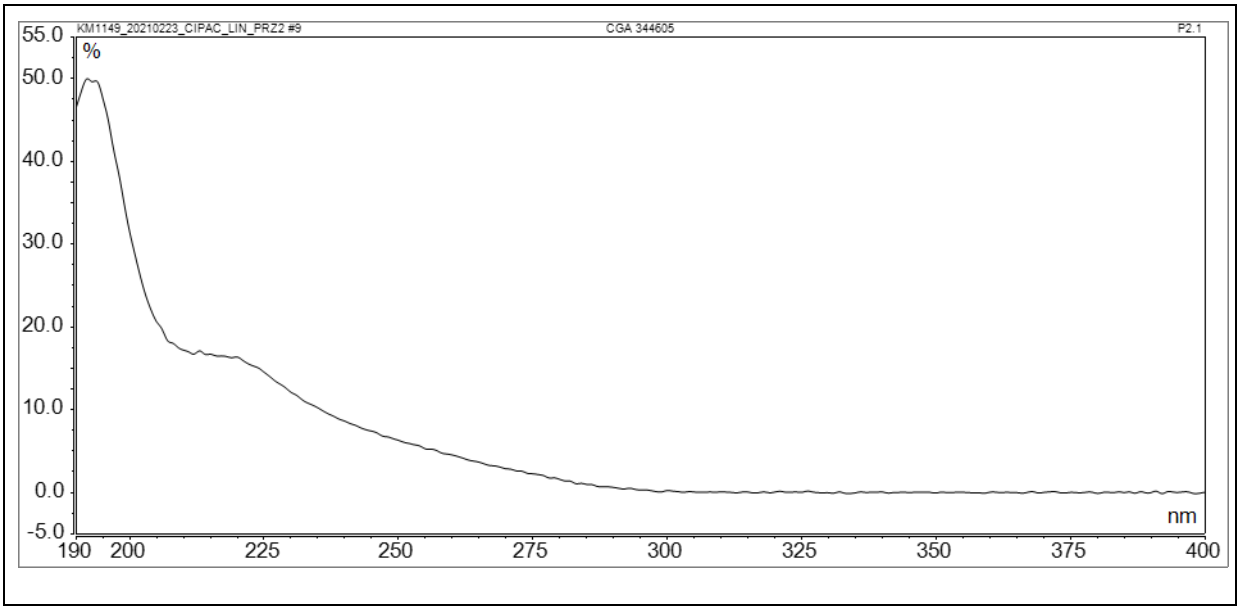


Figure 4: UV spectra of CGA 344605 in spiked test item fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L)

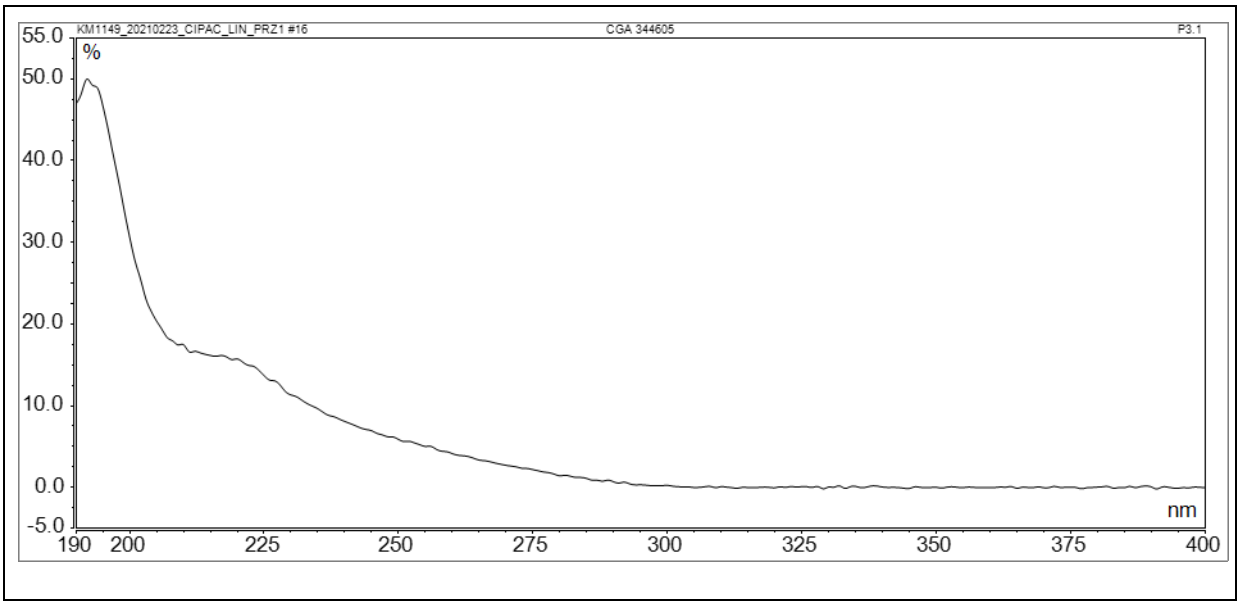
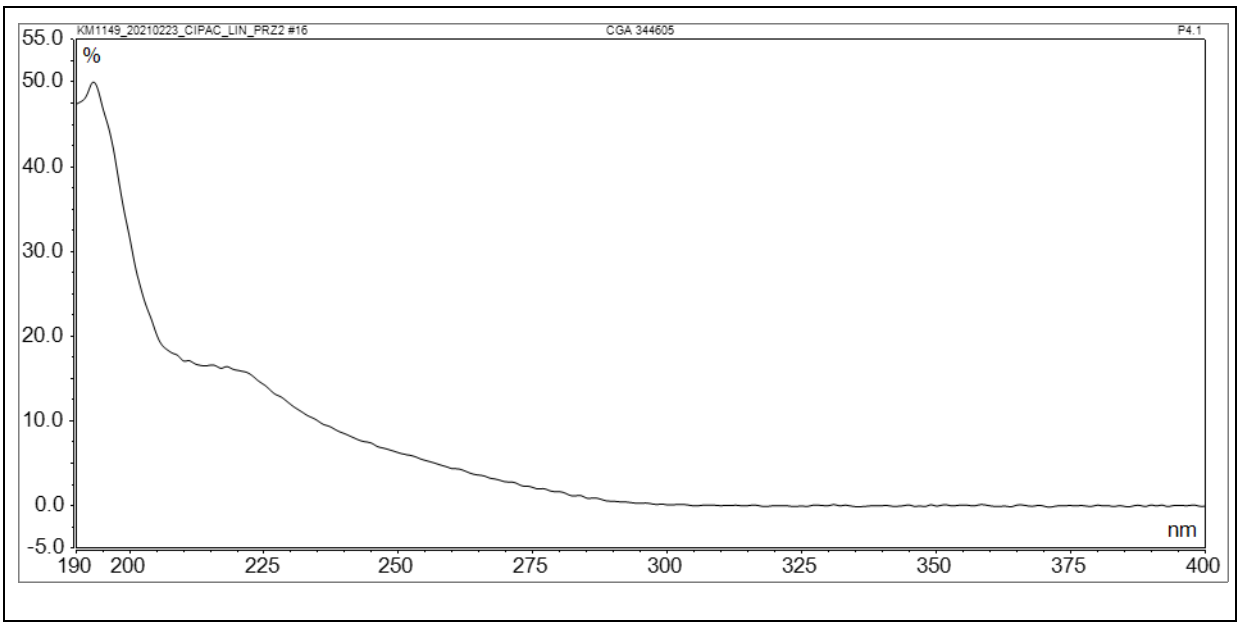


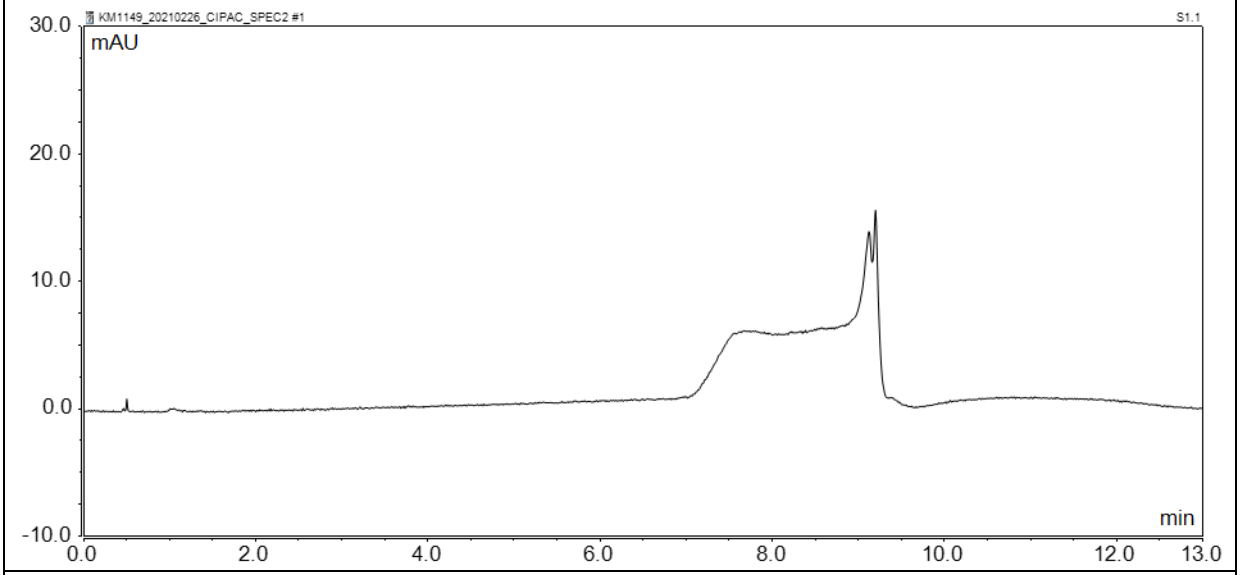
Figure 5: UV spectra of CGA 344605 in spiked test item trifloxystrobin WG 50 (50 g/kg)



6.3 Chromatograms

Figure 6: Chromatograms of sample solvent Developing Lab

acetonitrile



acetonitrile/water

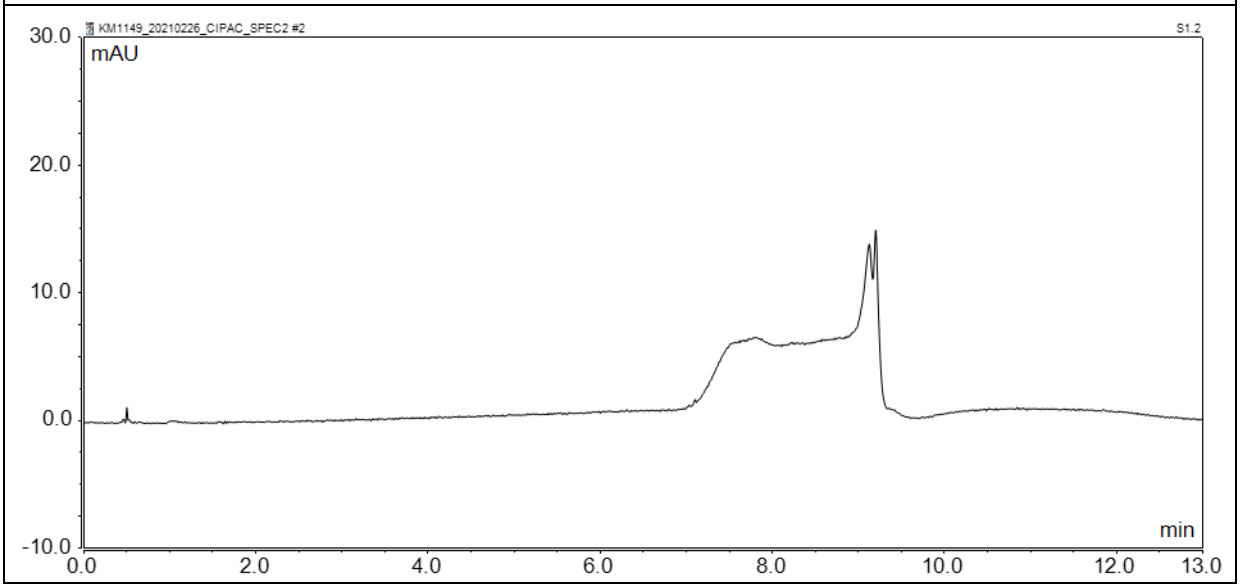
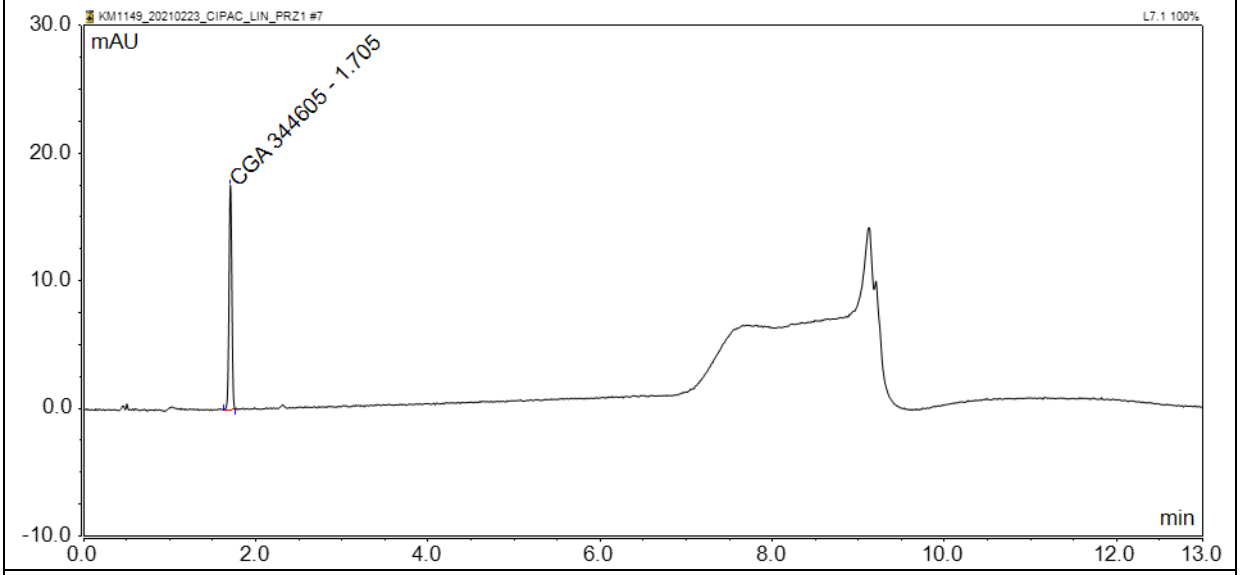


Figure 7: Chromatograms of reference standard CGA 344605 Developing Lab

reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile/water)

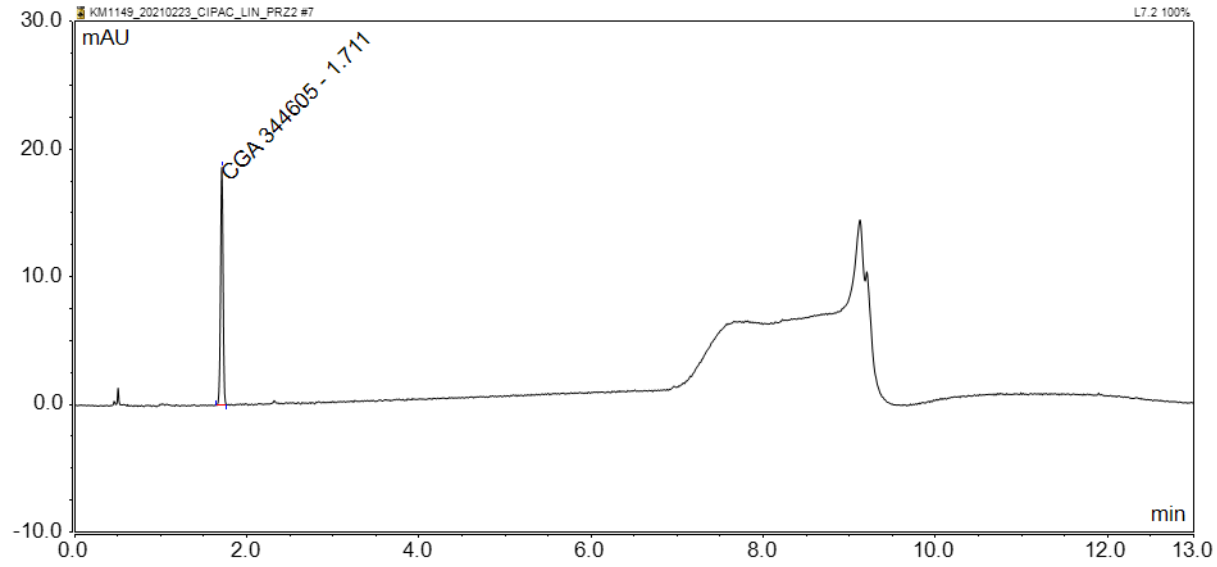
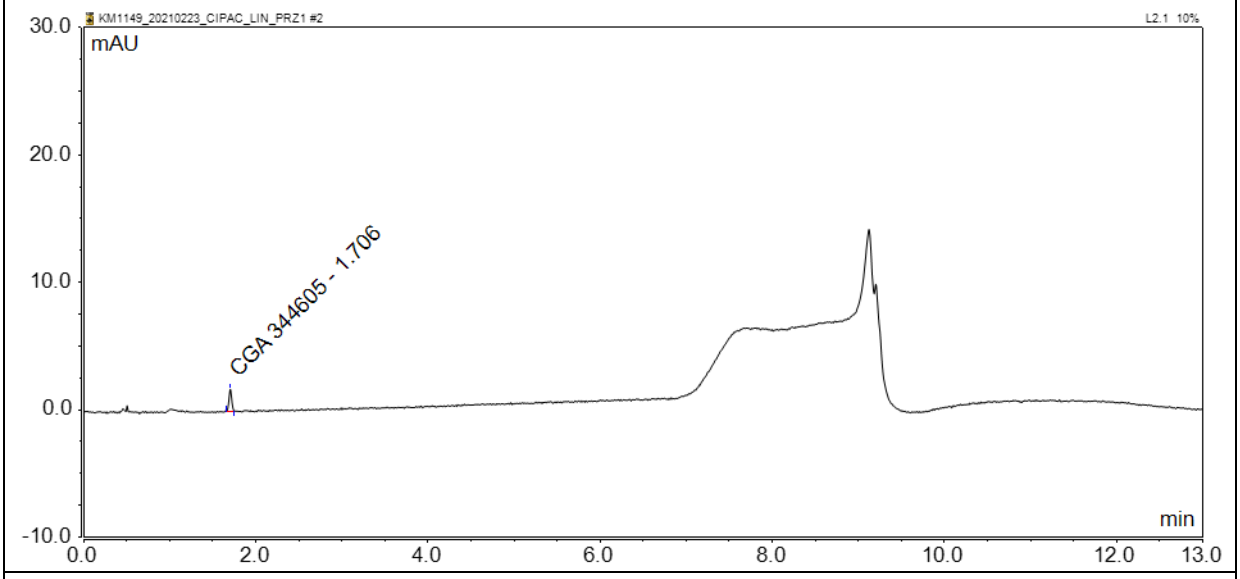


Figure 7. Continuation

reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile/water)

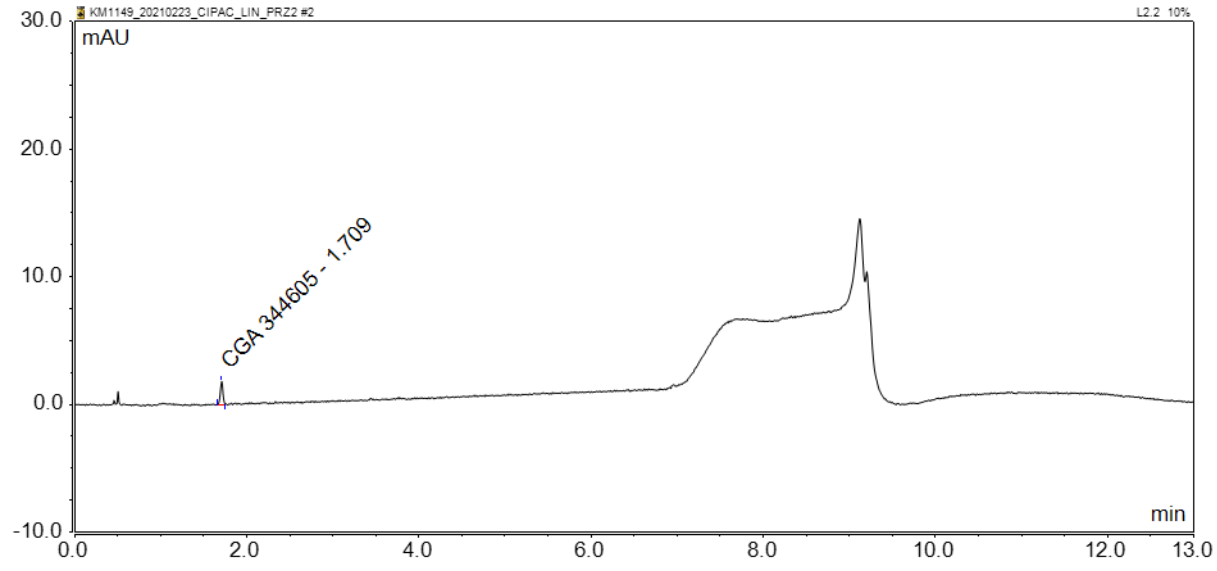
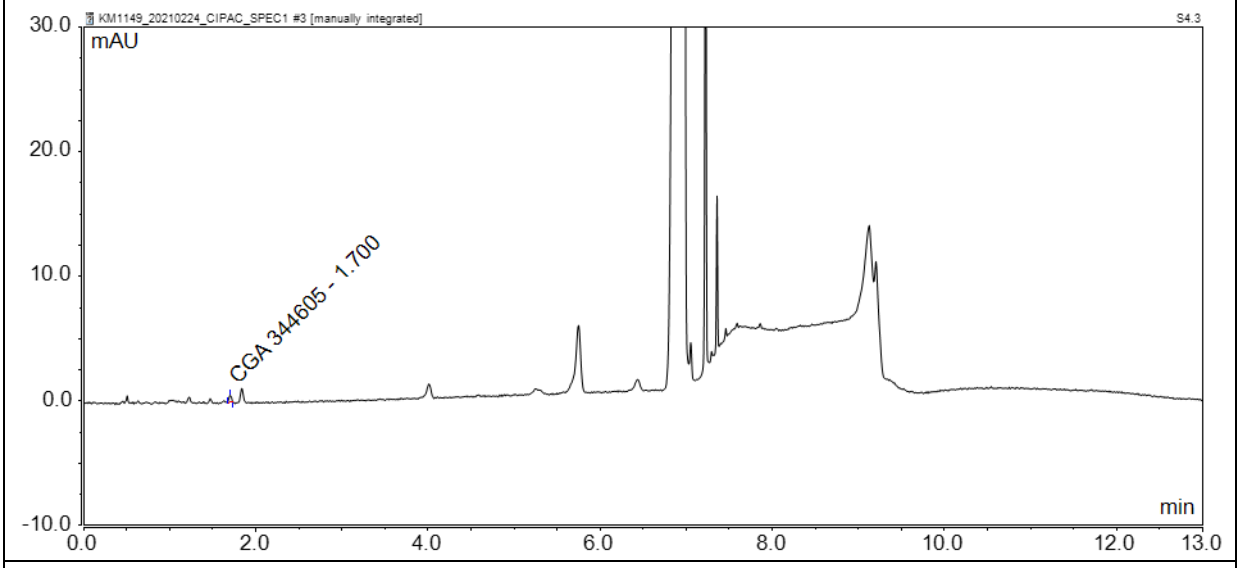


Figure 8: Chromatograms of reference standard of active substance trifloxystrobin. Developing Lab

reference standard of active substance trifloxystrobin in acetonitrile



reference standard of active substance trifloxystrobin in acetonitrile/water

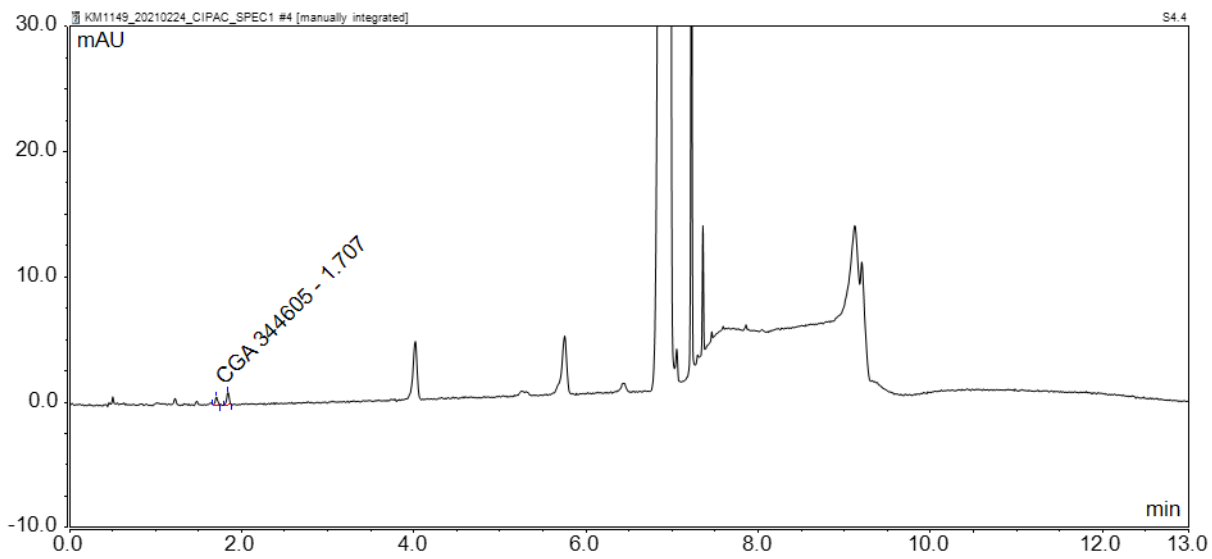


Figure 9: Chromatograms of reference standard of active substance fluoxastrobin. Developing Lab

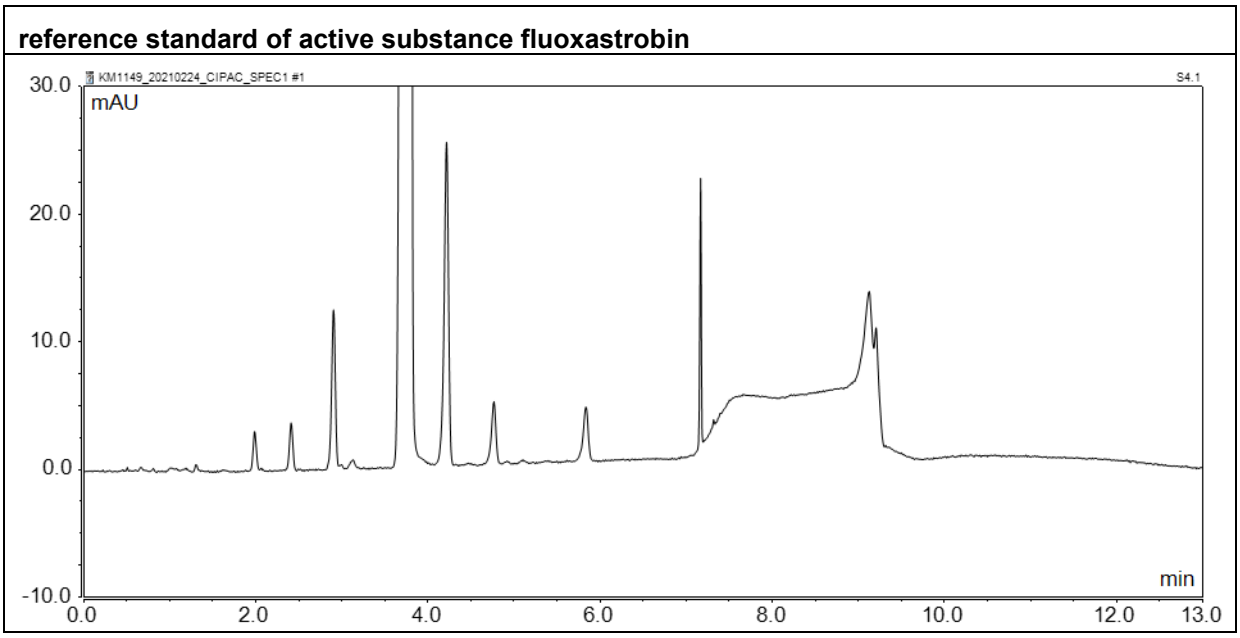


Figure 10: Chromatograms of reference standard of active substance prothioconazole. Developing Lab

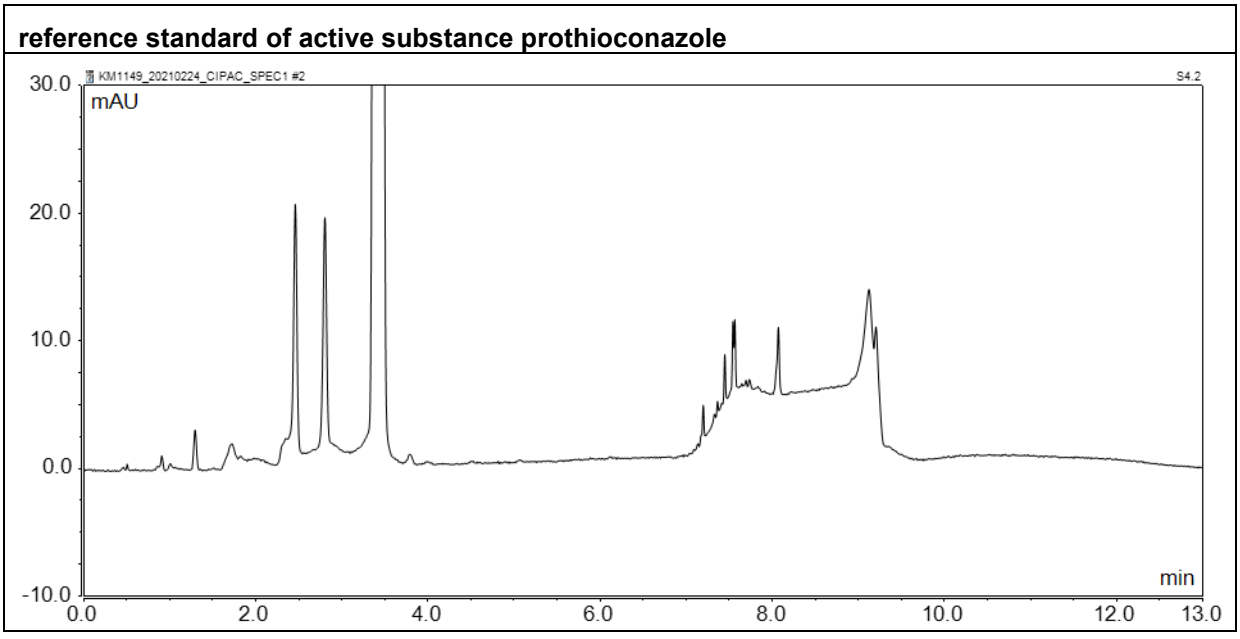
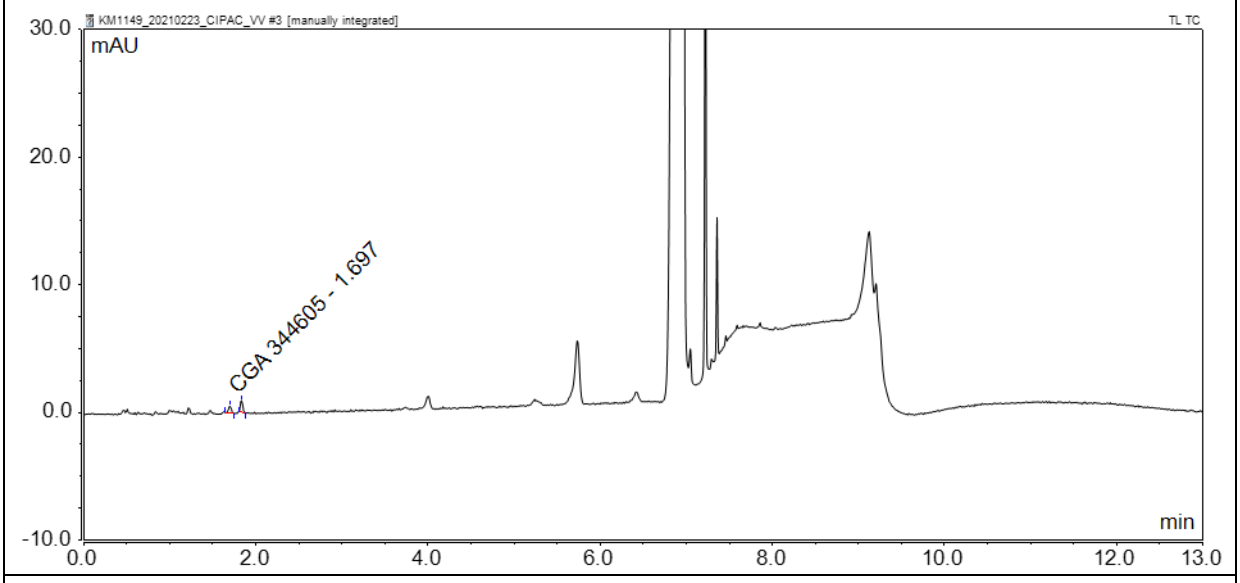


Figure 11: Chromatograms of trifloxystrobin TC Developing Lab

Technical material trifloxystrobin TC



Technical material trifloxystrobin TC spiked with reference standard CGA 344605

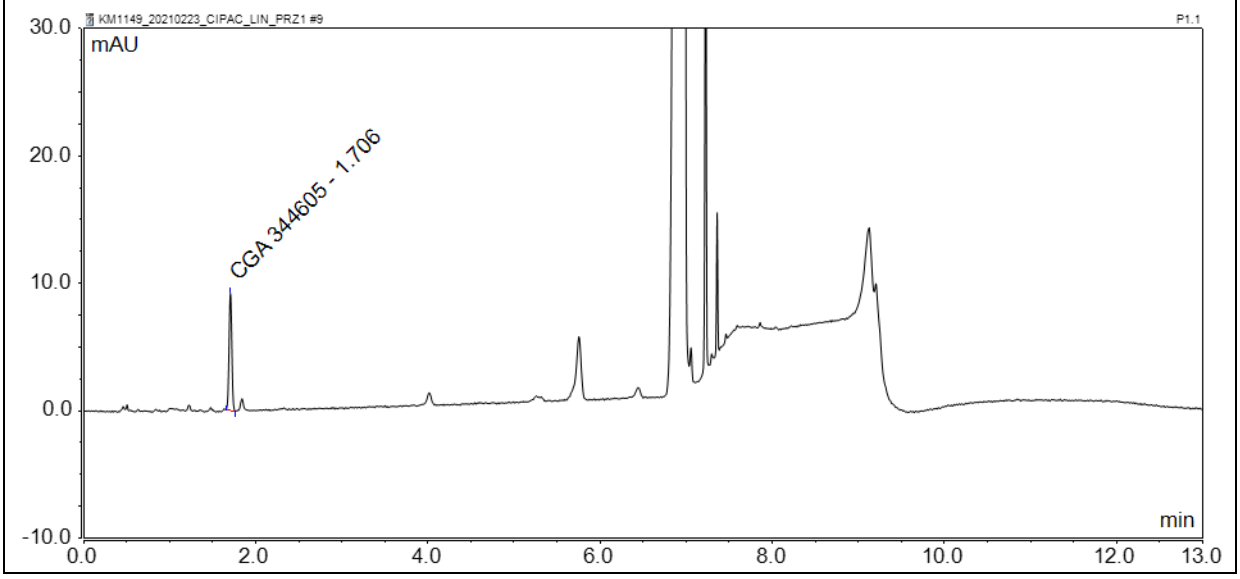
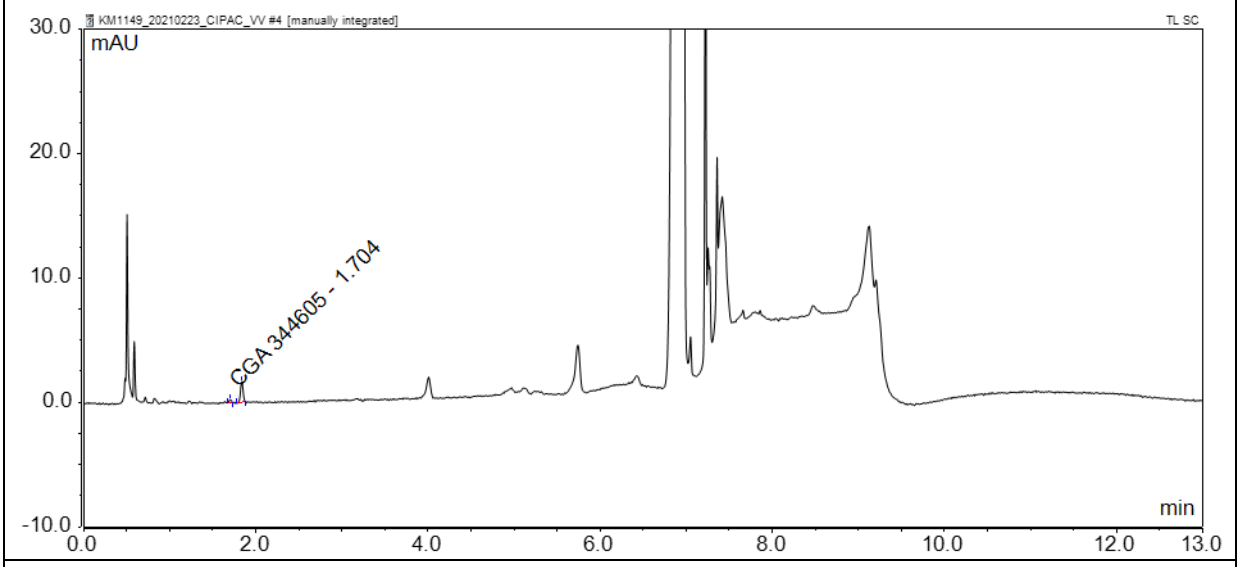


Figure 12: Chromatograms of trifloxystrobin SC 500 (500 g/L) Developing Lab.

