Rel. Impurities of Triflumuron

CIPAC Peer Validation

CIPAC Peer Validation on the Analytical Method of
1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline in
Triflumuron Technical and SC Formulation by
High Performance Liquid Chromatography

Report to CIPAC
by
Bayer AG, Crop Science Division
Alexandra Michel
Alfred-Nobel-Str. 50
40789 Monheim
Germany

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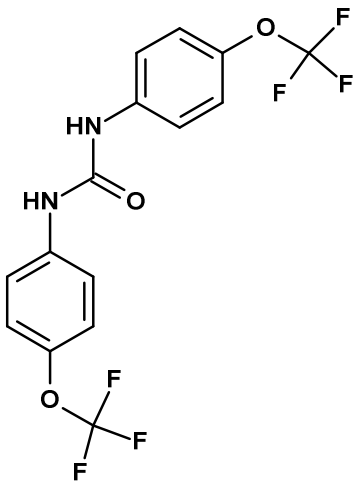
1. Introduction

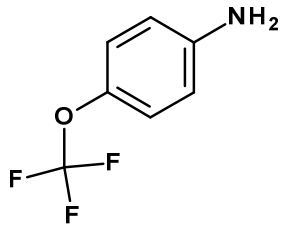
1.1 Scope

The results of peer validation on the analytical method for the determination of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline content within triflumuron Technical Grade Active Ingredient (TGAI) and triflumuron Suspension Concentrate (SC) formulation are reported.

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

1.2 Analytes

Report Name	1,3-bis(4-trifluoromethoxyphenyl)urea
Synonyms	N,N'-bis-[4-(trifluoromethoxy)phenyl]urea, AE B143886, BCS-AD26894
Three letter code	-
Structural formula	
Empirical Formula	$C_{15}\ H_{10}\ F_6\ N_2\ O_3$
Molecular Weight	380.2 g/mol
CAS no	78015-49-3

Report Name	4-(trifluoromethoxy)aniline
Synonyms	AE F069069, BCS-AC49934
Three letter code	-
Structural formula	
Empirical Formula	C7 H6 F3 N O
Molecular Weight	177.1 g/mol
CAS no	461-82-5

1.3 Samples

Two test samples and two analytical standards were sent to the participants:
1. Triflumuron technical (TC)
2. Triflumuron suspension concentrate (SC)

1,3-bis(4-trifluoromethoxyphenyl)urea, reference standard (purity 98.8%w/w)
4-trifluoromethoxyaniline, reference standard (purity 99.0%w/w)

1.4 Participants

Haustein, Michael	Currenta GmbH & Co. OHG ANT-PDA-PÜ3 HB 41538 Dormagen Germany
Koch, André	Eurofins Agroscience services EcoChem GmbH Eutinger Str. 24, 75223 Niefern-Öschelbronn Germany
Michel, Alexandra*	Bayer AG, Crop Science Division Formulation Technology Alfred-Nobel-Str. 50, 40789 Monheim am Rhein Germany
Wagner, Silke	Bayer AG, Crop Science Division Product Chemistry Analytics Alfred-Nobel-Str. 50, 40789 Monheim am Rhein Germany

* Developing lab

2. Analytical Method

2.1 Outline of the Method

1,3-bis(4-trifluoromethoxyphenyl)urea content and 4-trifluoromethoxyaniline content are determined using isocratic reversed High Performance Liquid Chromatography incorporating UV detection with an external standard calibration.

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

UV/VIS spectrum of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline were obtained for standard and sample solutions under the operating conditions described in CIPAC/5091/m.

2) Specificity

Retention times of the ingredients in triflumuron technical material and formulants in each formulation were checked with the solutions of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline standards, triflumuron technical material and SC formulation to check non-analyte interference.

3) Linearity

Calibration solutions were prepared using 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline standards. The solutions were analyzed and the peak area ratios of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline were plotted against the concentration of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline to make a calibration line.

4) Precision

Six separate sub-samples from a sample of triflumuron technical material and SC formulation were analyzed. Appropriate amounts of 1,3-bis(4-trifluoromethoxyphenyl)urea or 4-trifluoromethoxyaniline were spiked to the samples, if the contents of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline in the technical material and SC formulation were not enough to evaluate repeatability. Mean and relative standard deviations (RSD) were calculated from the analytical values.

5) Accuracy

The stock solution at an appropriate concentration of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline was fortified to triflumuron technical material and SC formulation so that the fortified concentration of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline were at the level of specification. These solutions were analyzed, and the recoveries were calculated by the following equation:

$R = \frac{C - C_0}{C_S}$

where, R : recovery (%)
C : observed concentration (g/kg) of 1,3-bis(4-trifluoromethoxyphenyl)urea or 4-trifluoromethoxyaniline
C_0 : initial concentration (g/kg) of 1,3-bis(4-trifluoromethoxyphenyl)urea or 4-trifluoromethoxyaniline in triflumuron technical material or SC formulation
C_S : fortified concentration (g/kg) of 1,3-bis(4-trifluoromethoxyphenyl)urea or 4-trifluoromethoxyaniline

6) Limit of Quantitation (LOQ)

Concentrations of 1,3-bis(4-trifluoromethoxyphenyl)urea and of 4-trifluoromethoxyaniline at the lowest tested fortification level were analyzed. The LOQ was defined at this level as acceptable accuracy and precision results were successfully demonstrated.

2.3 Peer Validation

The peer validation was conducted with four independent laboratories through the network of DAPA. The participants are shown in 1.4. We requested the collaborators to conduct peer validation according to the prescribed protocol, describe operating conditions in detail, and attach the calibration curve and all chromatograms for each sample.
The investigated items are specificity, linearity, precision, accuracy and LOQ.
The details of each procedure are the same as those described in 2.2.

3. Remarks of the Participants

Laboratory 1	Column:	Nucleosil 120-3 C18, 3 µm, 125 x 4 mm
	Remarks:	None
Laboratory 2	Column:	Nucleosil 120-3 C18, 3 µm, 125 x 4 mm
	Remarks:	None
Laboratory 3	Column:	Nucleosil 120-3 C18, 3 µm, 125 x 4 mm
	Remarks:	None
Laboratory 4	Column:	Nucleosil 120-3 C18, 3 µm, 125 x 4 mm
	Remarks:	None

4. Results and Discussion

4.1 Peer Validation

1) Confirmation of Analyte Identification

The DAD spectra of the 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline standard solution and sample solutions are shown in Figure 1 to Figure 6.

2) Specificity

The peaks of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline were separated from the peak of triflumuron and its related compounds in Triflumuron TC and Triflumuron SC. The peaks were also separated from the peaks from formulants in the Triflumuron SC formulation (Figure 7 to Figure 21).

3) Linearity

The calibration lines are shown in Figure 22 to Figure 29. The equation of the calibration line and the correlation factor (r) are reported in addition. The correlation factor was satisfactory, and the response of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline was linear over the concentration range of 0.0025 to 0.2 mg/50 mL.

4) Precision

The repeatability of this method was satisfactory with RSD values of 0.23 – 5.8 % as shown in Table 1 and Table 2. All RSD values were found to be smaller than 20%.

5) Accuracy

The recoveries were satisfactory as shown in Table 3 to Table 6.

6) LOQ

Concentrations of:
0.0164 mg/50 mL 1,3-bis(4-trifluoromethoxyphenyl)urea and of
0.0168 mg/50 mL 4-trifluoromethoxyaniline
were the lowest tested fortification levels for which acceptable accuracy and precision under repeatability conditions were successfully demonstrated.

These concentrations are equal to:
TC: 0.010% (w/w) of 1,3-bis(4-trifluoromethoxyphenyl)urea
0.011% (w/w) of 4-trifluoromethoxyaniline

SC: 0.004% (w/w) of 1,3-bis(4-trifluoromethoxyphenyl)urea
0.004% (w/w) of 4-trifluoromethoxyaniline

5. CONCLUSION

For all samples, the analytical method was peer-validated in terms of specificity, linearity, precision, accuracy and quantitation limit. The RSDs of repeatability for technical material and SC formulation were found to be smaller than 20% for all laboratories participated in this peer validation.

In conclusion, the proposed method was successfully peer-validated and was considered appropriate for the determination of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-trifluoromethoxyaniline in technical material and SC formulations.

