Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Chlorinated cobalt alkyne complexes derived from acetylsalicylic acid as new specific antitumor agents

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Synthesis and characterization of intermediate products

6-Chlorosalicylic acid

From 4.0 g 2-chloro-6-fluorobenzoic acid (22.9 mmol), 3.7 g NaOH (91.7 mmol) and 45 ml anhydrous DMSO as solvent (12 h, 130 °C). After cooling to room temperature, the reaction mixture was diluted with 100 ml water and acidified with 1M HCl (pH 1). Water phase was extracted four times with 100 mL diethyl ether. Collected ether phases were washed with water and saturated NaCl solution and dried over $NaSO_4$. Diethyl ether was removed under reduced pressure. Purification: recrystallization from toluene; white solid; yield: 2.34 g (13.56 mmol, 59%); mp 122 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.37 (dd, ^{3}J = 8.1 Hz, 1H, ArH-4), 7.02 (dd, ^{3}J = 8.0 Hz, ^{4}J = 0.8 Hz, 1H, ArH), 6.96 (dd, ^{3}J = 8.0 Hz, ^{4}J = 0.8 Hz, 1H, ArH); IR (ν cm^{-1}): 3100-2300 br (OH), 1630 s (C=O), 1590 s, 1564 s (C=C), 1223 s, 1192 s (C-O).

General procedure for the synthesis of chloroacetylsalicylic acids

1.0 g of chlorosalicylic acid (5.8 mmol) was dissolved in 20 ml of THF and added dropwise under cooling to a mixture of 20 ml of THF, 58 mmol acetic anhydride and 11.6 mmol of TEA. The mixture was stirred at room temperature until reaction was completed. 80 ml water was added and the reaction mixture was acidified with 1M HCl, then extracted three times with 50 ml of diethyl ether. Collected ether phases were washed with water and saturated NaCl solution and removed under reduced pressure. If necessary, product was purified with column chromatography prior to recrystallization. The product was recrystallized from toluene or a toluene-petroleum mixture.

3-Chloroacetylsalicylic acid (3-Cl-ASS)

Reaction time: 22.5 h; purification: recrystallization from petroleum/toluene (2:1); white solid; yield: 0.94 g (4.38 mmol, 76%); mp 122 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.02 (dd, ^{3}J = 8.0 Hz, ^{4}J = 1.8 Hz, 1H, ArH-6), 7.70 (dd, ^{3}J = 8.4 Hz, ^{4}J = 1.8 Hz, 1H, ArH-4), 7.31 (dd, ^{3}J = 7.8 Hz, 1H, ArH-5), 2.40 (s, 3H, CH_3); IR (ν cm^{-1}): 3100-2400 br (OH), 1774 s, 1686 s (C=O), 1592 m, 1568 w (C=C), 1366 m (δ CH_3), 1274 m, 1181 s (C-O).

4-Chloroacetylsalicylic acid (4-Cl-ASS)

Reaction time: 19 h; purification: recrystallization from petroleum/toluene (2:1); white solid; yield: 1.06 g (4.94 mmol, 85%); mp 142 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.06 (d, ^{3}J = 8.6 Hz, 1H, ArH-6), 7.34 (dd, ^{3}J = 8.6 Hz, ^{4}J = 1.8 Hz, 1H, ArH-5), 7.17 (d, ^{4}J = 1.8 Hz, 1H, ArH-3), 2.34 (s, 3H, CH_3); IR (ν cm^{-1}): 3100-2400 br (OH), 1759 s, 1684 s (C=O), 1594 m (C=C), 1366 m (δ CH3), 1278 s, 1188 s (C-O).

5-Chloroacetylsalicylic acid (5-Cl-ASS)

Reaction time: 3 h; purification: recrystallization from toluene; white solid; yield: 0.84 g (3.91 mmol, 68%); mp 142 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.09 (d, ^4J = 2.4 Hz, 1H, ArH-6), 7.58 (dd, ^3J = 8.4 Hz, ^4J = 2.4 Hz, 1H, ArH-4), 7.10 (d, ^3J = 8.4 Hz, 1H, ArH-3), 2.34 (s, 3H, CH_3); IR (ν cm^{-1}): 3100-2400 br (OH), 1751 s, 1688 s (C=O), 1600 m, 1562 m (C=C), 1364 m (δ CH3), 1303 s, 1193 s, 1102 s (C-O).

6-Chloroacetylsalicylic acid (6-Cl-ASS)

Reaction time: 20 h; purification: column chromatography using ethyl acetate/petroleum/glacial acetic acid (8:6:0.5), recrystallization from petroleum/toluene (2:1); white solid; yield: 0.92 g (4.29 mmol, 74%); mp 121 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.43 (dd, ^3J = 8.0 Hz, 1H, ArH-4), 7.36 (dd, ^3J = 8.0 Hz, ^4J = 1.8 Hz, 1H, ArH-5), 7.12 (dd, ^3J = 7.6 Hz, ^4J = 1.8 Hz, 1H, ArH-3), 2.31 (s, 3H, CH_3); IR (ν cm^{-1}): 3100-2700 br (OH), 1771 s, 1716 s, 1651 m (C=O), 1596 s, 1576 m (C=C), 1365 m (δ CH_3), 1258 s, 1179 s (C-O).

General procedure for the synthesis of chloroacetylsalicylic acid propargyl ester

The Cl-ASS derivative was dissolved in 10 ml anhydrous DCM. 0.2 equivalent DMAP and 1.1 equivalent propargyl alcohol were added under stirring, followed by the addition of 1.1 equivalent DCC under ice cooling. After 5 min of vigorous stirring, the ice bath was removed and reaction mixture was stirred at room temperature for 2 – 3 h. A white precipitate was built (dicyclohexylurea, DCU), which was removed by filtration. The basic solvent was evaporated, the residue was dissolved in DCM und if necessary filtrated again. DCM phase was washed twice with 0.5M HCl and saturated $NaHCO_3$ solution, dried over $NaSO_4$ and evaporated under reduced pressure. As resulting yellowish oil is a mixture of the salicylic acid ester and the acetylsalicylic acid ester, acetylation was carried out again.

General procedure for the (re-)acetylation of chlorosalicylic acid propargyl ester

The (re-)acetylation was conducted analogously to the synthesis of chloroacetylsalicylic acids. Although, the starting material was a mixture of salicylic acid and acetylsalicylic acid ester, calculation of reaction mixture was calculated based on the pure chlorosalicylic acid ester. Workup was performed as described in the *General procedure for the synthesis of chloroacetylsalicylic acids*, except for the acidification before extraction which was not necessary. After removal of the solvent under reduced pressure, possibly generated DCU crystals were removed by filtration after dissolution in toluene. Toluene was removed under reduced pressure, a yellowish solid crystallizes, which was resuspended in petroleum. The product was collected on a frit and washed with petroleum.

2-Acetoxy-3-chlorobenzoic acid prop-2-inyl ester (3-Cl-ASS propargyl ester)

From 0.70 g 3-chloroacetylsalcylic acid (3.26 mmol), 0.21 ml propargyl alcohol (3.59 mmol), 0.74 g DCC (3.59 mmol) and 0.08 g DMAP (0.65 mmol). White crystalline solid; yield: 0.59 g (2.34 mmol, 67%); mp 61 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.01 (d, ^3J = 8.8 Hz, 1H, ArH-6), 7.31 (dd, ^3J = 8.4 Hz, ^4J = 2.2 Hz, 1H, ArH-5), 7.15 (d, ^3J = 2.2 Hz, 1H, ArH-3), 4.87 (d, ^4J = 2.6 Hz, 2H, CH_2), 2.54 (t, ^4J = 2.4 Hz, 1H, CH), 2.37 (s, 3H, CH_3); IR (ν cm^{-1}): 3249 m (C≡C-H), 3081 w (Aryl-C-H), 2944 w, 2855 w (C-H), 2129 w (C≡C), 1761 s, 1718 s (C=O), 1600 s, 1568 m (C=C), 1372 m (δ -CH_3), 1262 s, 1202 s, 1099 s (C-O).

2-Acetoxy-4-chlorobenzoic acid prop-2-inyl ester (4-Cl-ASS propargyl ester)

From 0.70 g 4-chloroacetylsalcylic acid (3.26 mmol), 0.21 ml propargyl alcohol (3.59 mmol), 0.74 g DCC (3.59 mmol) and 0.08 g DMAP (0.65 mmol). White crystalline solid; yield: 0.50 g (1.98 mmol, 51%); mp 49 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.01 (d, ^3J = 8.8 Hz, 1H, ArH-6), 7.31 (dd, ^3J = 8.4 Hz, ^4J = 2.2 Hz, 1H, ArH-5), 7.15 (d, ^3J = 2.2 Hz, 1H, ArH-3), 4.87 (d, ^4J = 2.6 Hz, 2H, CH_2), 2.54 (t, ^4J = 2.4 Hz, 1H, CH), 2.37 (s, 3H, CH_3); IR (ν cm^{-1}): 3249 m (C≡C-H), 3081 w (Aryl-C-H), 2944 w, 2855 w (C-H), 2129 w (C≡C), 1761 s, 1718 s (C=O), 1600 s, 1568 m (C=C), 1372 m (δ -CH_3), 1262 s, 1202 s, 1099 s (C-O).