Formulation trifloxystrobin SC 500 (500 g/L)



Formulation trifloxystrobin SC 500 (500 g/L) spiked with reference standard CGA 344605

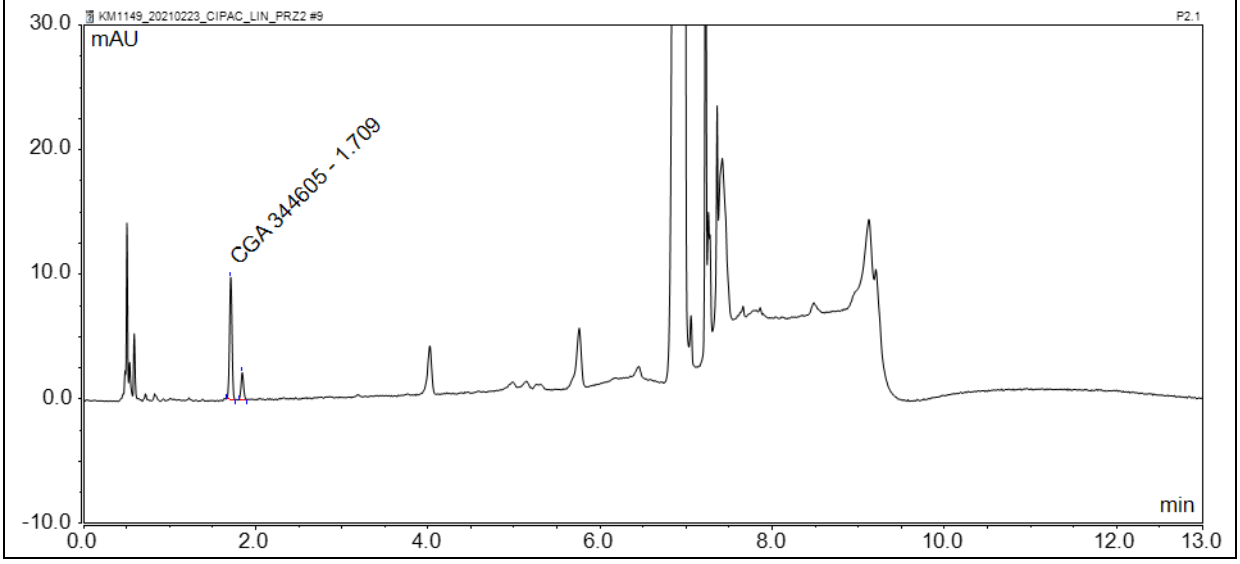
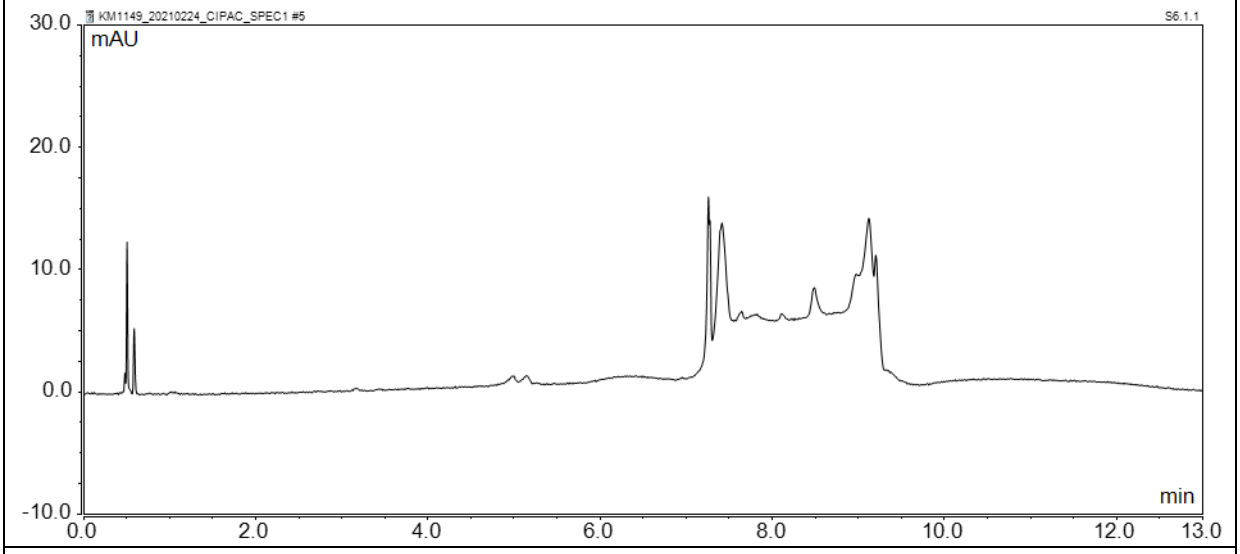


Figure 12. Continuation

Blank formulation trifloxystrobin SC 0 (0 g/L)



Blank formulation trifloxystrobin SC 0 (0 g/L) – three-fold weight

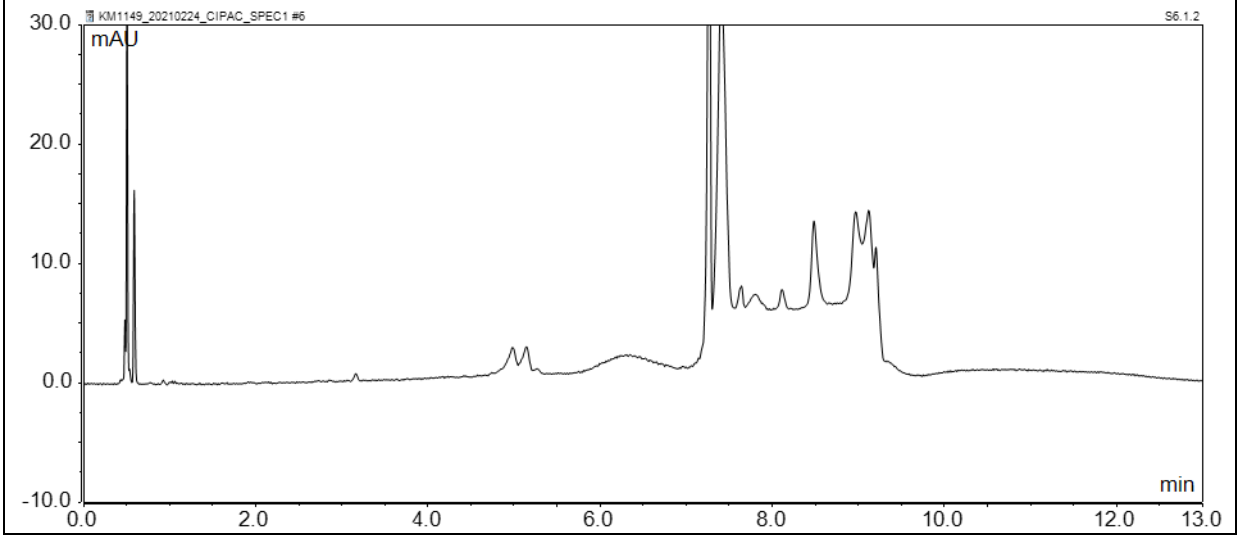
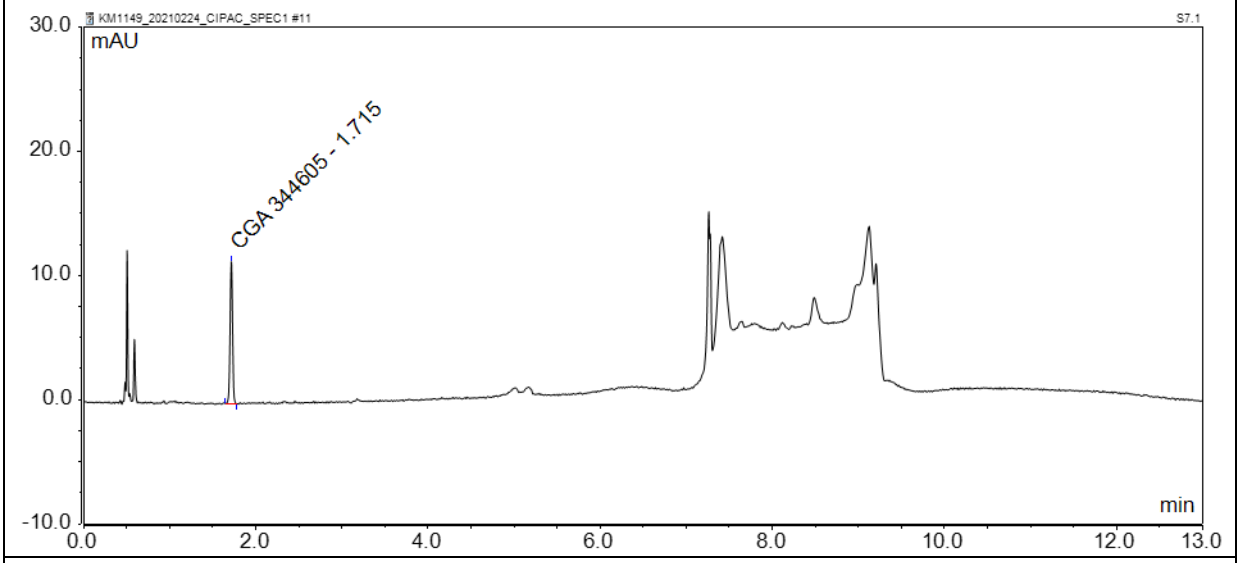


Figure 12. Continuation

**Blank formulation trifloxystrobin SC 0 (0 g/L)
spiked with reference standard CGA 344605**



**Blank formulation trifloxystrobin SC 0 (0 g/L)
spiked with reference standard trifloxystrobin**

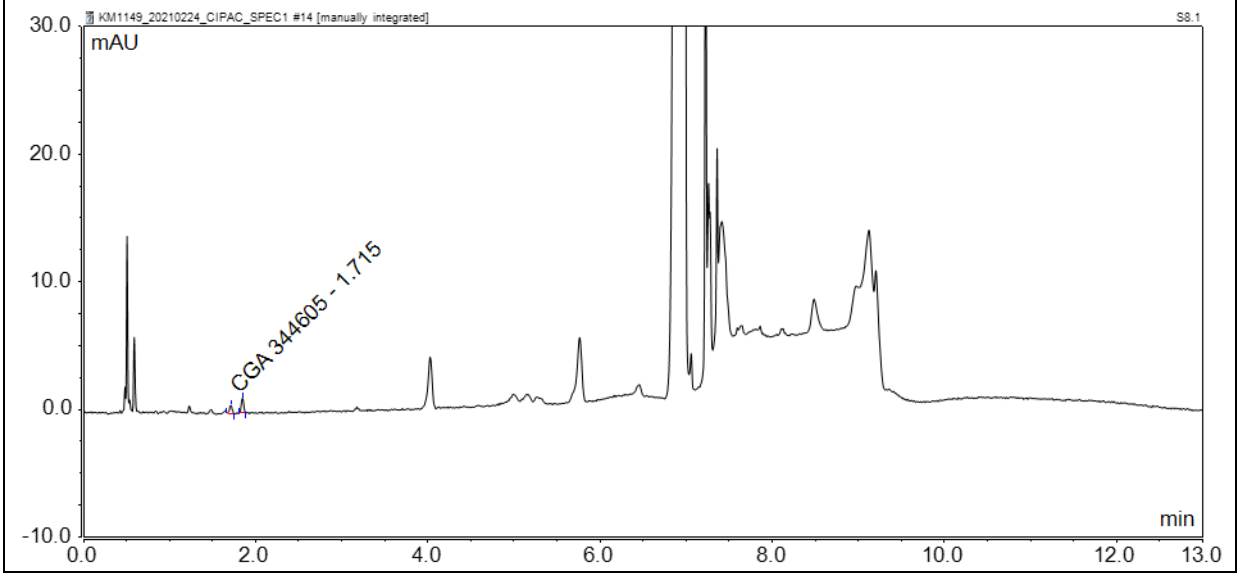
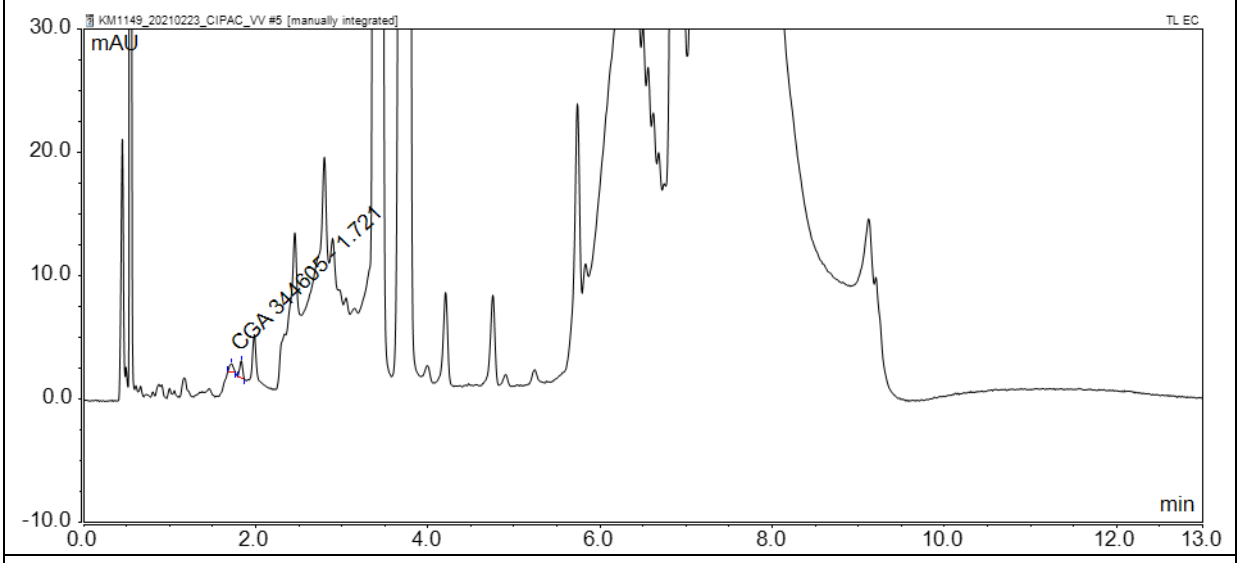


Figure 13: Chromatograms of fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) Developing Lab

Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L)



Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) spiked with reference standard CGA 344605

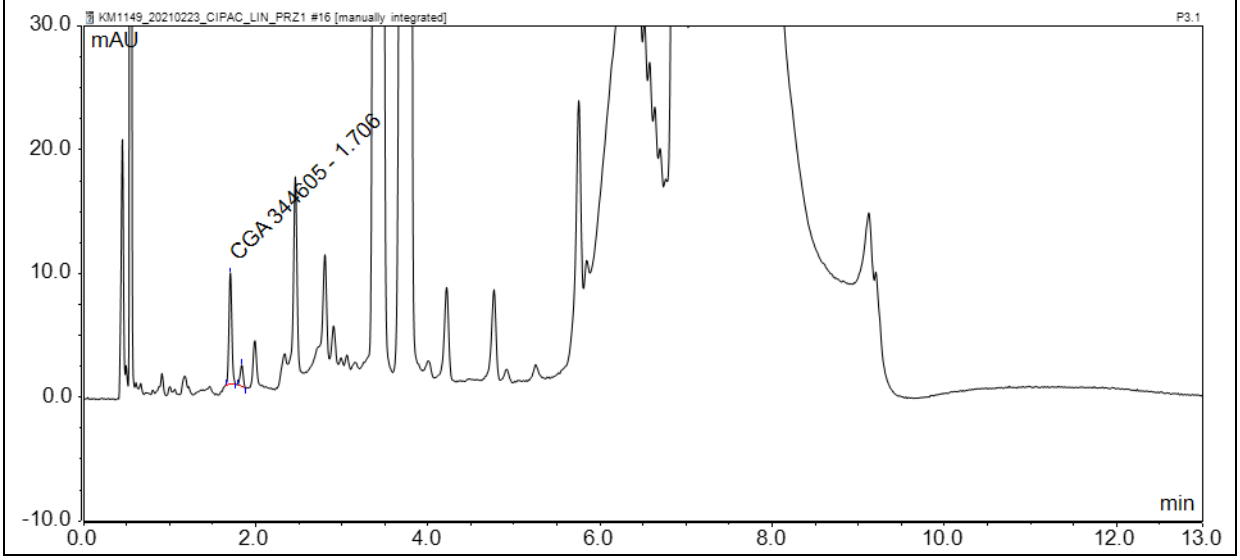
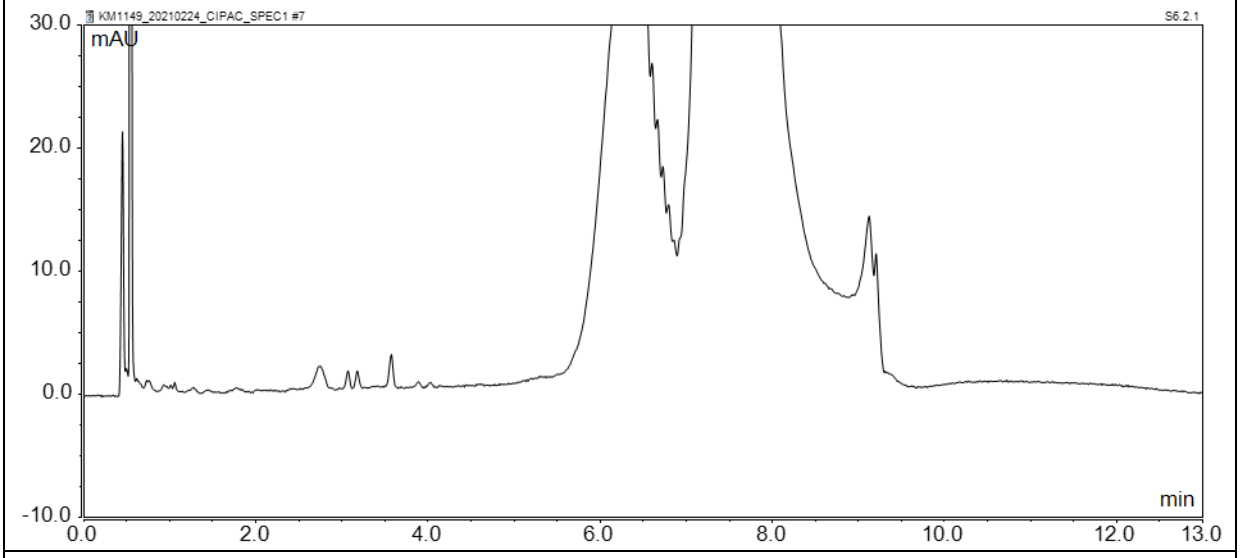


Figure 13. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L)



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) three-fold weigth

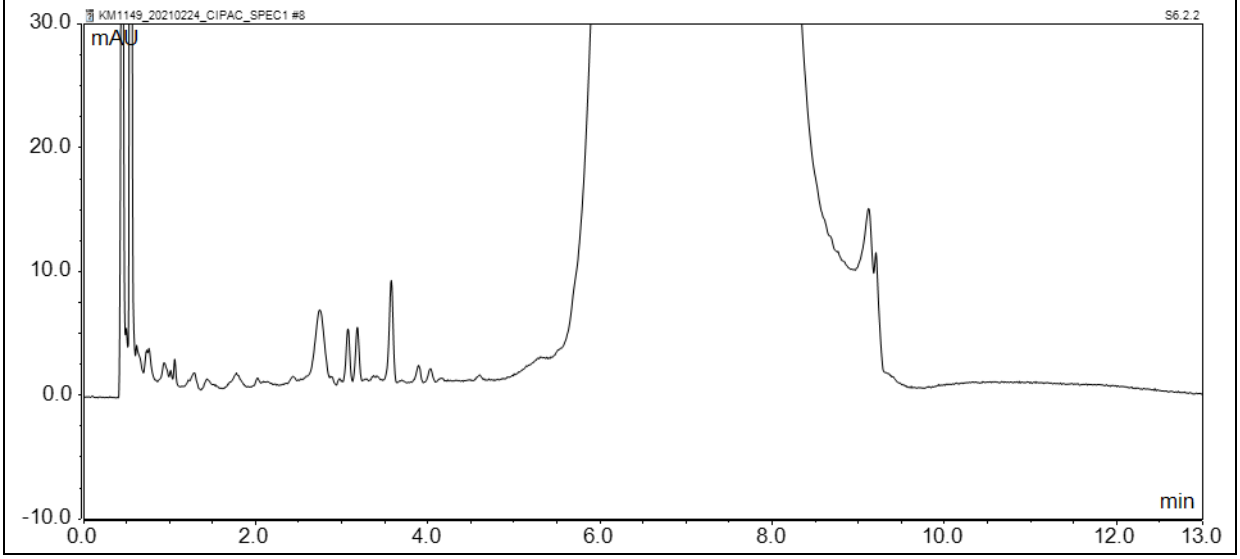
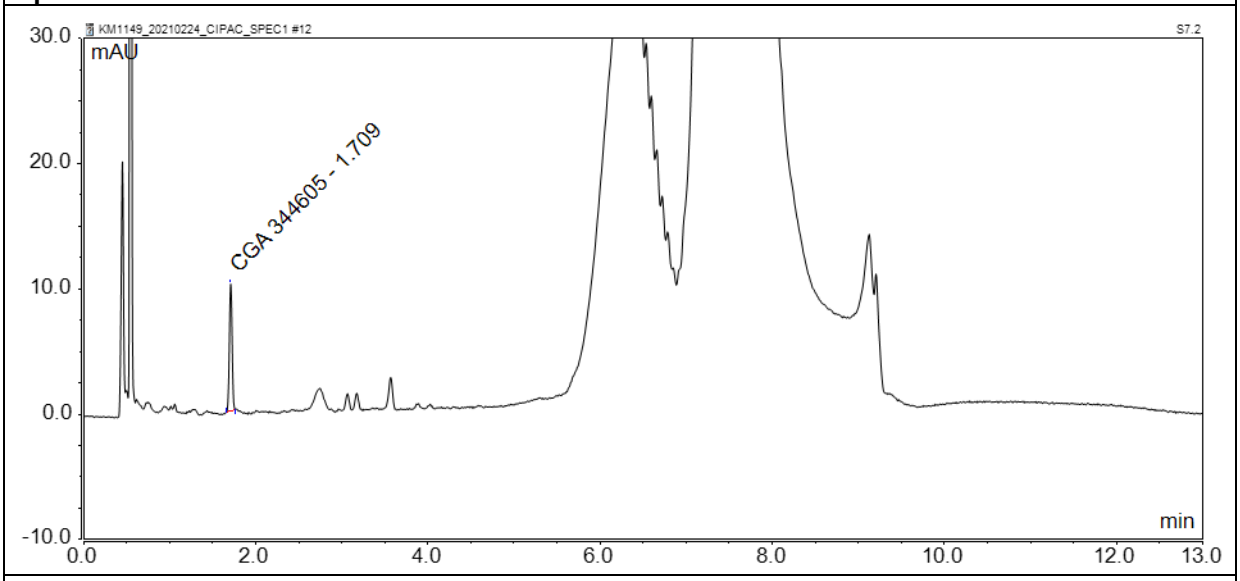


Figure 13. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) spiked with reference standard CGA 344605



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) spiked with reference standard fluoxastrobin. prothioconazole and trifloxystrobin

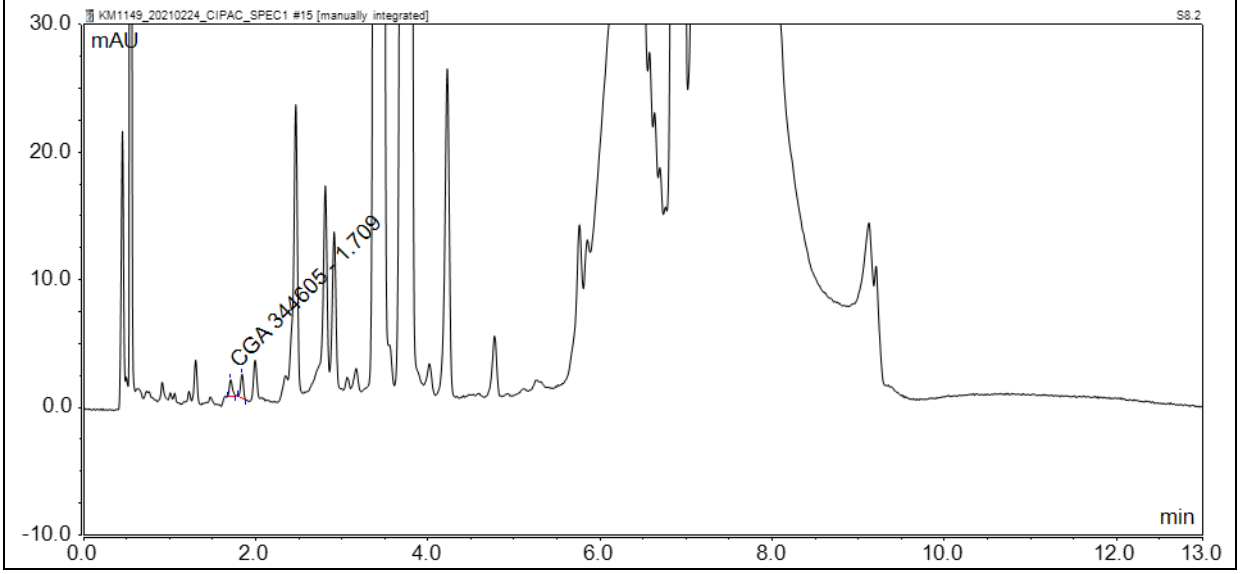
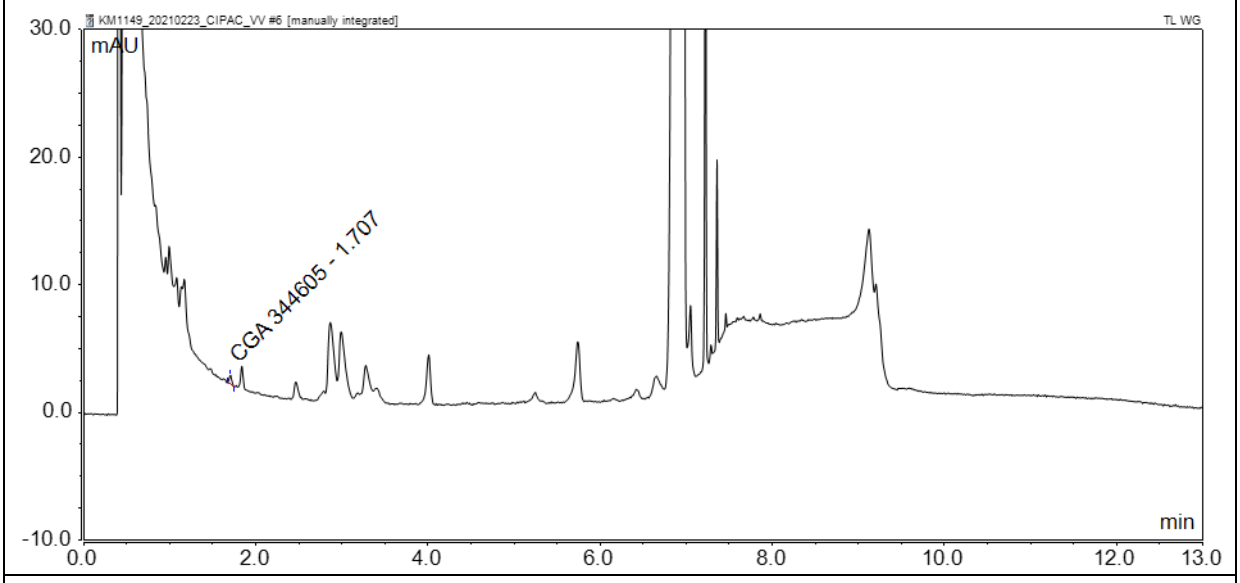


Figure 14: Chromatogram of trifloxystrobin WG 50 (50 g/kg) Developing Lab

Formulation trifloxystrobin WG 50 (50 g/kg)



Formulation trifloxystrobin WG 50 (50 g/kg) spiked with reference standard CGA 344605

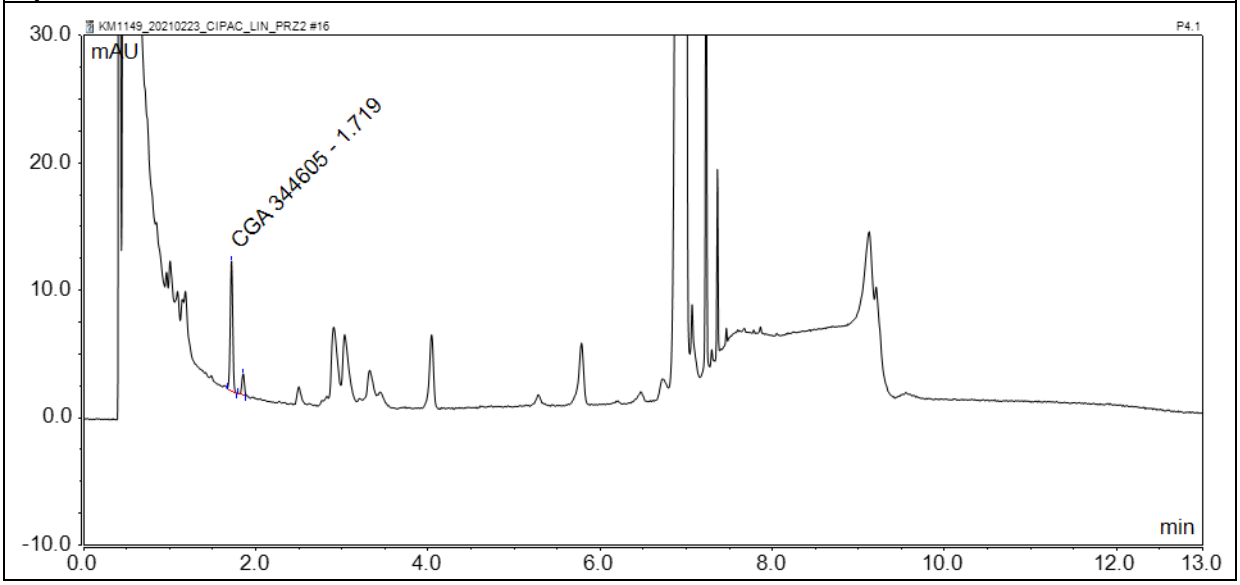
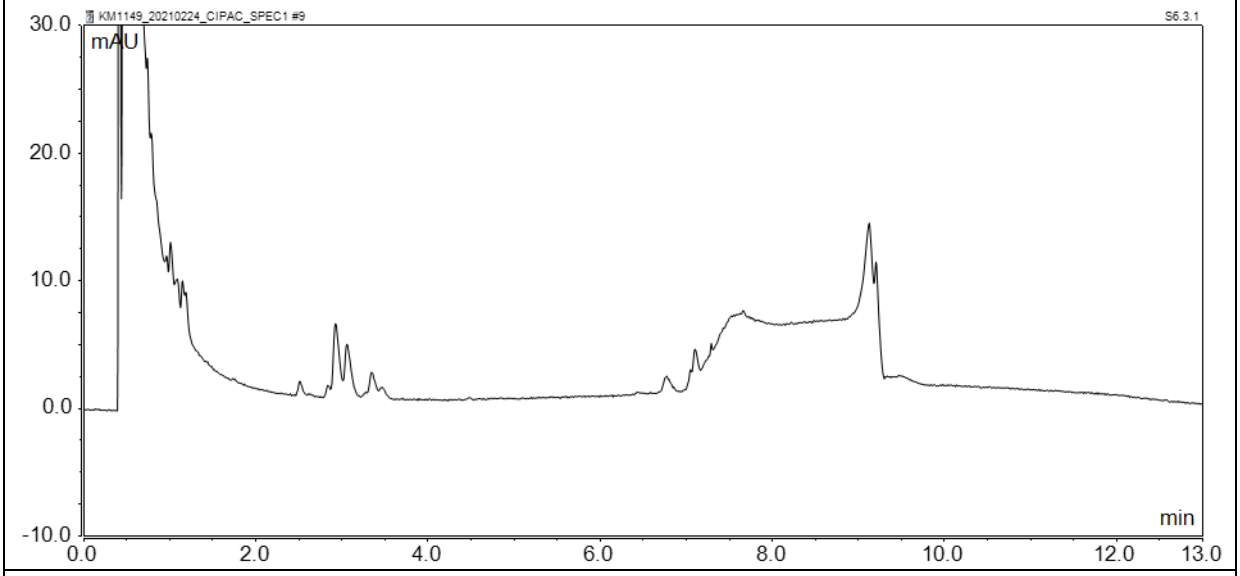


Figure 14. Continuation

Blank Formulation trifloxystrobin WG 0 (0 g/Kg)



Blank Formulation trifloxystrobin WG 0 (0 g/Kg) - three-fold weight

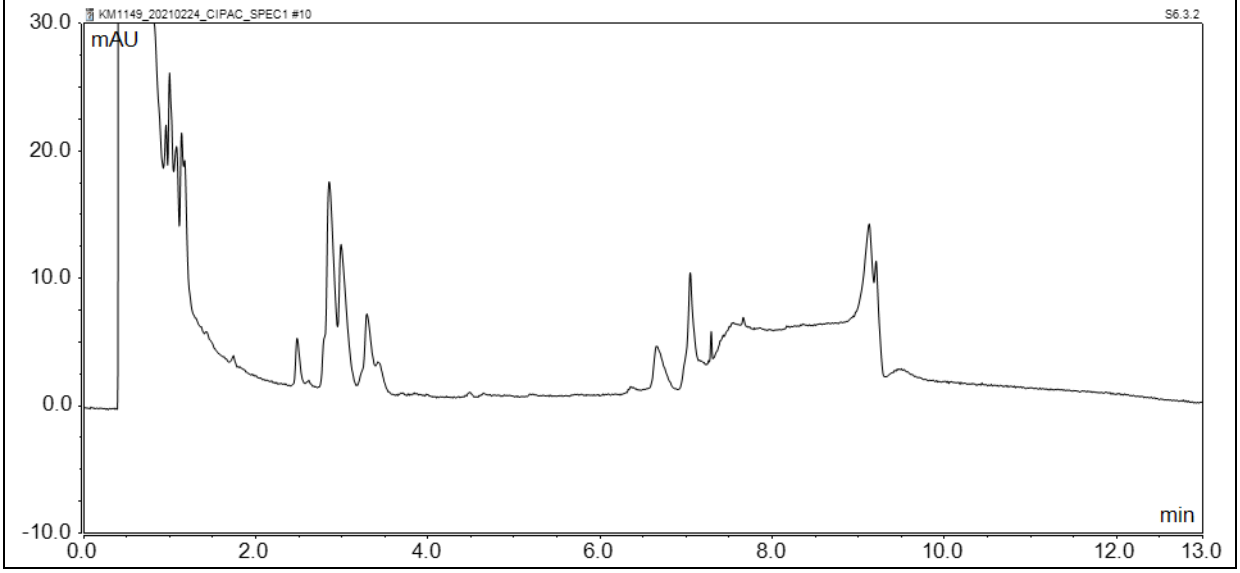
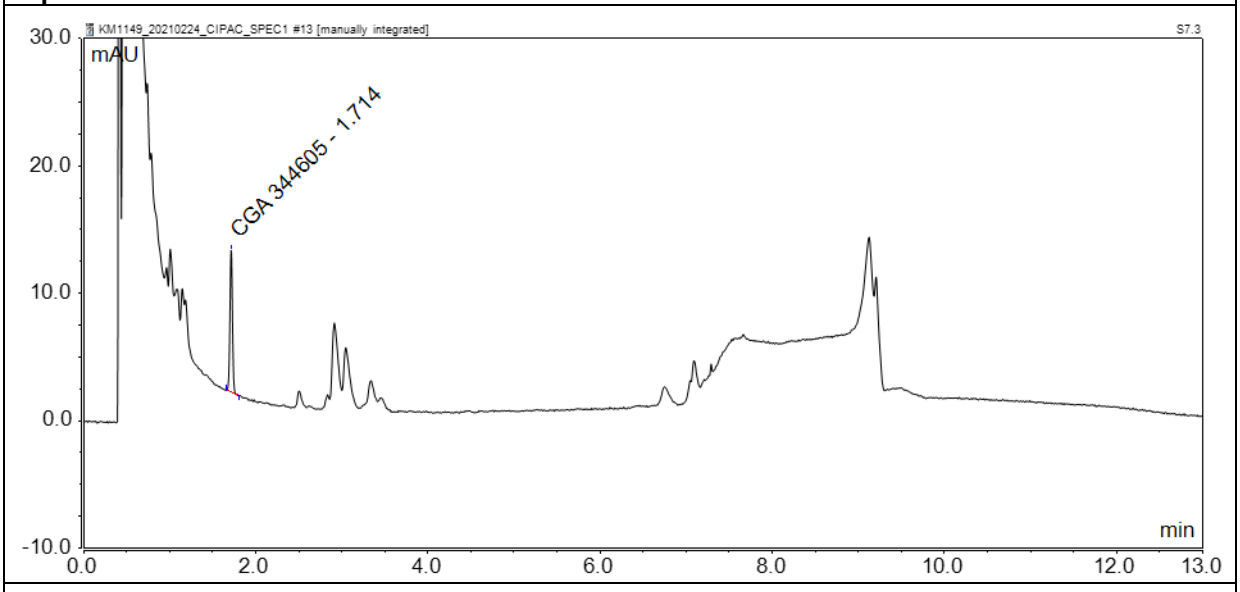