Determination of relevant Impurities in Triflumuron

Table 1 – Results Triflumuron TC (Precision)

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	0.0172	0.0149	0.0150	0.0158	0.0235	0.0210	0,0205	0.0217
Weighing no. 2	0.0164	0.0151	0.0154	0.0149	0.0238	0.0215	0,0257*	0.0217
Weighing no. 3	0.0162	0.0148	0.0155	0.0156	0.0238	0.0212	0,0208	0.0218
Weighing no. 4	0.0167	0.0151	0.0151	0.0156	0.0247	0.0213	0,0199	0.0216
Weighing no. 5	0.0161	0.0151	0.0152	0.0159	0.0243	0.0212	0,0206	0.0216
Weighing no. 6	0.0163	0.0151	0.0149	0.0169	0.0238	0.0214	0,0193	0.0216
Mean value	0.0165	0.0150	0.0152	0.0158	0.0240	0.0213	0.0202	0.0217
SD	0.0004	0.0001	0.0002	0.0006	0.0004	0.0002	0.0006	0.0001
RSD [%]	2.47	0.87	1.53	4.11	1.82	0.82	3.04	0.38
Horwitz-Value RSD $(r)_{max}$	4.97	5.04	5.03	5.00	4.70	4.78	4.82	4.77
Outliers	no	no	no	no	no	no	yes* (eliminated)	no

Table 2 – Results Triflumuron SC (Precision)

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	0.0270	0.0200	0.0215	0.0200	0.0216	0.0203	0.0166	0.0198
Weighing no. 2	0.0267	0.0200	0.0225	0.0199	0.0211	0.0203	0.0176	0.0196
Weighing no. 3	0.0271	0.0197	0.0225	0.0199	0.0221	0.0200	0.0177	0.0197
Weighing no. 4	0.0276	0.0200	0.0217	0.0199	0.0223	0.0202	0.0169	0.0196
Weighing no. 5	0.0277	0.0198	0.0226	0.0199	0.0218	0.0199	0.0177	0.0196
Weighing no. 6	0.0267	0.0201	0.0223	0.0200	0.0214	0.0206	0.0174	0.0197
Mean value	0.0271	0.0199	0.0222	0.0199	0.0217	0.0202	0.0173	0.0197
SD	0.0004	0.0002	0.0005	0.0001	0.0004	0.0002	0.0005	0.0001
RSD [%]	1.59	0.87	2.10	0.26	2.05	1.24	2.67	0.42
Horwitz-Value RSD $(r)_{max}$	4.61	4.83	4.75	4.83	4.77	4.82	4.93	4.84
Outliers	no	no	no	no	no	no	no	no

Table 3 – Results Triflumuron TC – Accuracy Level I

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	98.6	100.4	94.8	97.8	99.2	99.2	102.5	94.8
Weighing no. 2	100.2	100.8	99.2	99.4	100.3	100.7	102.3	95.1
Weighing no. 3	101.8	99.7	95.0	99.1	100.3	100.2	103.9	95.1
Weighing no. 4	102.3	101.4	93.1	99.4	103.7	99.7	101.5	96.5
Weighing no. 5	102.4	100.0	91.5	97.2	102.4	100.9	105.9	94.8
Weighing no. 6	98.6	94.1	93.3	97.2	100.3	100.1	97.6	94.8
Mean value	100.6	99.4	94.5	98.4	101.0	100.1	102.3	95.2
SD	1.79	2.66	2.64	1.07	1.67	0.63	2.77	0.66
RSD [%]	1.78	2.68	2.80	1.09	1.65	0.63	2.71	0.70

Single and mean recovery rates within the requested range of 75 – 125 %

Table 4 – Results Triflumuron TC – Accuracy Level II

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	94.1	98.6	98.6	99.2	96.1	100.0	98.6	96.1
Weighing no. 2	98.9	99.6	98.4	99.5	102.6	99.8	98.8	96.1
Weighing no. 3	98.5	98.4	97.7	99.9	102.8	99.8	98.7	96.3
Weighing no. 4	101.7	99.2	98.1	99.3	104.3	100.1	98.2	96.6
Weighing no. 5	95.5	97.5	98.5	99.1	98.0	99.7	98.1	96.8
Weighing no. 6	97.2	98.3	99.9	99.9	98.8	100.7	97.4	97.1
Mean value	97.7	98.6	98.5	99.5	100.4	100.0	98.3	96.5
SD	2.68	0.73	0.74	0.35	3.27	0.37	0.54	0.41
RSD [%]	2.75	0.75	0.75	0.35	3.26	0.37	0.55	0.42

Single and mean recovery rates within the requested range of 75 – 125 %

Table 5 – Results Triflumuron SC – Accuracy Level I

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	105.0	102.2	95.1	99.4	97.5	103.9	97.8	96.9
Weighing no. 2	103.9	101.7	94.5	99.4	100.6	103.7	91.9	98.1
Weighing no. 3	106.7	102.3	88.2	98.8	101.9	104.4	91.5	96.9
Weighing no. 4	102.8	102.0	91.3	98.8	96.9	103.5	90.5	97.5
Weighing no. 5	109.4	101.9	92.2	99.4	99.4	103.5	96.4	98.1
Weighing no. 6	106.1	101.8	90.8	98.8	100.0	103.4	91.2	98.1
Mean value	105.6	102.0	92.0	99,1	99.4	103.7	93.2	97.6
SD	2.34	0.23	2.52	0.33	1.90	0.37	3.07	0.59
RSD [%]	2.21	0.23	2.74	1.34	1.91	0.36	3.29	0.60

Single and mean recovery rates within the requested range of 75 – 125 %

Table 6 – Results Triflumuron SC – Accuracy Level II

	1,3-bis(4-trifluoromethoxyphenyl)urea [% w/w]				4-trifluoromethoxyaniline [% w/w]			
	Lab I	Lab II	Lab III	Lab IV	Lab I	Lab II	Lab III	Lab IV
Weighing no. 1	103.3	99.2	97.3	99.9	102.1	99.1	95.1	103.2
Weighing no. 2	102.1	99.1	96.2	99.5	102.5	99.3	94.1	102.9
Weighing no. 3	102.2	99.6	96.9	99.9	102.9	99.9	95.2	102.6
Weighing no. 4	101.4	99.7	97.3	98.8	102.5	99.3	94.8	101.4
Weighing no. 5	102.3	99.8	96..1	99.9	104.4	99.7	94.4	102.0
Weighing no. 6	102.4	99.1	97.7	99.5	102.7	99.4	94.8	101.1
Mean value	102.3	99.4	96.9	99.6	102.8	99.5	94.7	102.2
SD	0.61	0.32	0.66	0.43	0.79	0.30	0.41	0.84
RSD [%]	0.60	0.32	0.68	0.43	0.77	0.30	0.43	0.82

Single and mean recovery rates within the requested range of 75 – 125 %

Fig. 1 – UV-Spectrum of 1,3-bis(4-trifluoromethoxyphenyl)urea – Standard Solution

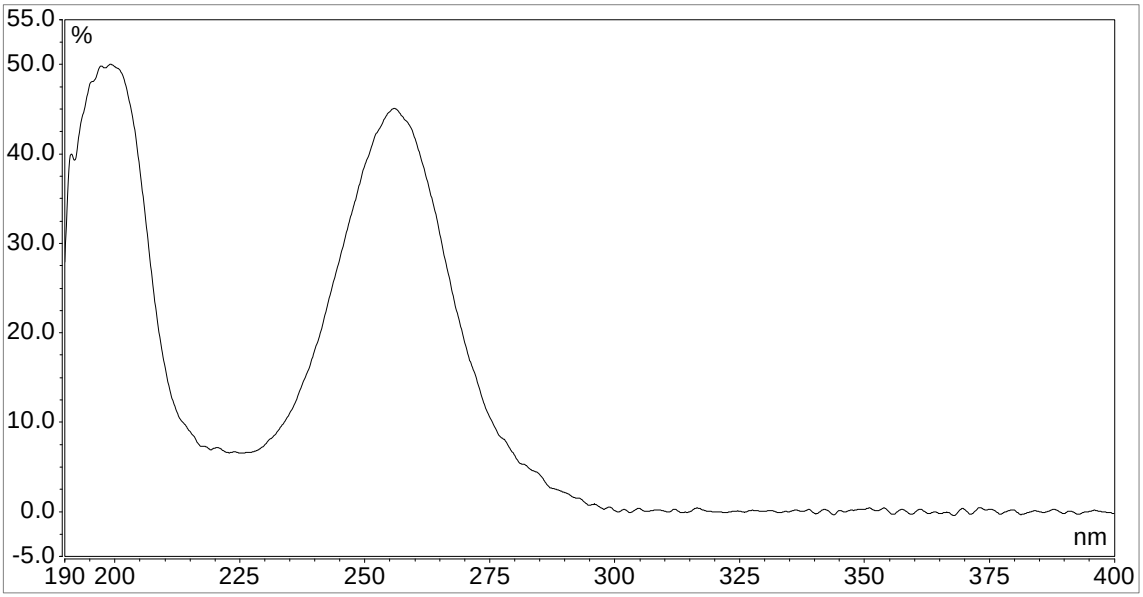


Fig. 2 – UV-Spectrum of 4-trifluoromethoxyaniline – Standard Solution

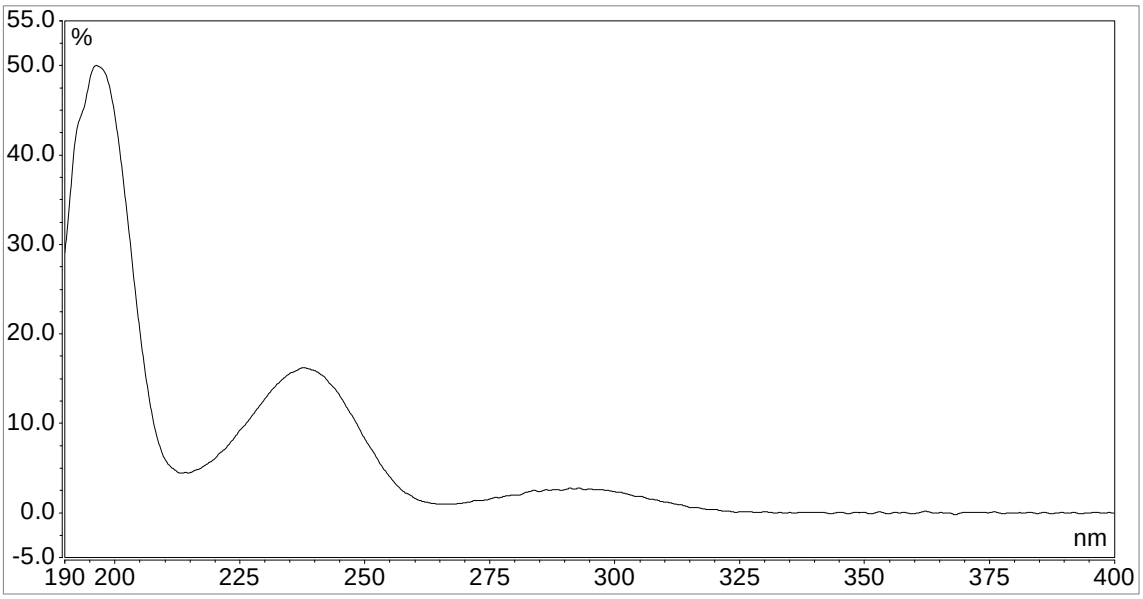


Fig. 3 – UV-Spectrum of 1,3-bis(4-trifluoromethoxyphenyl)urea – Triflumuron TC (spiked with reference item)