2-Acetoxy-5-chlorobenzoic acid prop-2-inyl ester (5-Cl-ASS propargyl ester)

From 0.80 g 5-chloroacetylsalcylic acid (3.73 mmol), 0.24 ml propargyl alcohol (4.10 mmol), 0.85 g DCC (4.10 mmol) and 0.09 g DMAP (0.65 mmol). White crystalline solid; yield: 0.60 g (2.37 mmol, 64%); mp 55 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 8.03 (d, ^4J = 3.0 Hz, 1H, ArH-6), 7.54 (dd, ^3J = 8.8 Hz, ^4J = 3.0 Hz, 1H, ArH-4), 7.07 (d, ^3J = 8.8 Hz, 1H, ArH-3), 4.88 (d, ^4J = 2.4 Hz, 2H, CH_2), 2.55 (t, ^4J = 2.5 Hz, 1H, CH), 2.37 (s, 3H, CH_3); IR (ν cm^{-1}): 3246 m (C≡C-H), 3116 w, 3068 w (Aryl-C-H), 2931 w, 2850 w (C-H), 2128 w (C≡C), 1749 s, 1720 s (C=O), 1601 w, 1574 w (C=C), 1370 m (δ -CH_3), 1202 s, 1081 s (C-O).

2-Acetoxy-6-chlorobenzoic acid prop-2-inyl ester (6-Cl-ASS propargyl ester)

From 0.47 g 6-chloroacetylsalcylic acid (2.19 mmol), 0.14 ml propargyl alcohol (2.41 mmol), 0.50 g DCC (2.41 mmol) and 0.05 g DMAP (0.44 mmol). White crystalline solid; yield: 0.23 g (0.91 mmol, 35%); mp 68 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.40 (dd, ^3J = 7.9 Hz, 1H, ArH-4), 7.32 (dd, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 1H, ArH-3), 7.11 (dd, ^3J = 8.0 Hz, ^4J = 1.6 Hz, 1H, ArH-5), 4.93 (d, ^4J = 2.6 Hz, 2H, CH_2), 2.55 (t, ^4J = 2.5 Hz, 1H, CH), 2.30 (s, 3H, CH_3); IR (ν cm^{-1}): 3252 m (C≡C-H), 3081 w (Aryl-C-H), 2960 w, 2833 w, 2850 w (C-H), 2125 w (C≡C), 1768 s, 1746 s (C=O), 1595 m, 1576 m (C=C), 1369 m (δ -CH_3), 1269 s, 1182 s (C-O).

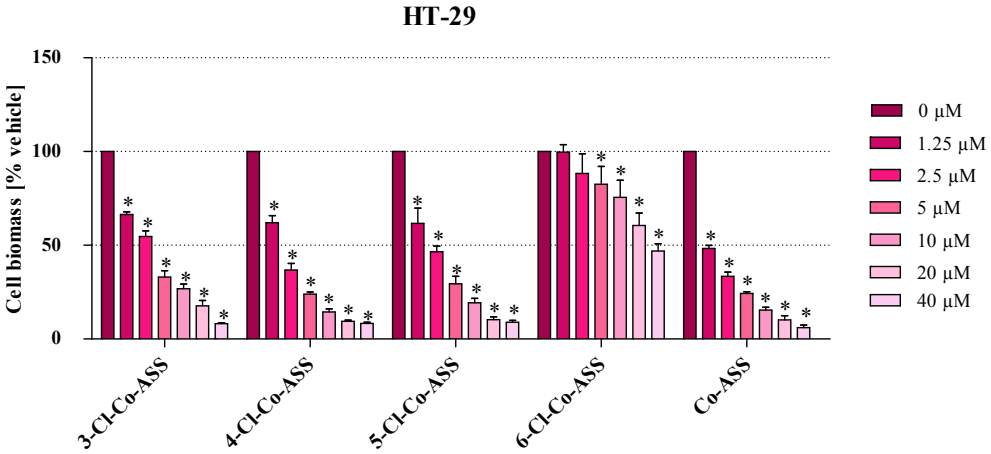
Table S1: Inhibition of COX-1/2

compound	concentration [µM]	COX-1 [%]	COX-2 [%]	PGE2 [%]
ASS	10 µM	28.8 ± 3.7	0.6 ± 2.8	2.3 ± 2.7
ASS	100 µM	73.3 ± 8.7	59.4 ± 8.7	25.4 ± 4.3
3-Cl-ASS	100 µM	27.0 ± 6.1	29.5 ± 3.5	n. t.
4-Cl-ASS	100 µM	60.2 ± 6.2	55.0 ± 2.7	n. t.
5-Cl-ASS	100 µM	56.4 ± 5.7	42.8 ± 0.4	n. t.
6-Cl-ASS	100 µM	30.0 ± 2.9	30.6 ± 3.2	n. t.
ASS-Propargyl	100 µM	25.4 ± 4.4	34.5 ± 4.2	n. t.
propargyl-3-Cl-ASS	100 µM	32.5 ± 1.9	52.2 ± 5.7	n. t.
propargyl-4-Cl-ASS	100 µM	24.0 ± 7.7	47.2 ± 5.3	n. t.
propargyl-5-Cl-ASS	100 µM	34.6 ± 0.5	52.0 ± 8.2	n. t.
propargyl-6-Cl-ASS	100 µM	37.3 ± 7.1	47.4 ± 6.0	n. t.
Co-ASS	10 µM	82.7 ± 3.2	78.5 ± 6.1	57.5 ± 3.0
3-Cl-Co-ASS	10 µM	24.9 ± 2.6	71.4 ± 8.3	37.7 ± 6.7
4-Cl-Co-ASS	10 µM	22.9 ± 1.7	69.9 ± 2.7	48.5 ± 6.8
5-Cl-Co-ASS	10 µM	25.0 ± 1.0	64.0 ± 7.5	41.9 ± 4.2
6-Cl-Co-ASS	10 µM	24.4 ± 3.0	70.5 ± 3.5	18.3 ± 1.9

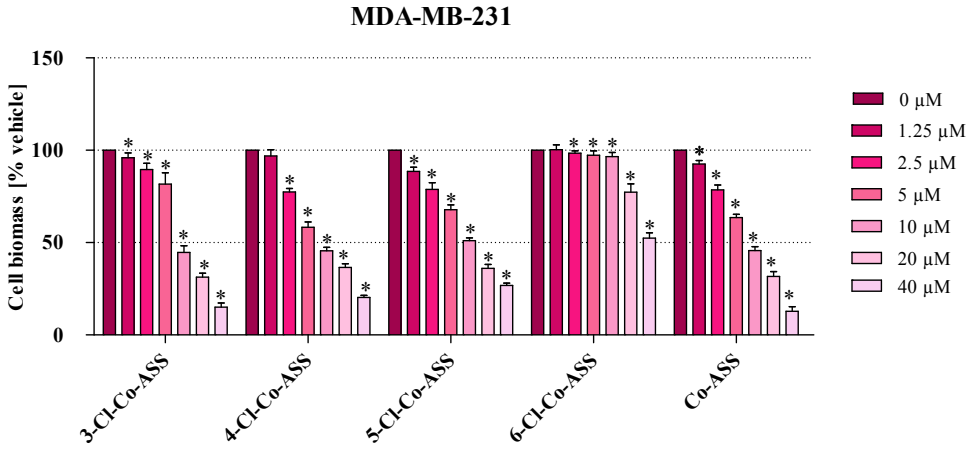
Table S2: Graphite furnace time-temperature program for Co analysis by HR CS AAS

step	operation	temperature	heating rate	hold time	argon flow
1	drying	80 °C	10 °C/s	10 s	maximal
2	drying	100 °C	10 °C/s	25 s	maximal
3	drying	120 °C	15 °C/s	10 s	maximal
4	pyrolysis	1100 °C	200 °C/s	20 s	maximal
5	auto-zero	1100 °C	0 °C/s	5 s	stop
6	atomization	2300 °C	1500 °C/s	5 s	stop
7	cleaning	2600 °C	500 °C/s	4 s	maximal

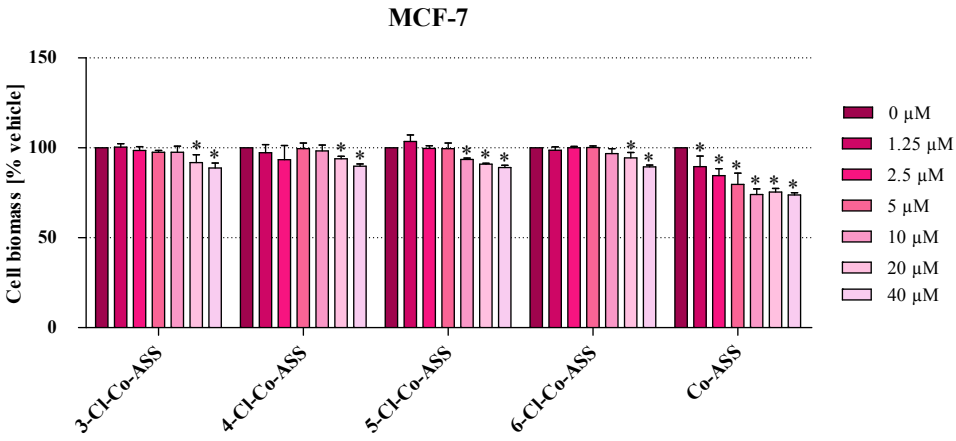
Supporting Figure 1: Proliferation of HT-29 cells