Figure 14. Continuation

Blank formulation Blank Formulation trifloxystrobin WG 0 (0 g/Kg) spiked with reference standard CGA 344605



Blank Formulation trifloxystrobin WG 0 (0 g/Kg) spiked with reference standard trifloxystrobin

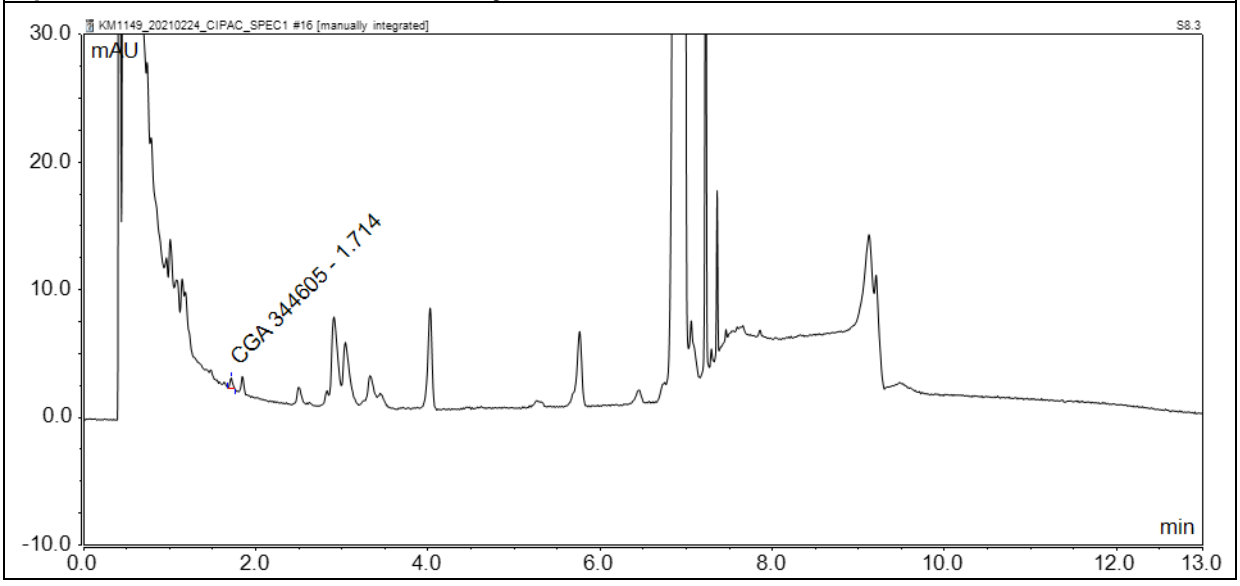
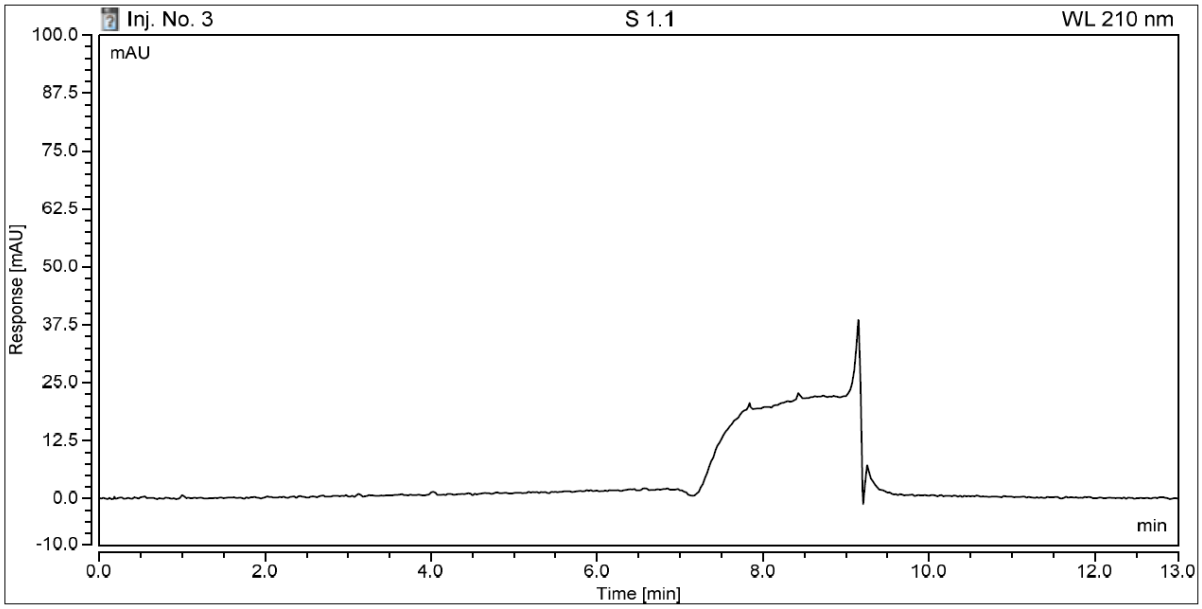


Figure 15: Chromatograms of sample solvent Lab 1

acetonitrile



acetonitrile/water

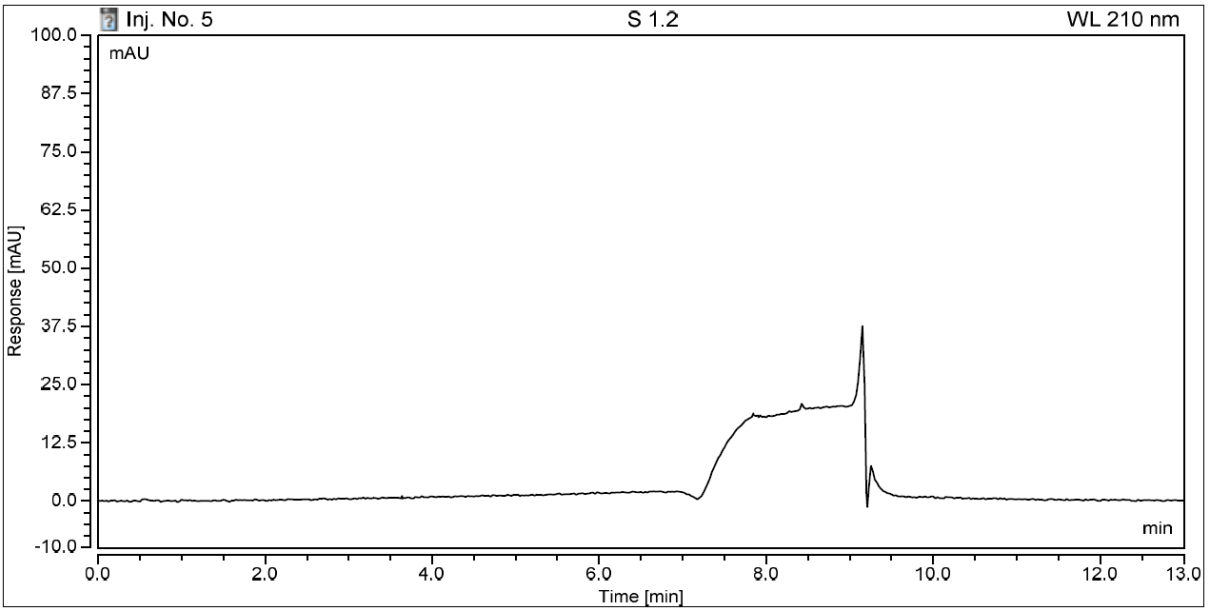
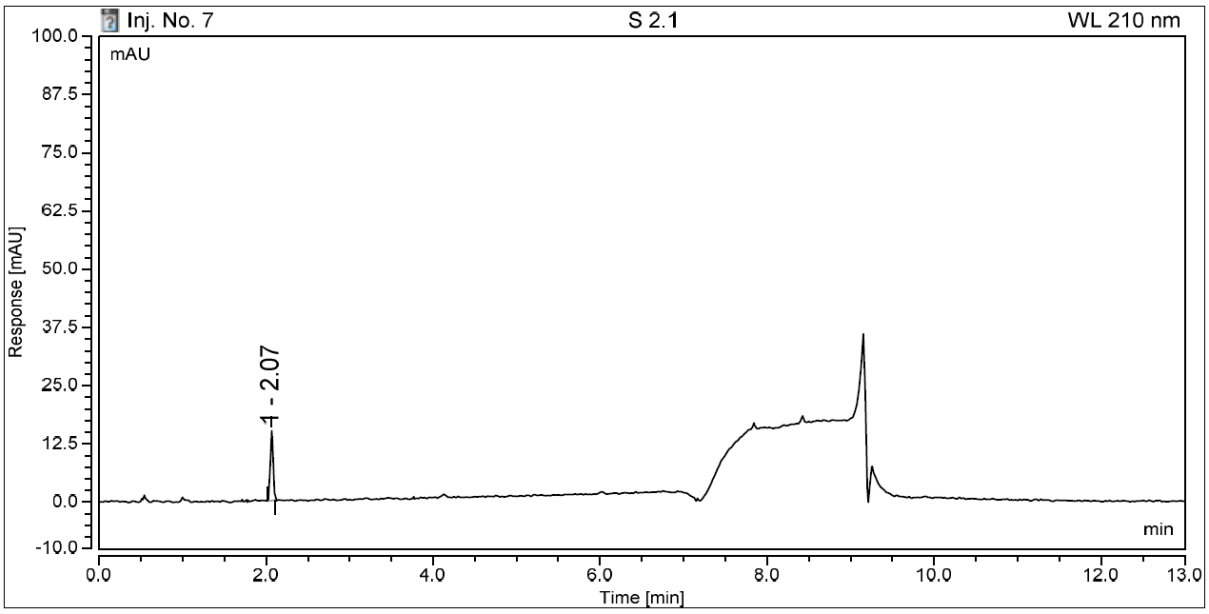


Figure 16: Chromatograms of reference standard CGA 344605 Lab 1

reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile/water)

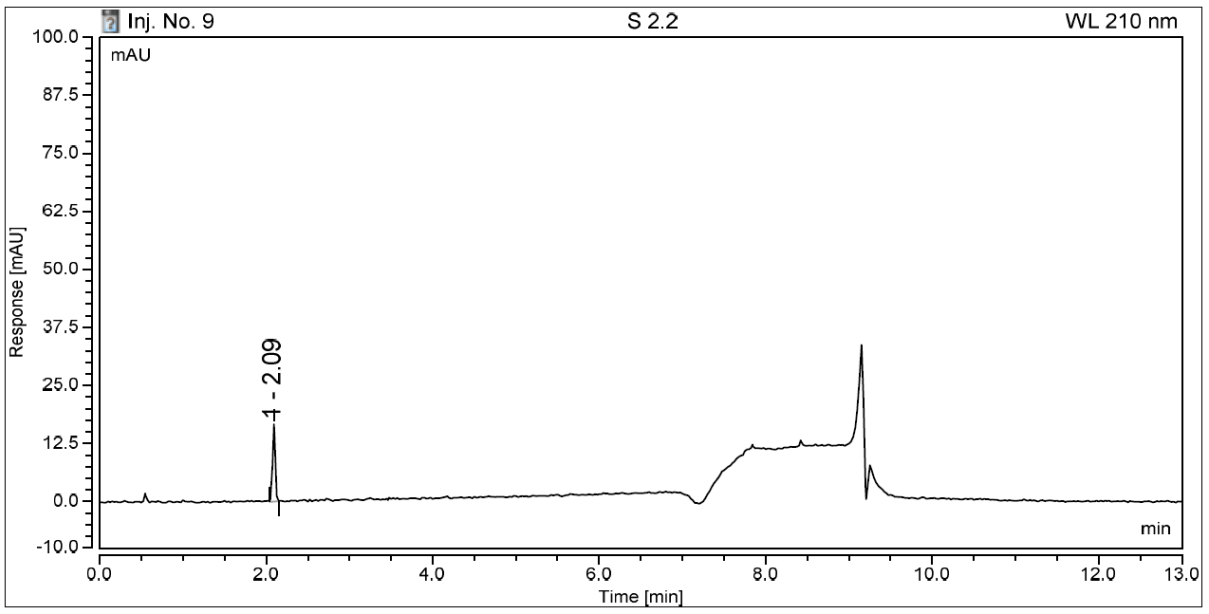
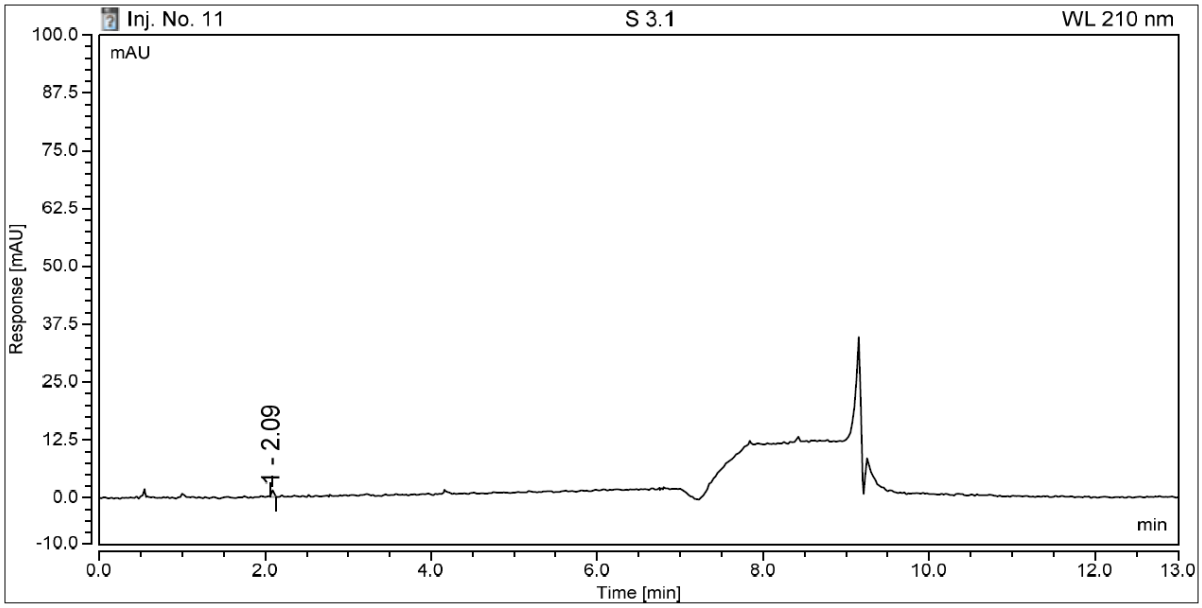


Figure 16. Continuation

reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile/water)

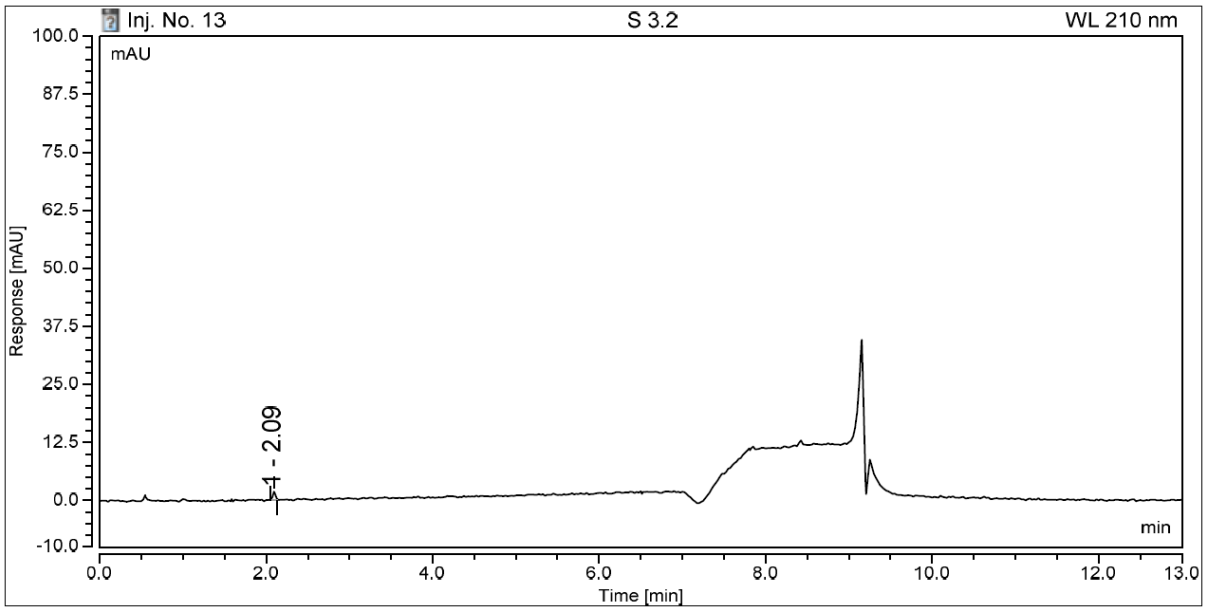
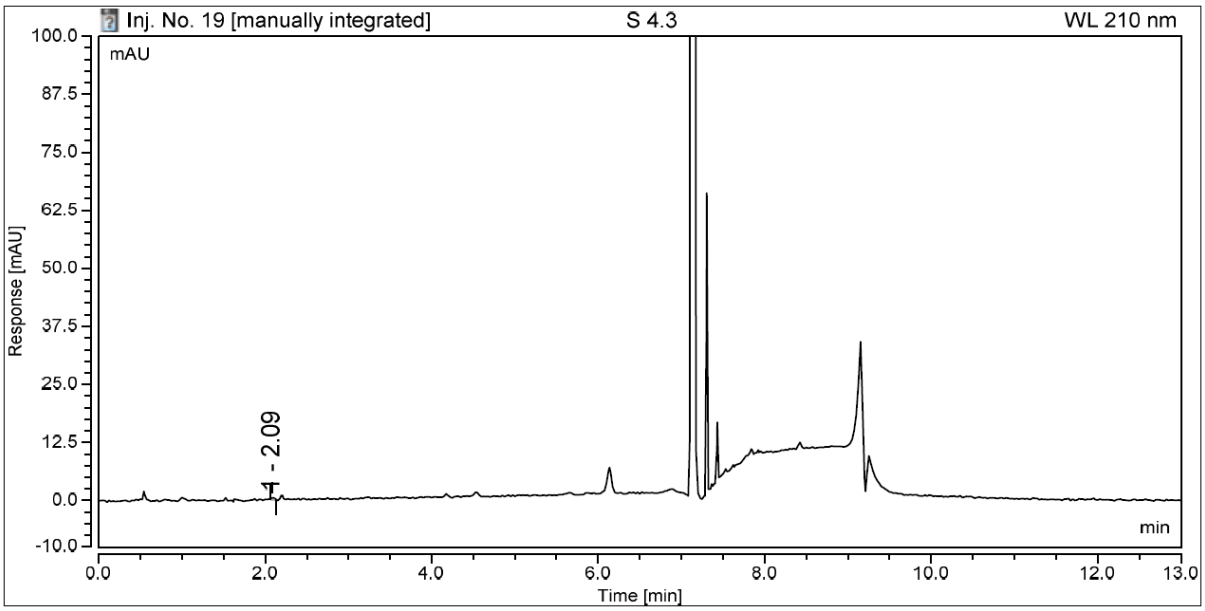


Figure 17: Chromatograms of active substance trifloxystrobin Lab 1

reference standard of active substance trifloxystrobin in acetonitrile



reference standard of active substance trifloxystrobin in acetonitrile/water

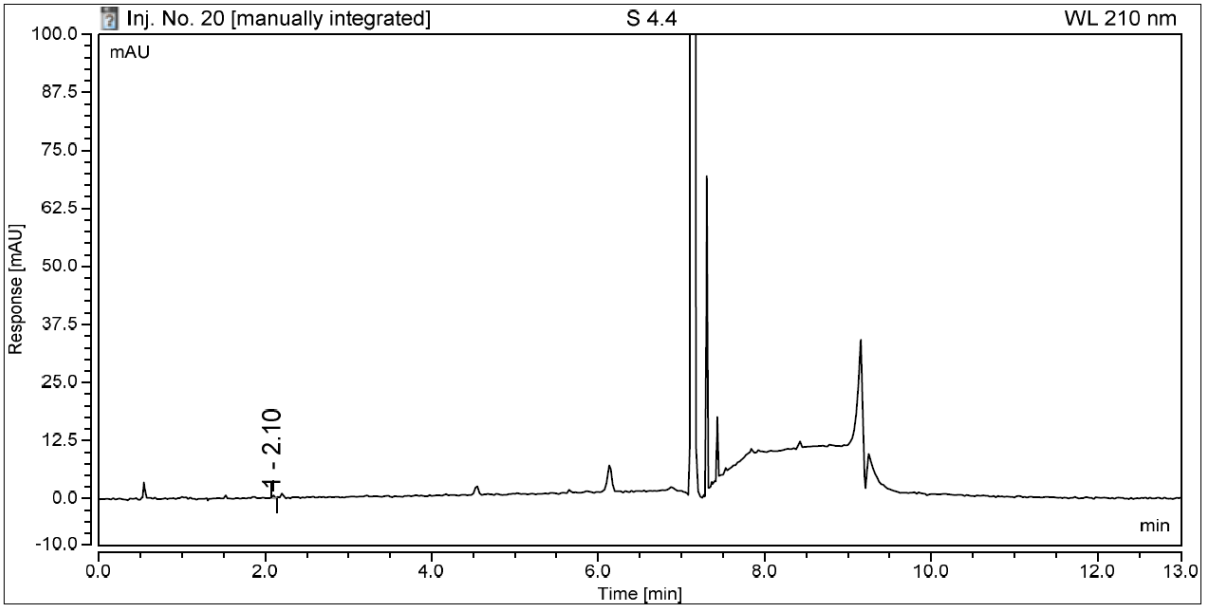


Figure 18: Chromatograms of active substance fluoxastrobin. Lab 1

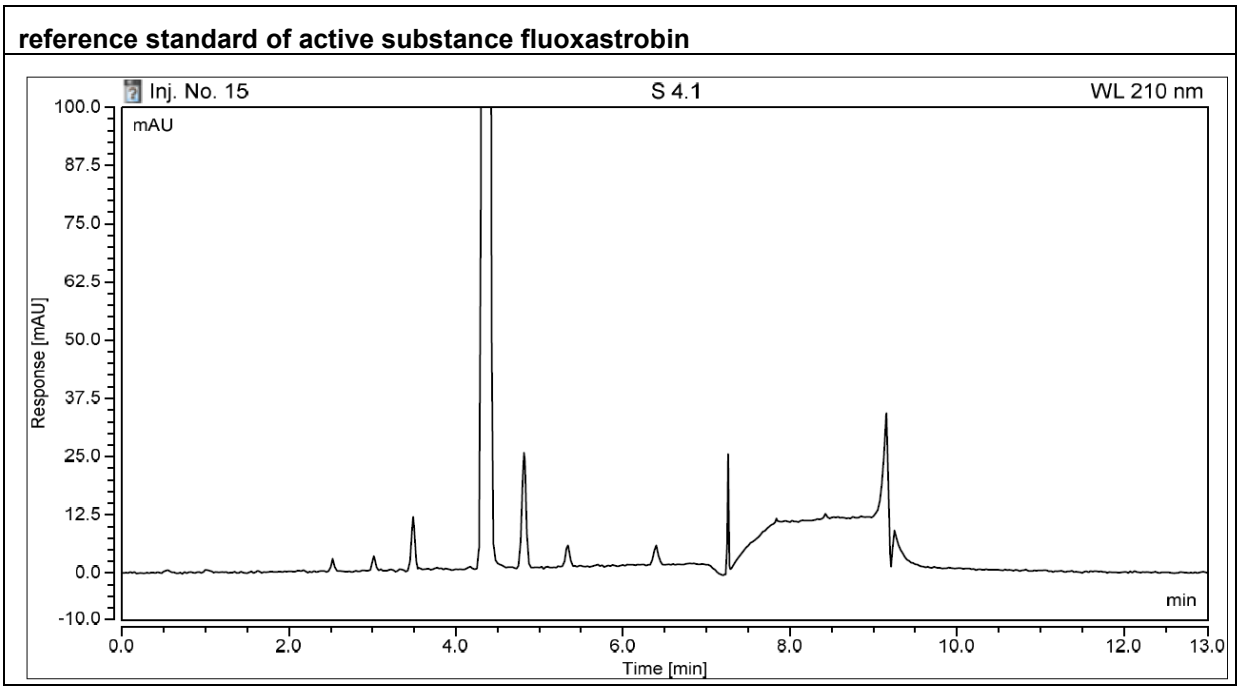


Figure 19: Chromatograms of active substance prothioconazole Lab 1

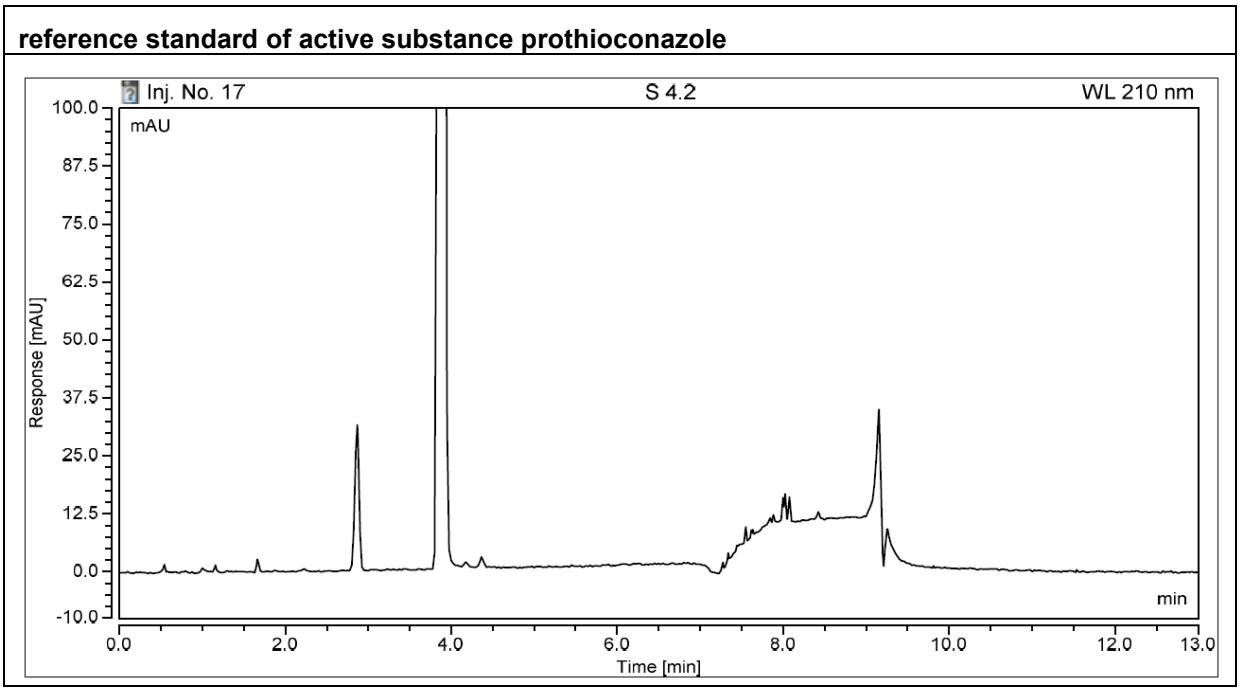


Figure 20: Chromatograms of trifloxystrobin TC Lab 1

Technical material trifloxystrobin TC spiked with reference standard CGA 344605

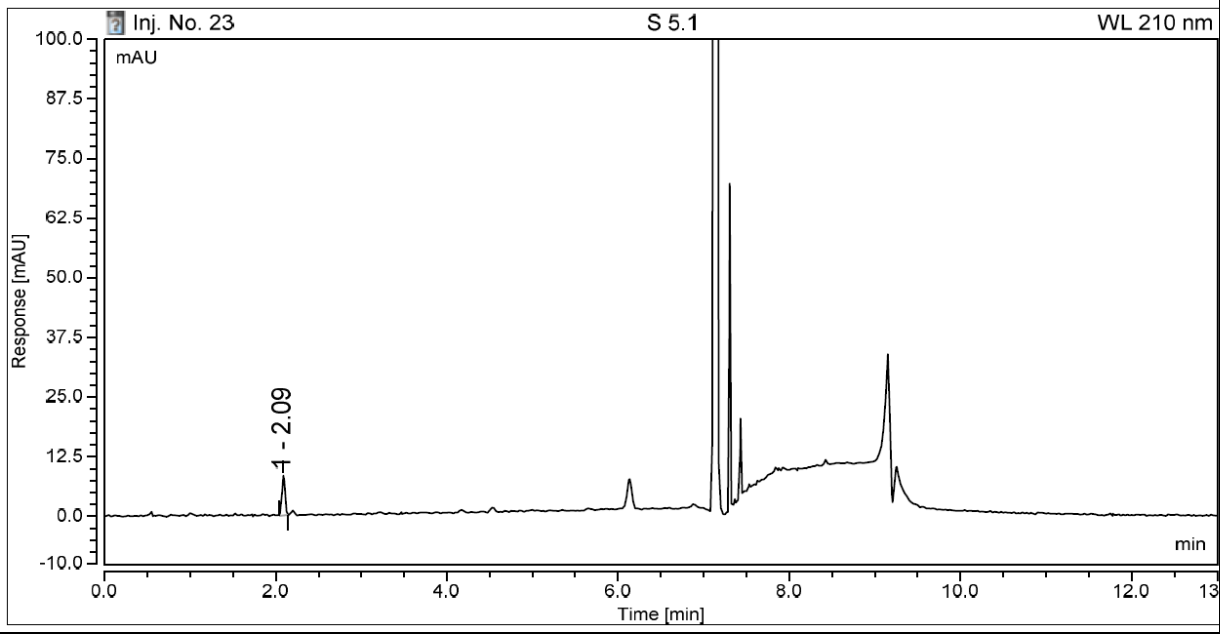
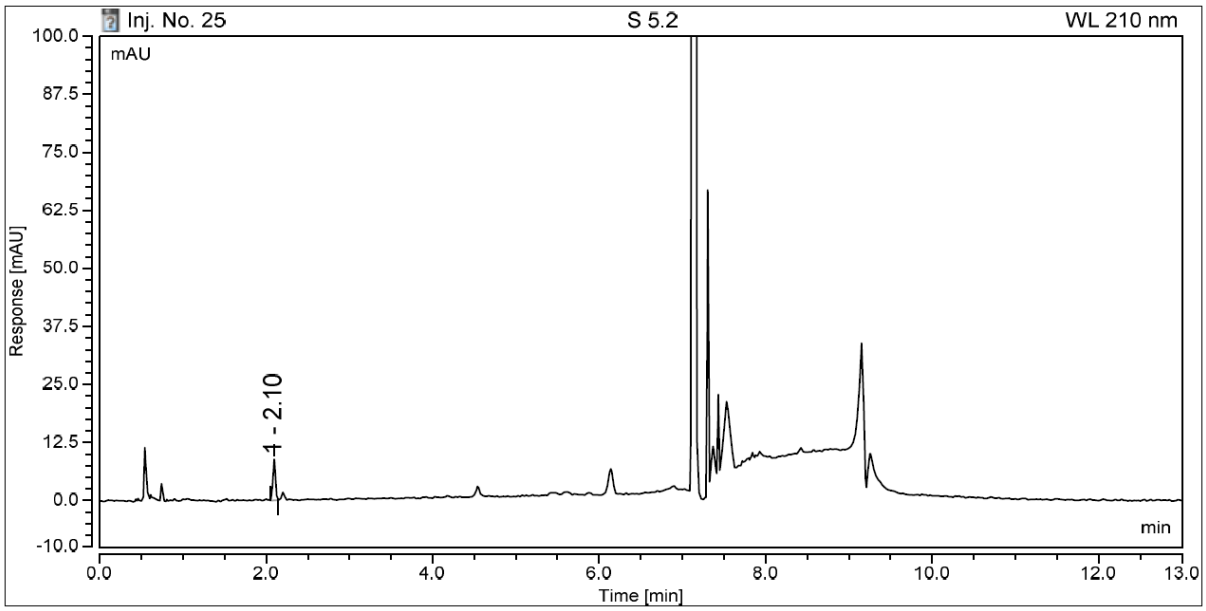


Figure 21: Chromatograms of trifloxystrobin SC 500 (500 g/L) Lab 1

Formulation trifloxystrobin SC 500 (500 g/L) spiked with reference standard CGA 344605



Blank formulation trifloxystrobin SC 0 (0 g/L)