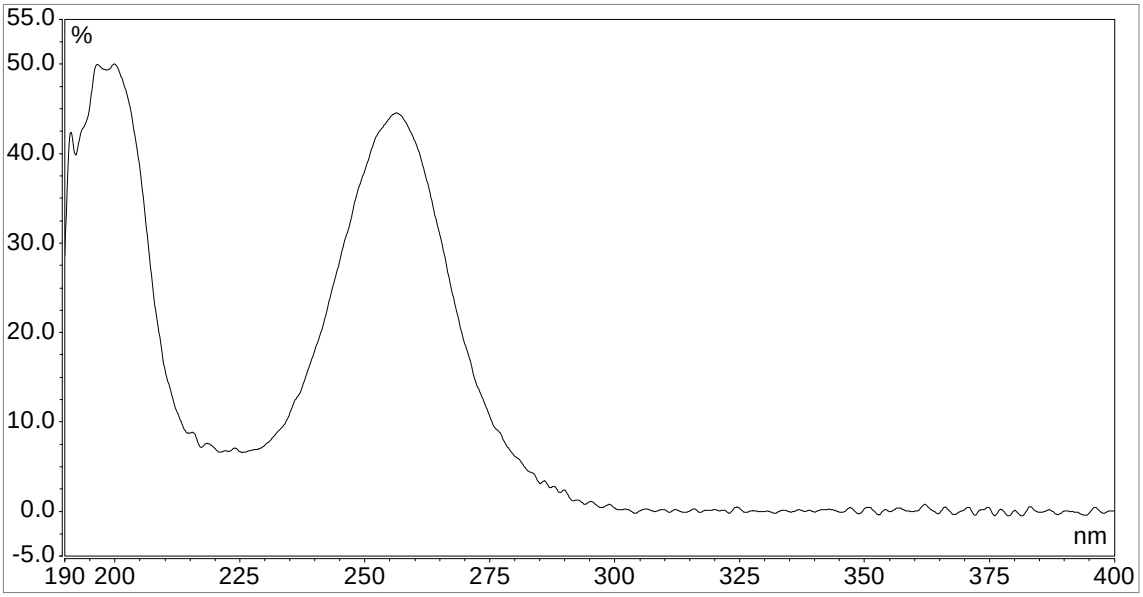


Fig. 4 – UV-Spectrum of 4-trifluoromethoxyaniline – Triflumuron TC (spiked with reference item)

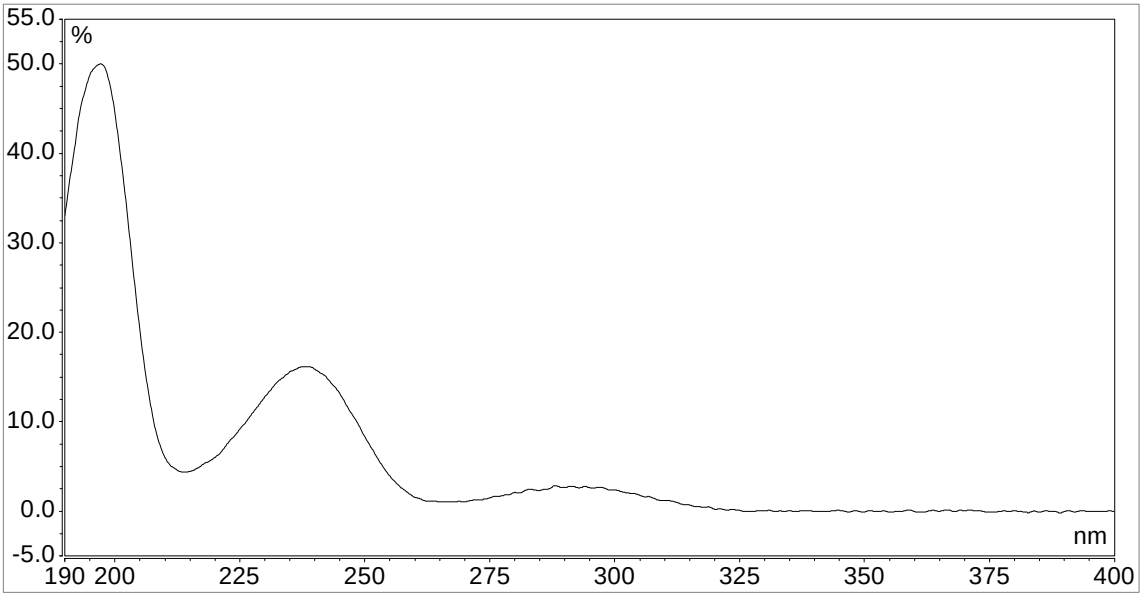


Fig. 5 – UV-Spectrum of 1,3-bis(4-trifluoromethoxyphenyl)urea – Triflumuron SC (spiked with reference item)

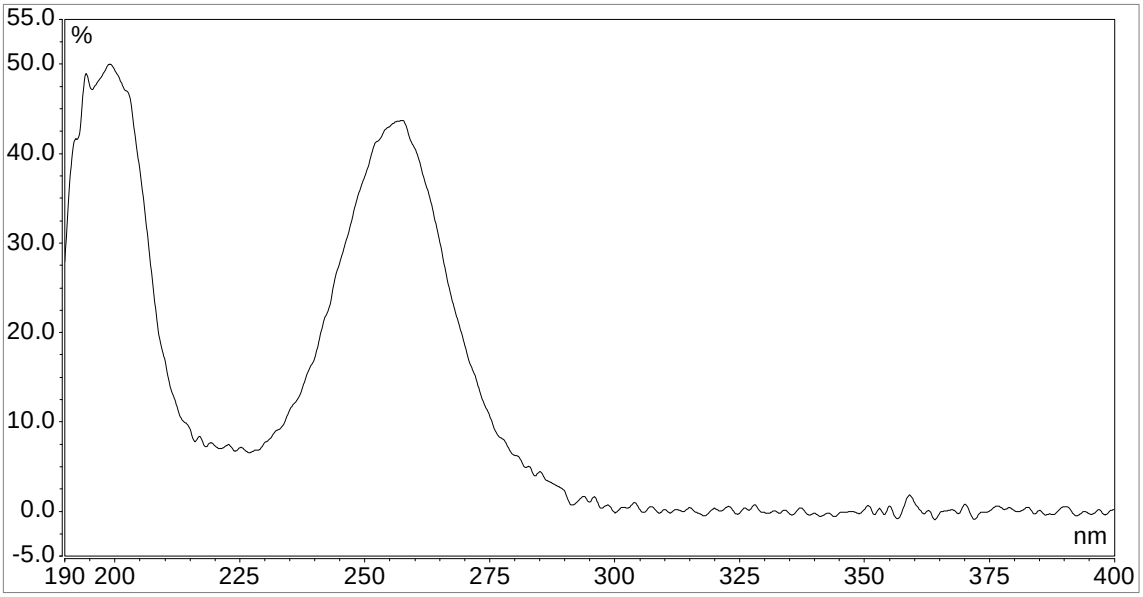


Fig. 6 – UV-Spectrum of 4-trifluoromethoxyaniline – Triflumuron SC (spiked with reference item)

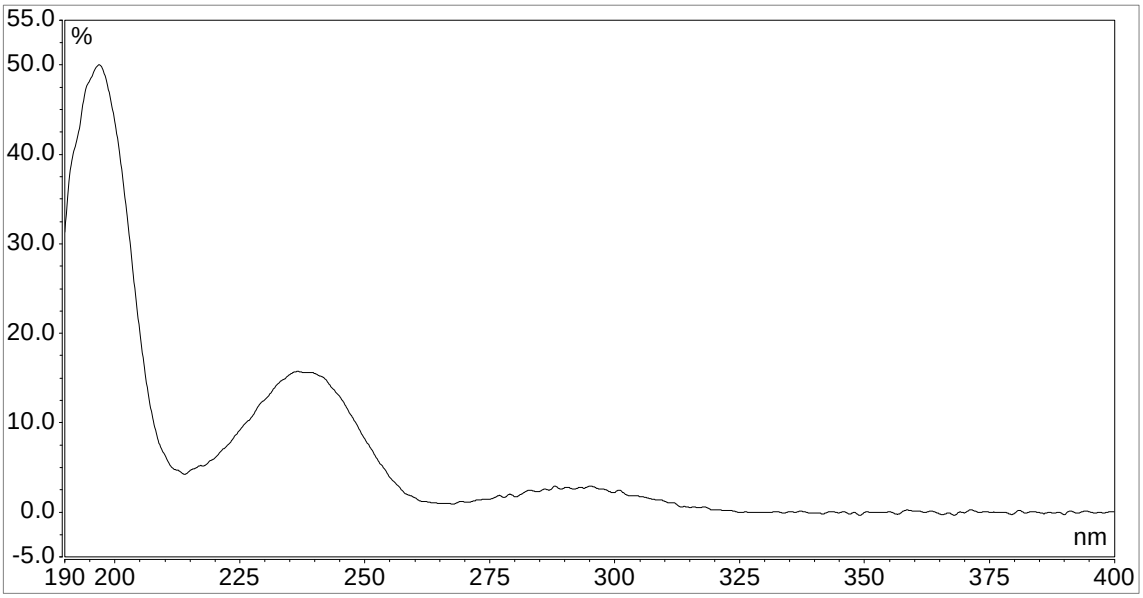


Fig. 7 Chromatogram of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline Analytical Standards – Lab I