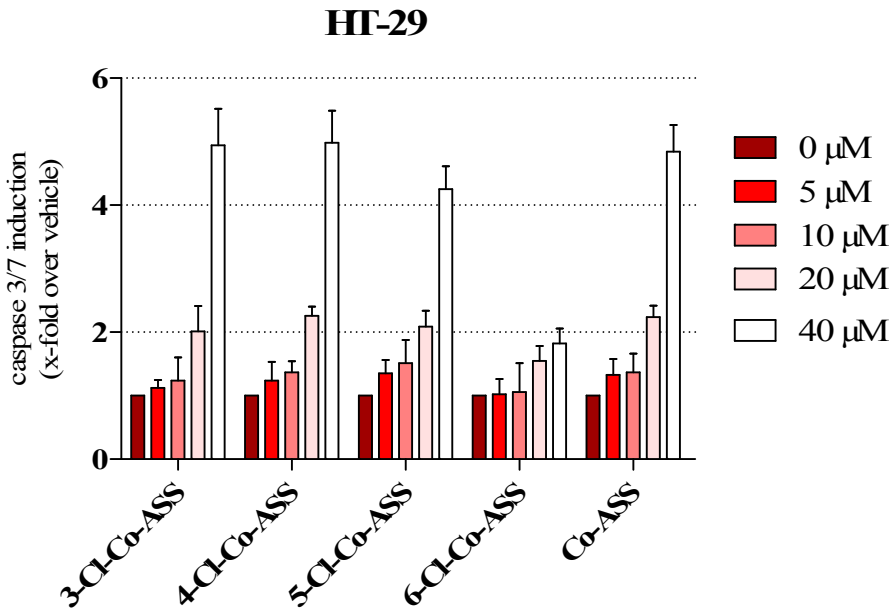
Supporting Figure 2: Proliferation of MDA-MB-231 cells



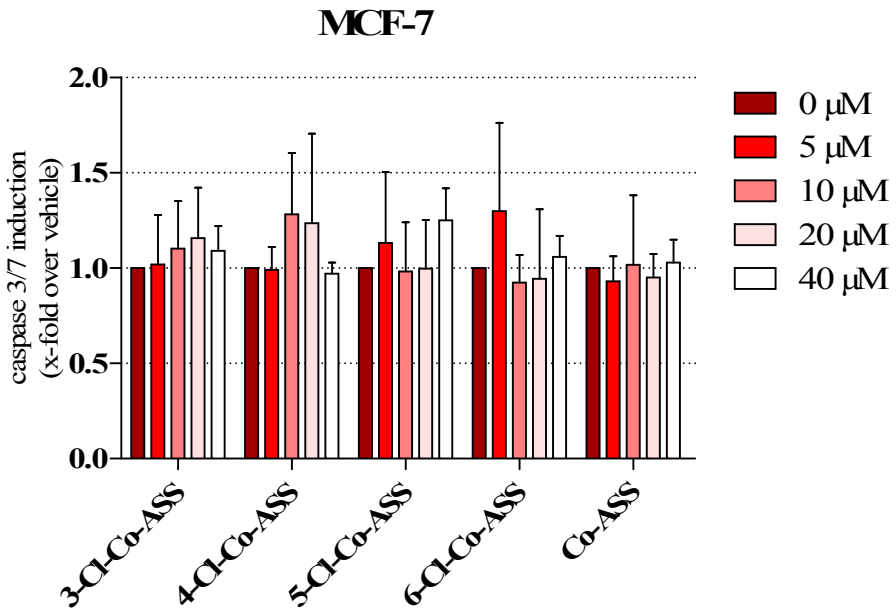
Supporting Figure 3: Proliferation of MCF-7 cells



Supporting Figure 4: Caspase-3/7 induction (HT-29)



Supporting Figure 5: Caspase-3/7 induction (MCF-7)



Supporting Figure 6: Caspase-3/7 induction (MDA-MB-231)

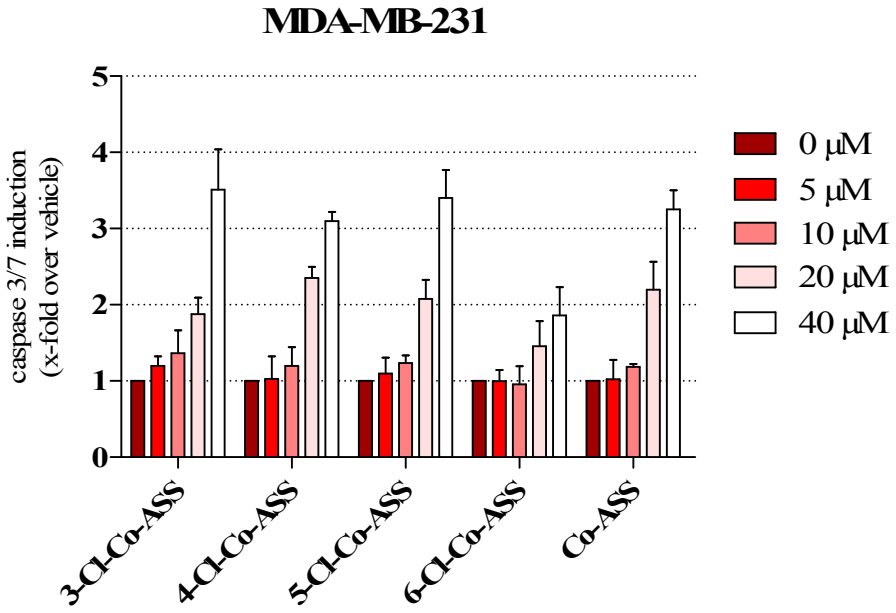
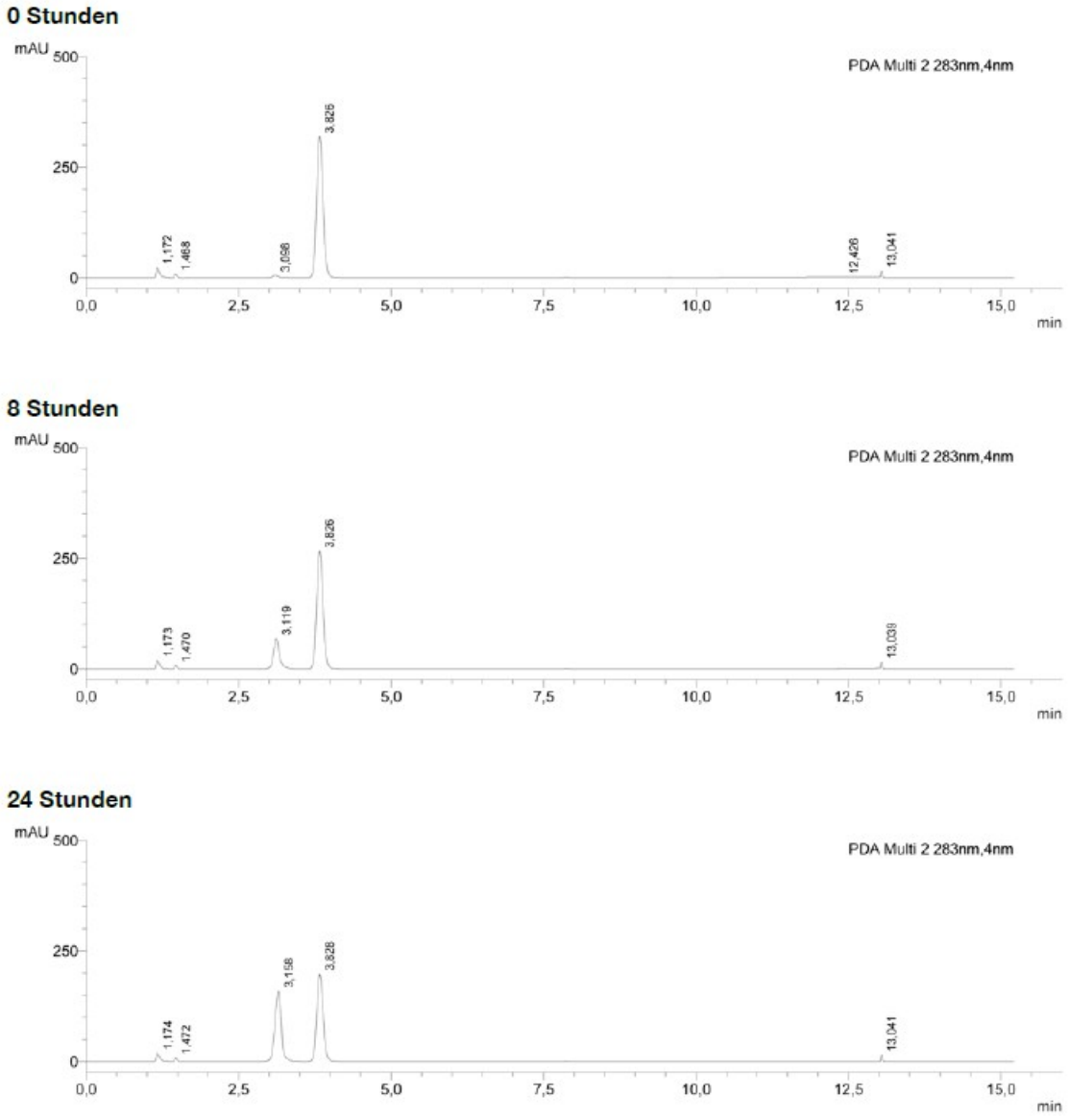


Table S3: Solvent gradient for stability analysis with HPLC

compound	Time [min]	ACN [%]	AcOH (1%) [%]
3-Cl-ASS, 4-Cl-ASS, 5-Cl-ASS, 3-Cl-ASS propargyl ester, 4-Cl-ASS propargyl ester, 5-Cl-ASS propargyl ester	0 – 2	40	60
	2 – 6	40 – 60	60 – 40
	6 – 11	60 – 100	40 – 0
	11 – 11.5	100	0
	11.5 – 15	40	60
6-Cl-ASS, 6-Cl-ASS propargyl ester	0 – 2	20	80
	2 – 6	20 – 40	80 – 60
	6 – 11	40 – 90	60 – 10
	11 – 11.5	90 – 20	10 – 80
	11.5 – 15	20	80