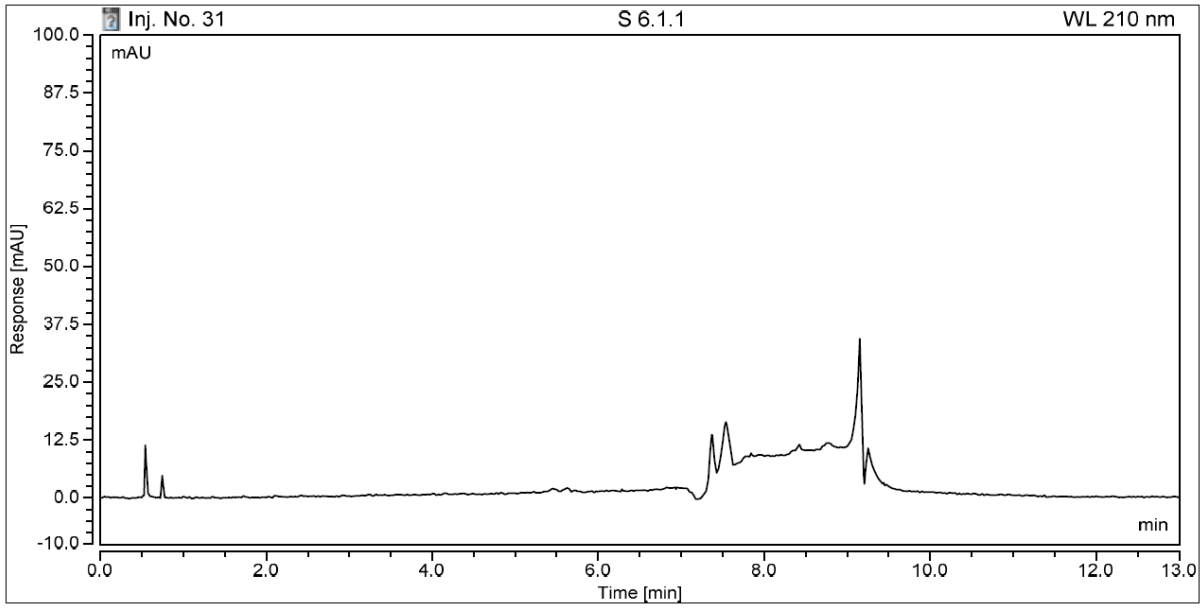


Figure 21. Continuation

Blank formulation trifloxystrobin SC 0 (0 g/L) – three-fold weight

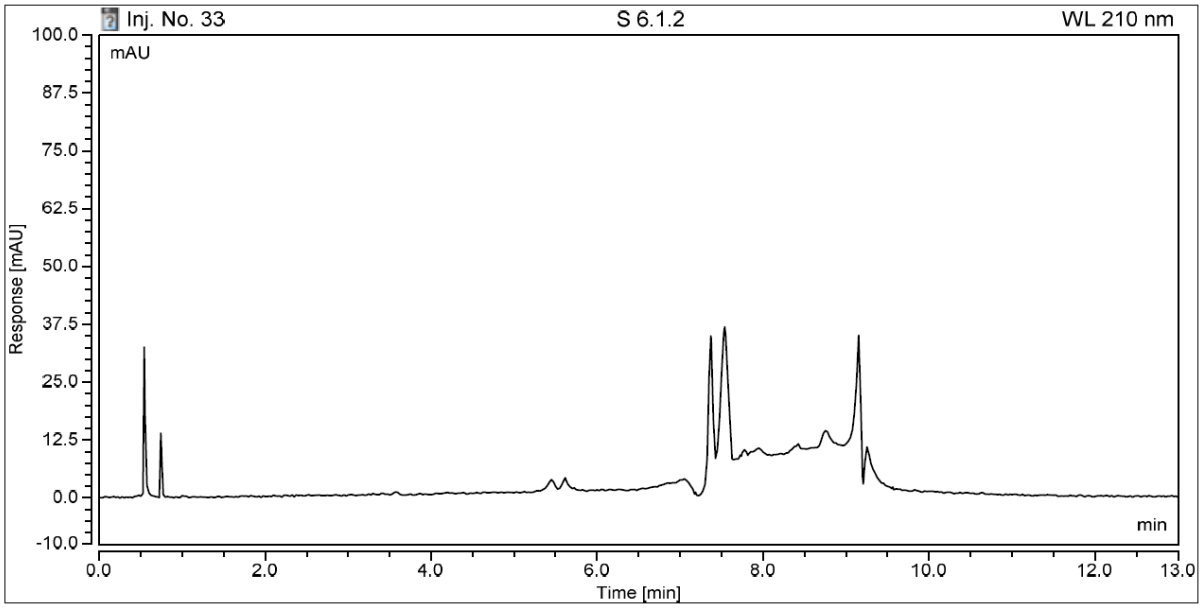
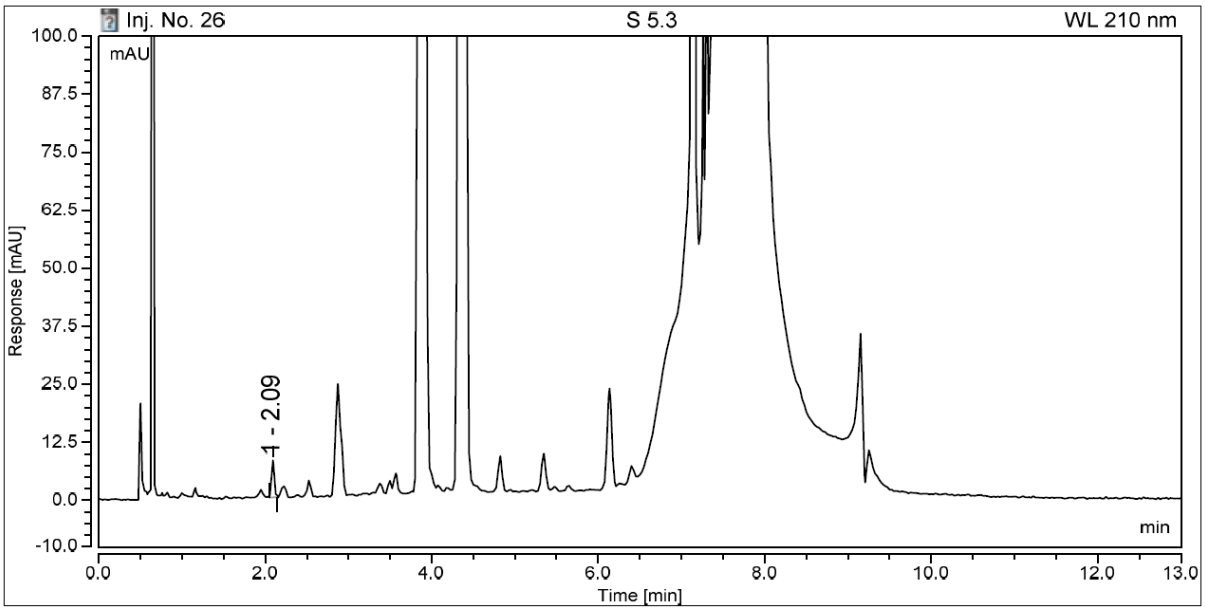


Figure 22: Chromatograms of fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) Lab 1

Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) spiked with reference standard CGA 344605



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L)

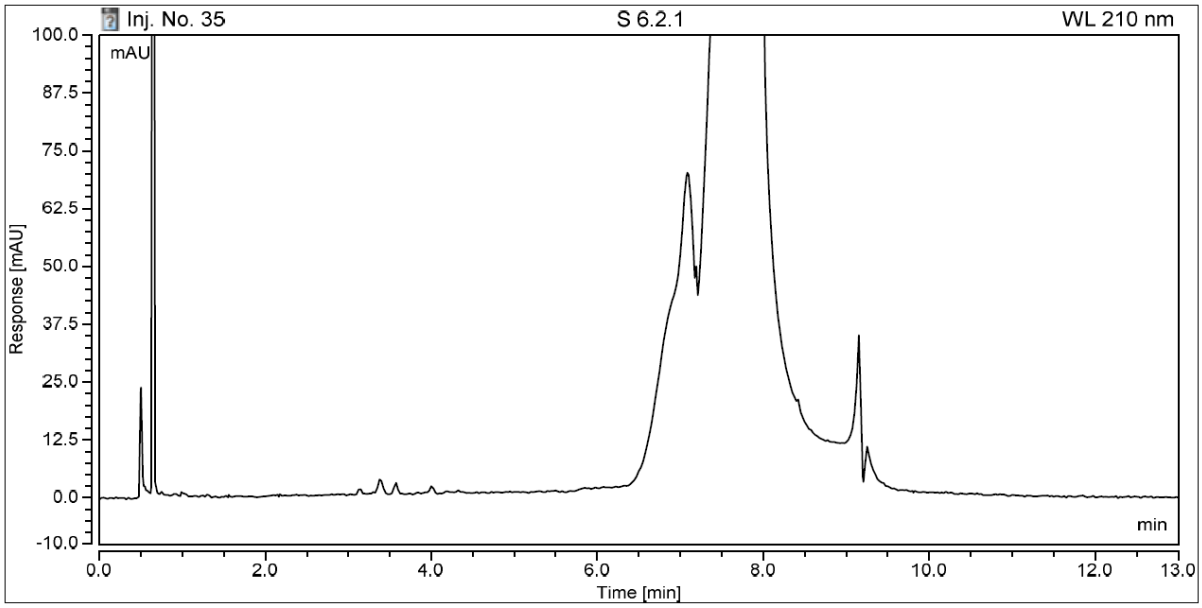


Figure 22. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) three-fold weigth

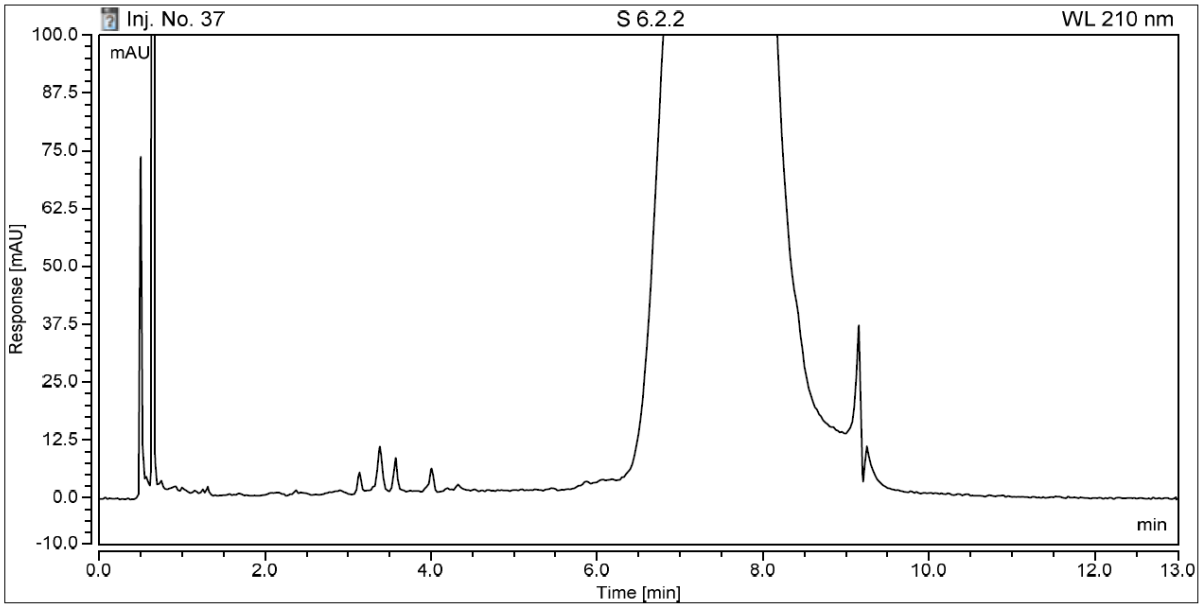
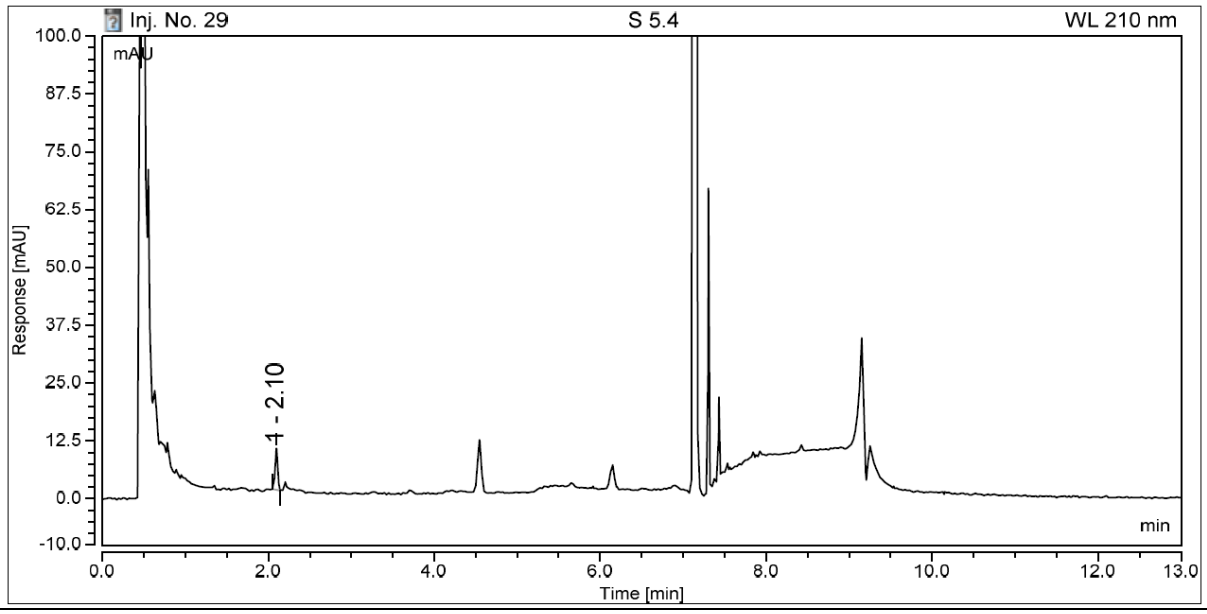


Figure 23: Chromatogram of trifloxystrobin WG 50 (50 g/kg) Lab 1

Formulation trifloxystrobin WG 50 (50 g/kg) spiked with reference standard CGA 344605



Blank Formulation trifloxystrobin WG 0 (0 g/Kg)

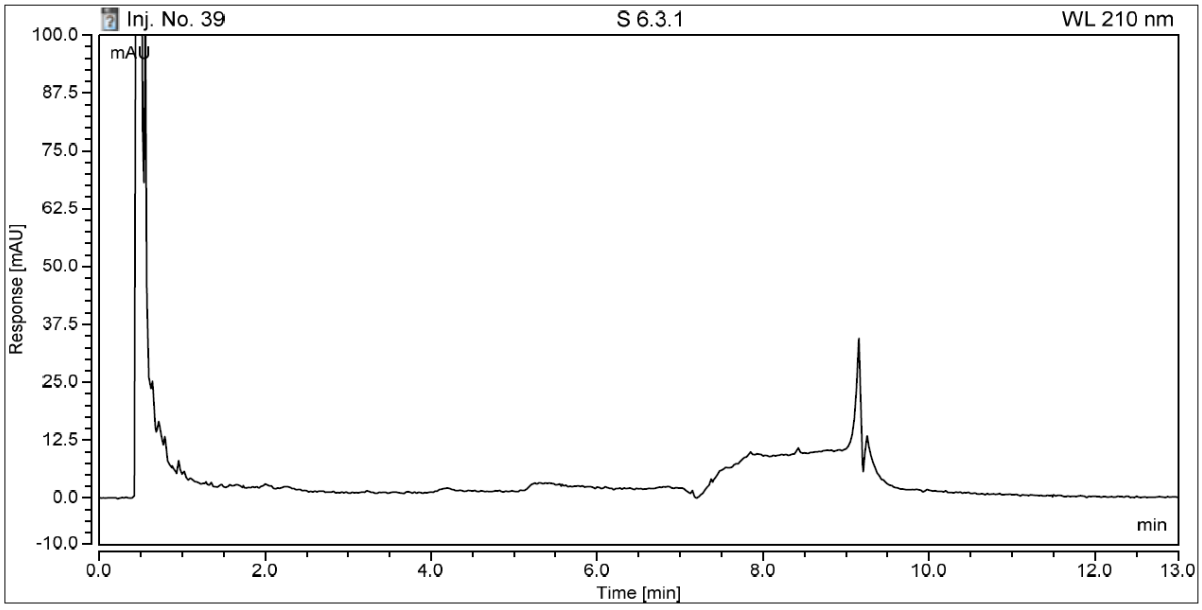


Figure 23. Continuation

Blank Formulation trifloxystrobin WG 0 (0 g/Kg) - three-fold weight

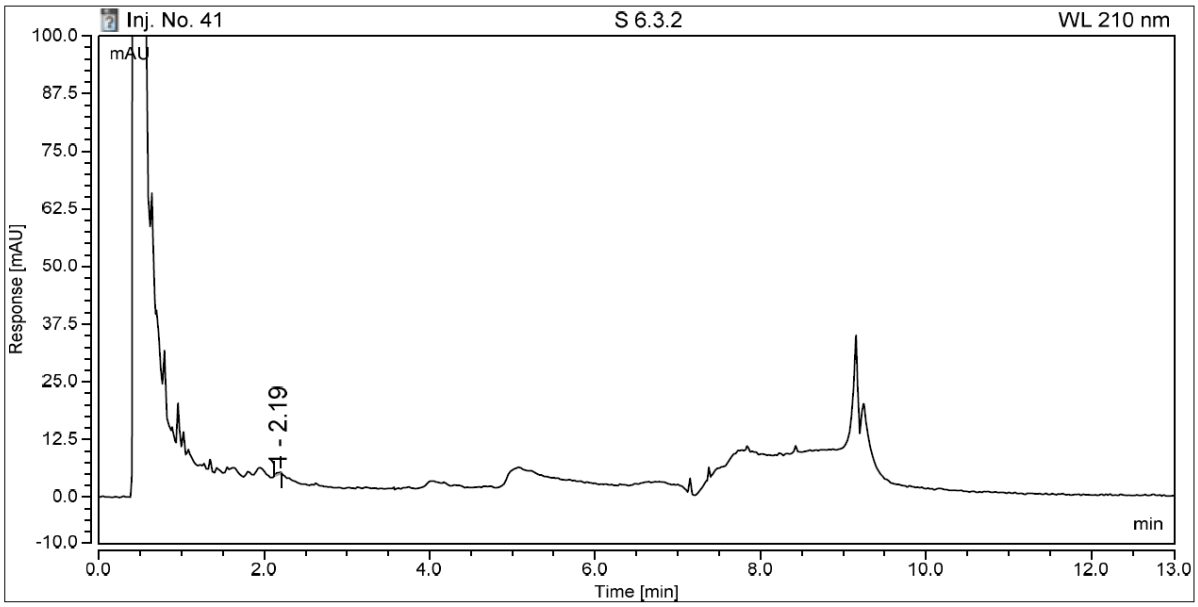
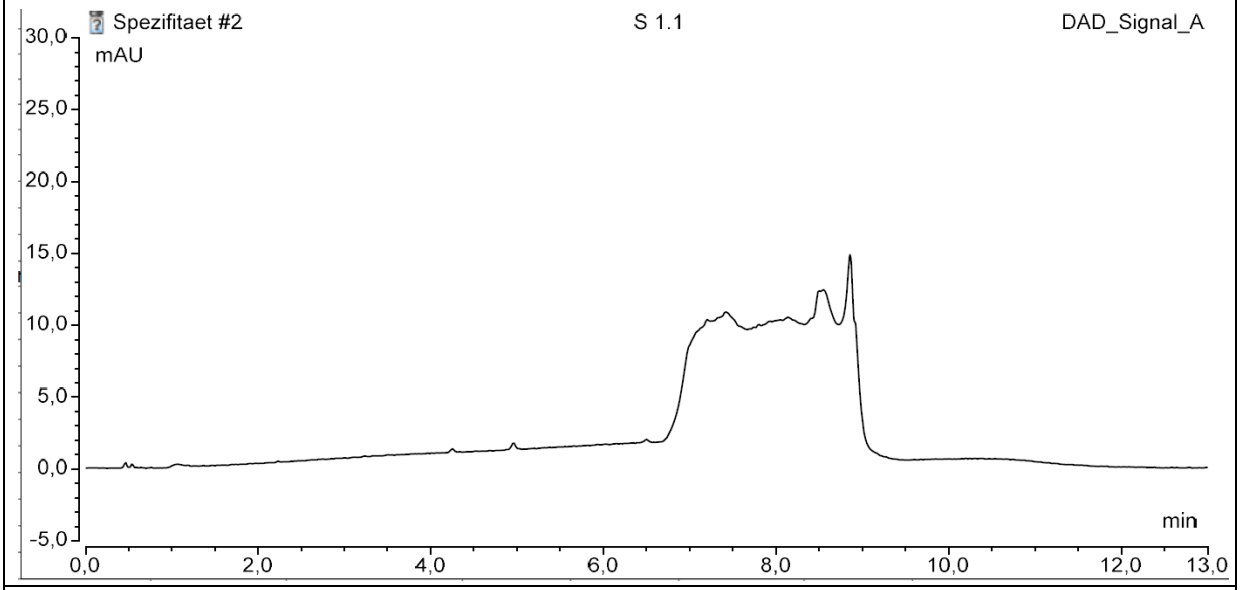


Figure 24: Chromatograms of sample solvent Lab 2

acetonitrile



acetonitrile/water

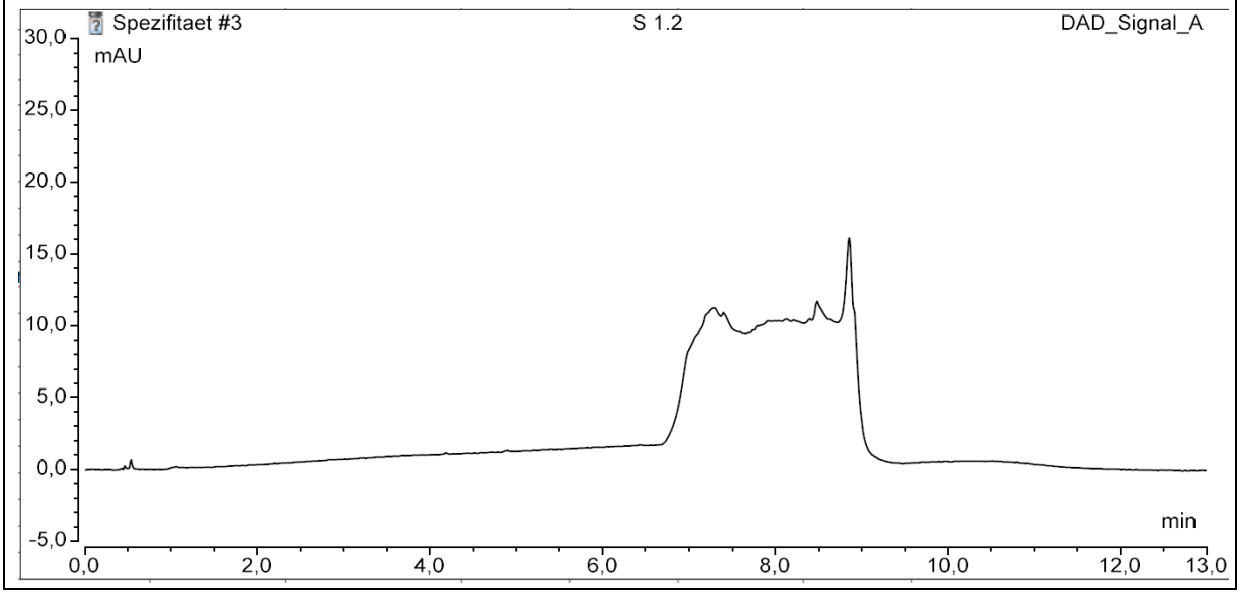
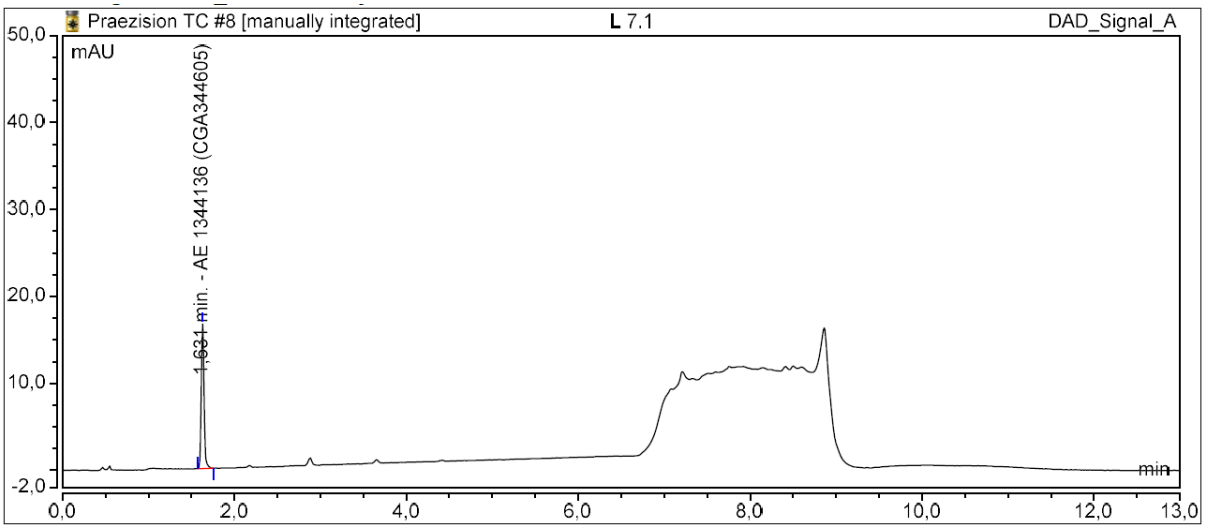


Figure 25: Chromatograms of reference standard CGA 344605 Lab 2

reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile/water)

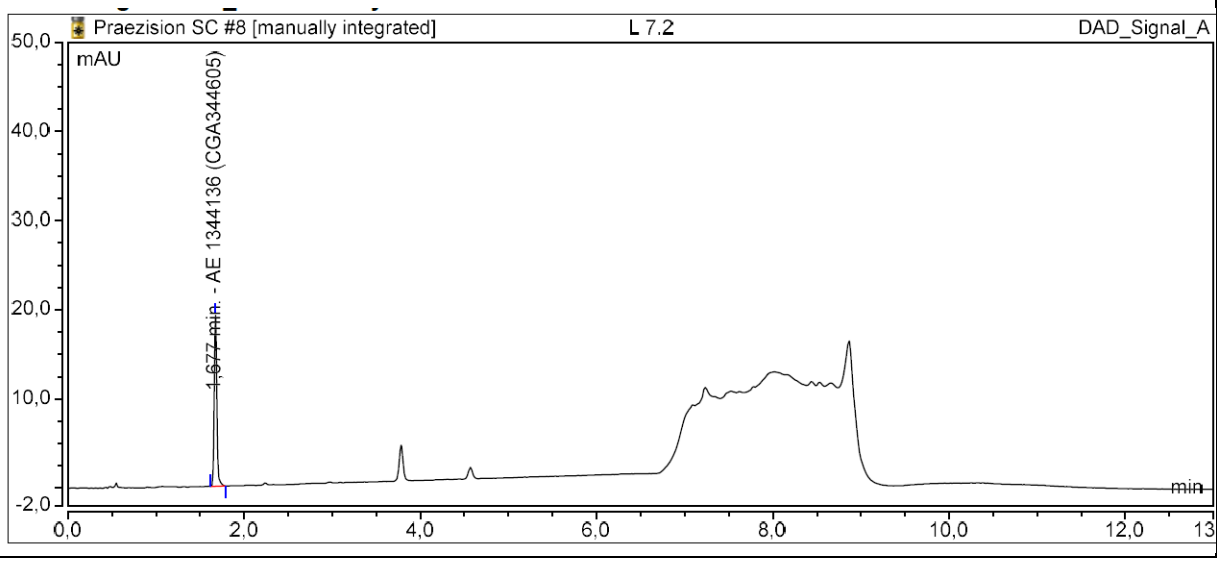
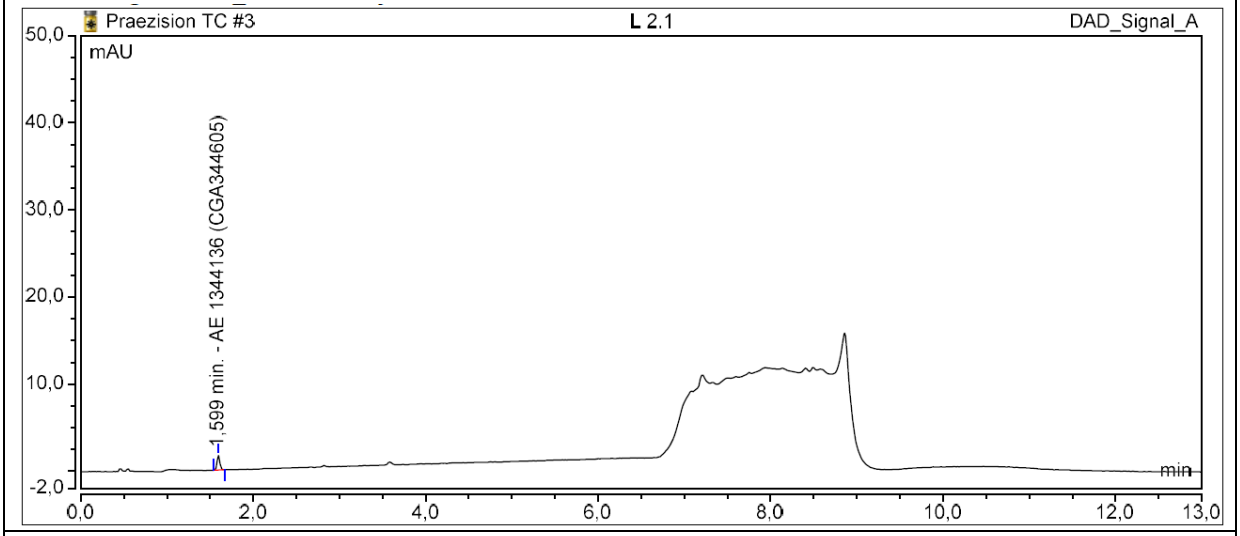


Figure 25. Continuation

reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile/water)

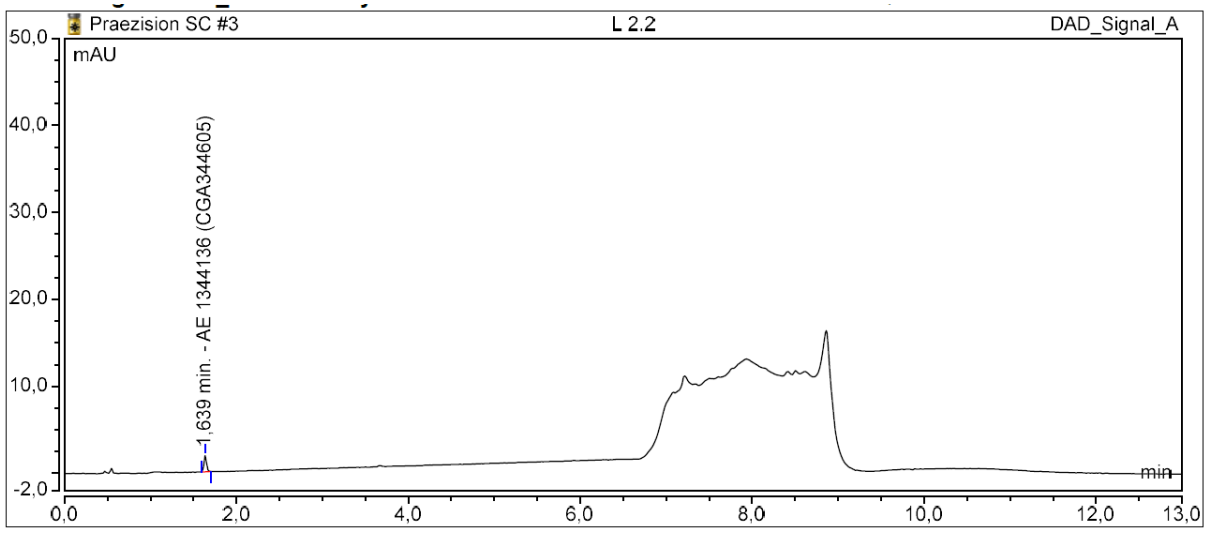


Figure 26: Chromatograms of trifloxystrobin TC Lab 2

Technical material trifloxystrobin TC spiked with reference standard CGA 344605

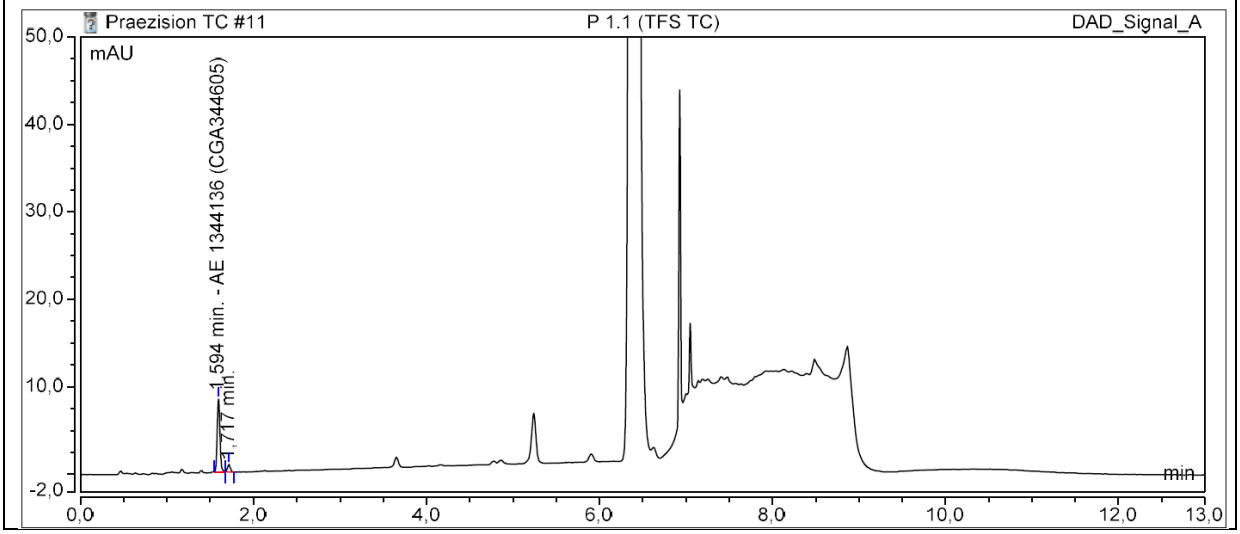
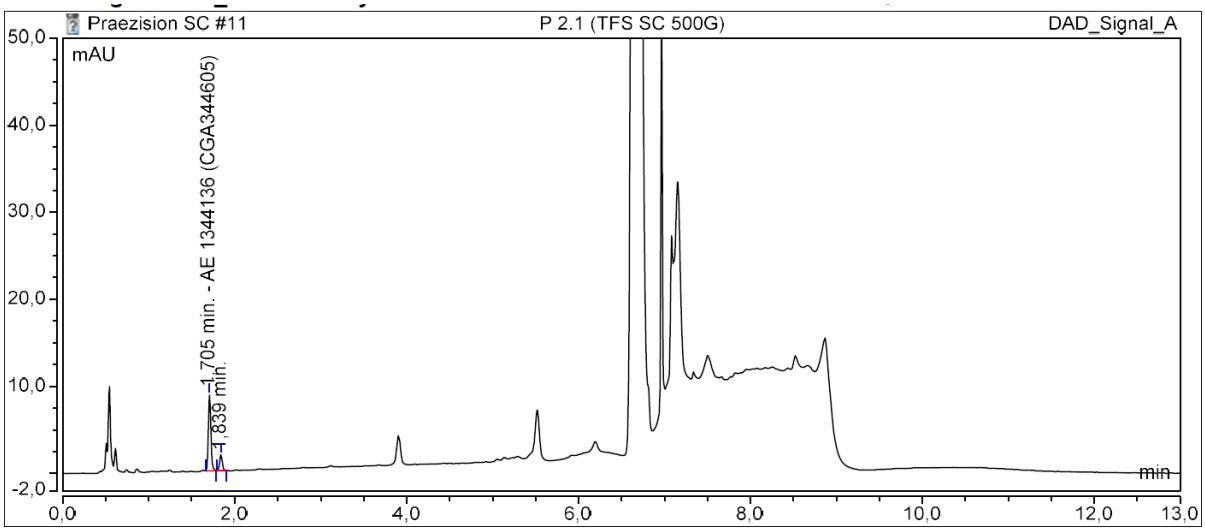


Figure 27: Chromatograms of trifloxystrobin SC 500 (500 g/L) Lab 2

Formulation trifloxystrobin SC 500 (500 g/L) spiked with reference standard CGA 344605



Blank formulation trifloxystrobin SC 0 (0 g/L)

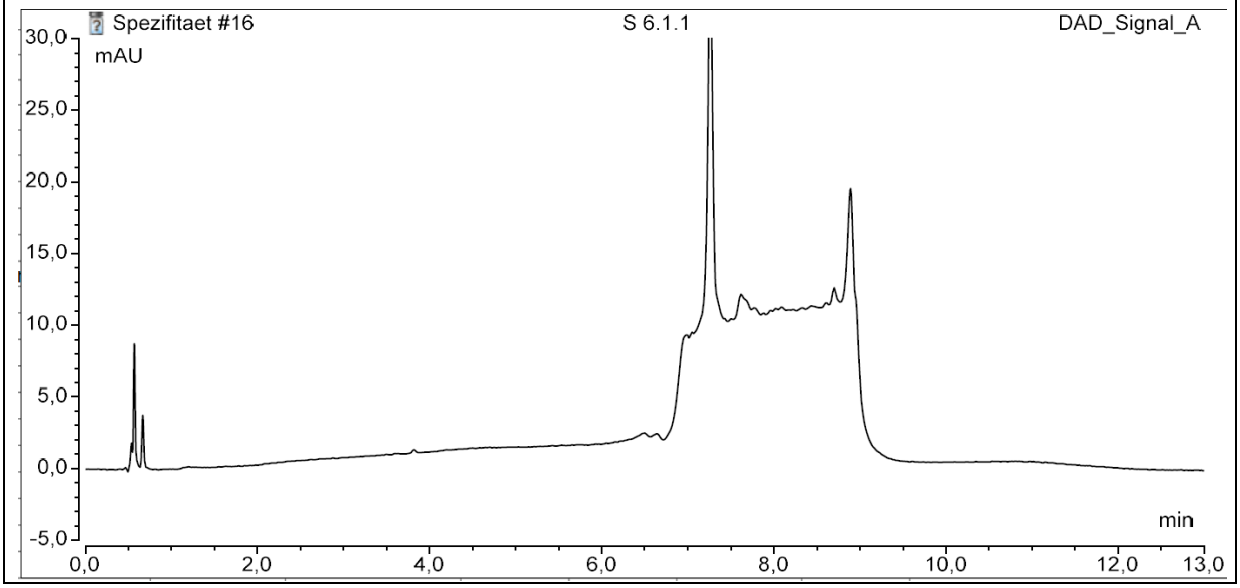


Figure 27. Continuation

Blank formulation trifloxystrobin SC 0 (0 g/L) – three-fold weight

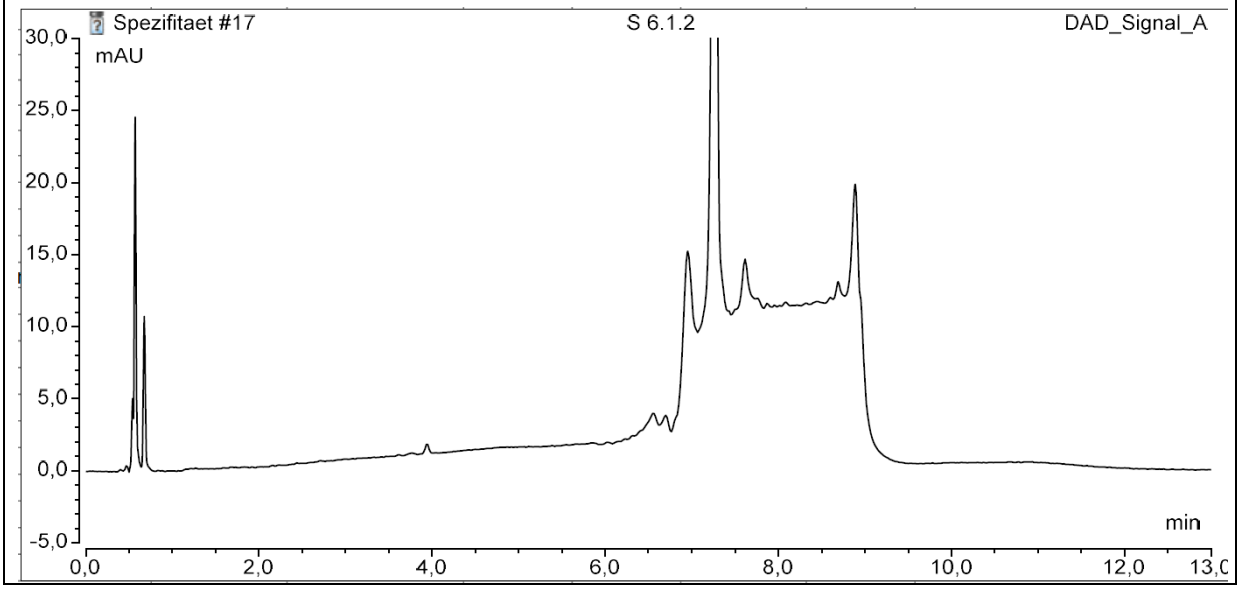
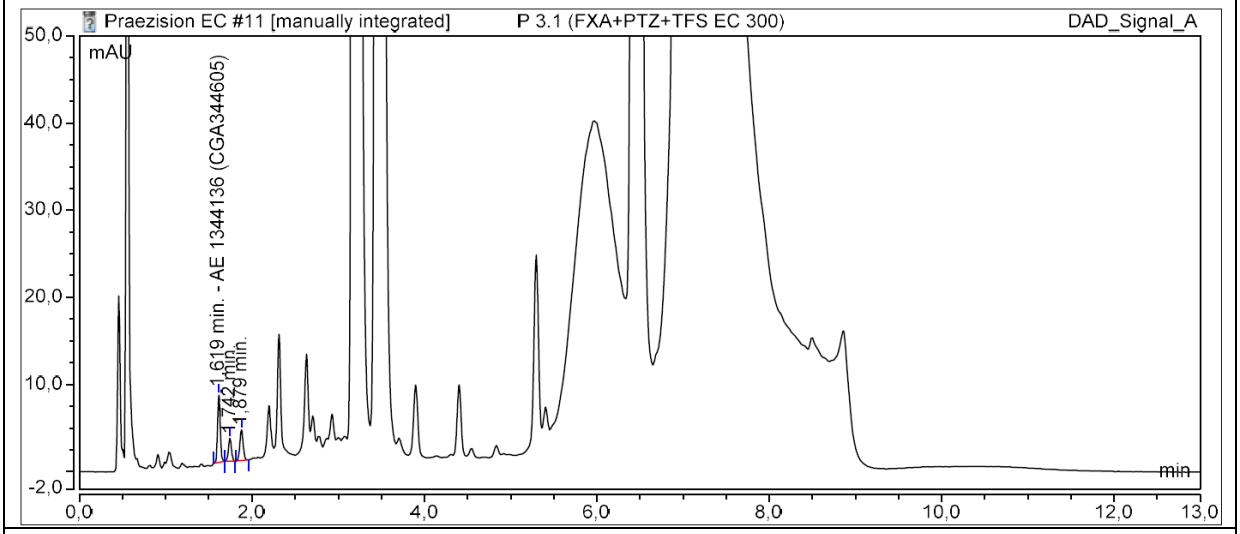