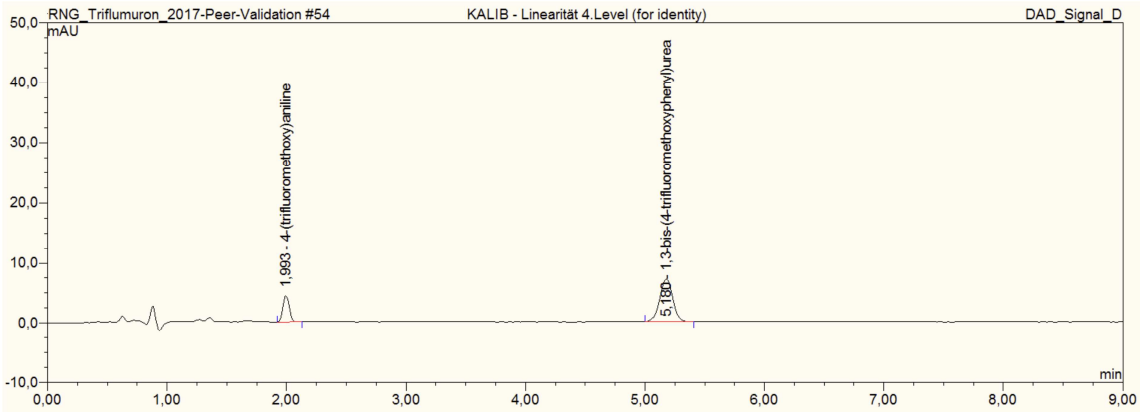


Fig. 8 Chromatogram of Triflumuron TC – Lab I

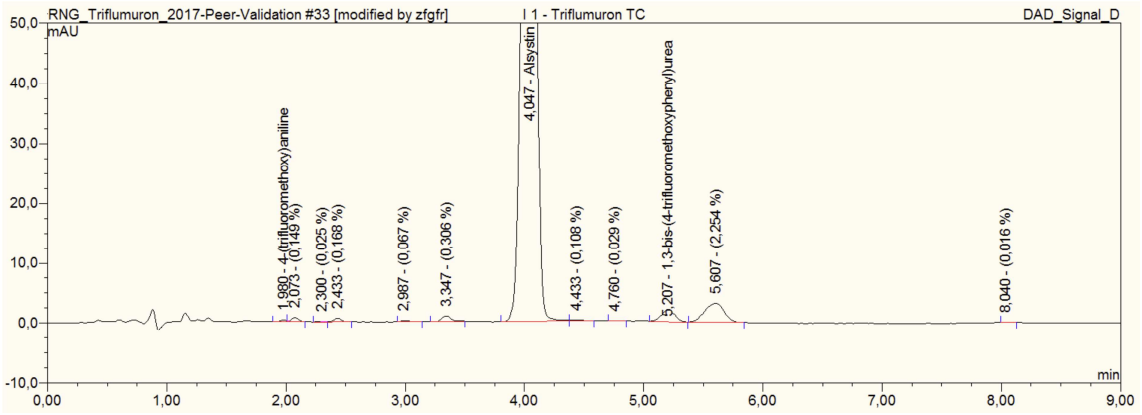


Fig. 9 Chromatogram of Triflumuron SC – Lab I

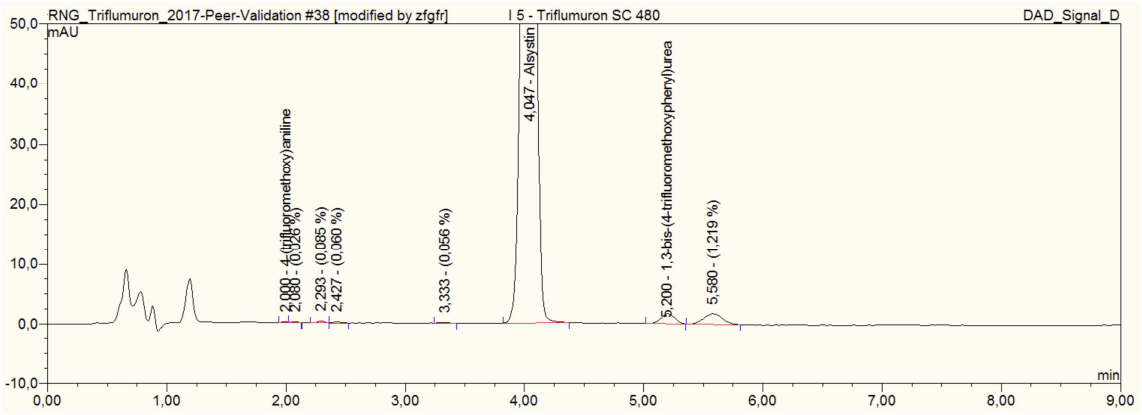


Fig. 10 Chromatogram of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline Analytical Standards – Lab II