Supporting Figure 7: Stability of 3-Cl-ASS



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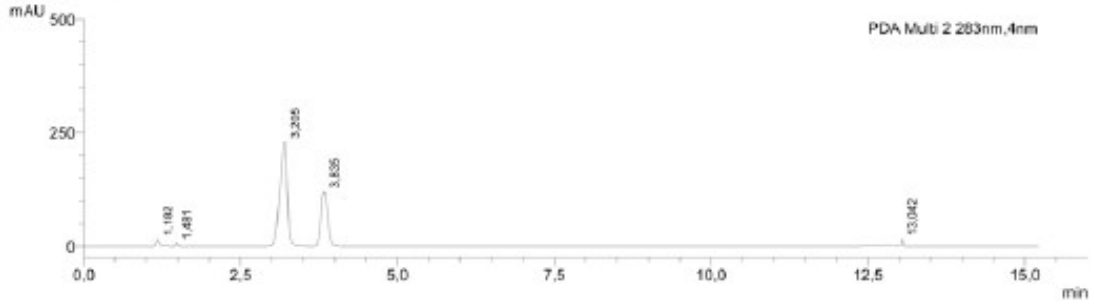

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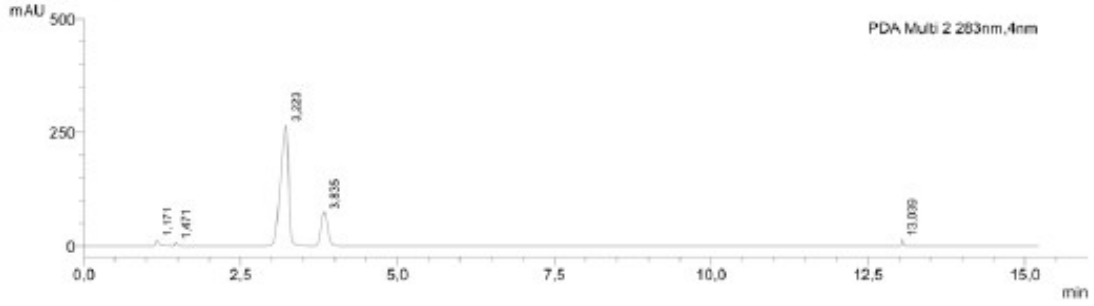

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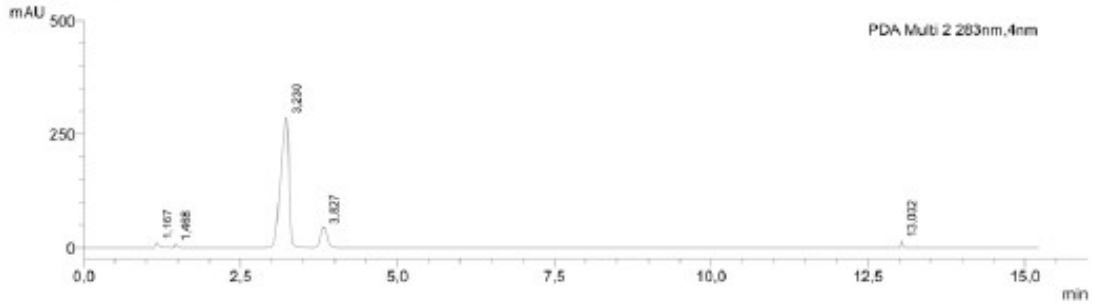

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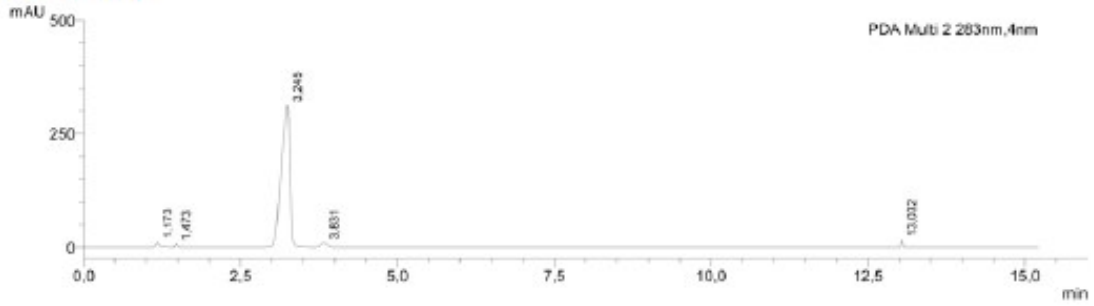
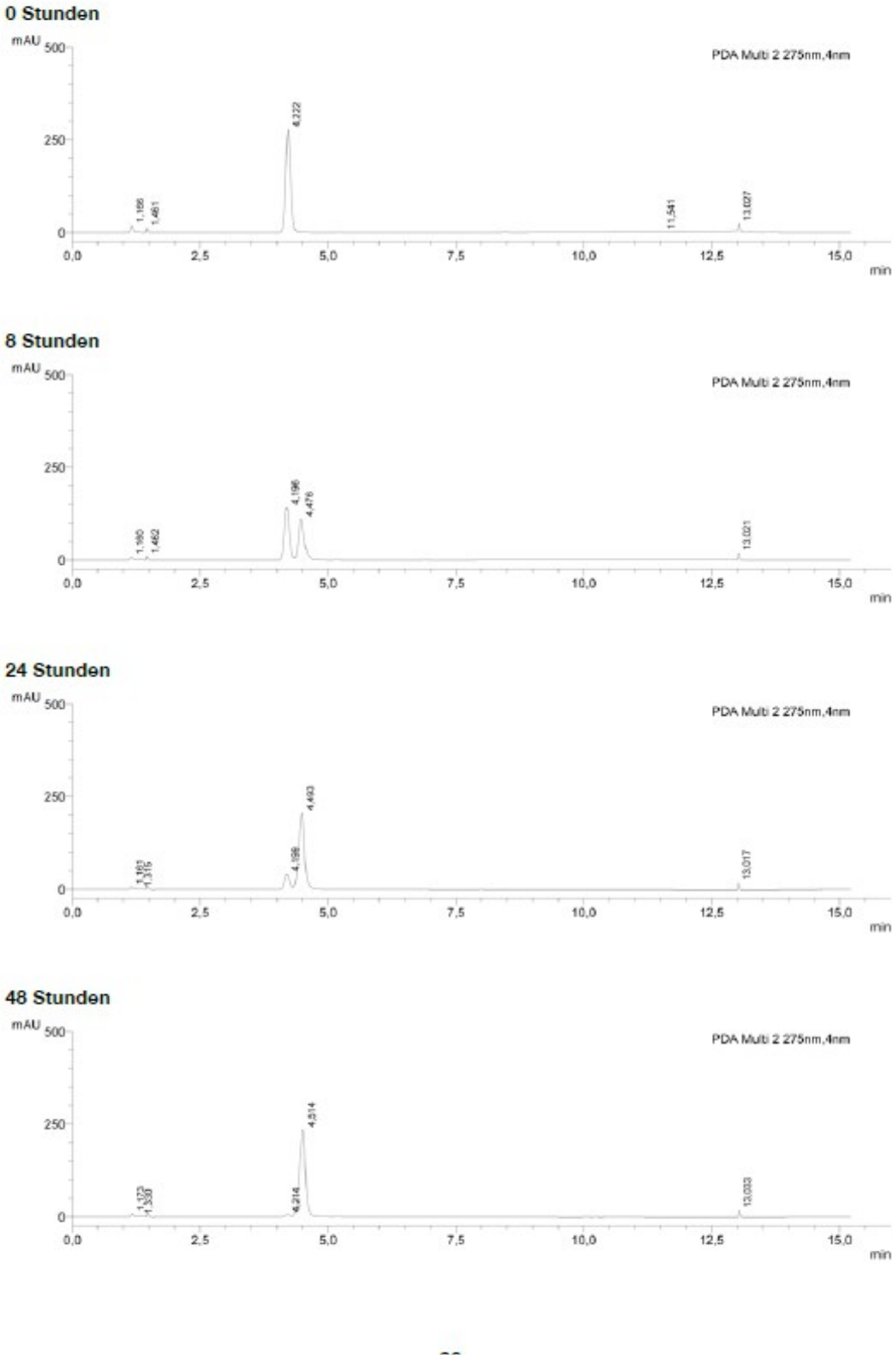


Supporting Figure 8: Stability of 4-Cl-ASS



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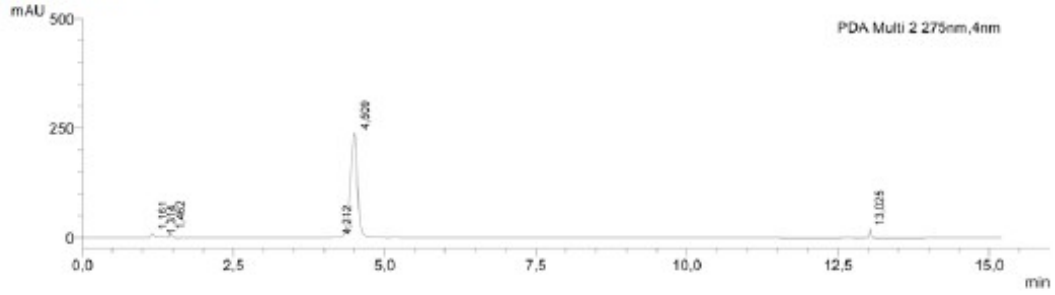