Figure 28: Chromatograms of fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) Lab 2

Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) spiked with reference standard CGA 344605



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L)

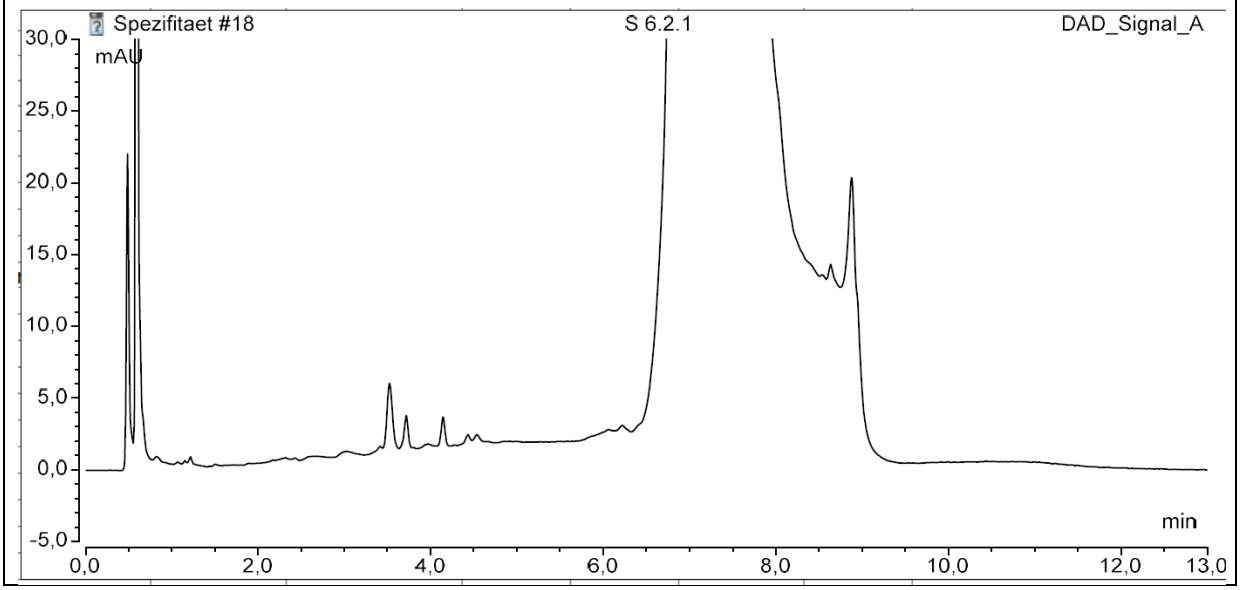


Figure 28. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) three-fold weigth

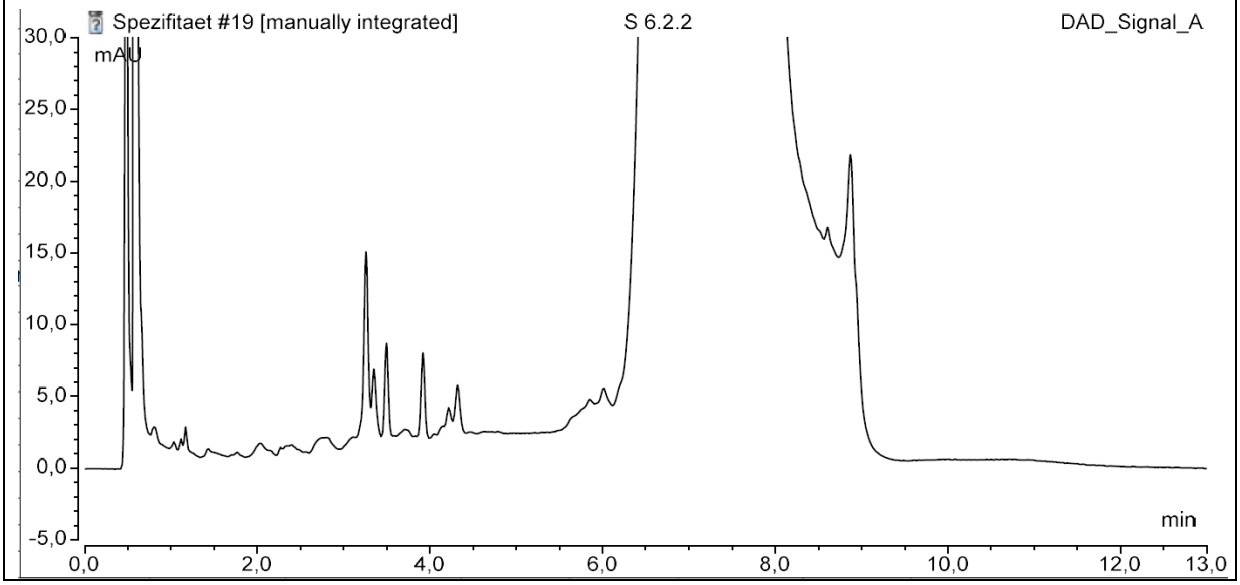
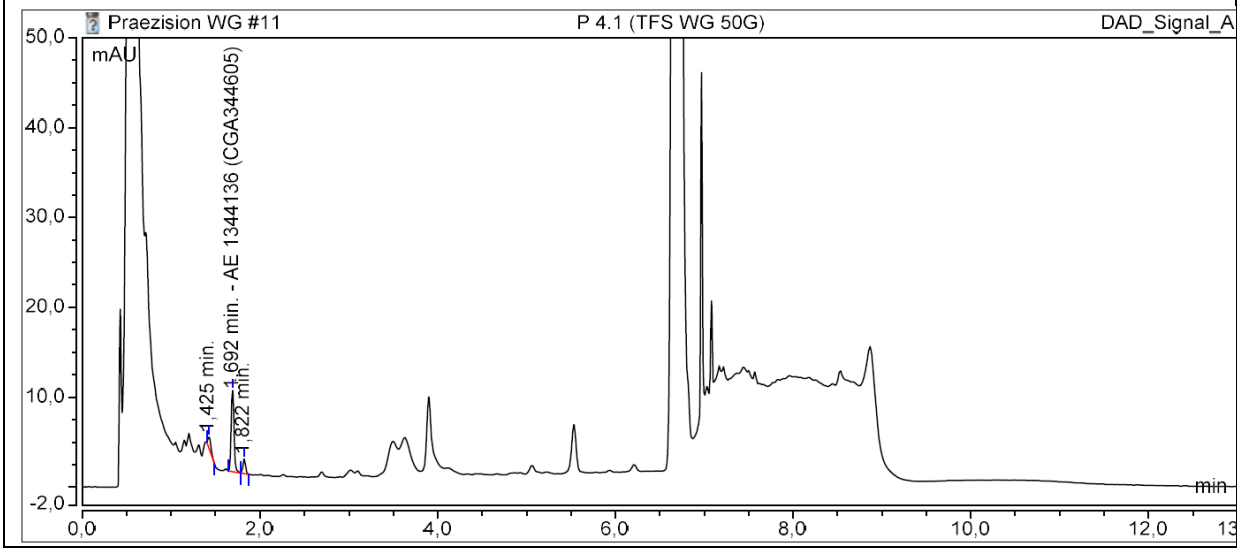


Figure 29: Chromatogram of trifloxystrobin WG 50 (50 g/kg) Lab 2

Formulation trifloxystrobin WG 50 (50 g/kg) spiked with reference standard CGA 344605



Blank Formulation trifloxystrobin WG 0 (0 g/Kg)

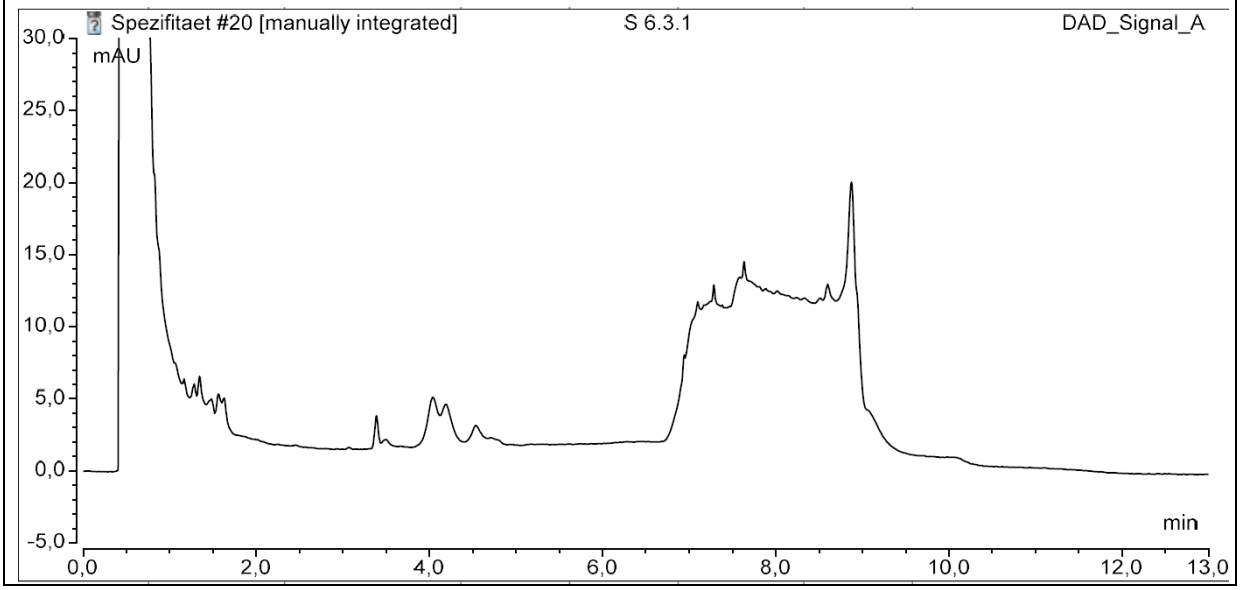


Figure 29. Continuation

Blank Formulation trifloxystrobin WG 0 (0 g/Kg) - three-fold weight

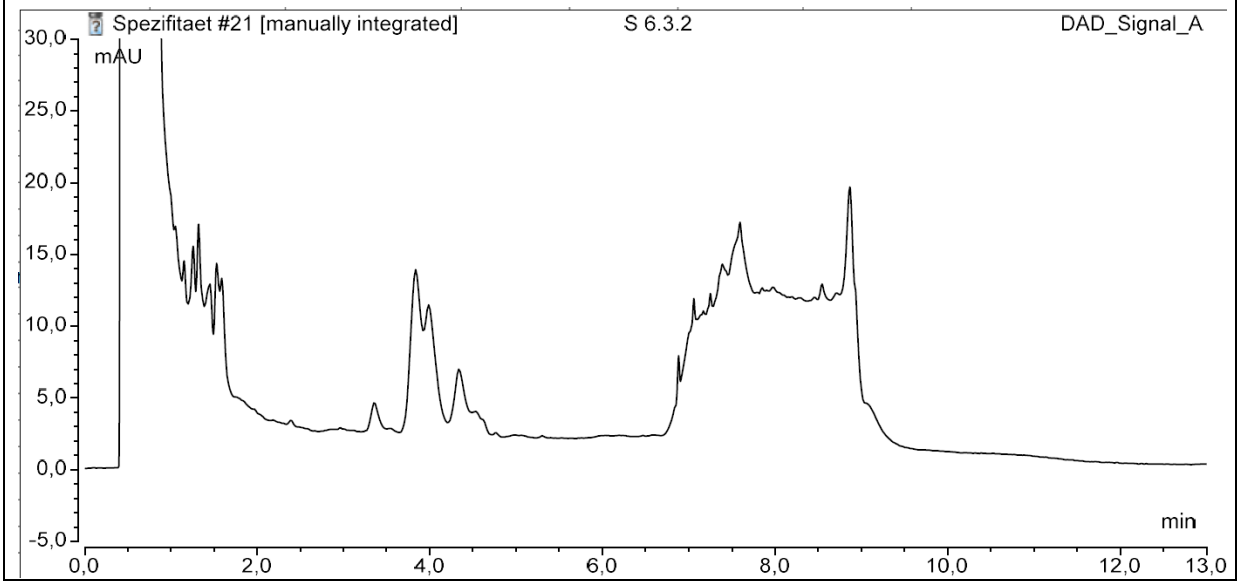
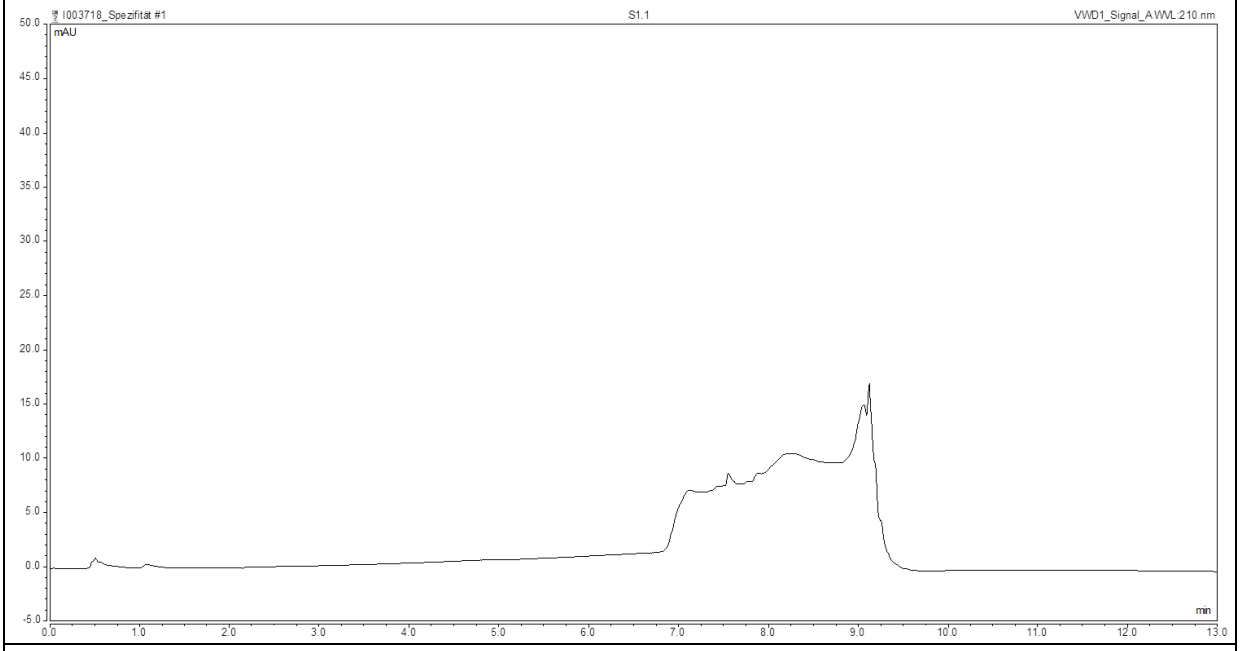


Figure 30: Chromatograms of sample solvent Lab 3

acetonitrile



acetonitrile/water

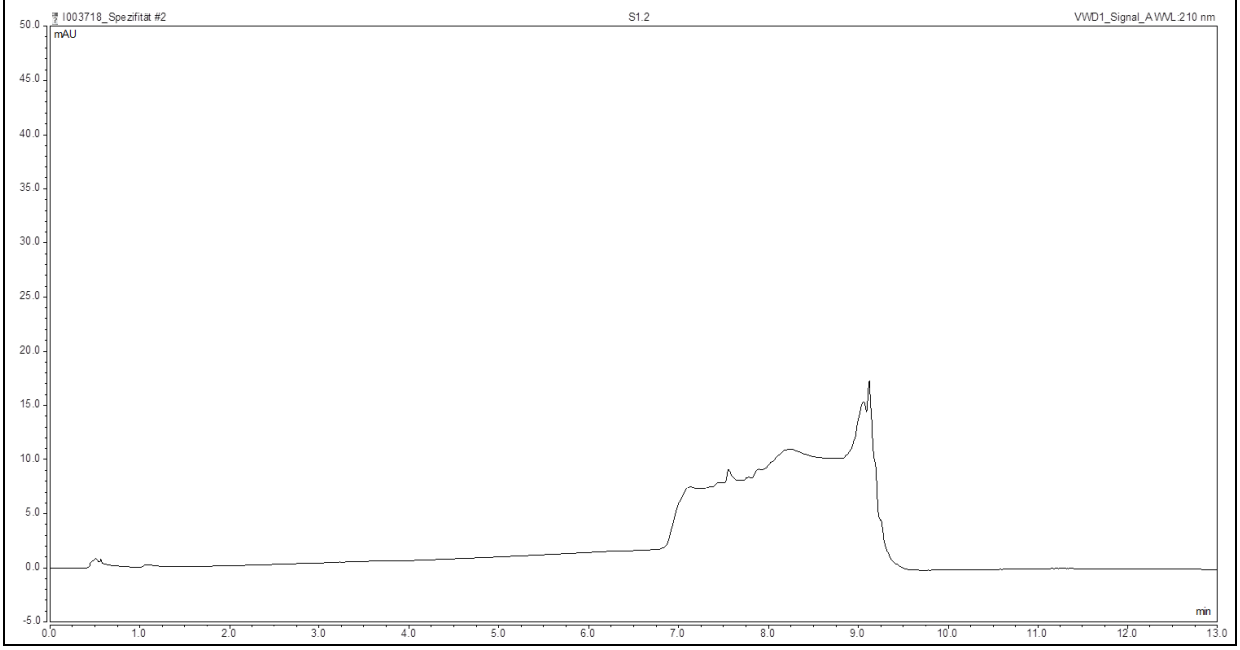
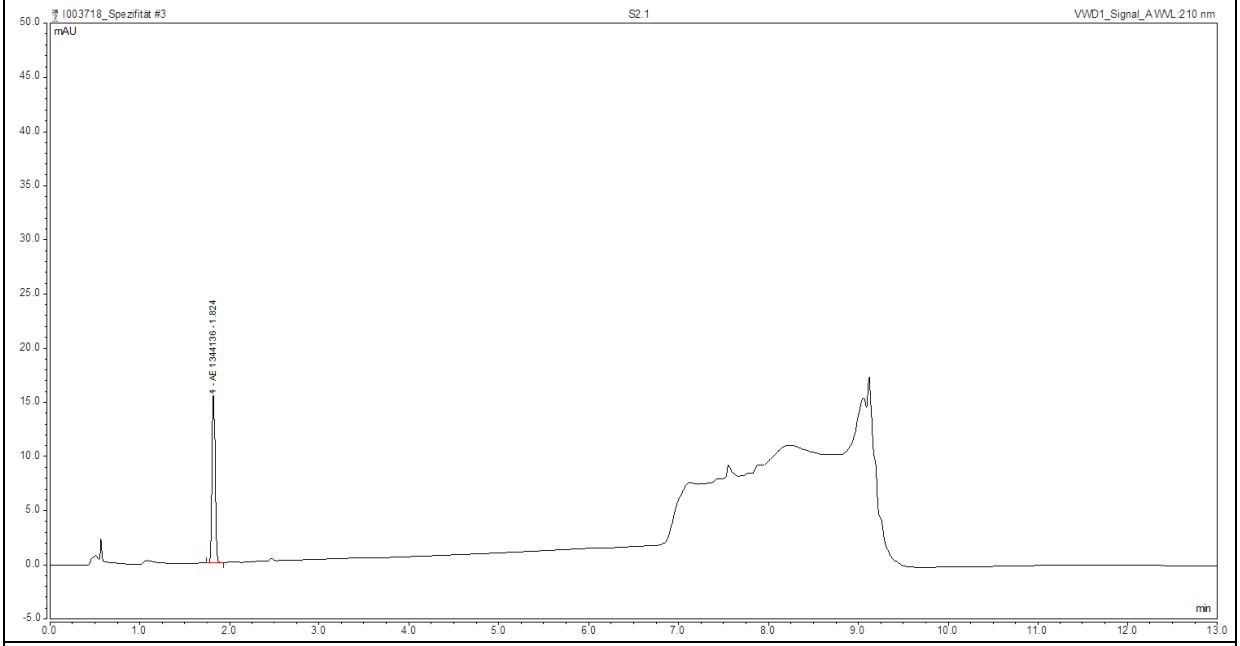


Figure 31: Chromatograms of reference standard CGA 344605 Lab 3

reference standard CGA 344605 (nominal 0.20 mg/50 mL acetonitrile)



reference standard CGA 344605 (nominal 0.20 mg/50 mL acetonitrile/water)

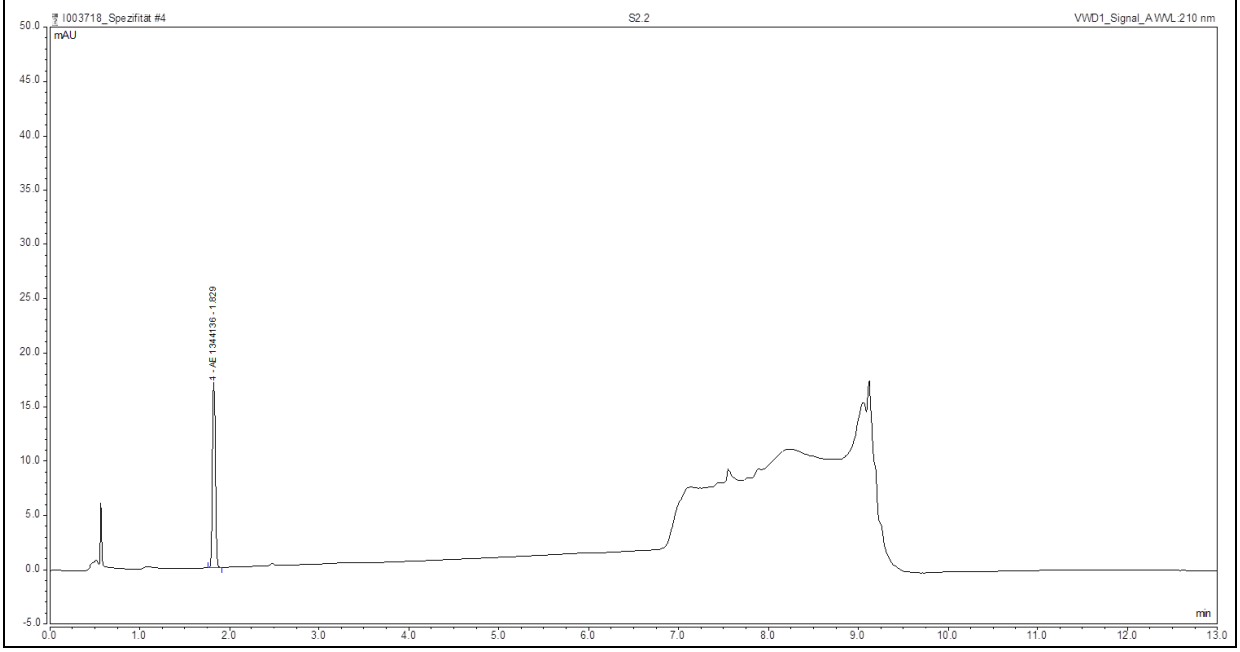
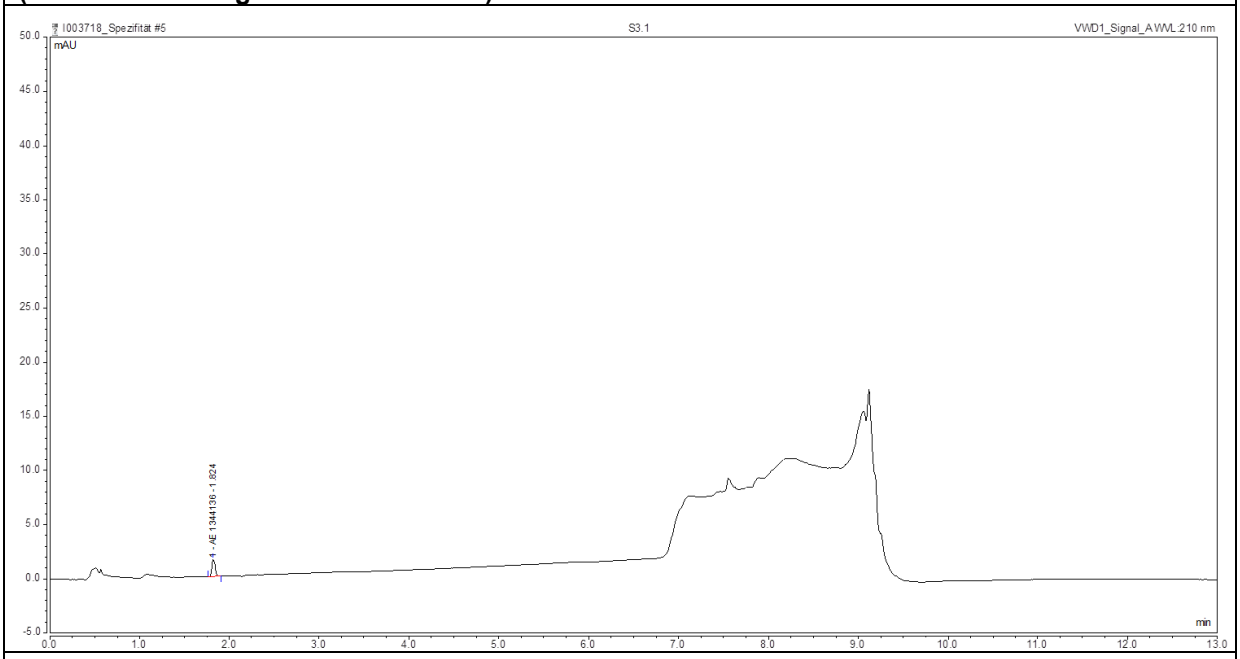


Figure 31. Continuation

reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile/water)

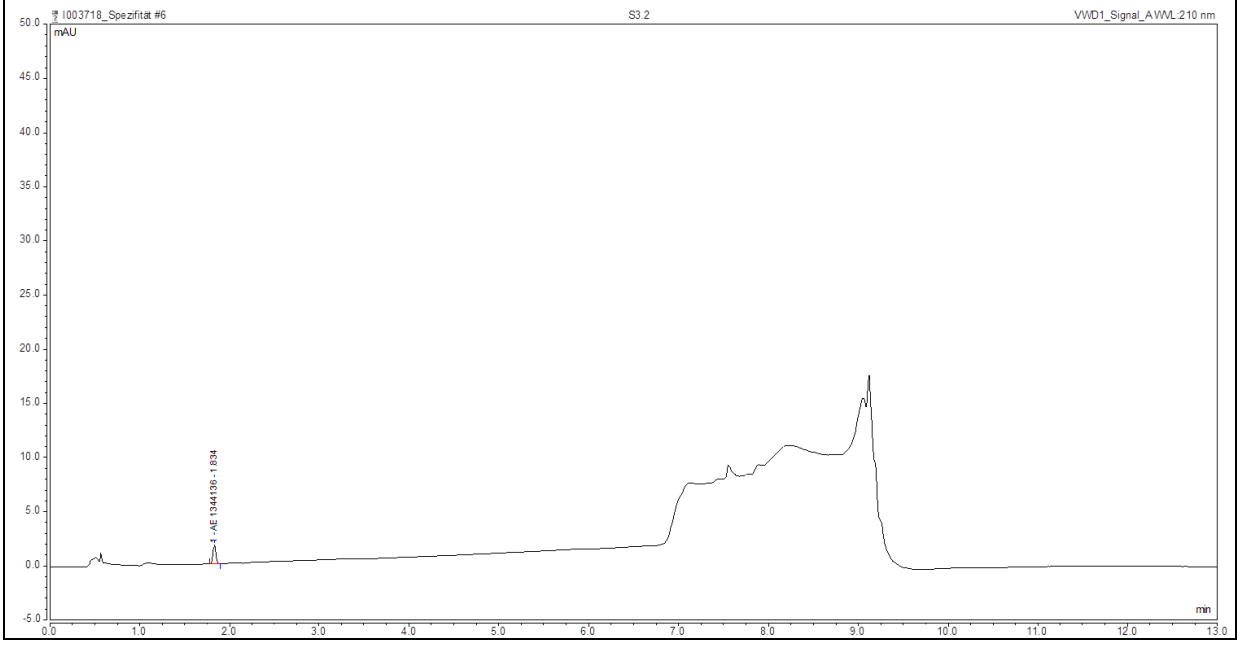
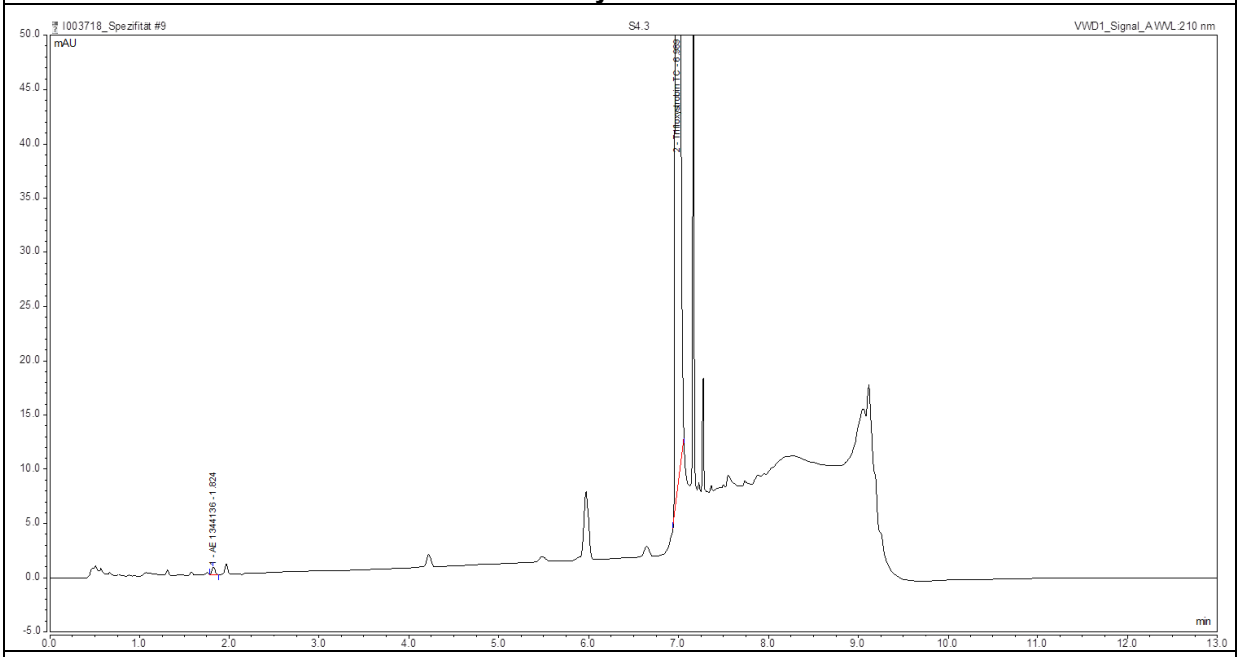