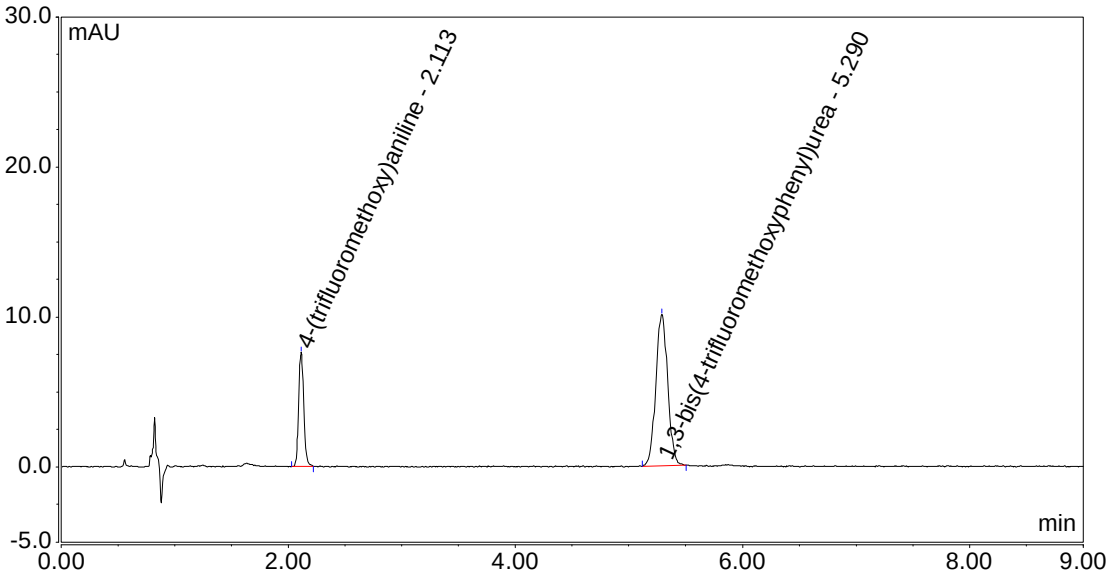


Fig. 11 Chromatogram of Triflumuron TC – Lab II

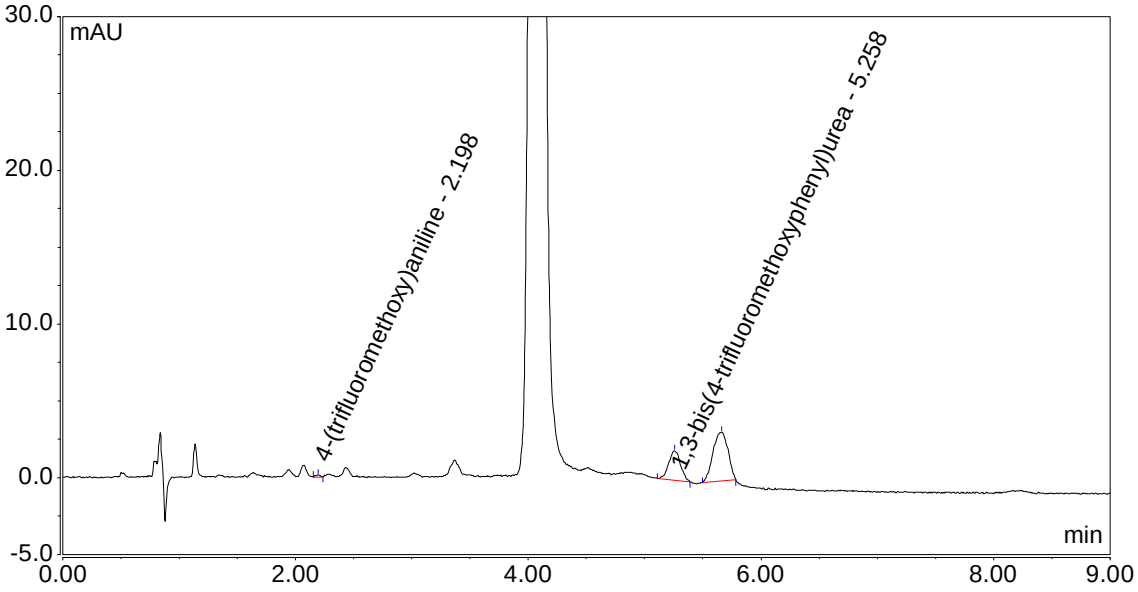


Fig. 12 Chromatogram of Triflumuron SC – Lab II

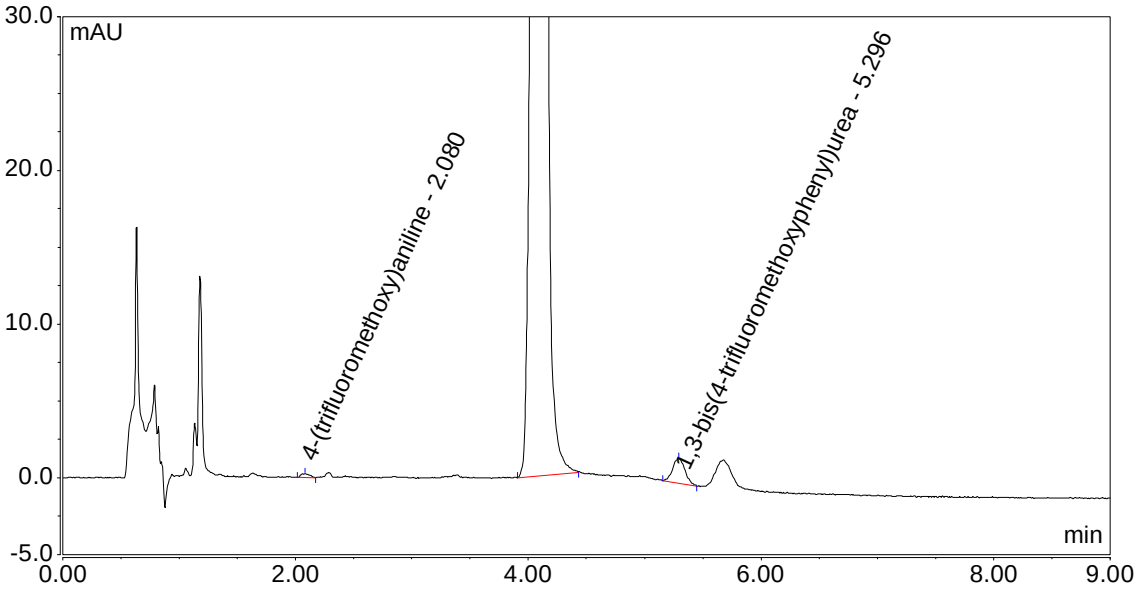


Fig. 13 Chromatogram of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline Analytical Standards – Lab III

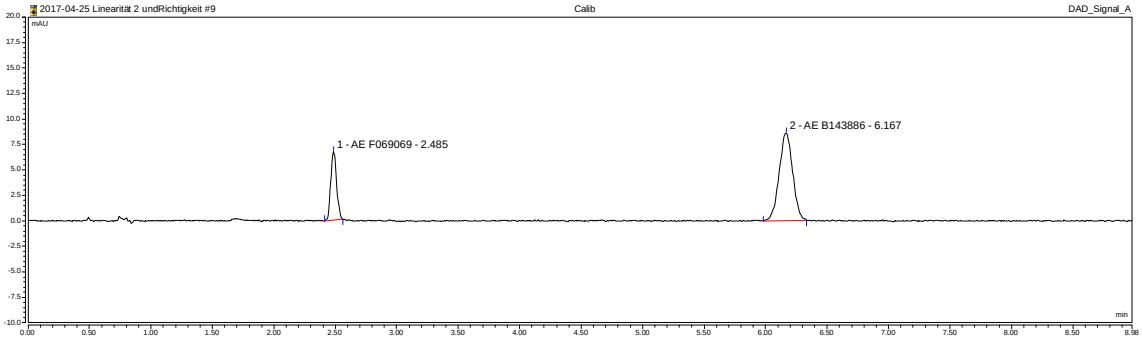


Fig. 14 Chromatogram of Triflumuron TC – Lab III

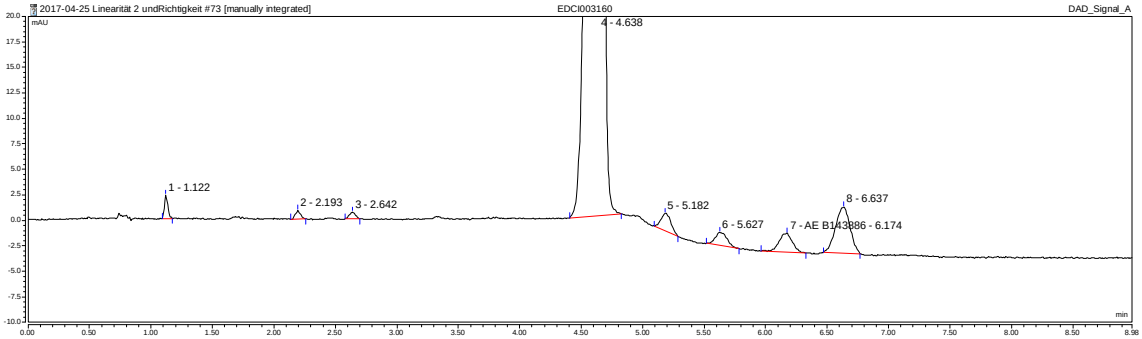


Fig. 15 Chromatogram of Triflumuron SC – Lab III

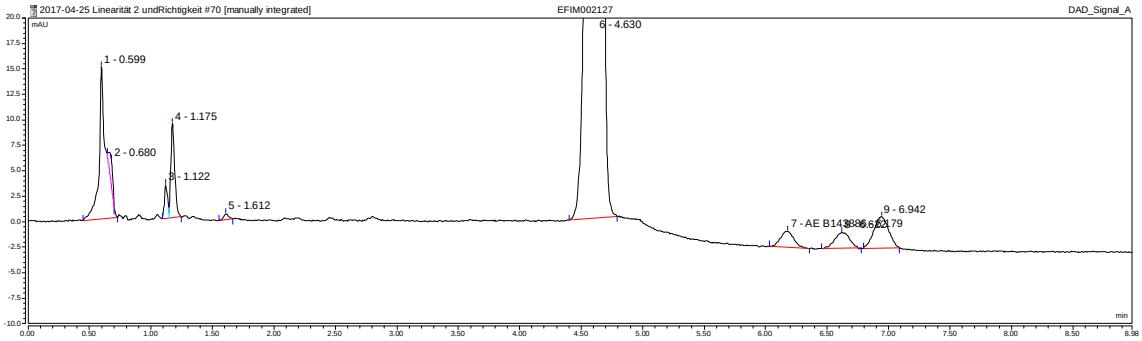


Fig. 16 Chromatogram of 1,3-bis(4-trifluoromethoxyphenyl)urea (258 nm) Analytical Standards – Lab IV

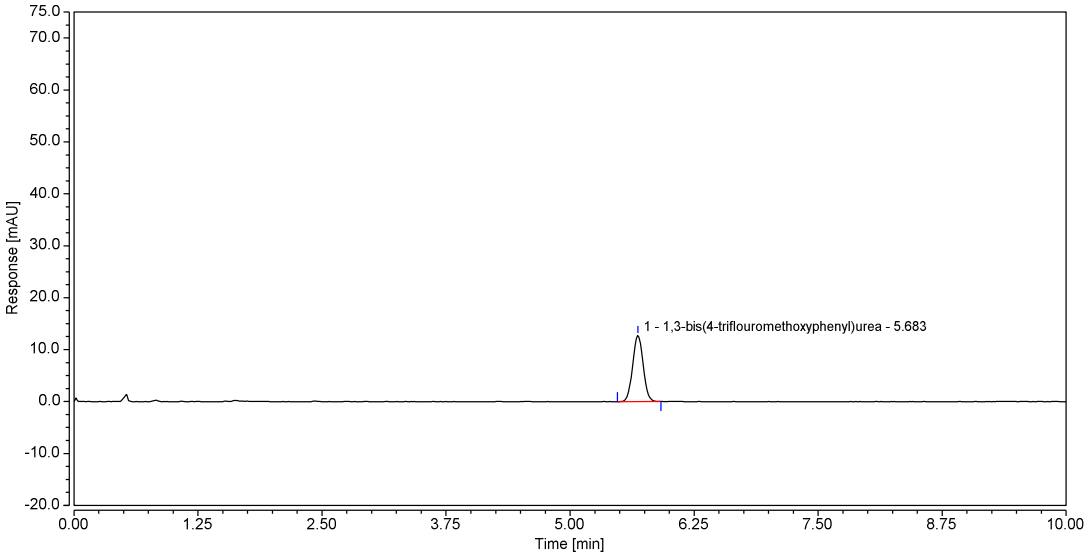


Fig. 17 Chromatogram of 4-(trifluoromethoxy)aniline (226 nm) Analytical Standards – Lab IV