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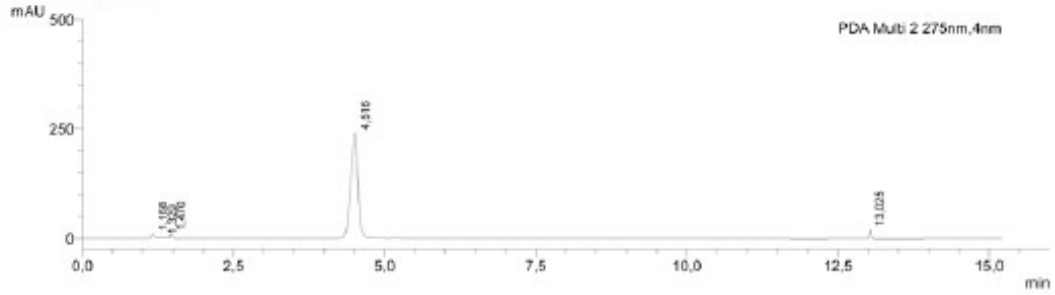
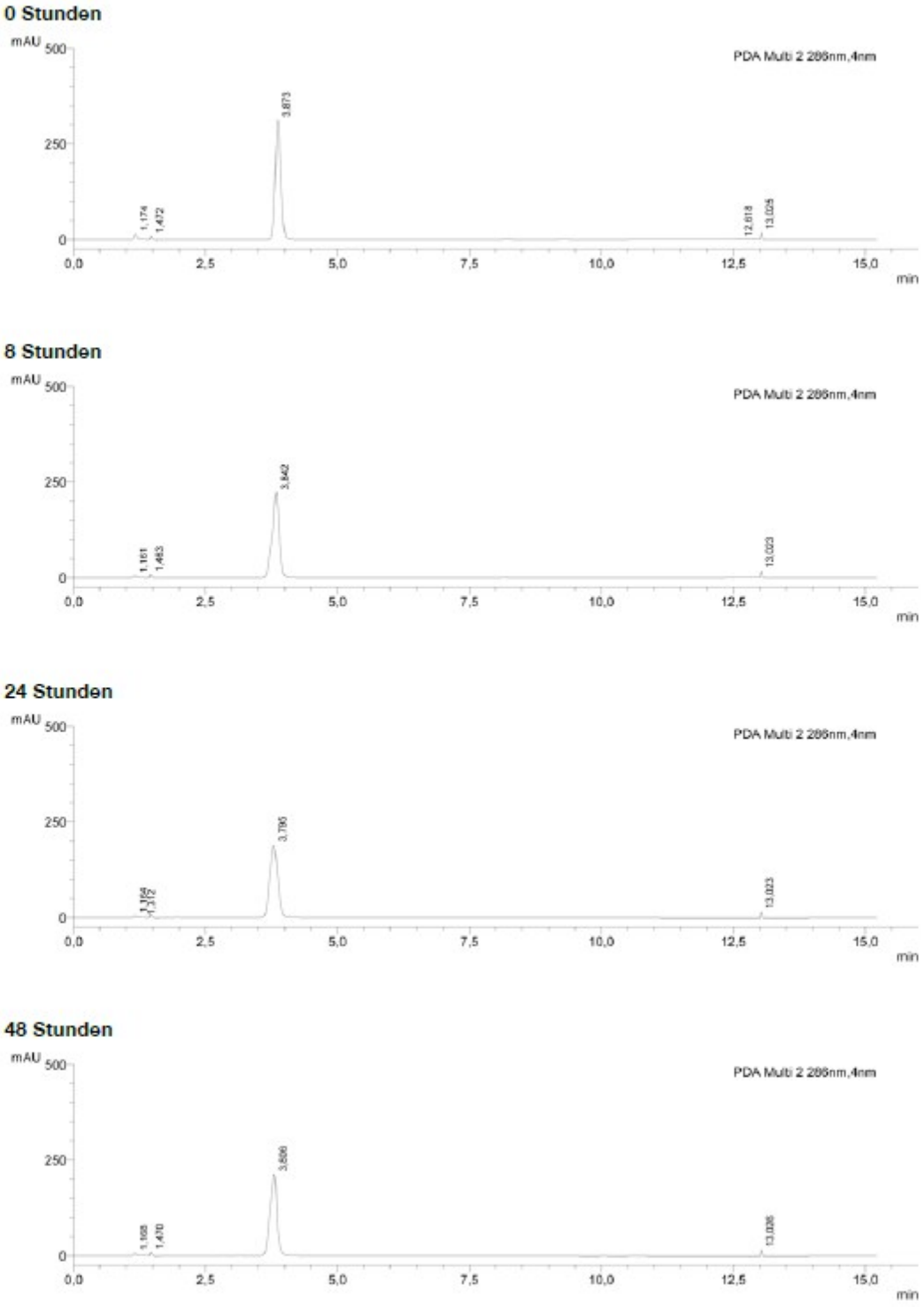


Supporting Figure 9: Stability of 5-Cl-ASS



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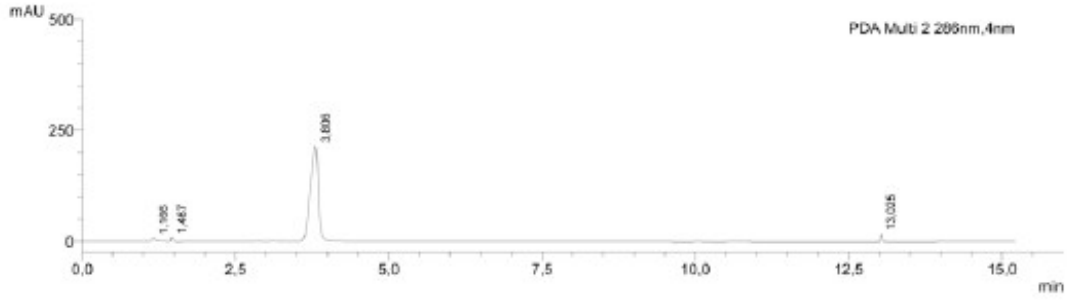

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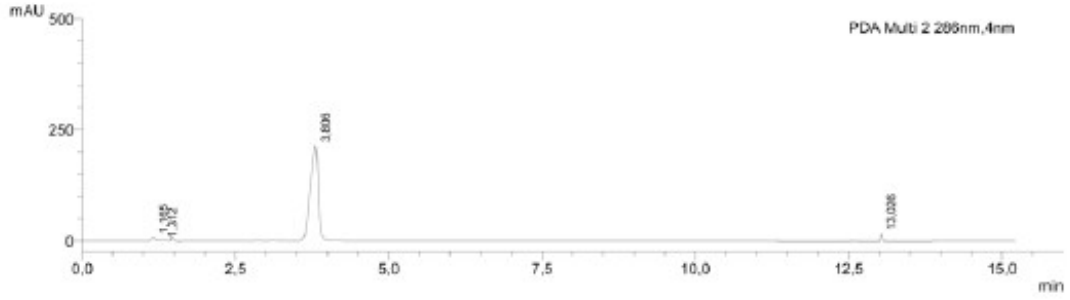
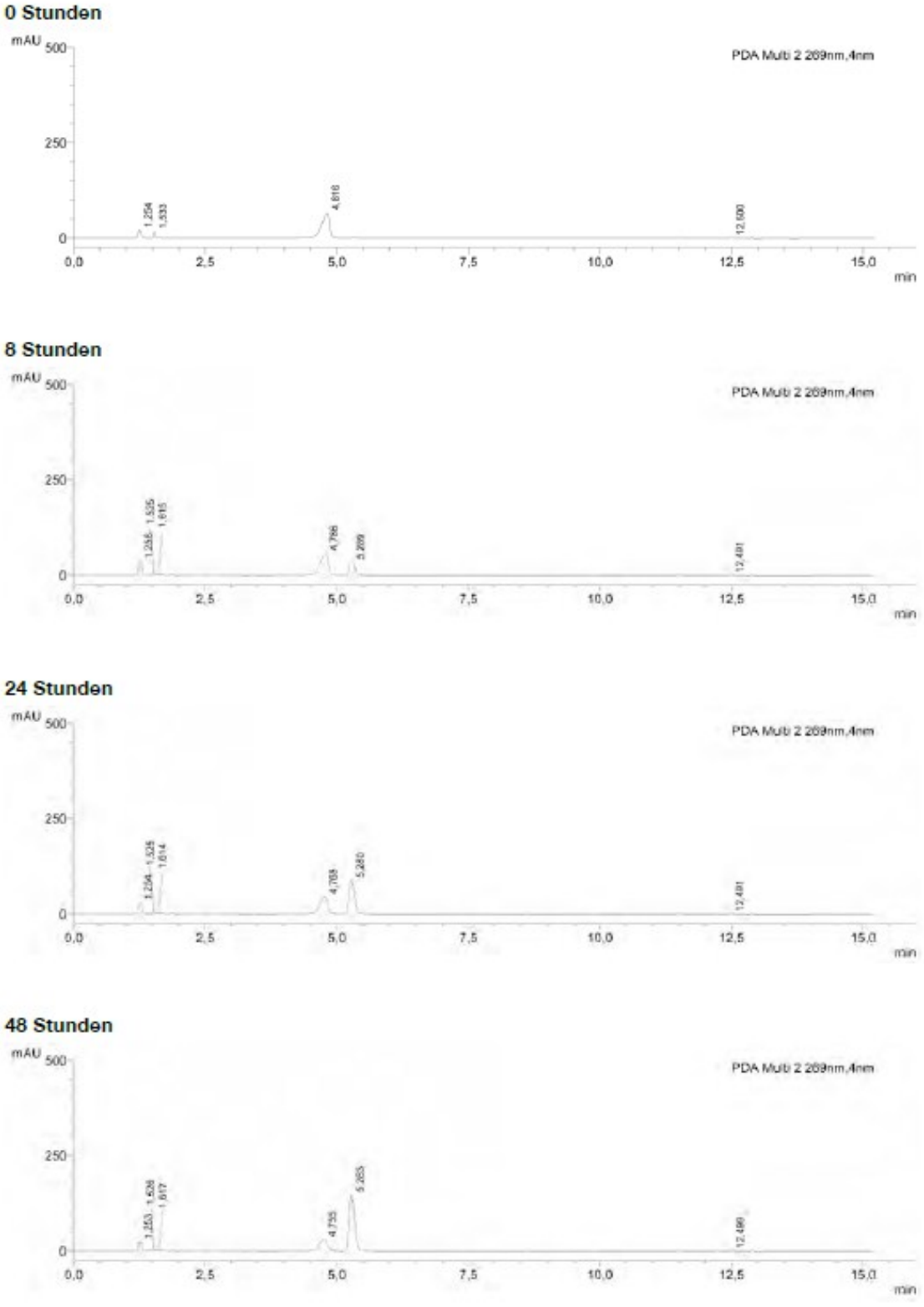