Figure 32: Chromatograms of active substance trifloxystrobin Lab 3

reference standard of active substance trifloxystrobin in acetonitrile



reference standard of active substance trifloxystrobin in acetonitrile/water

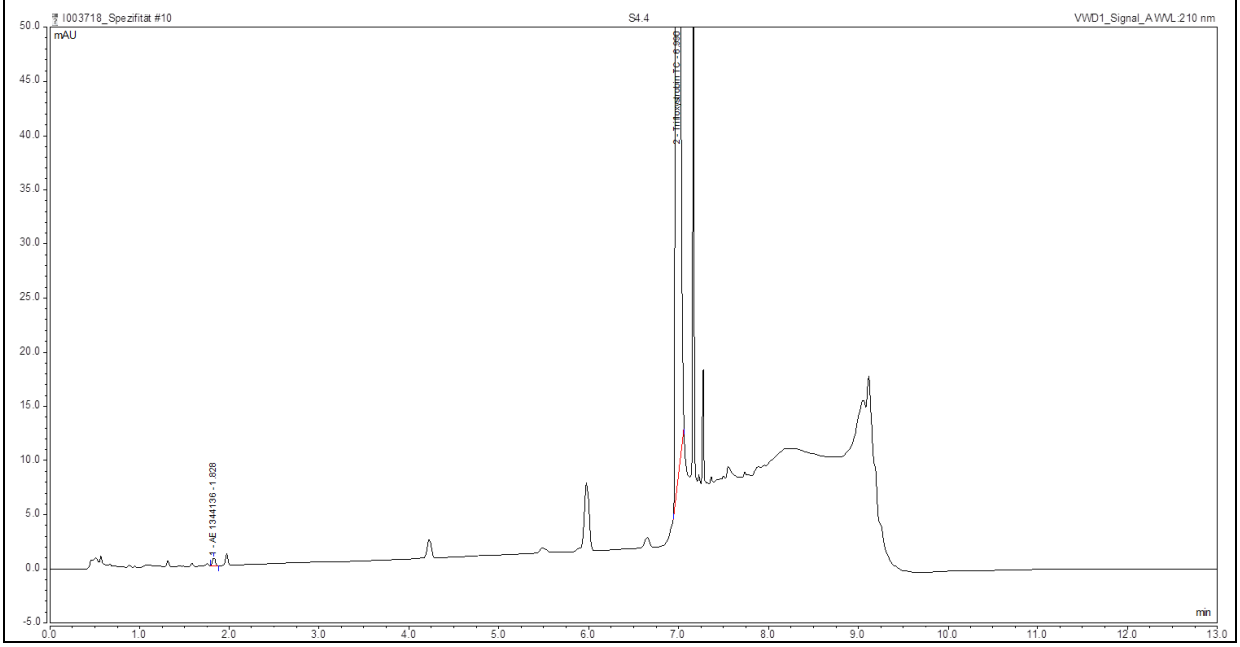


Figure 33: Chromatograms of active substance fluoxastrobin. Lab 3

reference standard of active substance fluoxastrobin

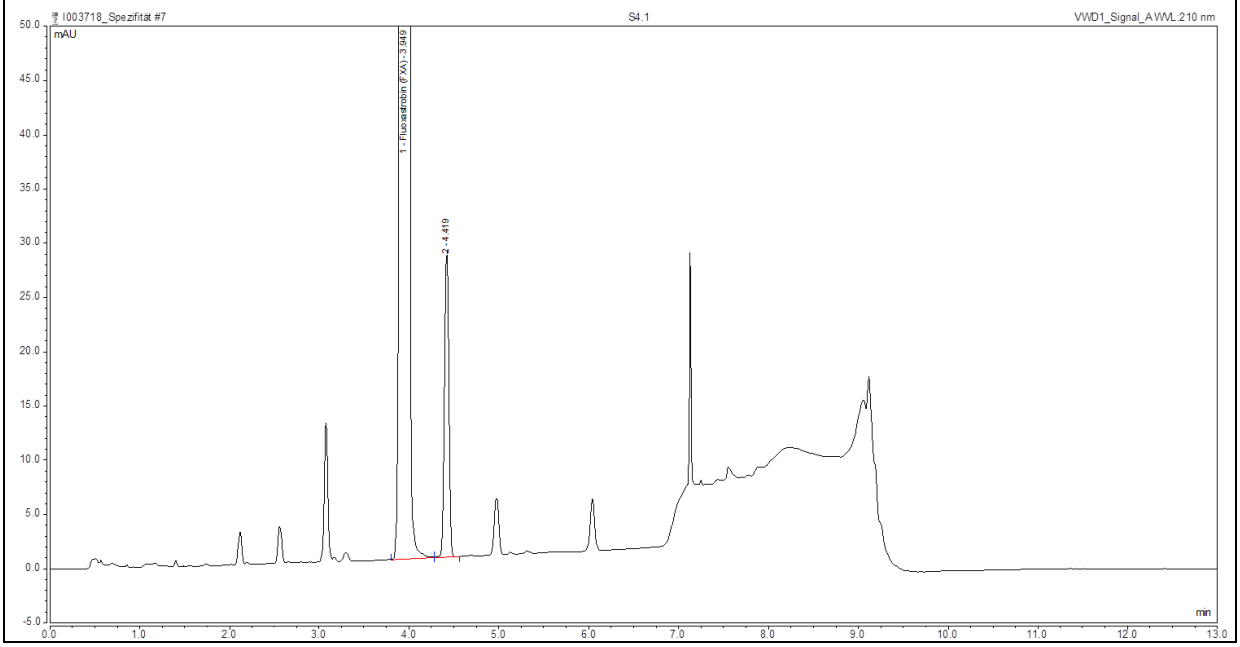


Figure 34: Chromatograms of active substance prothioconazole Lab 3

reference standard of active substance prothioconazole

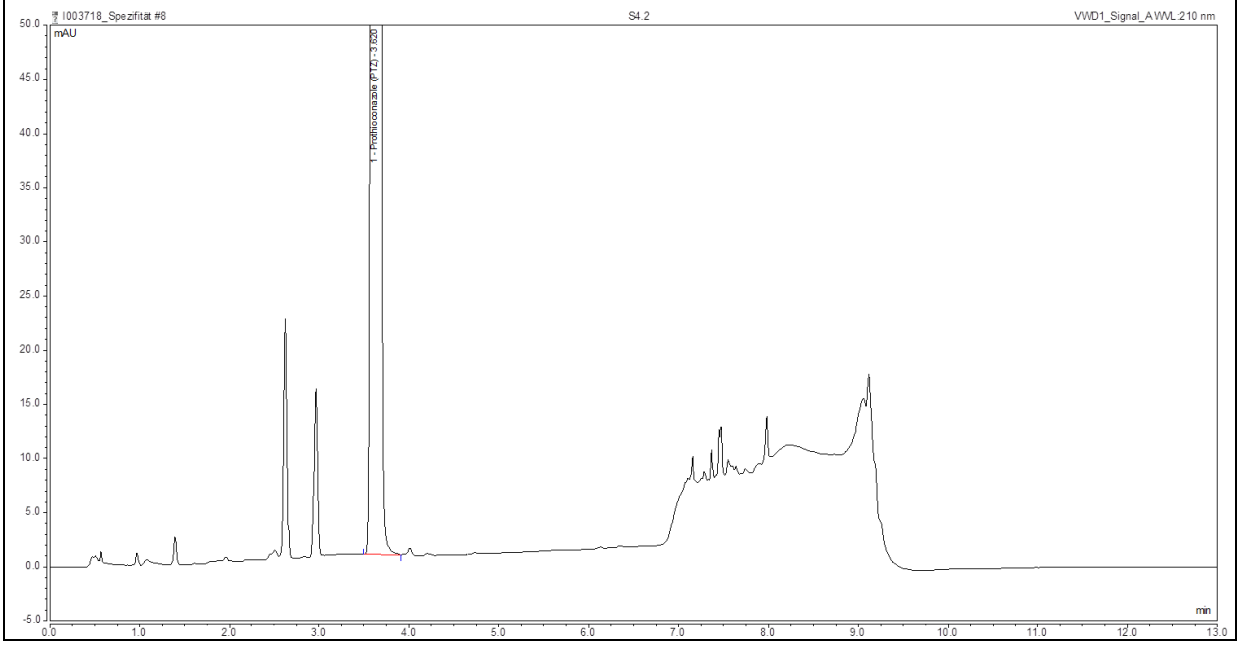


Figure 35: Chromatograms of trifloxystrobin TC Lab 3

Technical material trifloxystrobin TC spiked with reference standard CGA 344605

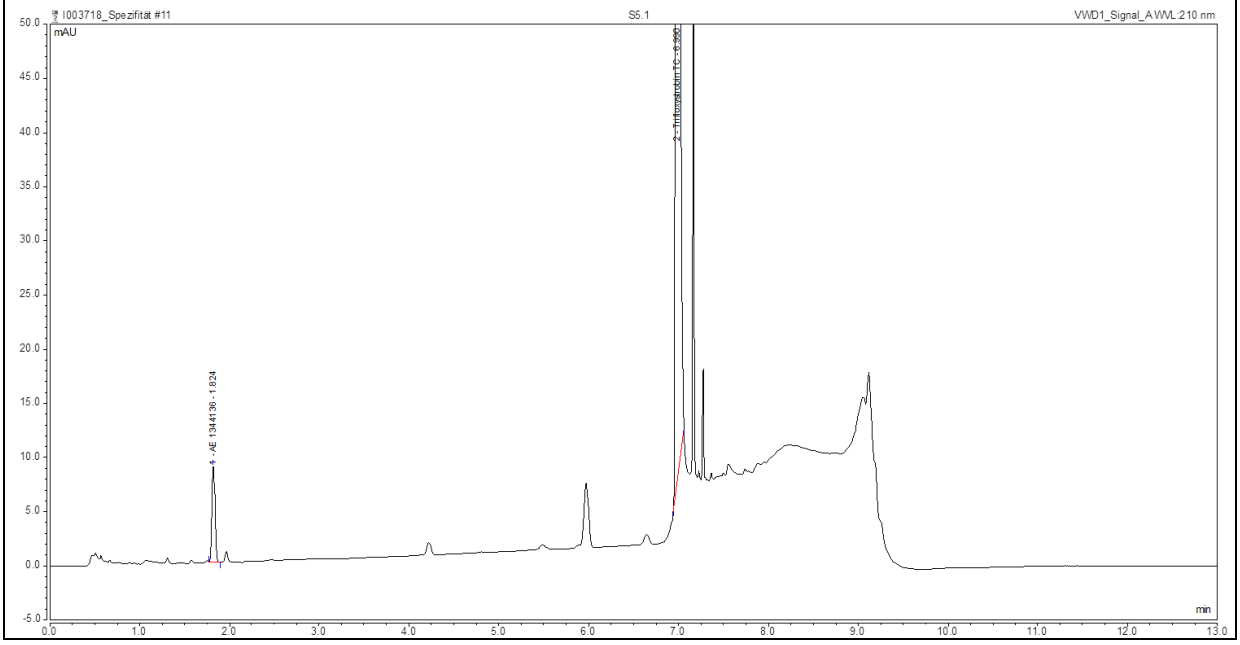
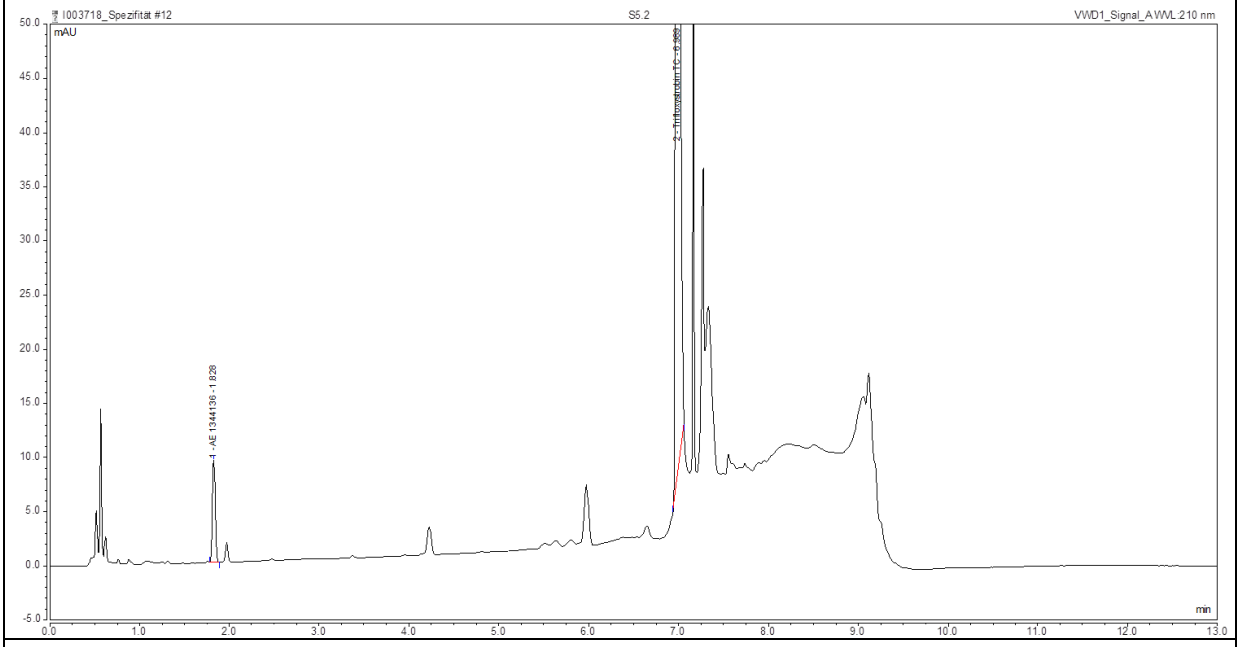


Figure 36: Chromatograms of trifloxystrobin SC 500 (500 g/L) Lab 3

Formulation trifloxystrobin SC 500 (500 g/L) spiked with reference standard CGA 344605



Blank formulation trifloxystrobin SC 0 (0 g/L)

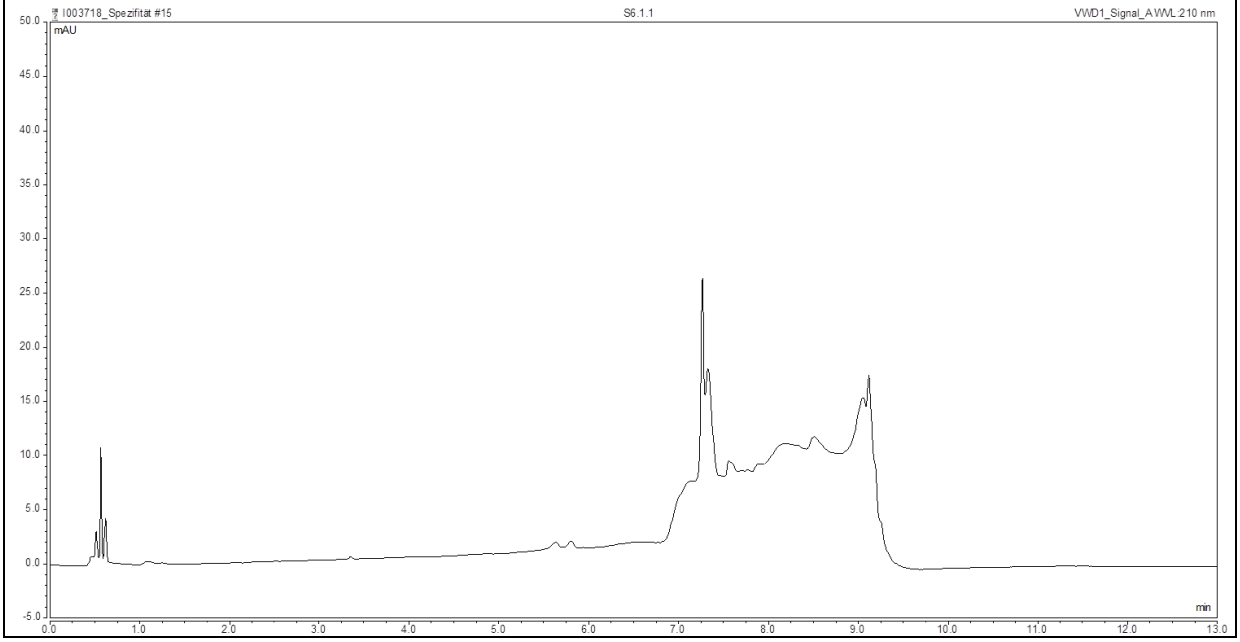


Figure 36. Continuation

Blank formulation trifloxystrobin SC 0 (0 g/L) – three-fold weight

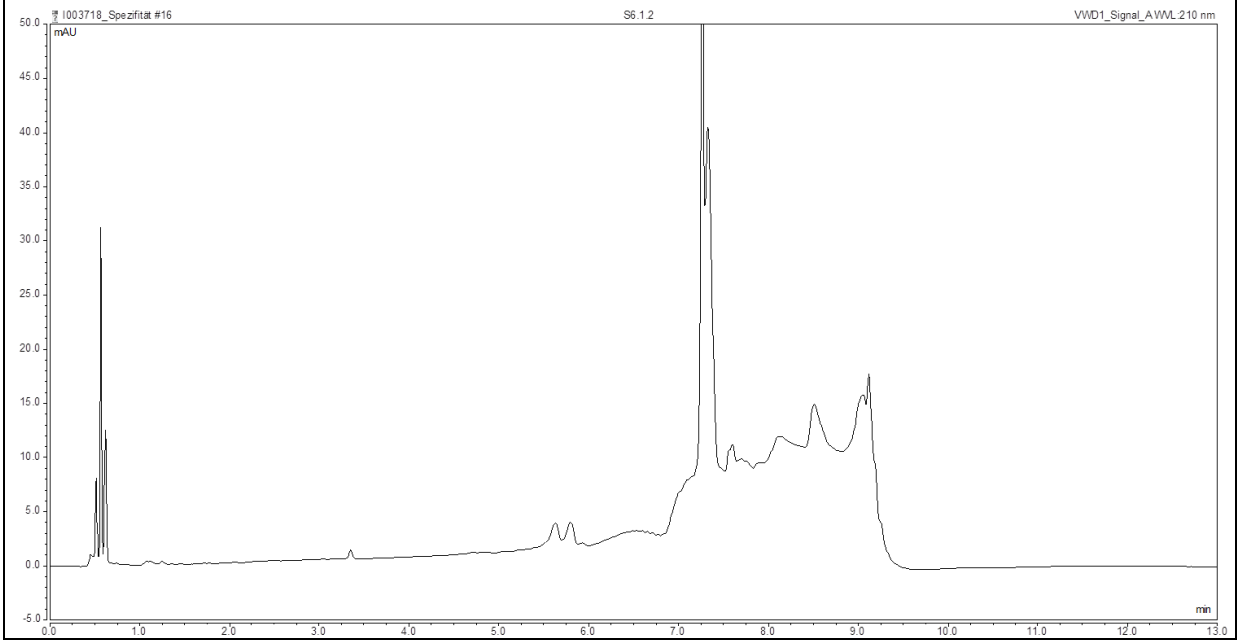
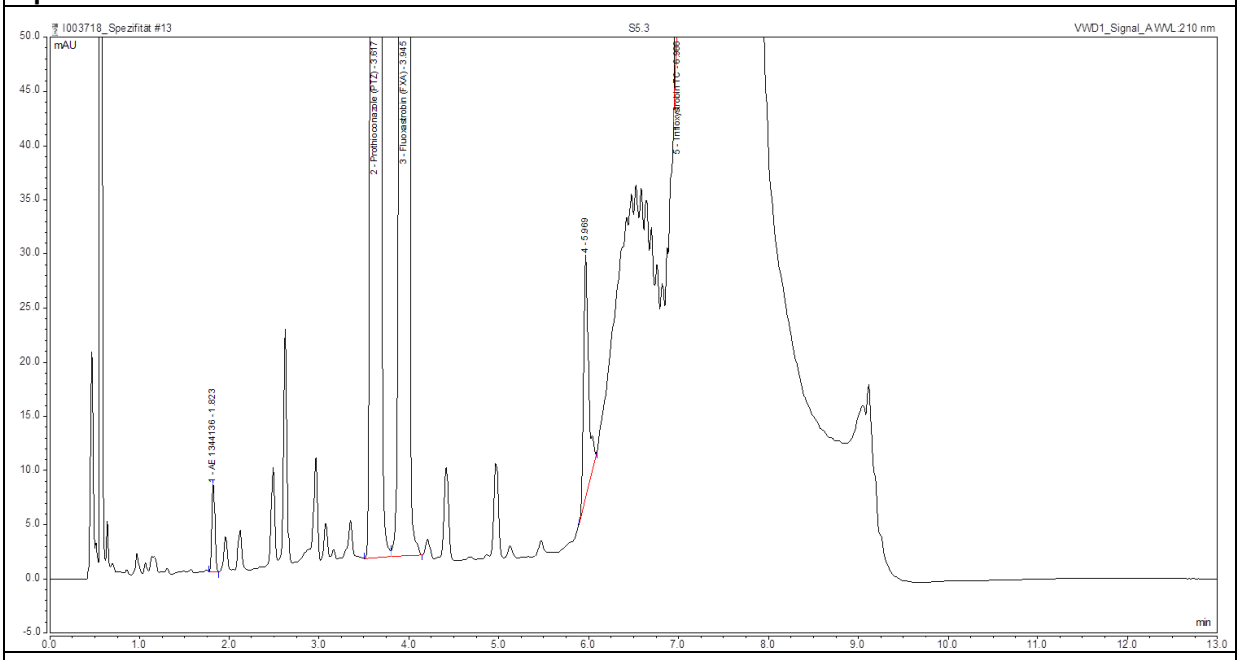


Figure 37: Chromatograms of fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) Lab 3

Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) spiked with reference standard CGA 344605



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L)

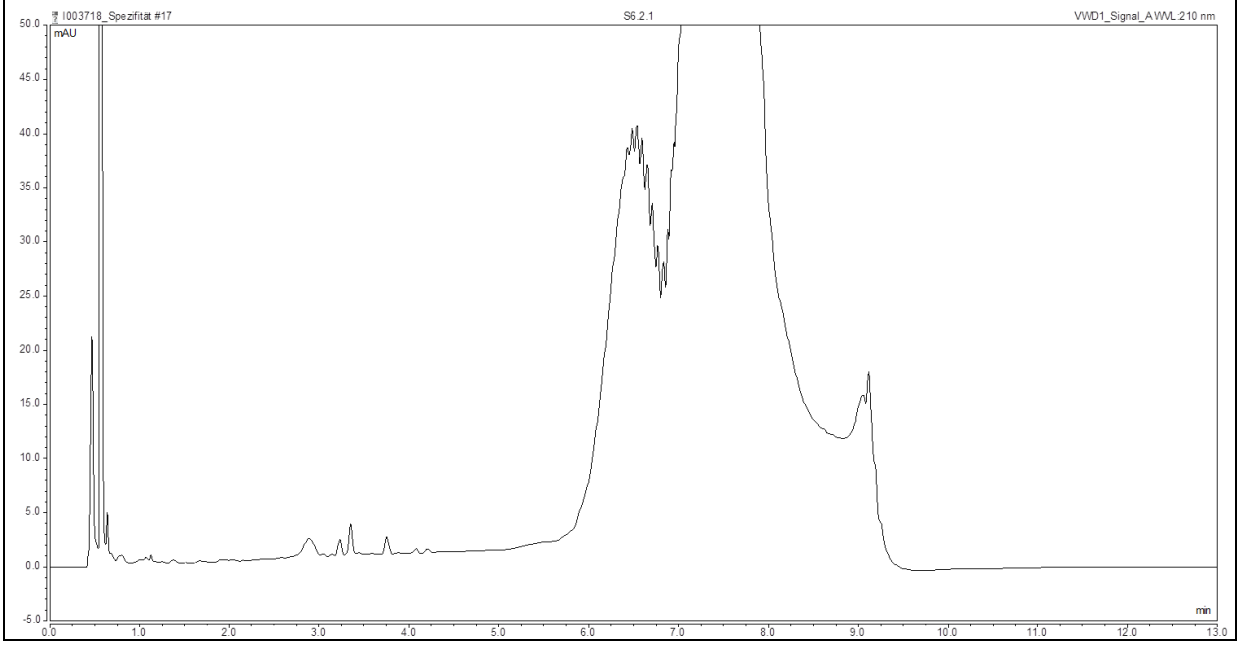


Figure 37. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) three-fold weigth

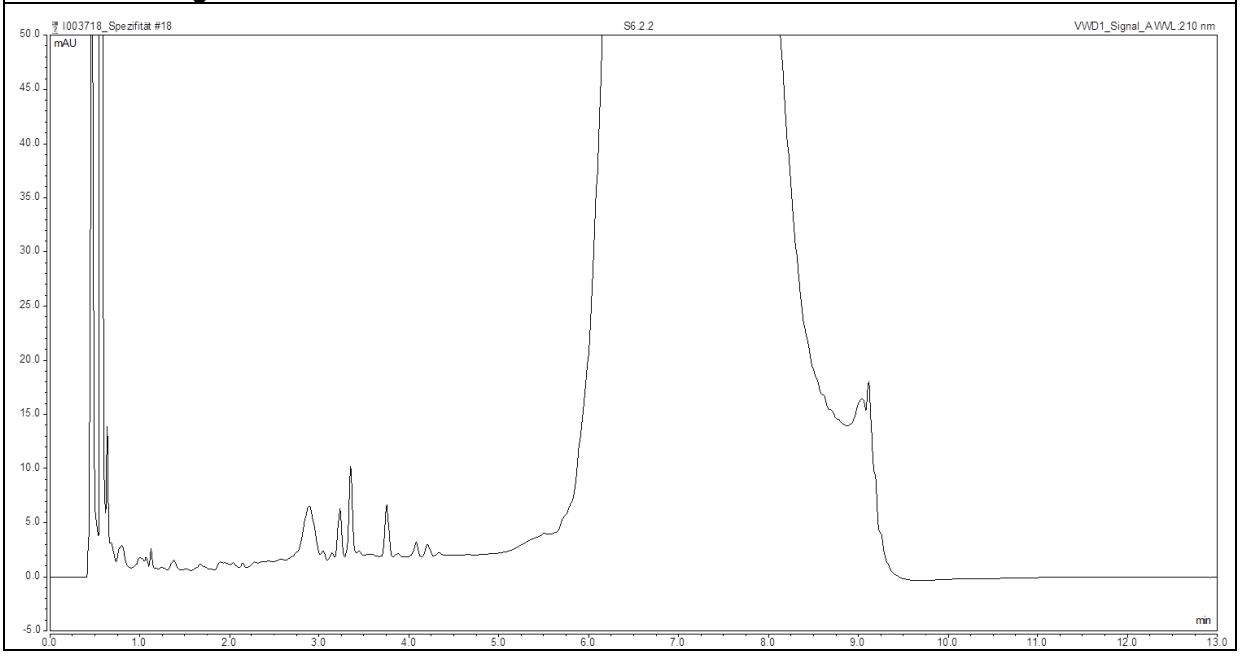
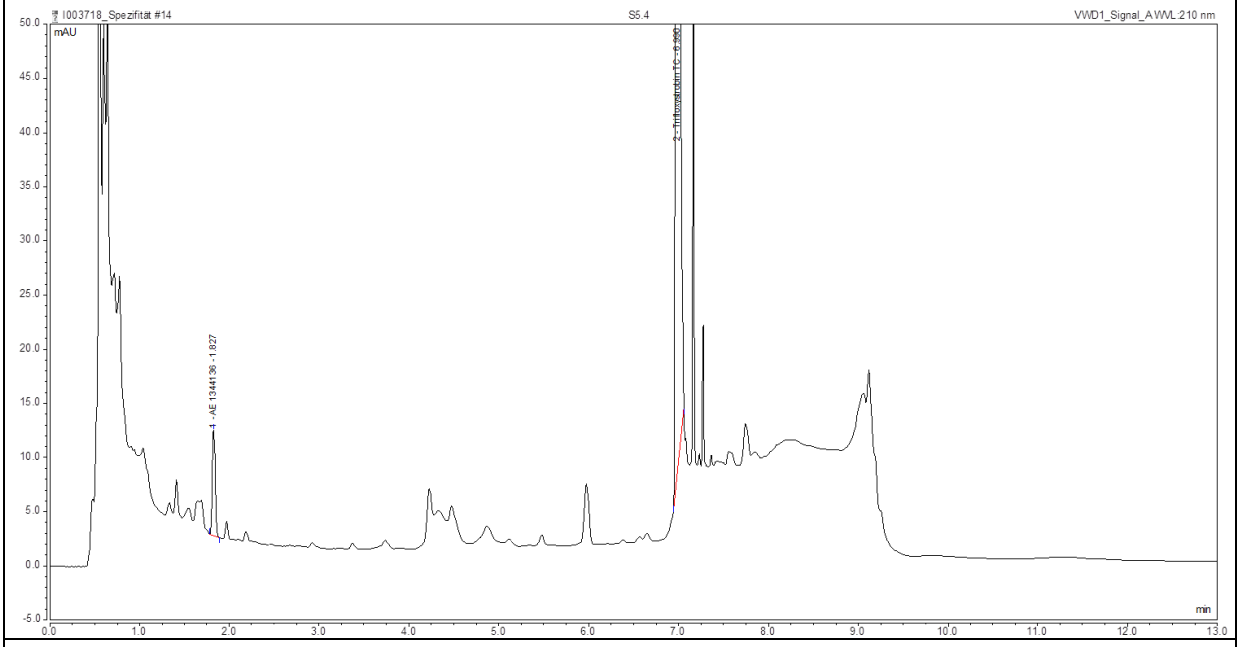


Figure 38: Chromatogram of trifloxystrobin WG 50 (50 g/kg) Lab 3

Formulation trifloxystrobin WG 50 (50 g/kg) spiked with reference standard CGA 344605



Blank Formulation trifloxystrobin WG 0 (0 g/Kg)

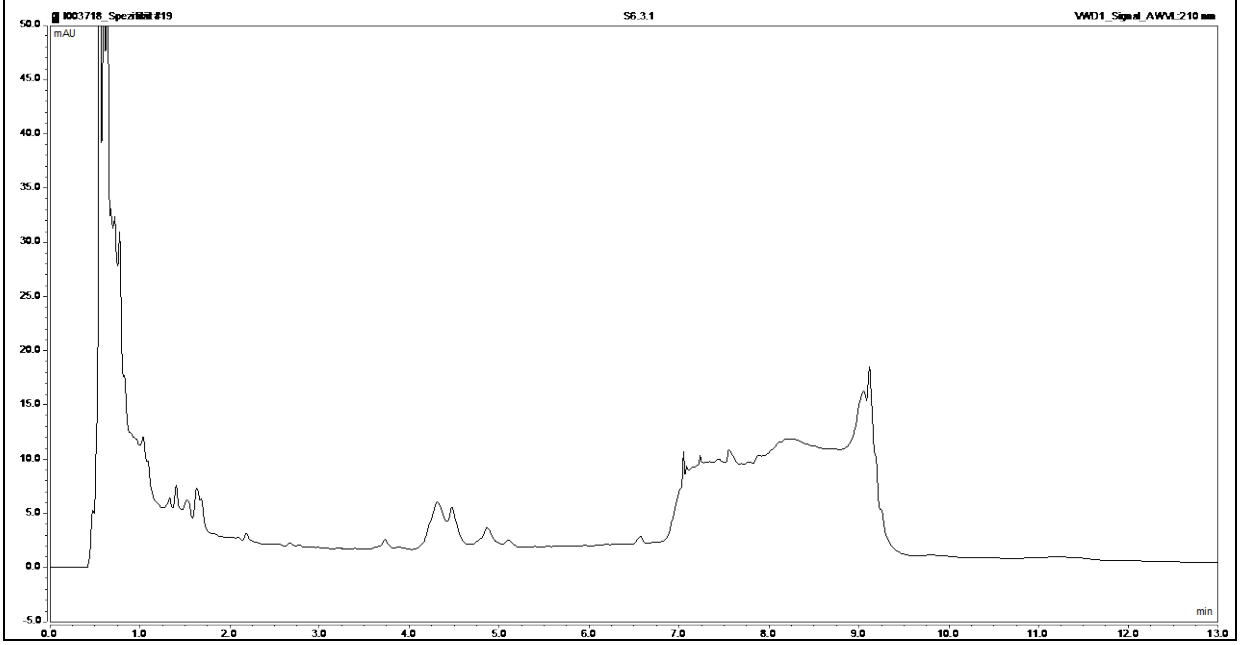


Figure 38. Continuation

Blank Formulation trifloxystrobin WG 0 (0 g/Kg) - three-fold weight

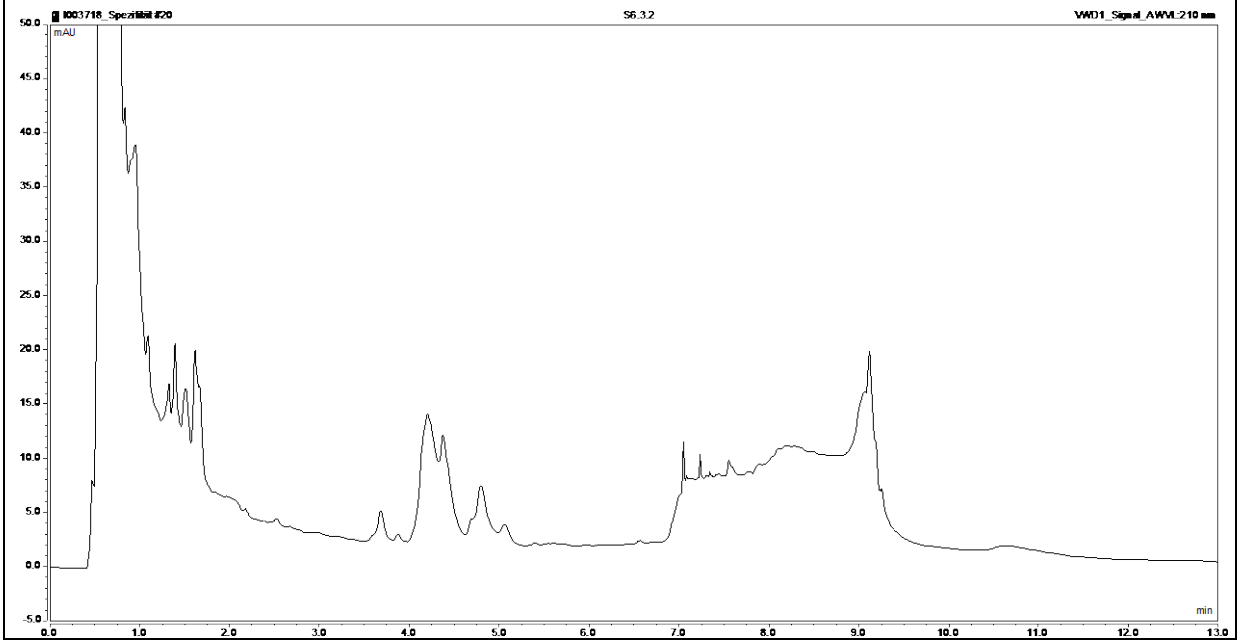
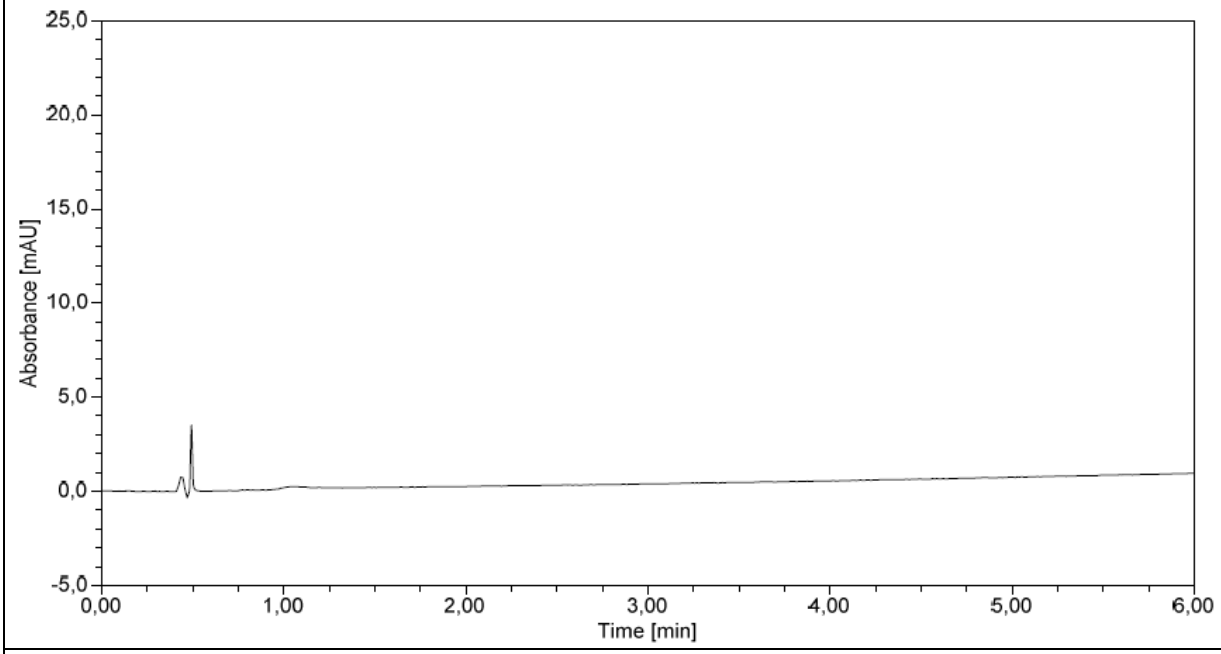