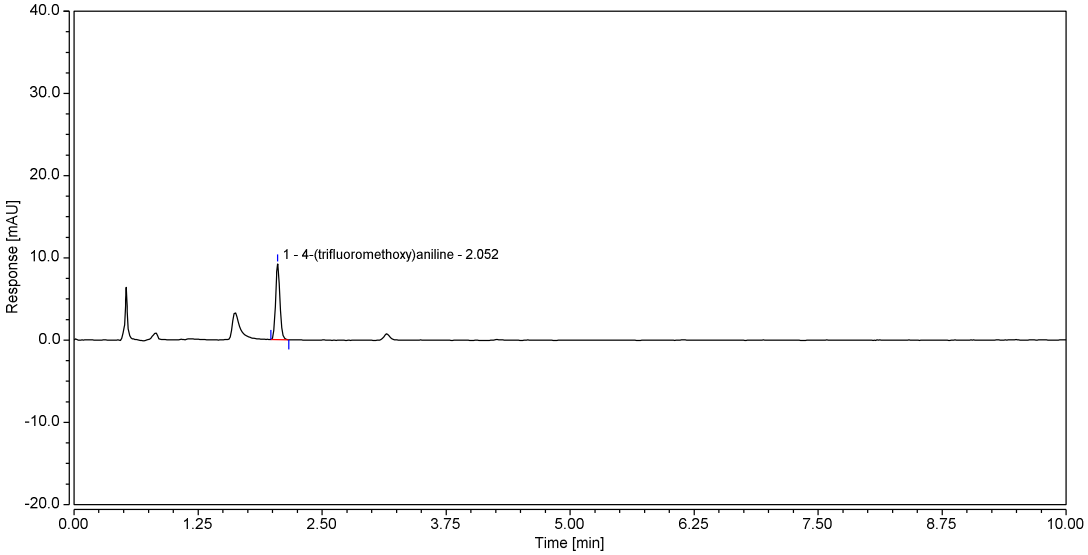


Fig. 18 Chromatogram of Triflumuron TC (258 nm) – Lab IV

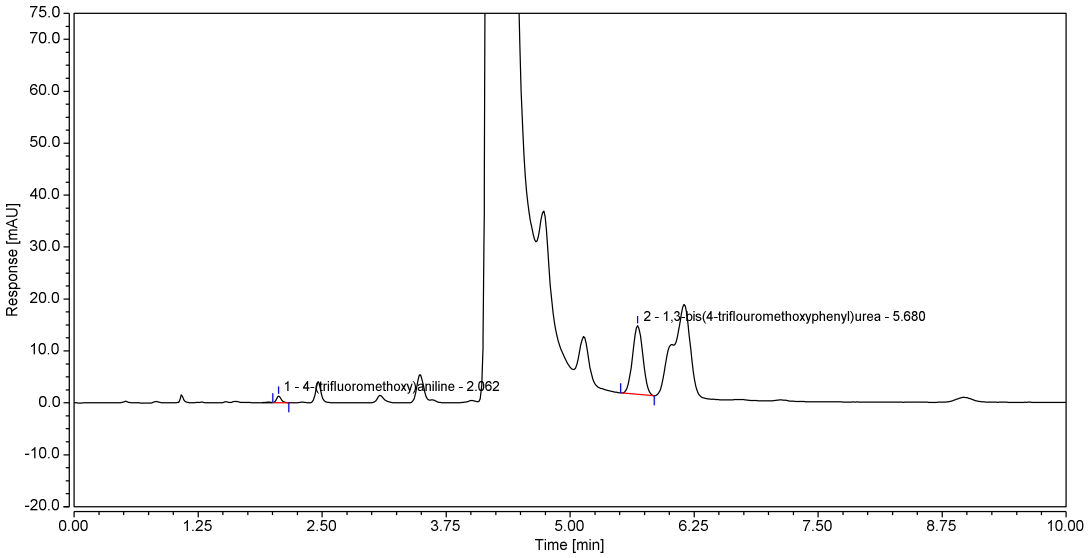


Fig. 19 Chromatogram of Triflumuron TC (226 nm) – Lab IV

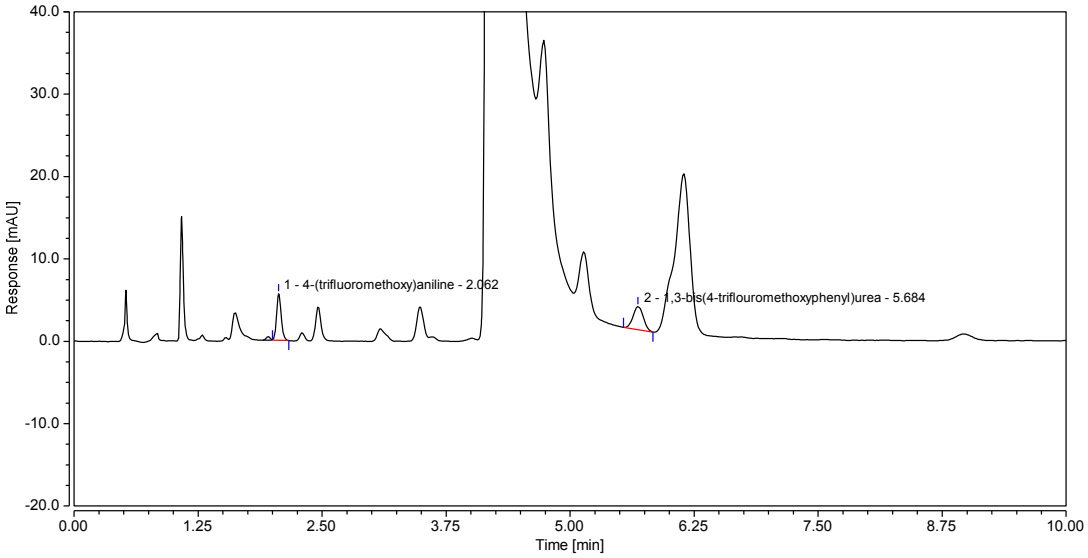


Fig. 20 Chromatogram of Triflumuron SC (258 nm) – Lab IV

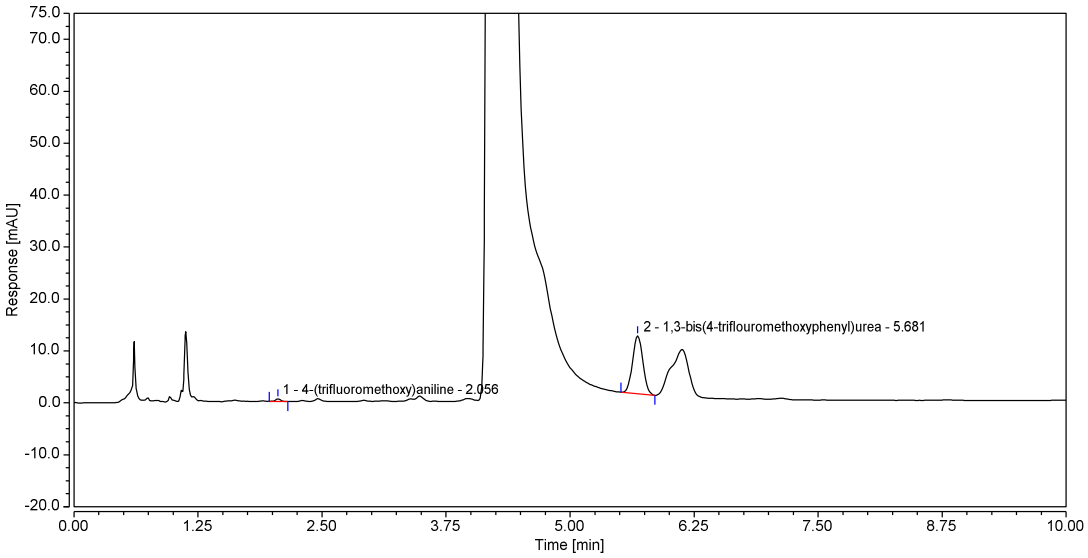


Fig. 21 Chromatogram of Triflumuron SC (226 nm) – Lab IV

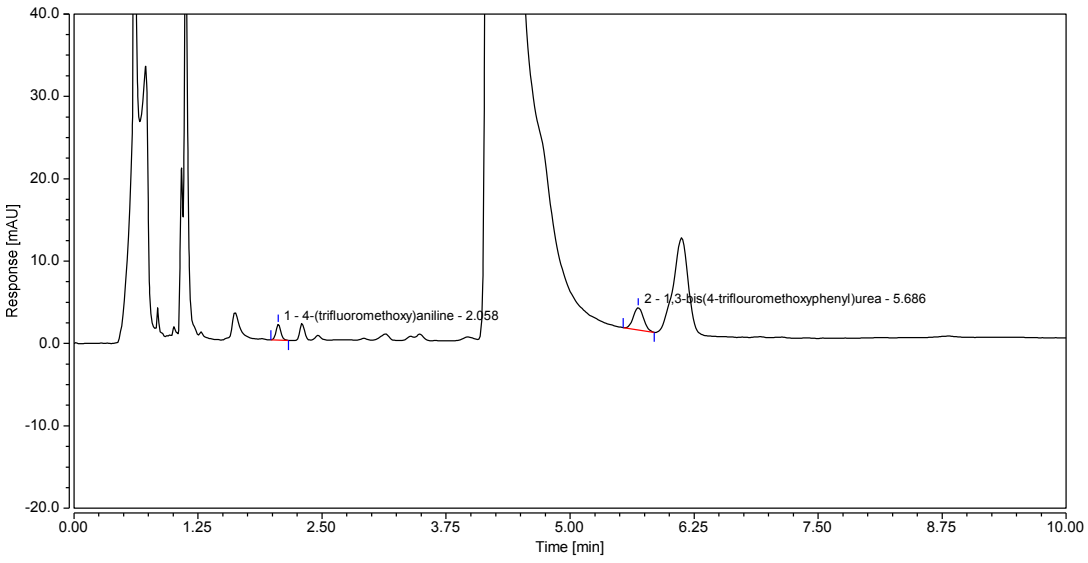
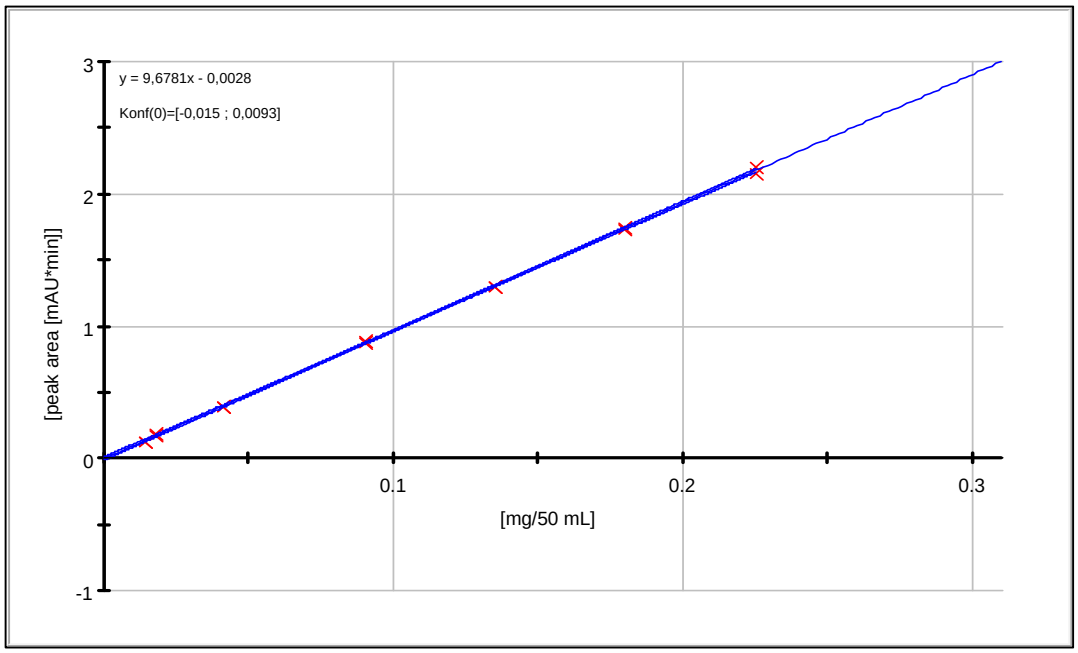