Supporting Figure 10: Stability of 6-Cl-ASS



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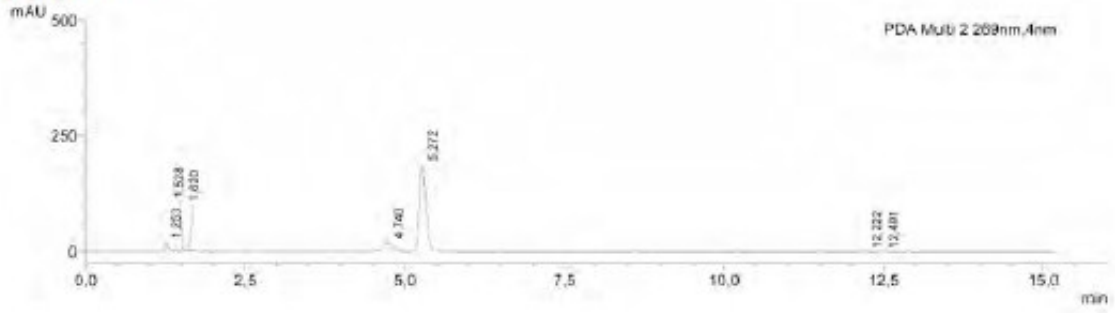

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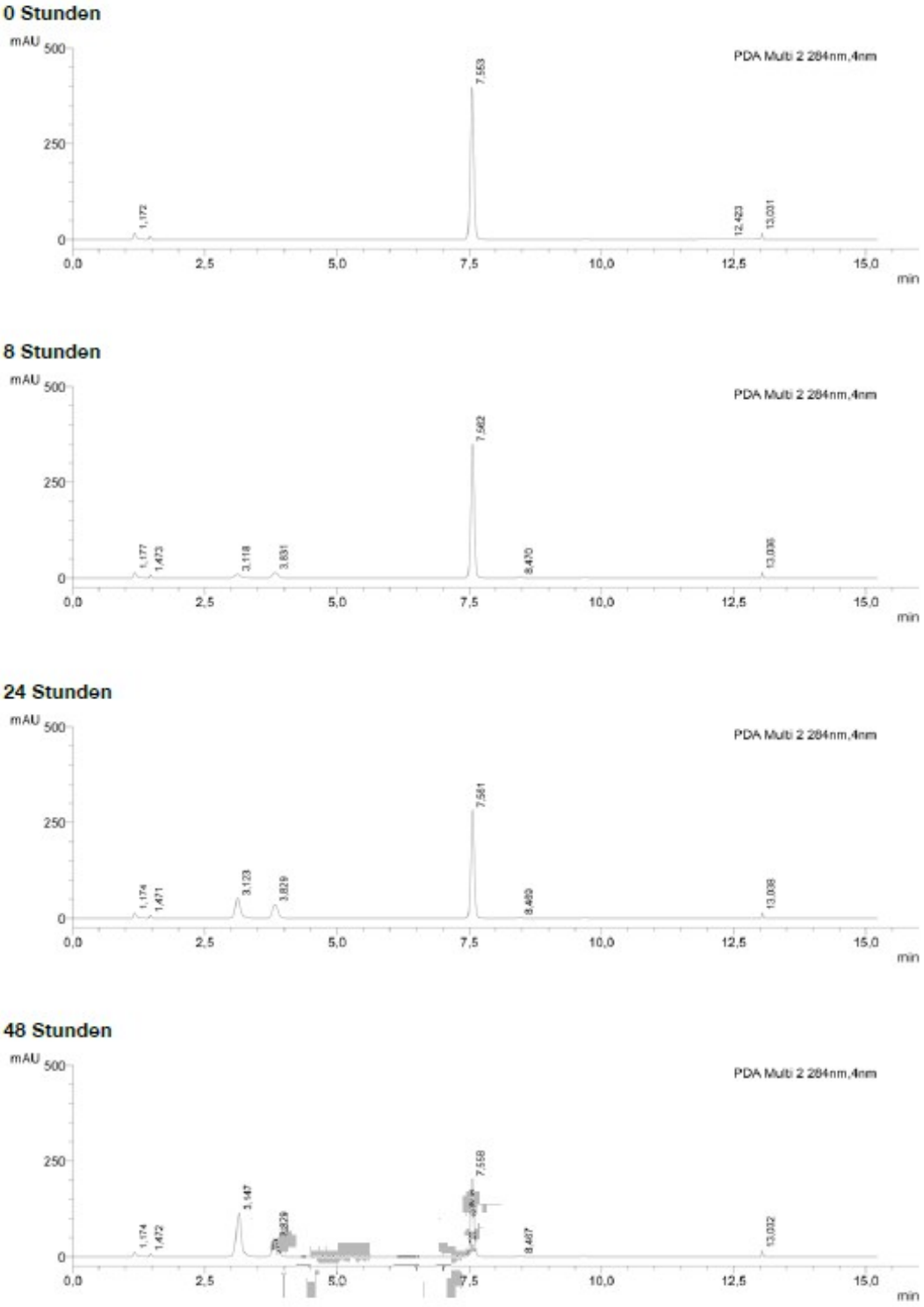
Supporting Figure 11: Stability of 3-Cl-ASS propargyl ester



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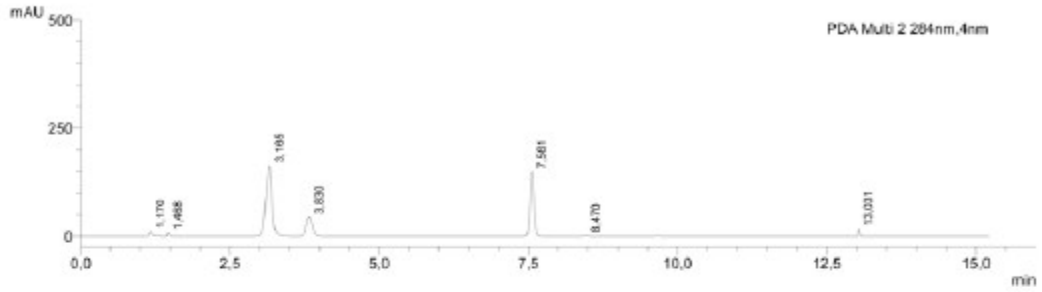

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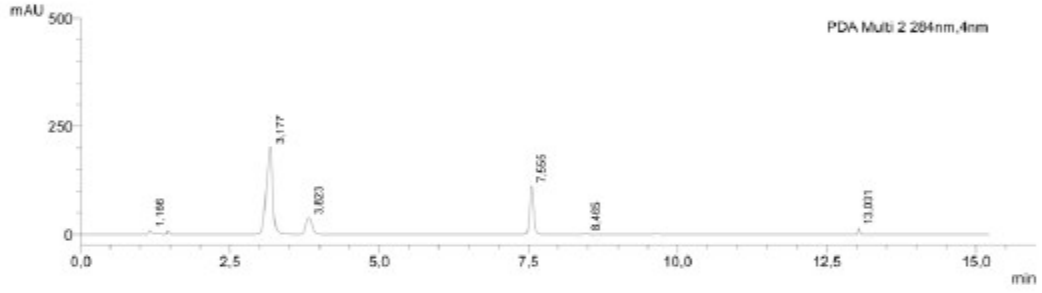