Figure 39: Chromatograms of sample solvent Lab 4

acetonitrile



acetonitrile/water

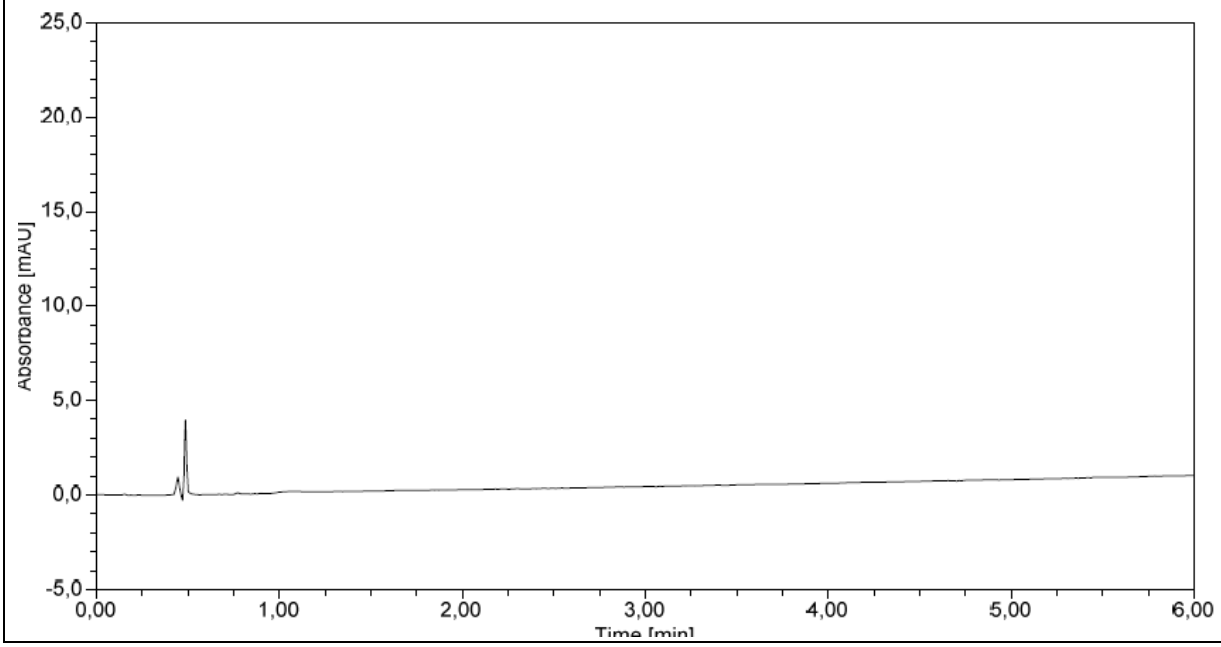
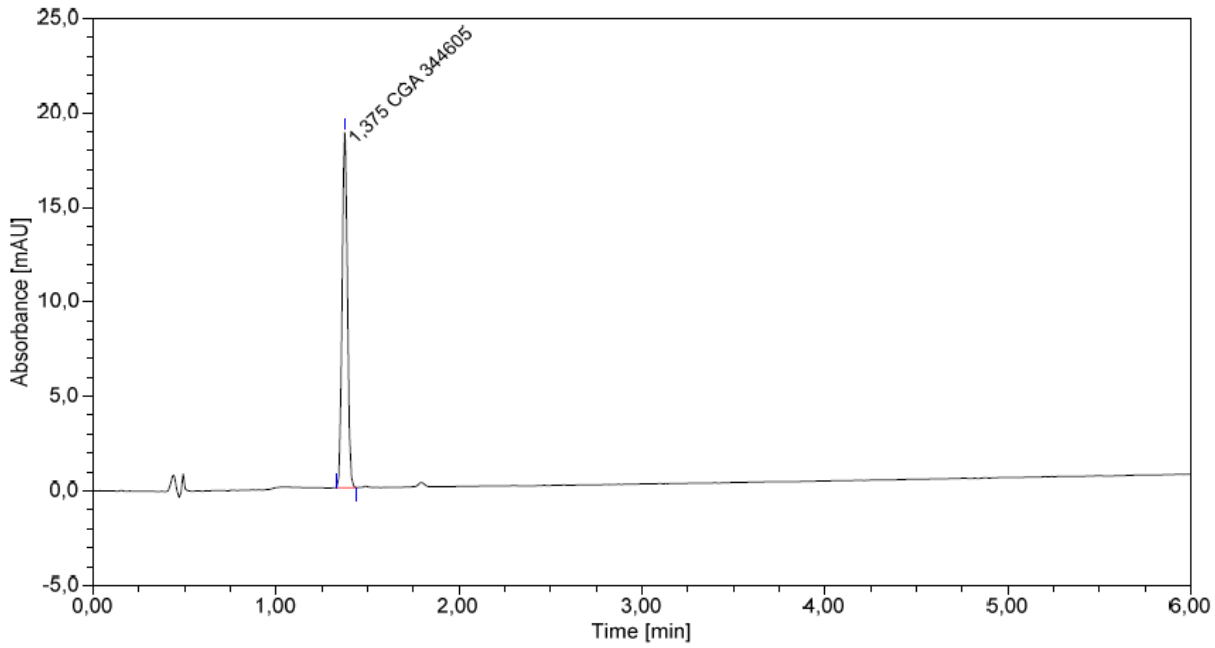


Figure 40: Chromatograms of reference standard CGA 344605 Lab 4

reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.20 mg/50 mL acetonitrile/water)

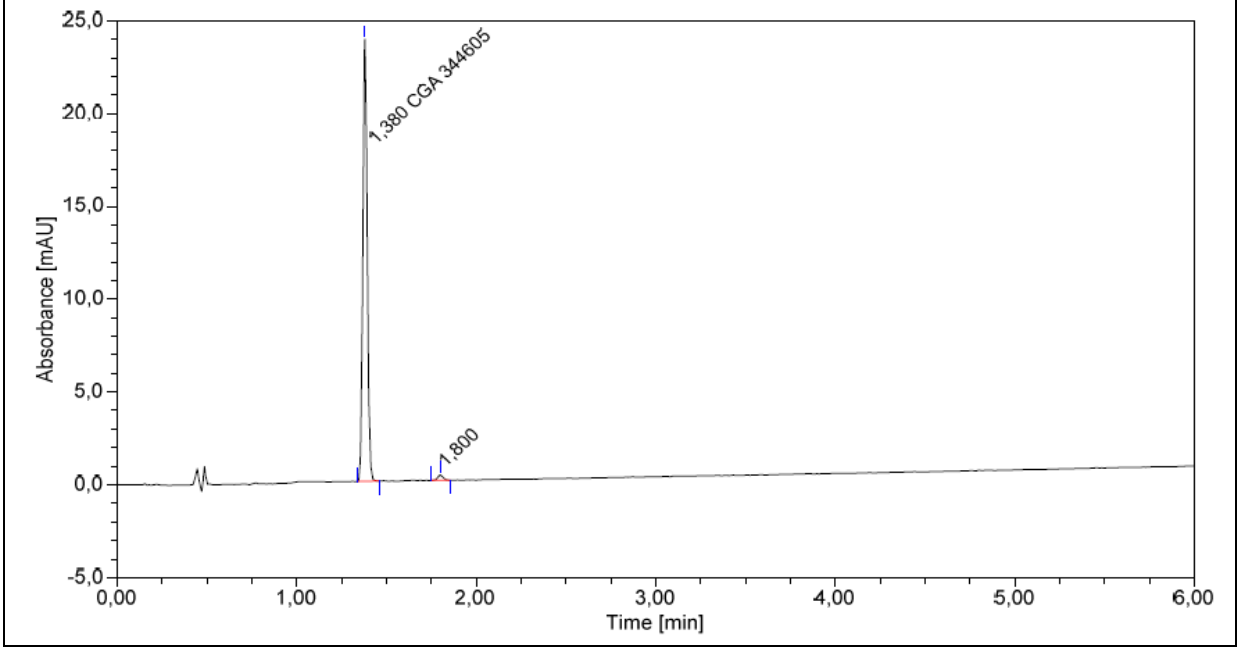
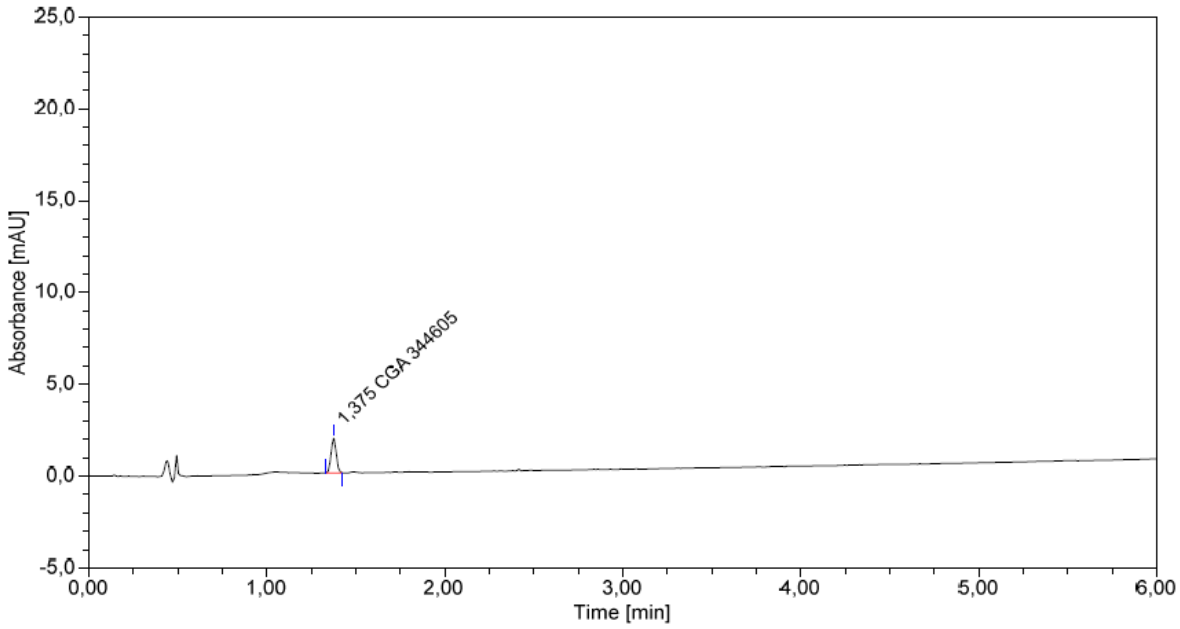


Figure 40. Continuation

reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile)



reference standard CGA 344605
(nominal 0.020 mg/50 mL acetonitrile/water)

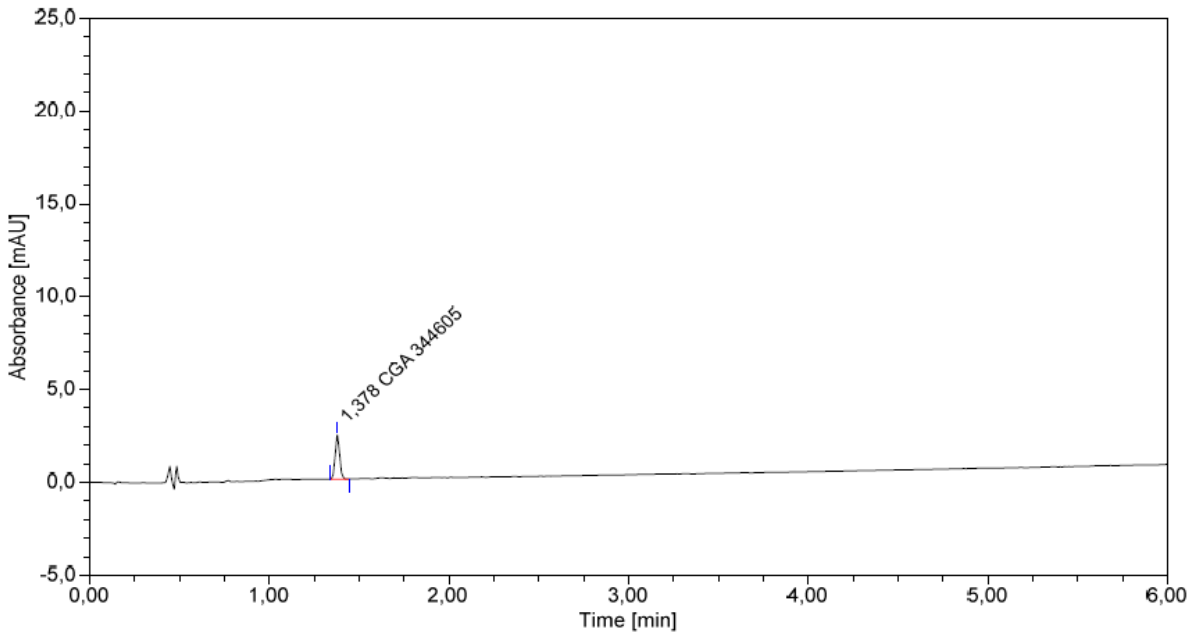
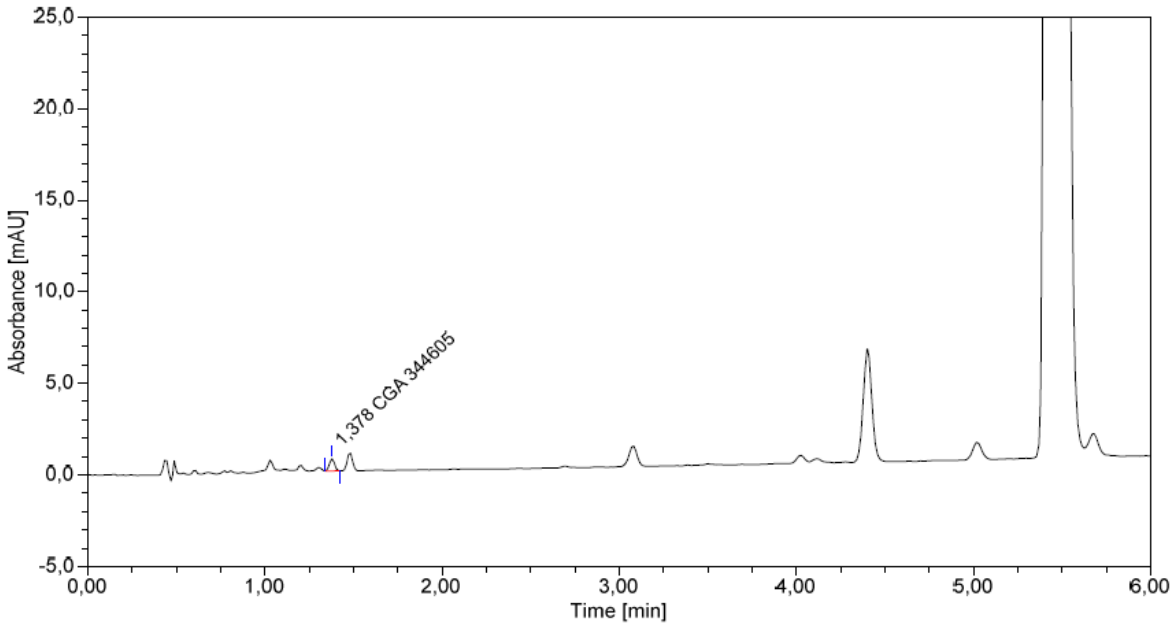


Figure 41: Chromatograms of active substance trifloxystrobin Lab 4

reference standard of active substance trifloxystrobin in acetonitrile



reference standard of active substance trifloxystrobin in acetonitrile/water

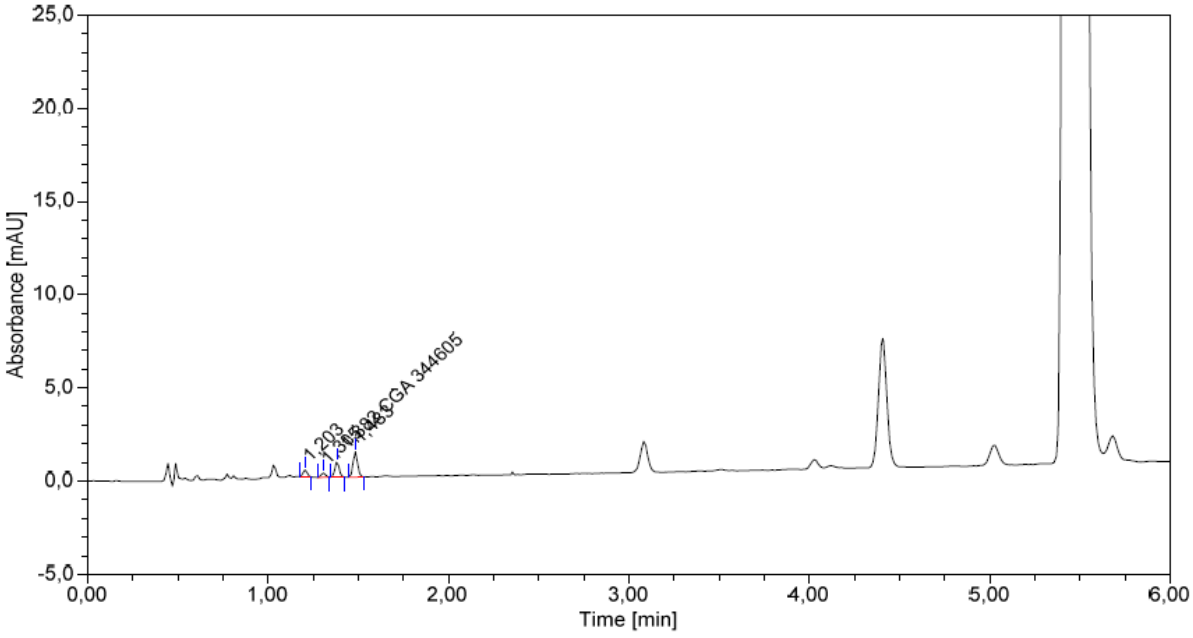


Figure 42: Chromatograms of active substance fluoxastrobin. Lab 4

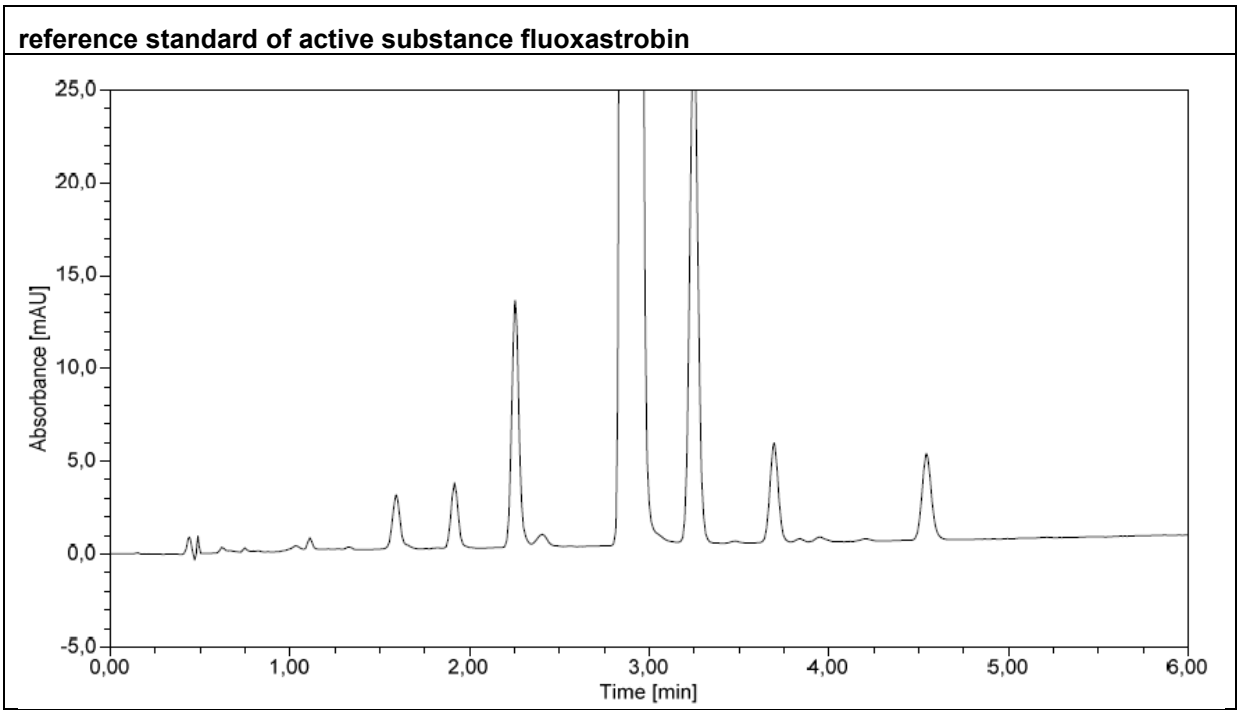


Figure 43: Chromatograms of active substance prothioconazole Lab 4

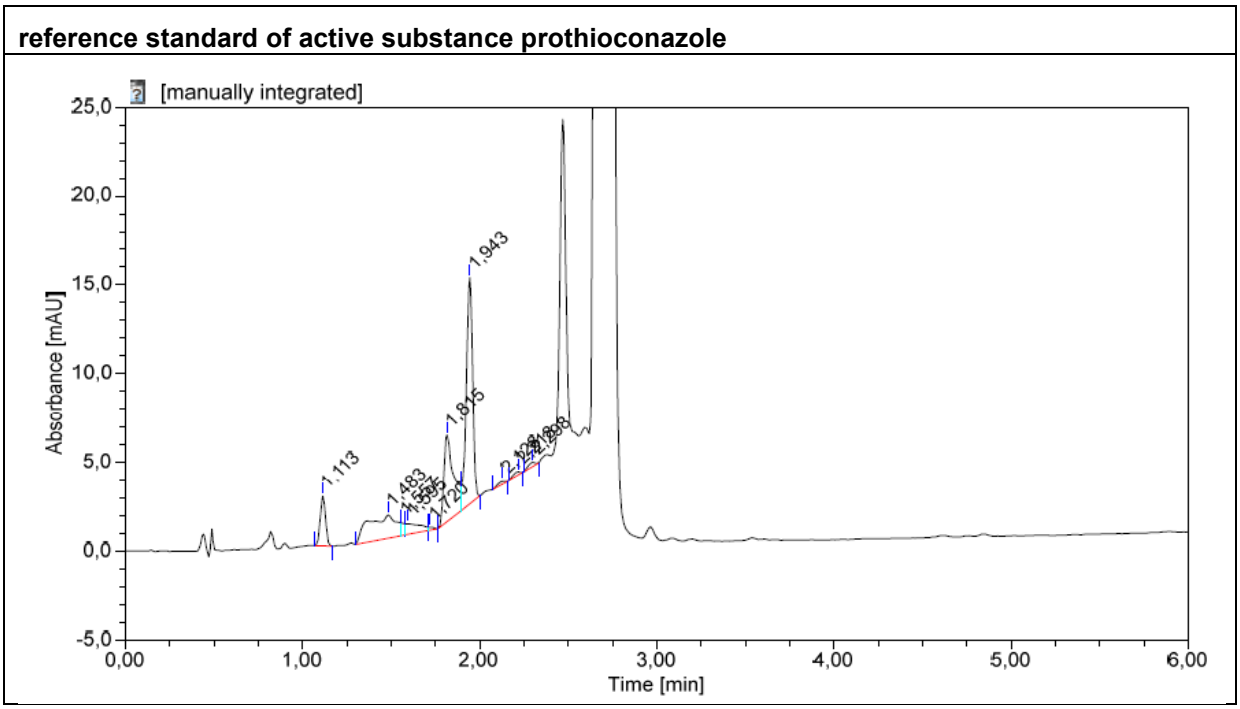


Figure 44: Chromatograms of trifloxystrobin TC Lab 4

Technical material trifloxystrobin TC spiked with reference standard CGA 344605

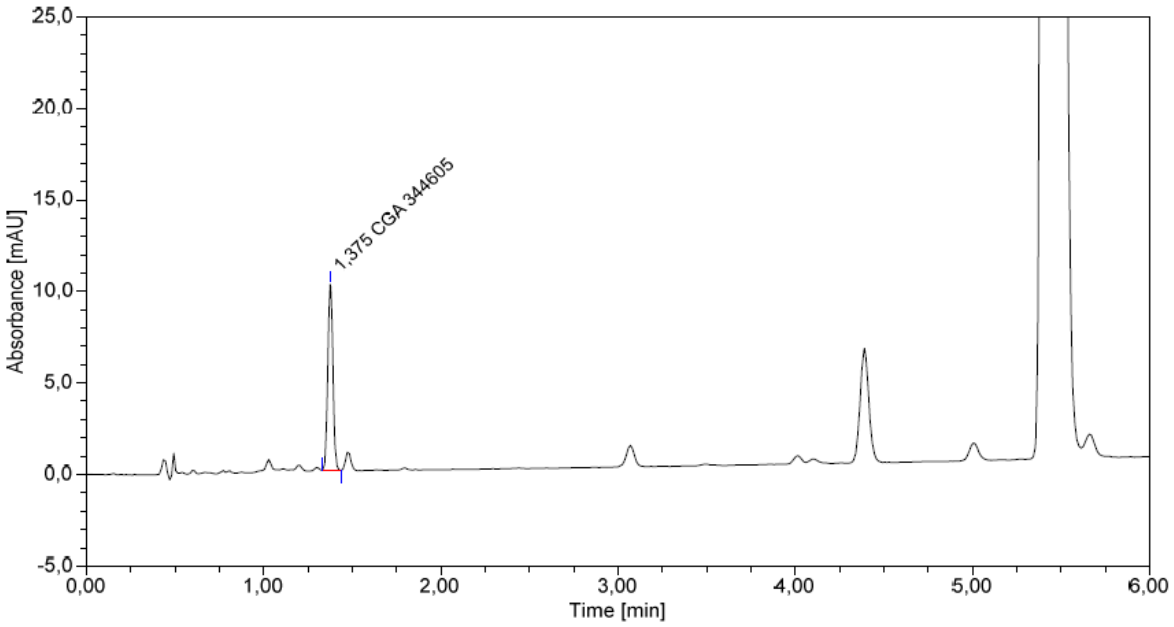
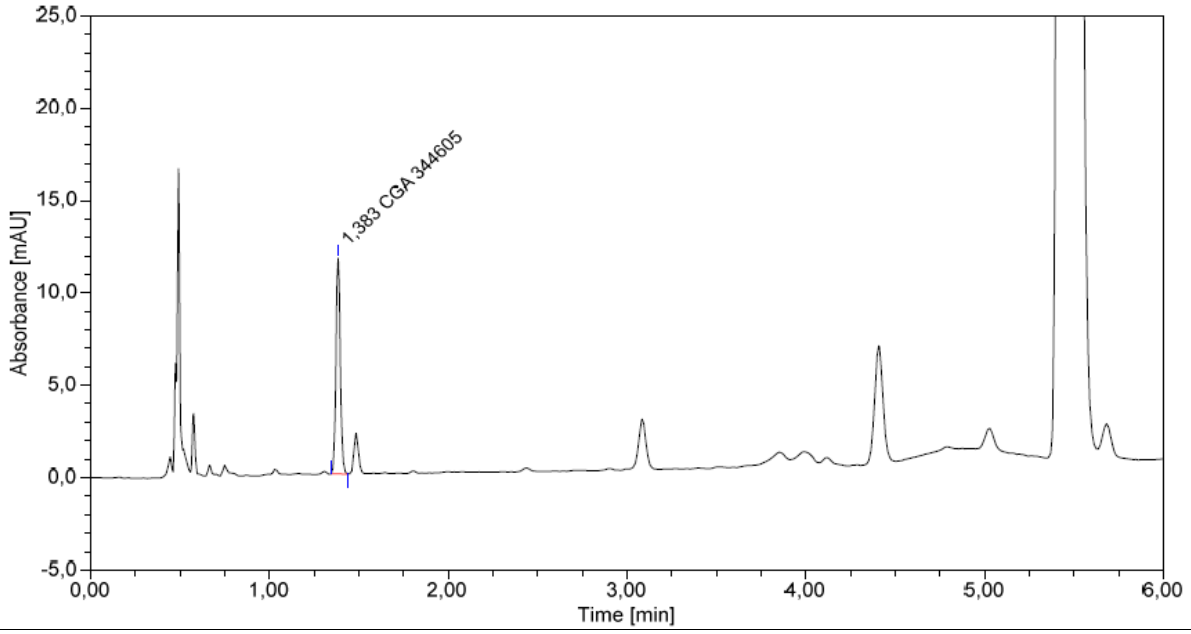


Figure 45: Chromatograms of trifloxystrobin SC 500 (500 g/L) Lab 4

Formulation trifloxystrobin SC 500 (500 g/L) spiked with reference standard CGA 344605 (0.03645 mg mg/50 mL)



Blank formulation trifloxystrobin SC 0 (0 g/L)

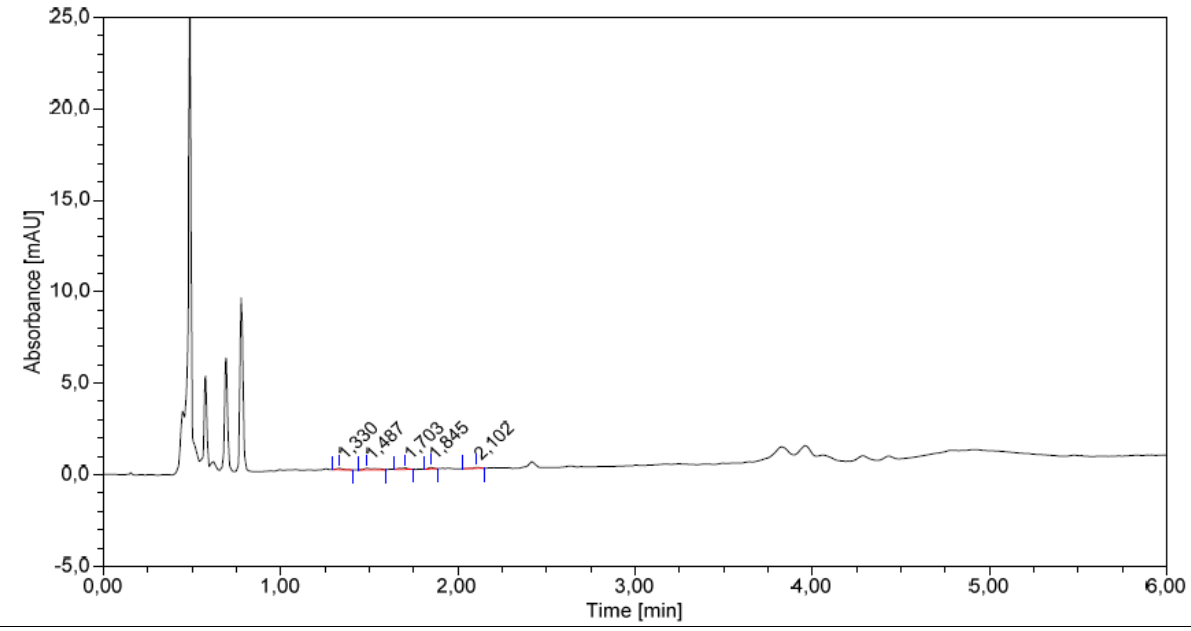


Figure 45. Continuation

Blank formulation trifloxystrobin SC 0 (0 g/L) – three-fold weight

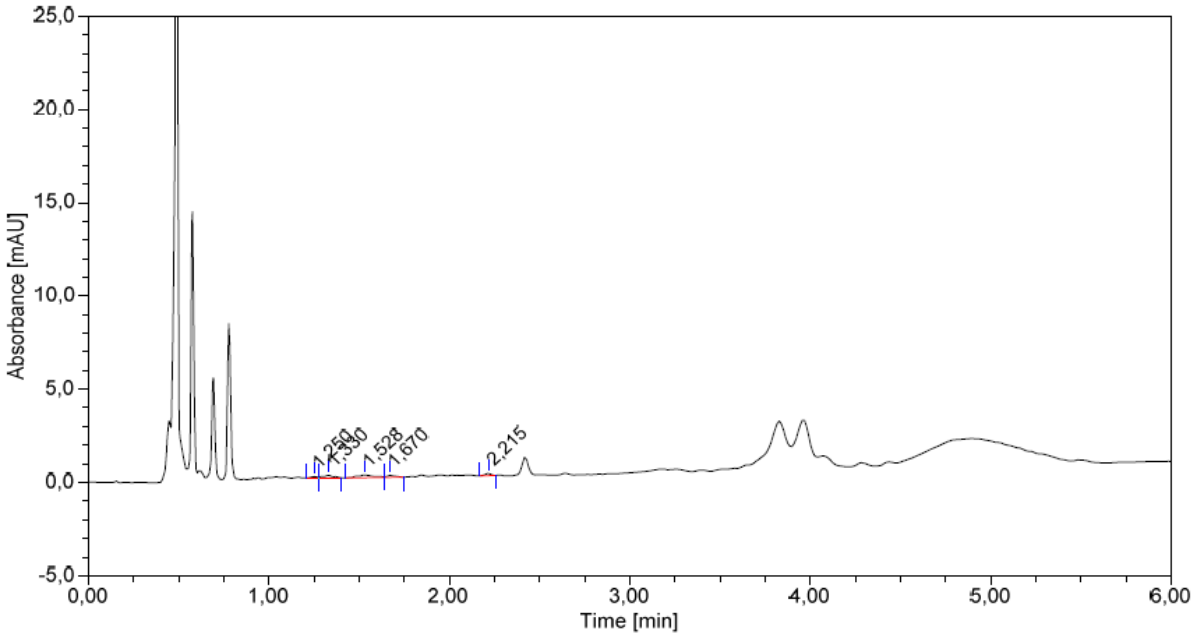
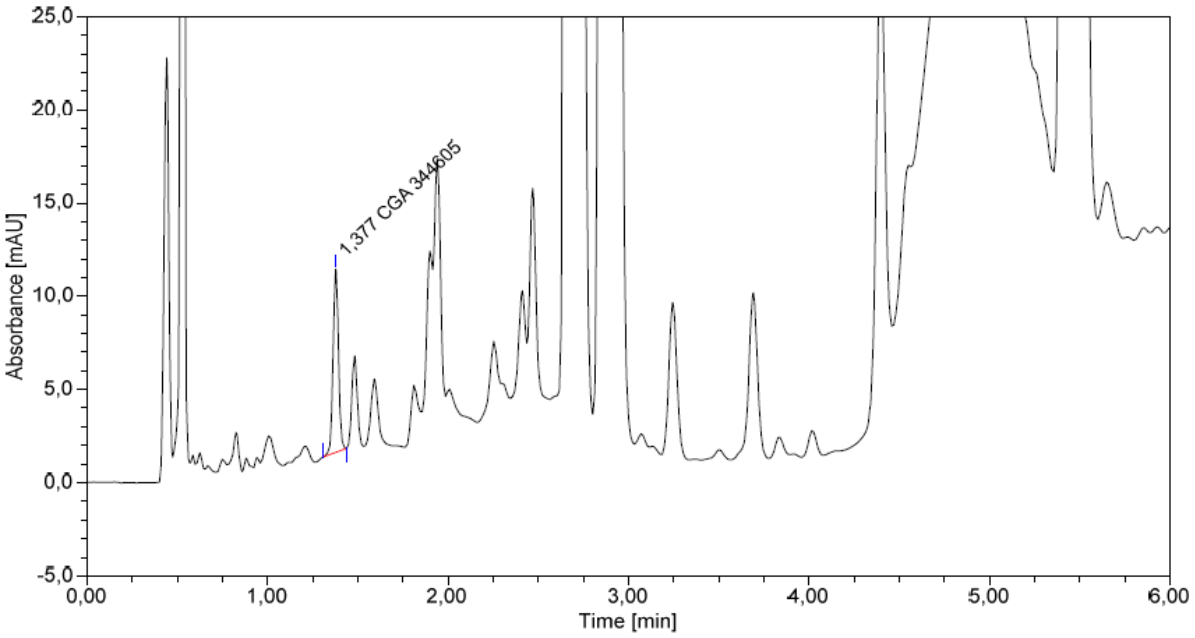


Figure 46: Chromatograms of fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) Lab 4

Formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L) spiked with reference standard CGA 344605



Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L)

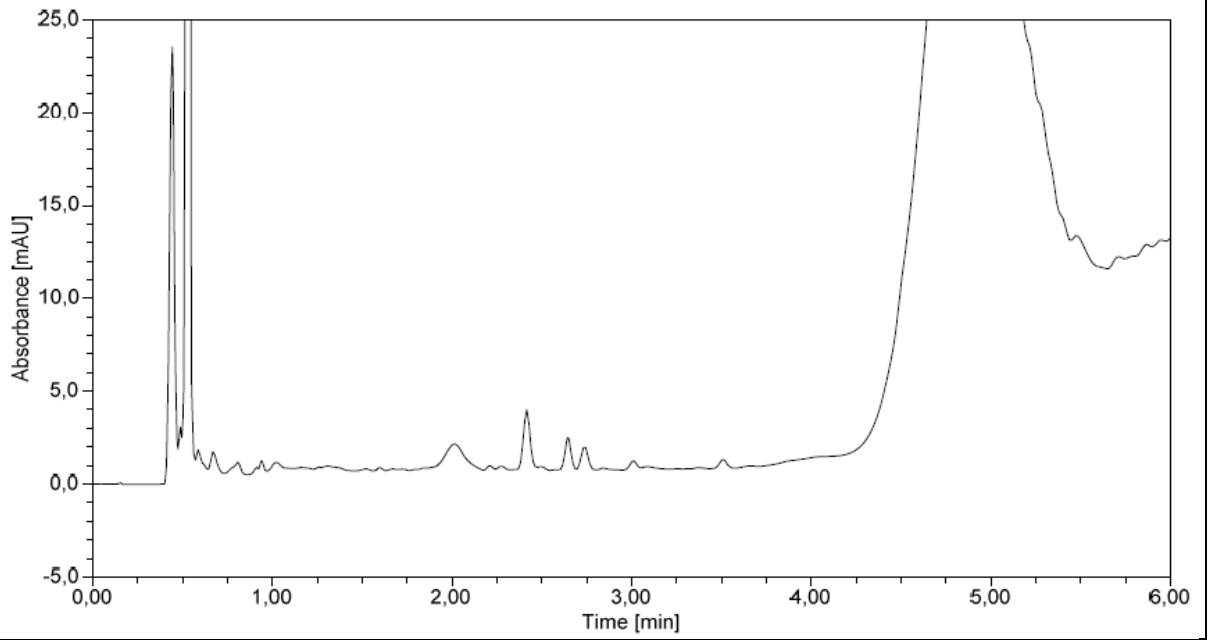


Figure 46. Continuation

Blank formulation fluoxastrobin + prothioconazole + trifloxystrobin EC 0 (0+0+0 g/L) three-fold weigth