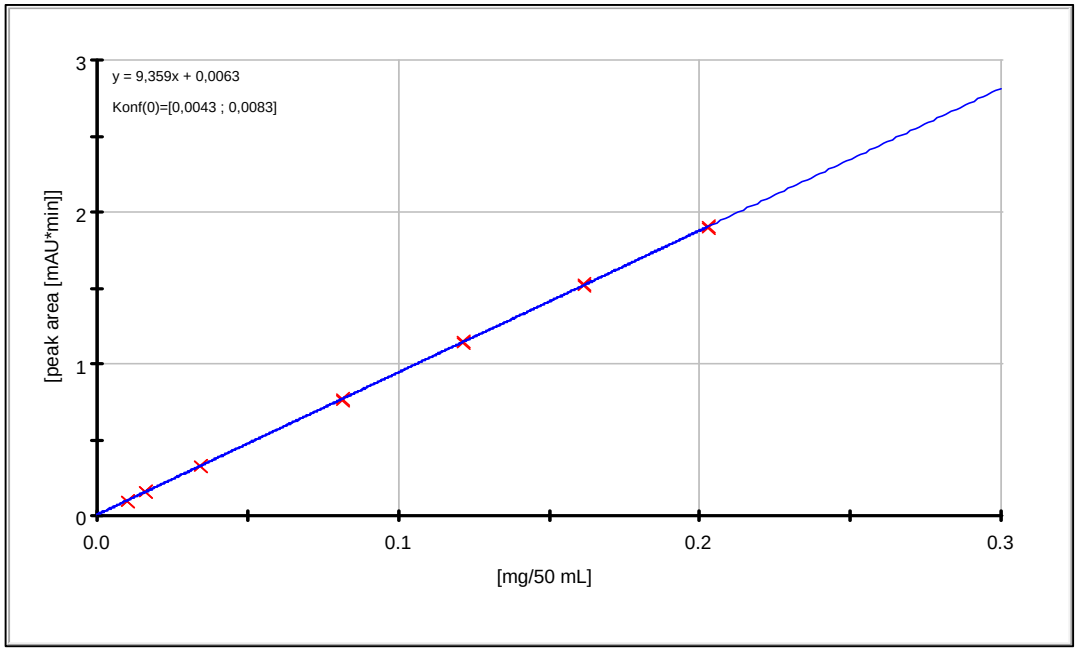


Fig. 22 – Linearity of 1,3-bis(4-trifluoromethoxyphenyl)urea – Lab I



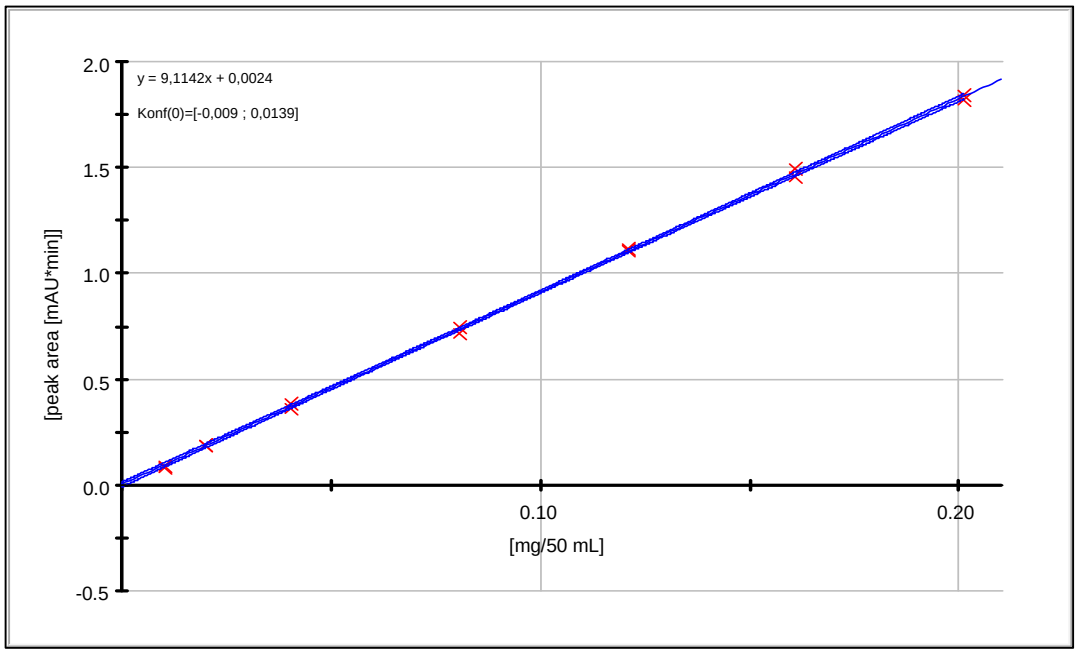
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = - 0.0028 + 9.6781 x
Correlation coefficient	**1.0000**

Fig. 23 – Linearity of 1,3-bis(4-trifluoromethoxyphenyl)urea – Lab II



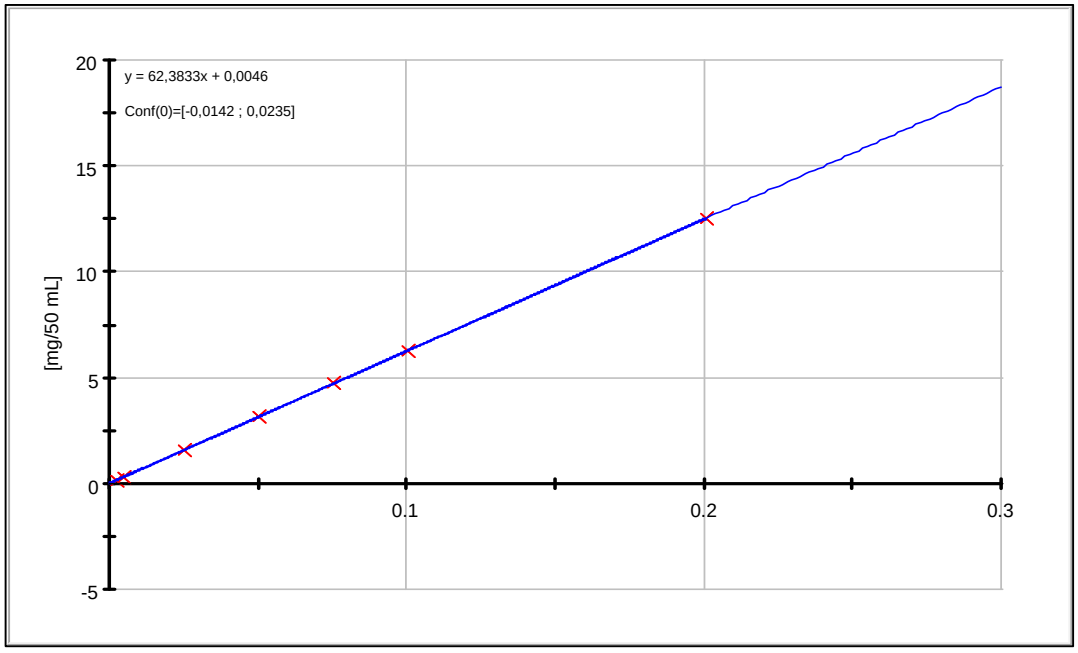
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = 0.0063 + 9.359 x
Correlation coefficient	**1.0000**