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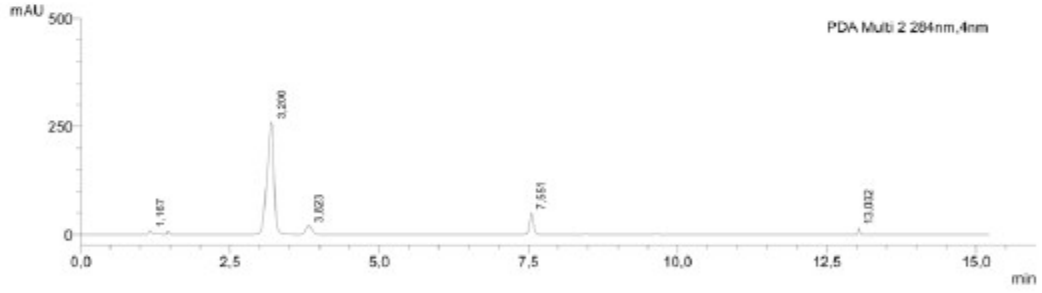
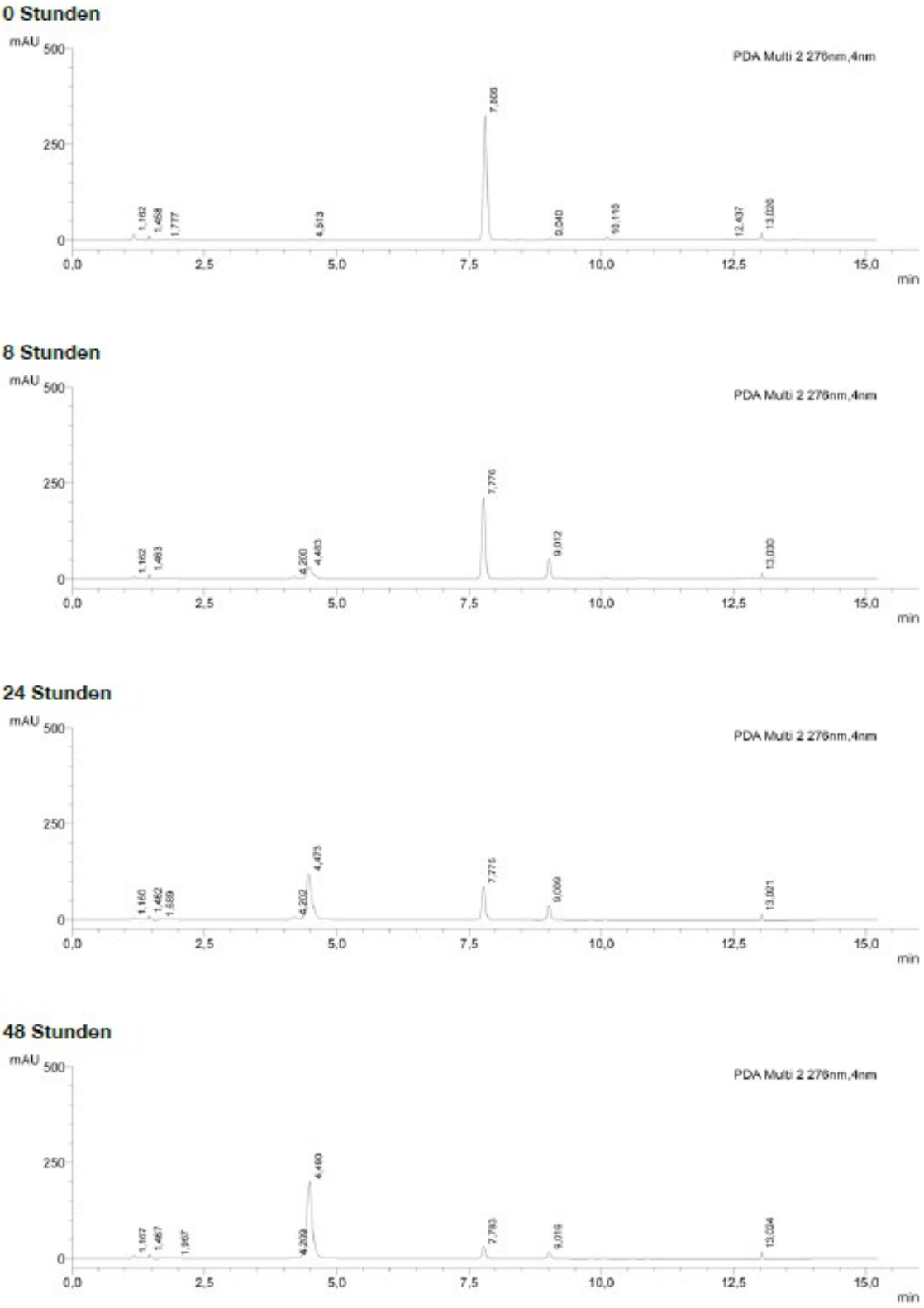


Supporting Figure 12: Stability of 4-Cl-ASS propargyl ester



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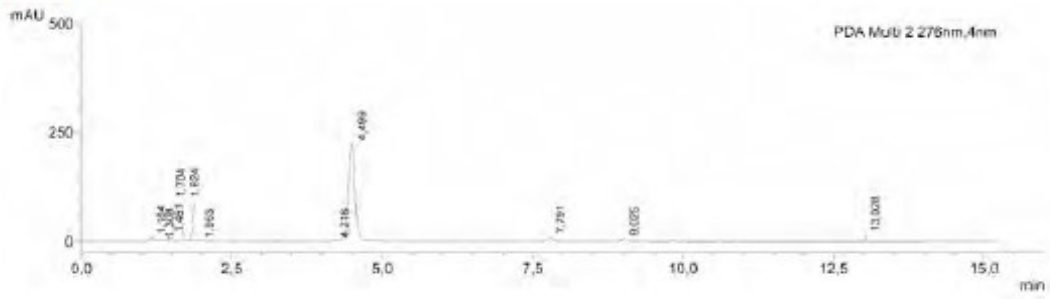
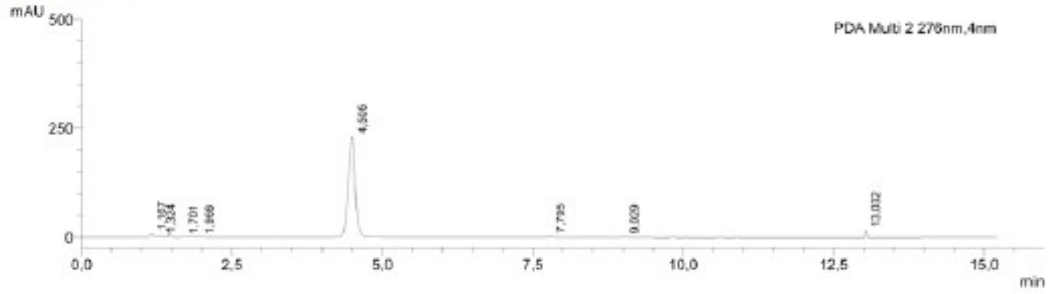
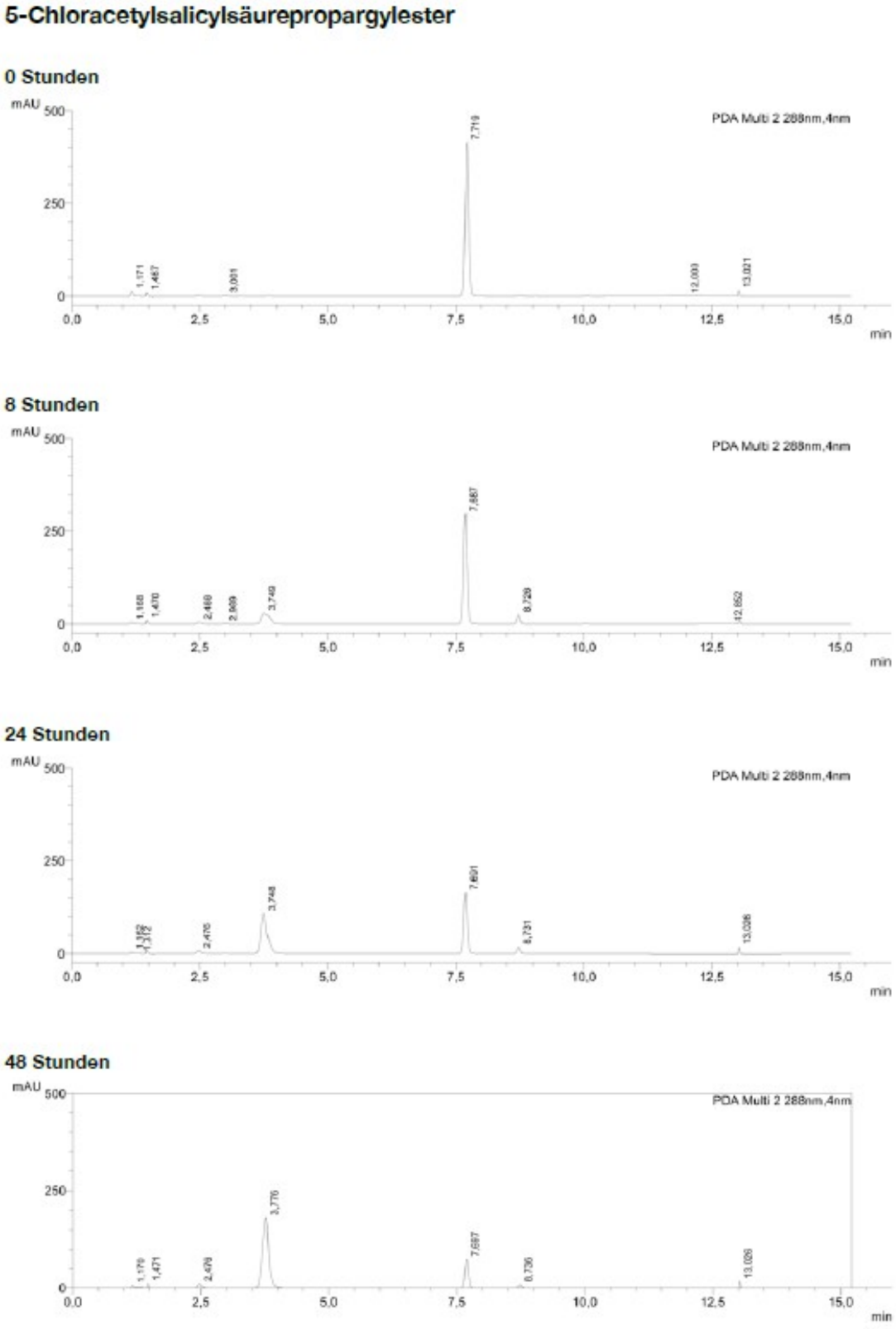