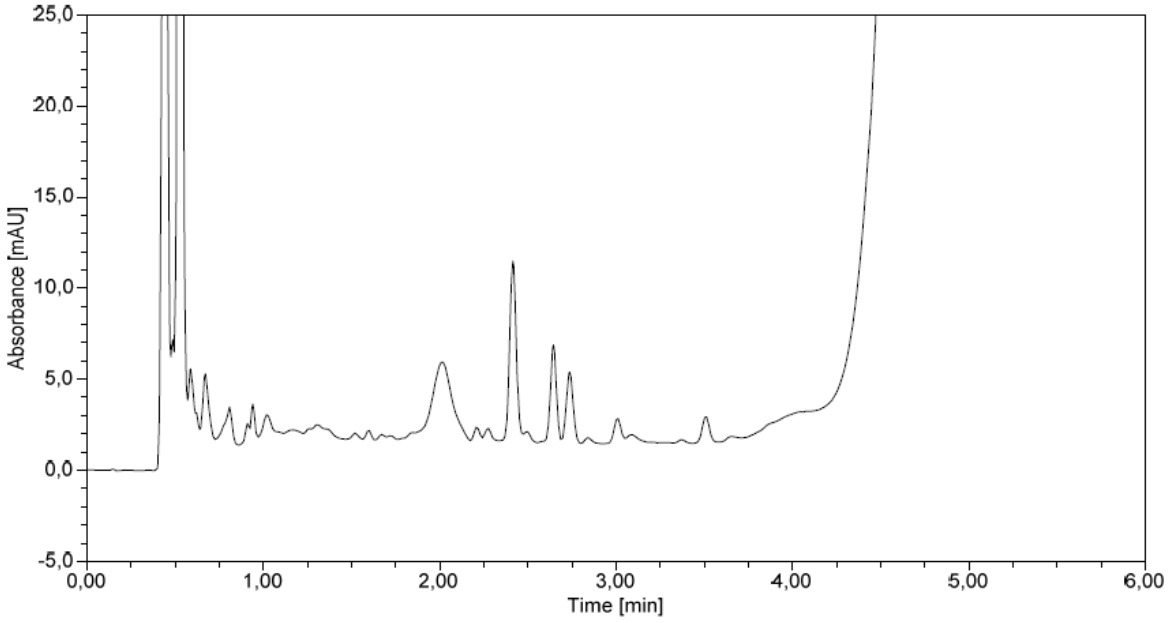
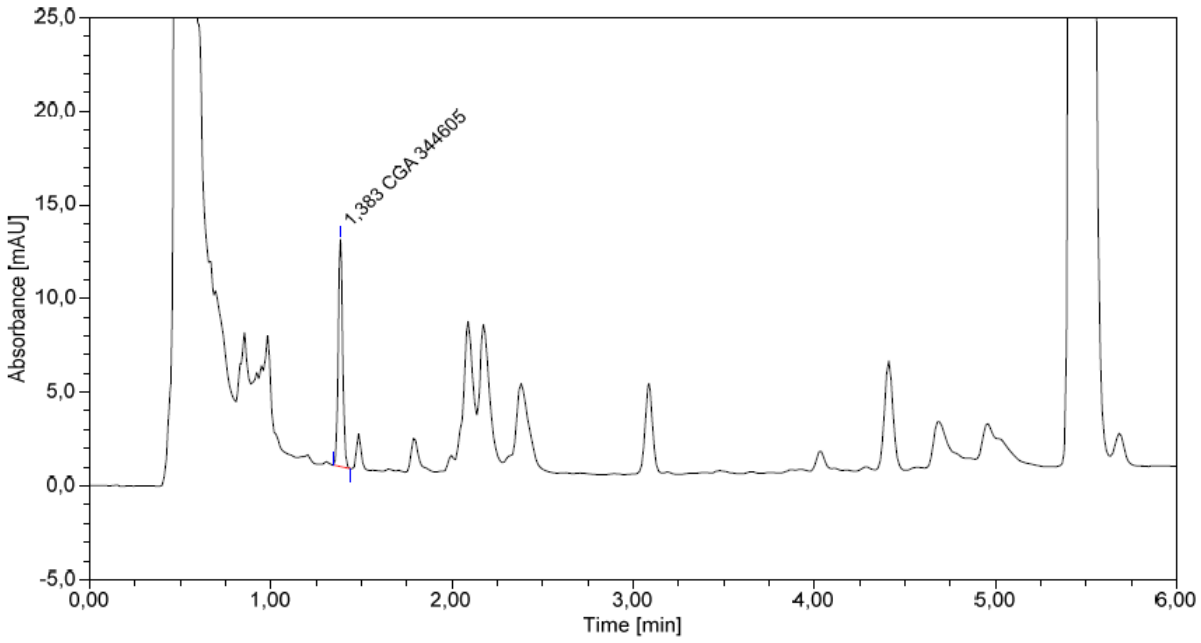


Figure 47: Chromatogram of trifloxystrobin WG 50 (50 g/kg) Lab 4

Formulation trifloxystrobin WG 50 (50 g/kg) spiked with reference standard CGA 344605



Blank Formulation trifloxystrobin WG 0 (0 g/Kg)

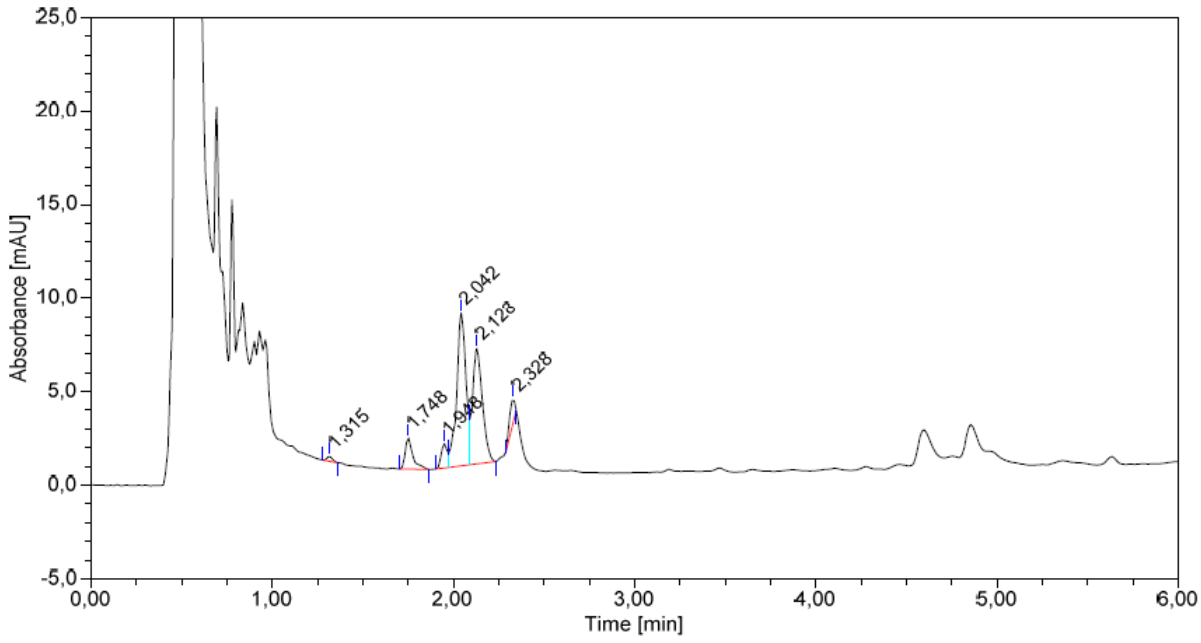
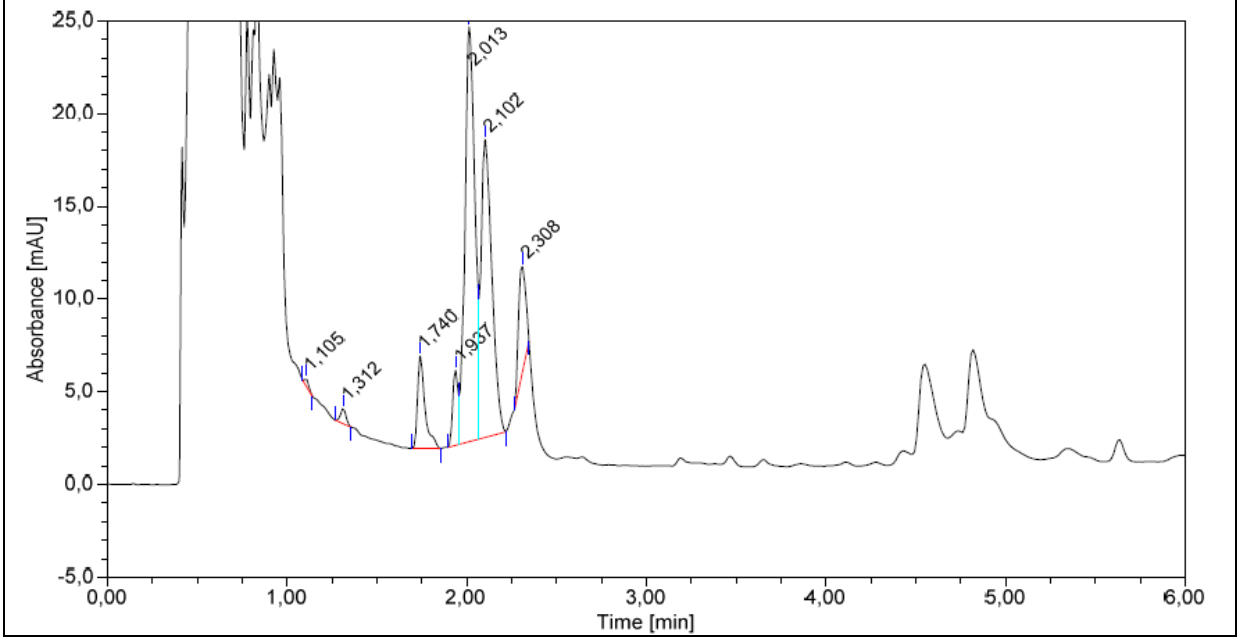


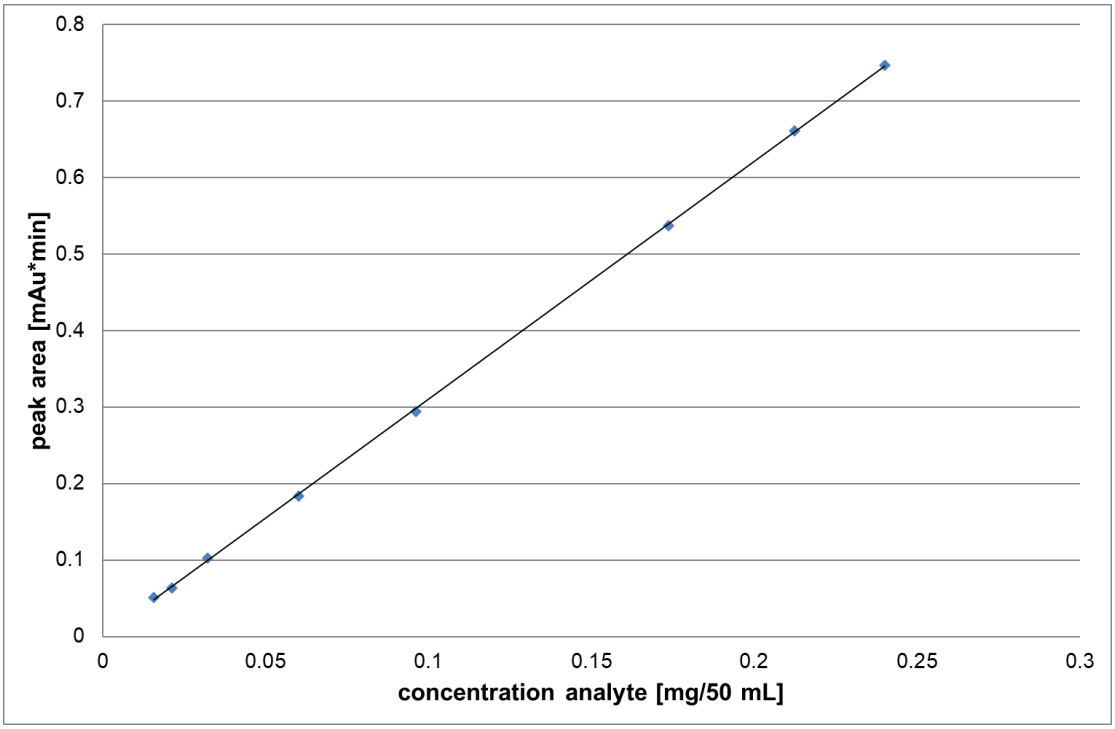
Figure 47. Continuation

Blank Formulation trifloxystrobin WG 0 (0 g/Kg) - three-fold weight



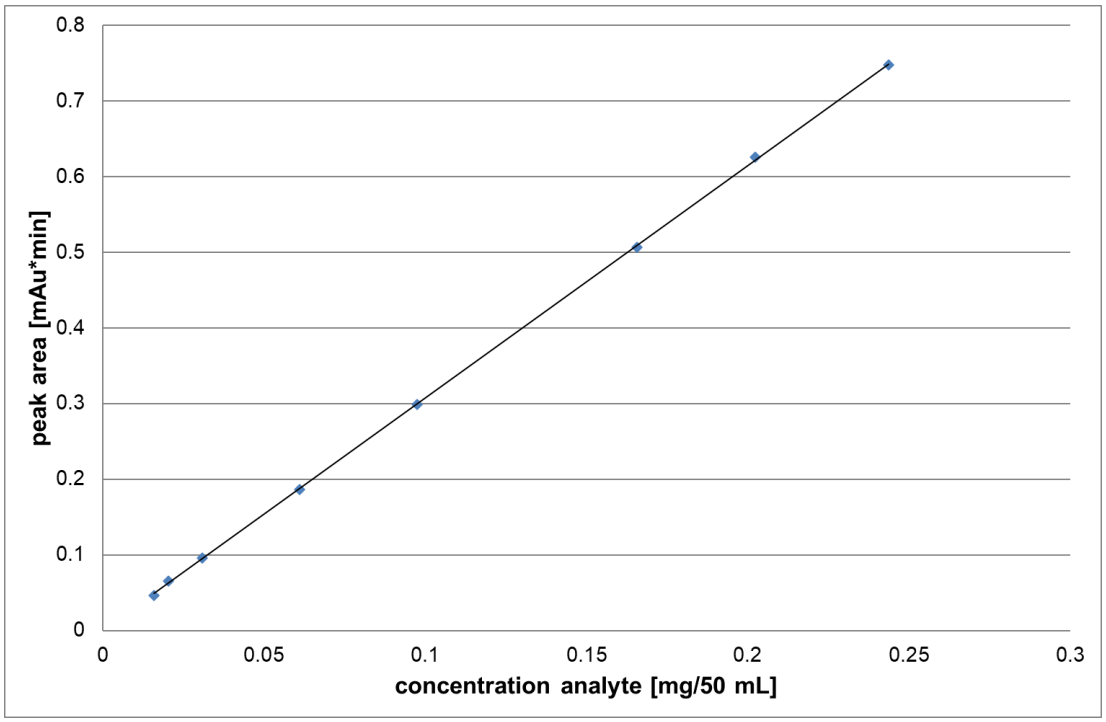
6.4 Calibration function (Linearity)

Figure 48: Calibration function of CGA 344605 in acetonitrile Developing Lab



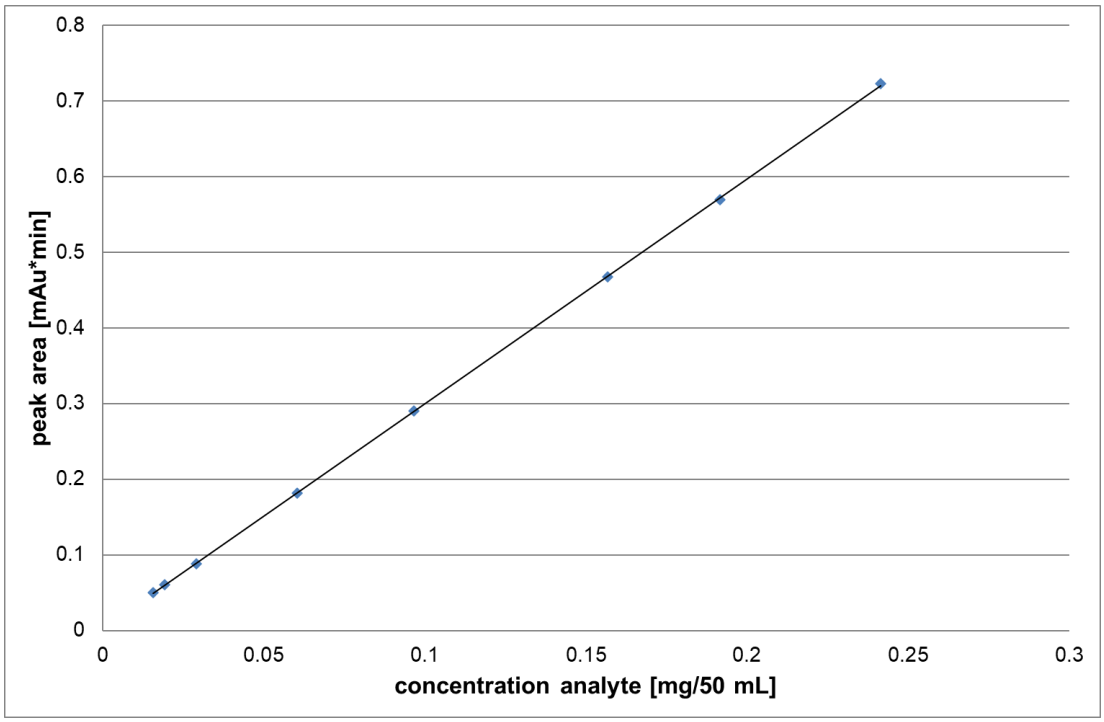
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = -0.000678 + 3.105749 x
Correlation coefficient	**= 0.99995**

Figure 49: Calibration function of CGA 344605 in acetonitrile/water Developing Lab



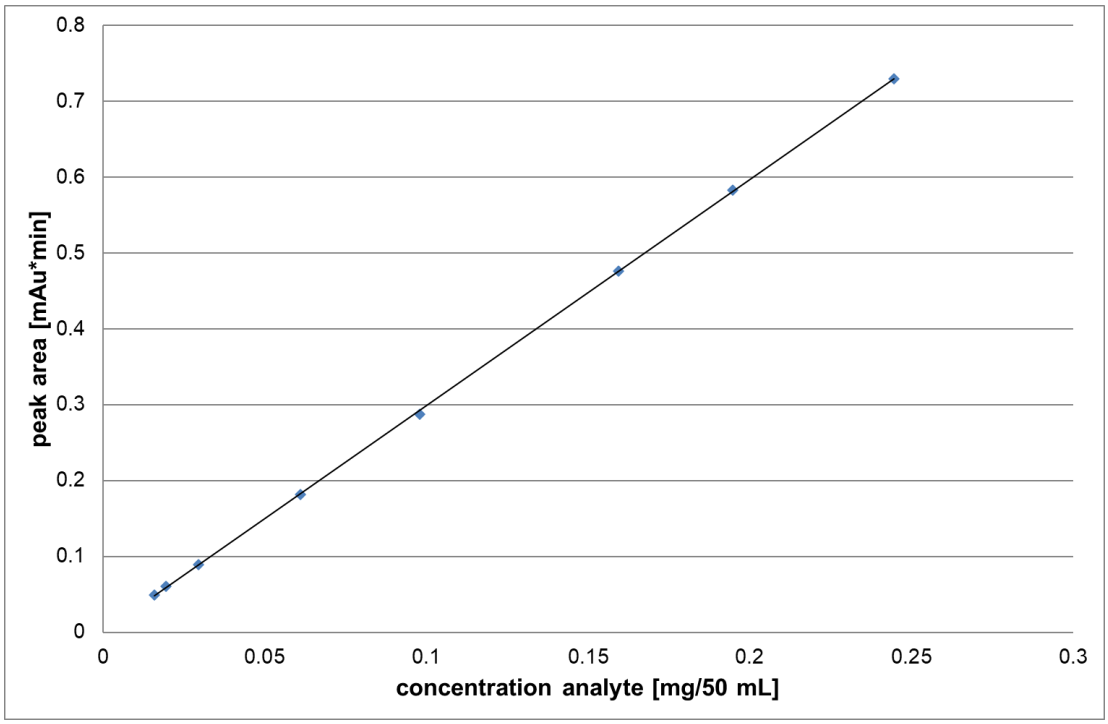
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.000502 + 3.070411 x
Correlation coefficient	**= 0.99996**

Figure 50: Calibration function of CGA 344605 in acetonitrile Lab 1



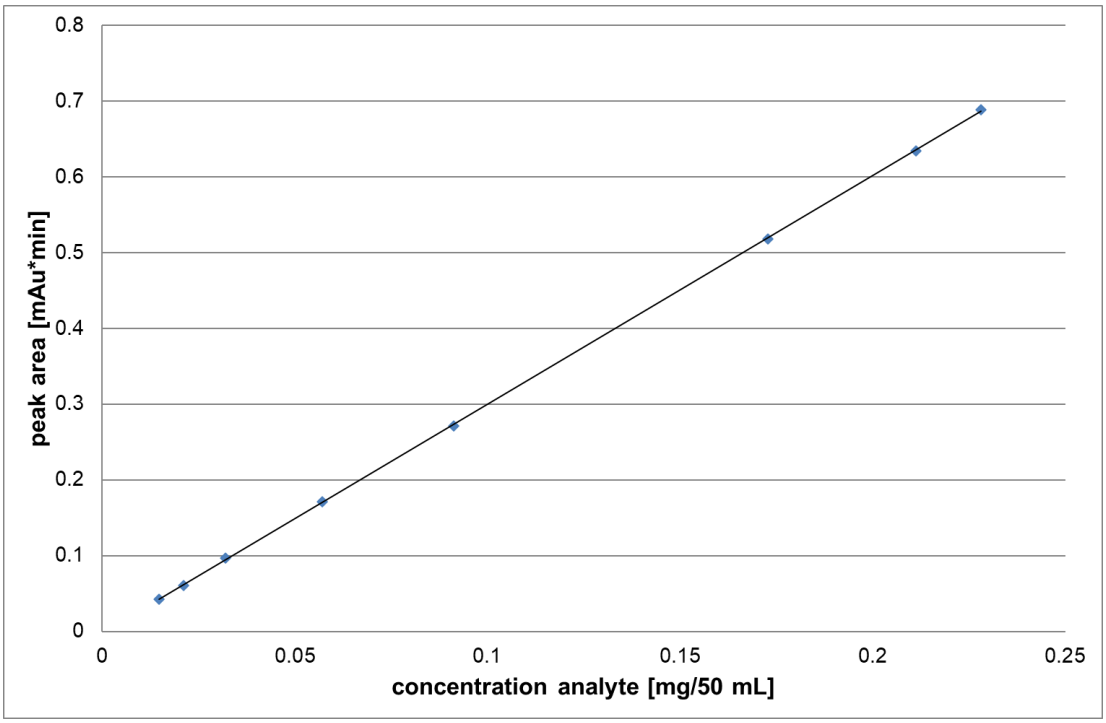
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.002356 + 2.972195 x
Correlation coefficient	**= 0.99998**

Figure 51: Calibration function of CGA 344605 in acetonitrile/water Lab 1



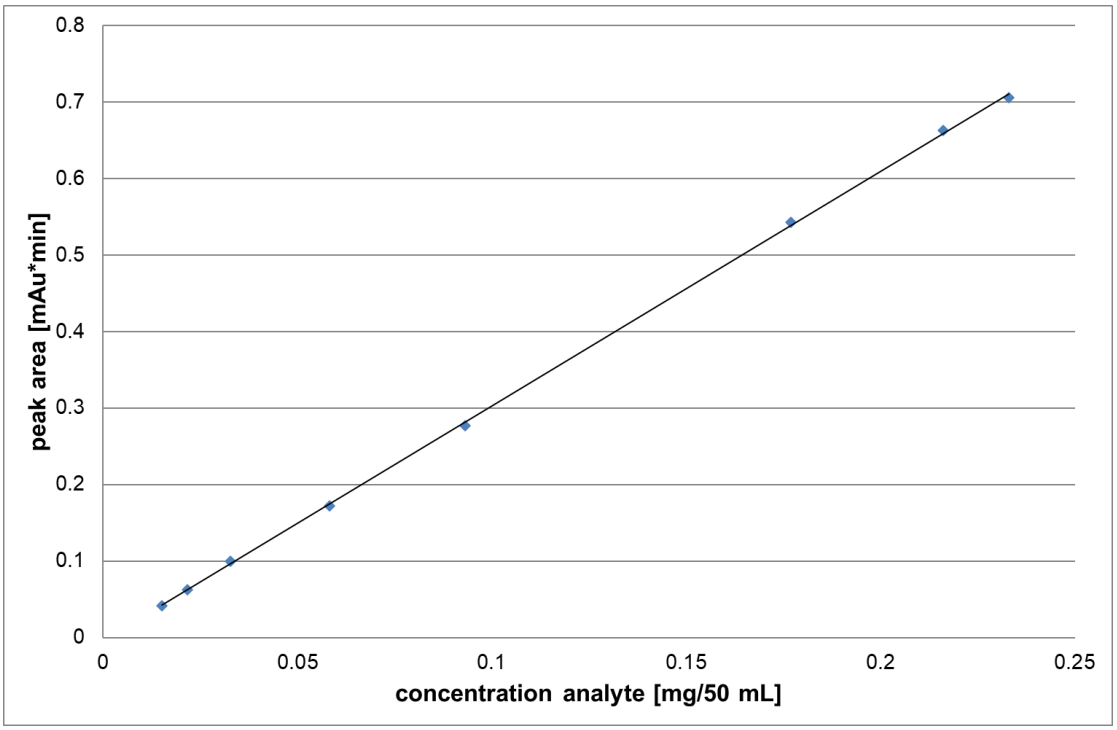
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.000736 + 2.978150 x
Correlation coefficient	**= 0.99997**

Figure 52: Calibration function of CGA 344605 in acetonitrile Lab 2



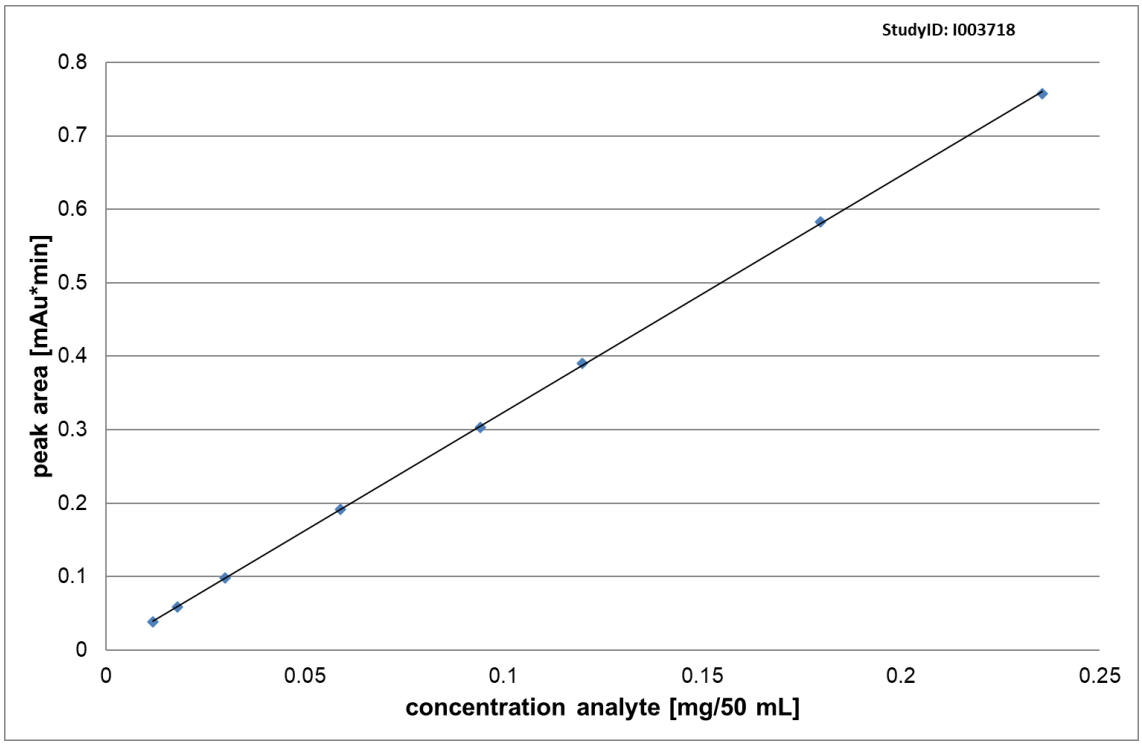
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = -0.002209 + 3.016589 x
Correlation coefficient	**= 0.99998**

Figure 53: Calibration function of CGA 344605 in acetonitrile/water Lab 2



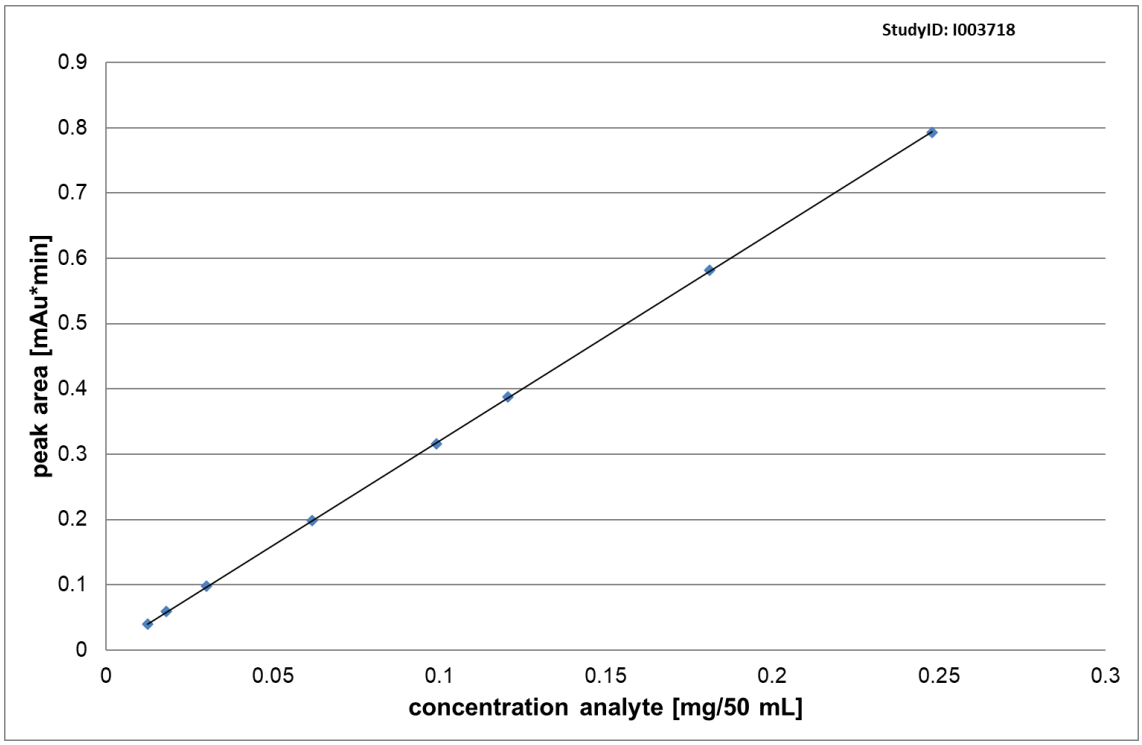
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = -0.004383 + 3.068154 x
Correlation coefficient	**= 0.99991**

Figure 54: Calibration function of CGA 344605 in acetonitrile Lab 3



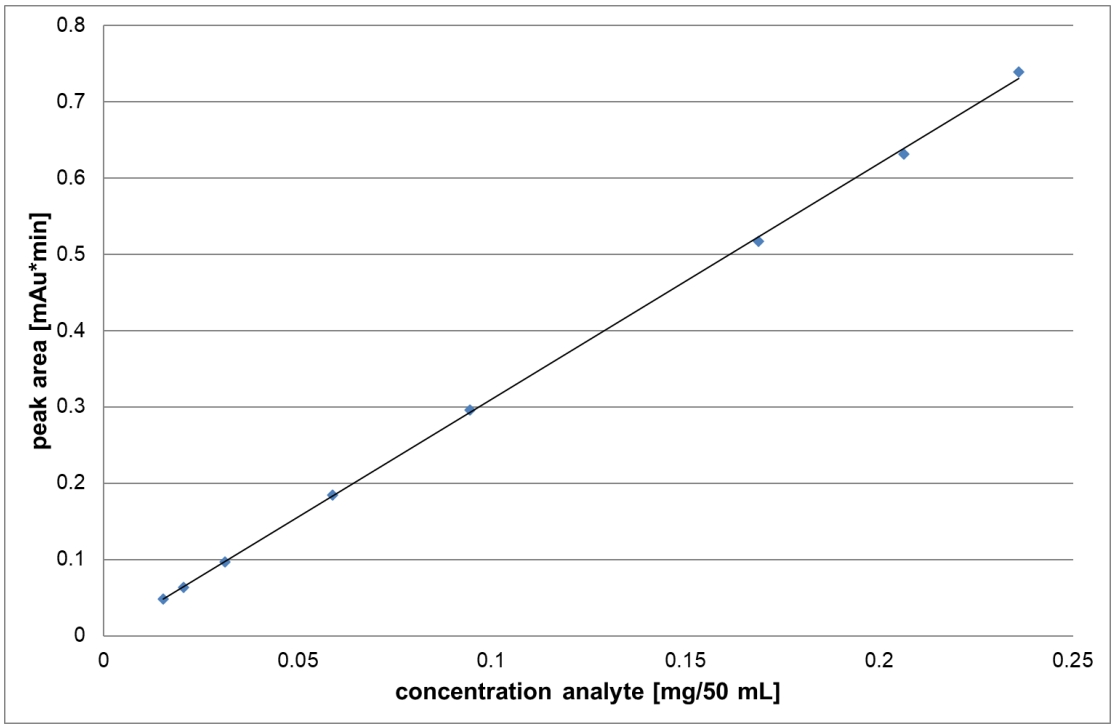
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.001541 + 3.220038 x
Correlation coefficient	**= 0.99997**

Figure 55: Calibration function of CGA 344605 in acetonitrile/water Lab 3



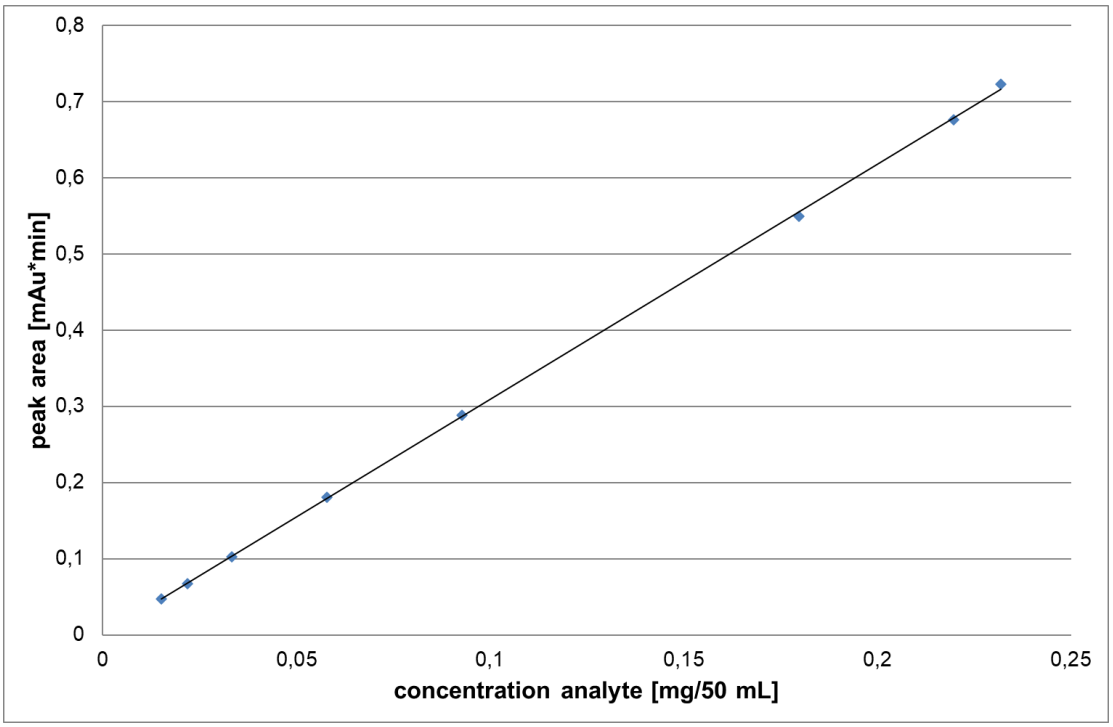
Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.000602 + 3.201252 x
Correlation coefficient	**= 0.99999**

Figure 56: Calibration function of CGA 344605 in acetonitrile Lab 4



Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.00038 + 3.09196 x
Correlation coefficient	**= 0.99982**

Figure 57: Calibration function of CGA 344605 in acetonitrile/water Lab 4



Number of values	n = 8
Regression equation	y = a + b x (1st order)
	y = 0.00031 + 3.08620 x
Correlation coefficient	**= 0.99992**

Appendix 1: Calculation of max. limit of CGA 344605 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 CGA 344605 in the technical grade active substance trifloxystrobin is 4 g/kg. equal to 0.4% (*w/w*).

Thus the MAL of CGA 344605 in the formulations is calculated as follow.

Formulation	$\mathrm{MAL}_{\mathrm{TGAS}}^{\mathrm{analyte}}$	$DC_{formulation}^{a.s.(TFS)}$	$MAL_{formulation}^{analyte}$	
	[g/kg]	[% (*w/w*)]	[g/kg]	[% (*w/w*)]
trifloxystrobin SC 500 (500 g/L)	4	43.9	1.76	0.176
fluoxastrobin + prothioconazole + trifloxystrobin EC 300 (75+150+75 g/L)	4	6.47	2.59	0.0259
trifloxystrobin WG 50 (50 g/kg)	4	50.0	2.00	0.200