Fig. 24 – Linearity of 1,3-bis(4-trifluoromethoxyphenyl)urea – Lab III



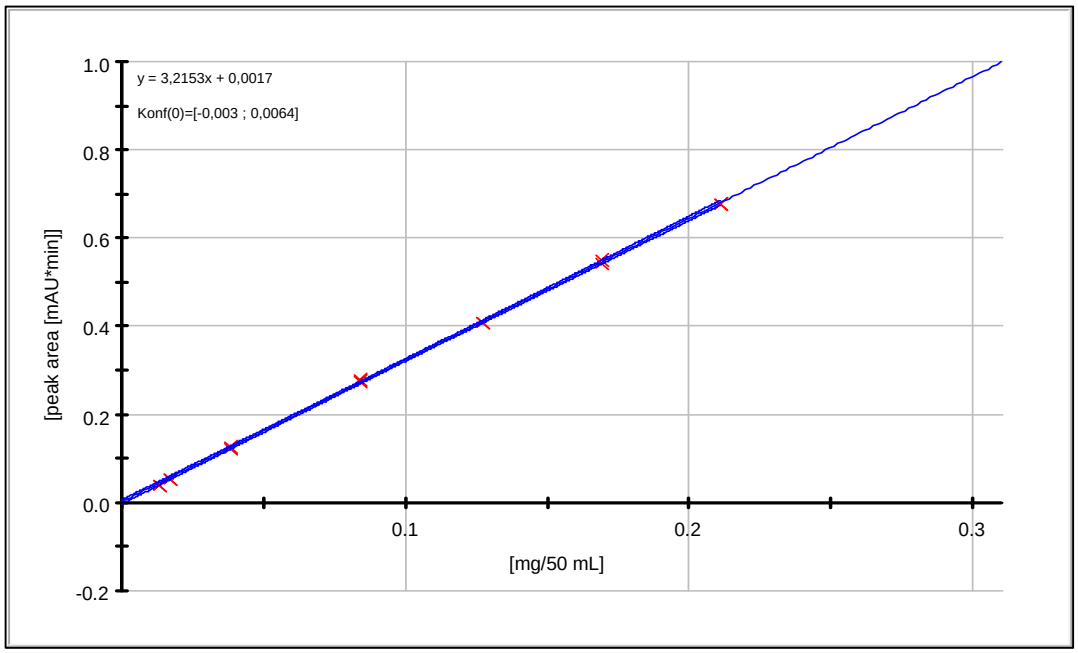
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = 0.0024 + 9.1142 x
Correlation coefficient	**1.0000**

Fig. 25 – Linearity of 1,3-bis(4-trifluoromethoxyphenyl)urea – Lab IV



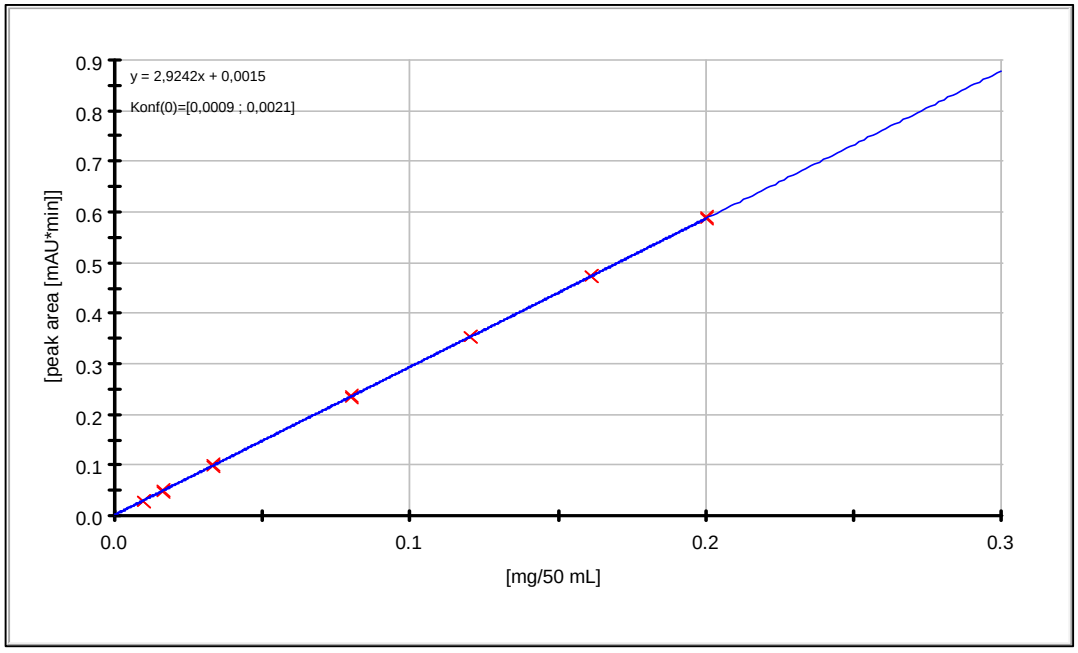
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = 0.0046 + 62.3833 x
Correlation coefficient	**1.0000**

Fig. 26 – Linearity of 4-trifluoromethoxyaniline – Lab I



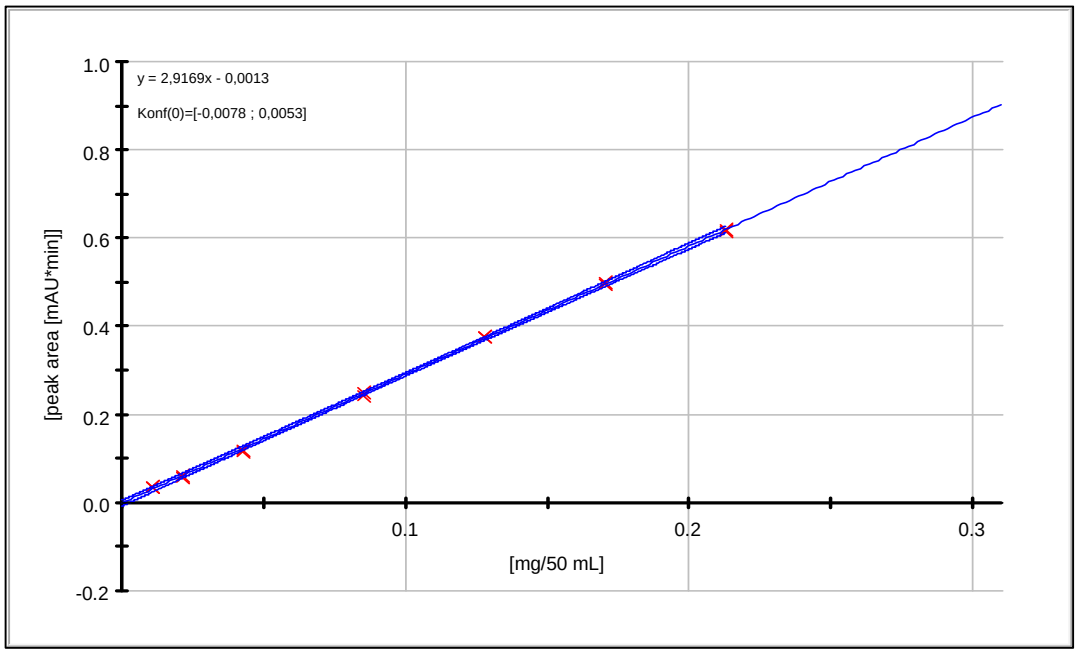
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = 0.0017 + 3.2153 x
Correlation coefficient	**0.9999**

Fig. 27 – Linearity of 4-trifluoromethoxyaniline – Lab II



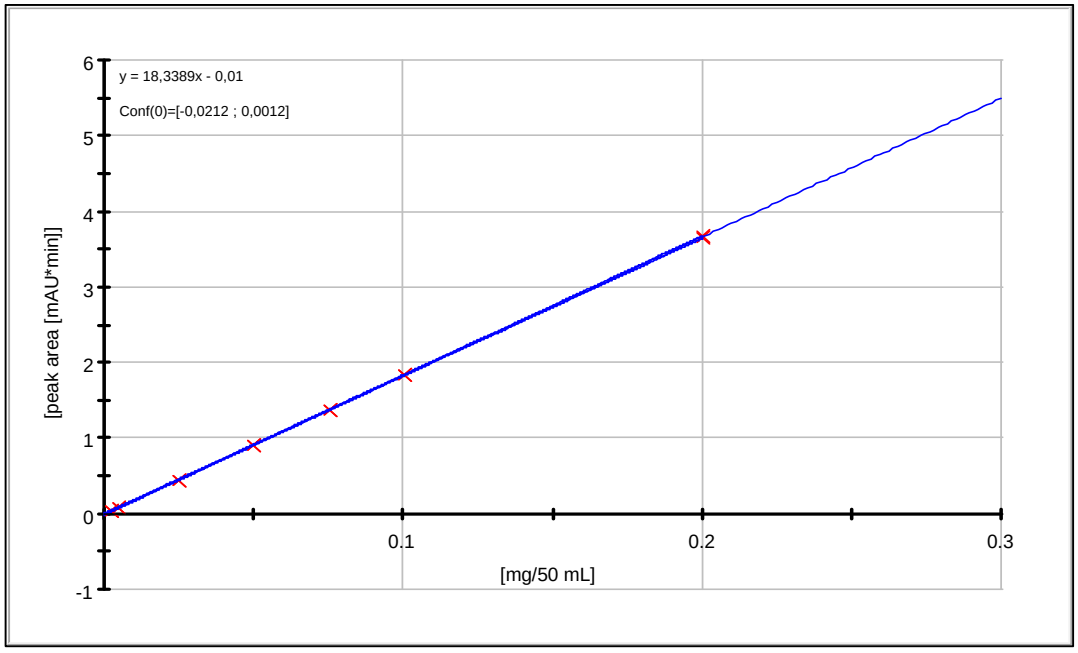
Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = 0.0015 + 2.9242 x
Correlation coefficient	**1.0000**

Fig. 28 – Linearity of 4-trifluoromethoxyaniline – Lab III



Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = - 0.0013 + 2.9169 x
Correlation coefficient	**0.9999**

Fig. 29 – Linearity of 4-trifluoromethoxyaniline – Lab IV



Number of values	**n = 7**
Regression equation	**y = a + b x (1st order)**
	y = - 0.01 + 18.3389 x
Correlation coefficient	**1.0000**