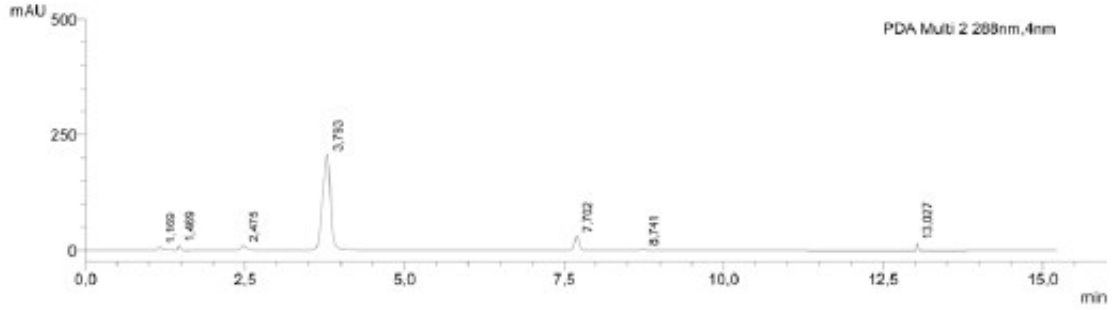
96 Stunden



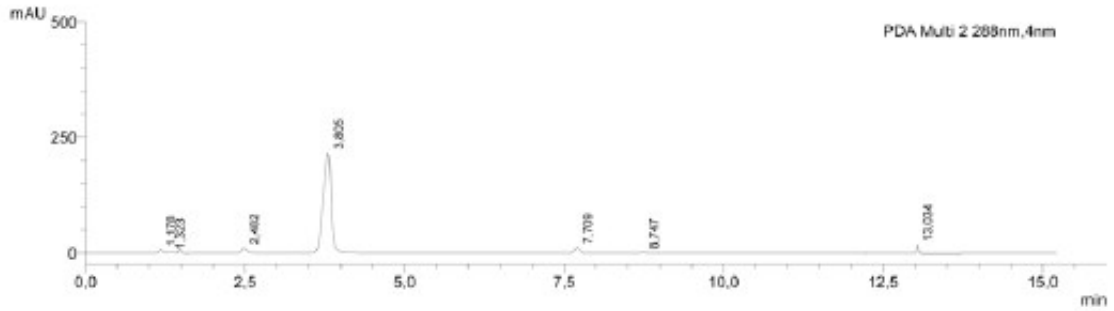
Supporting Figure 13: Stability of 5-Cl-ASS propargyl ester



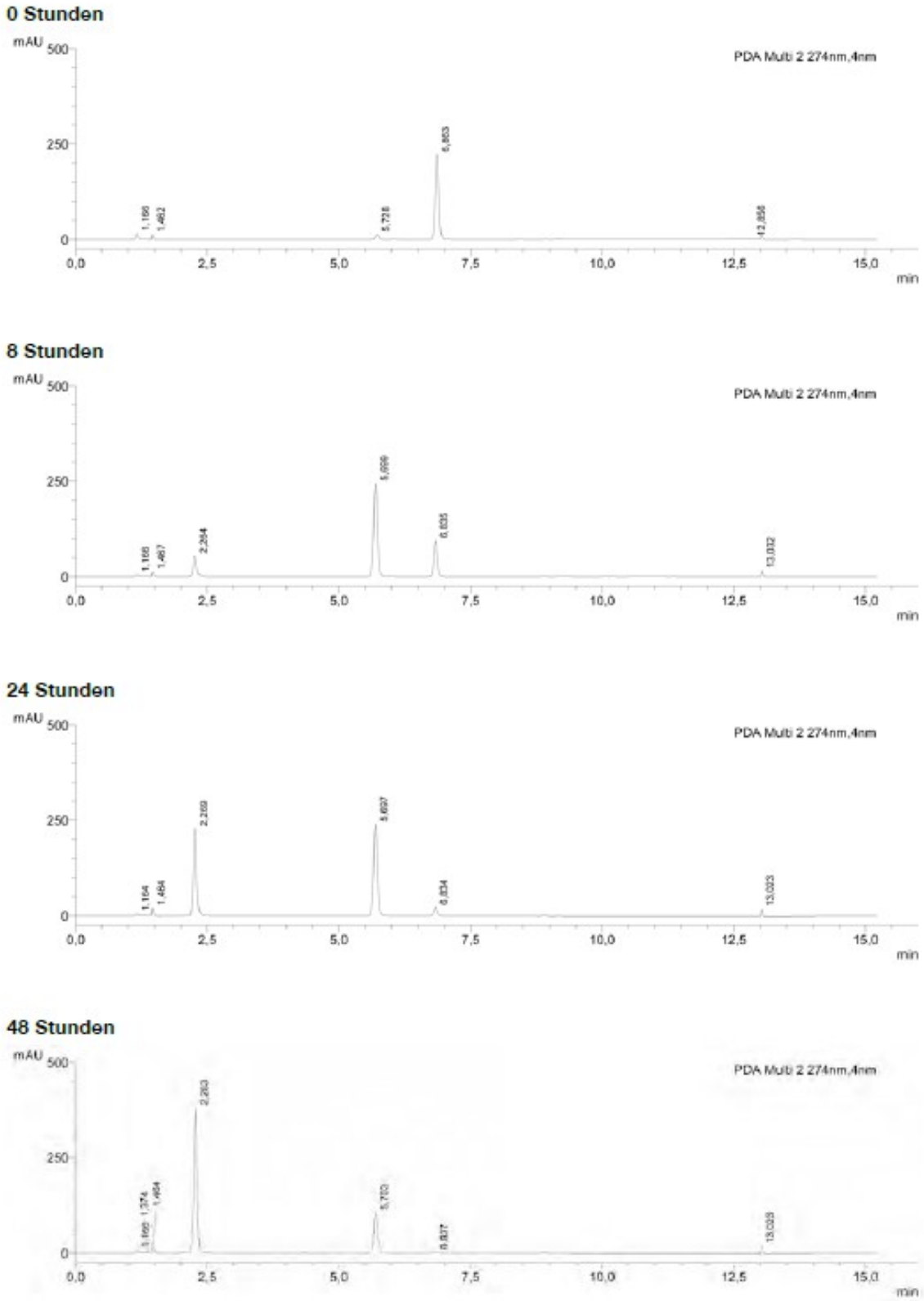
72 Stunden



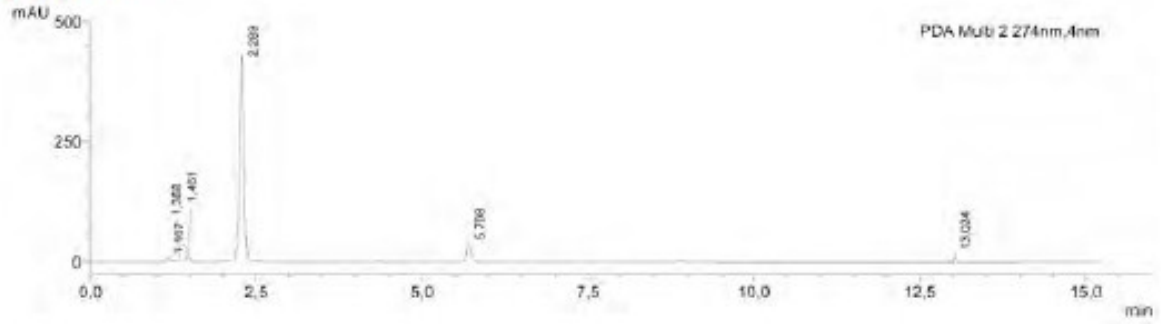
96 Stunden



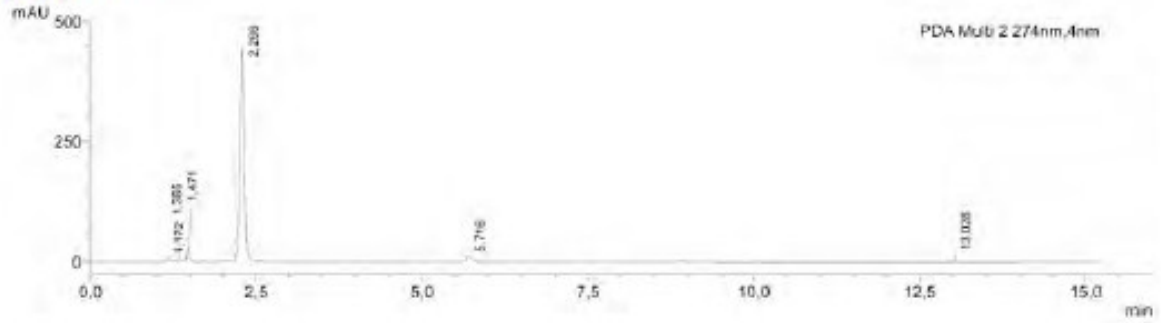
Supporting Figure 14: Stability of 6-Cl-ASS propargyl ester



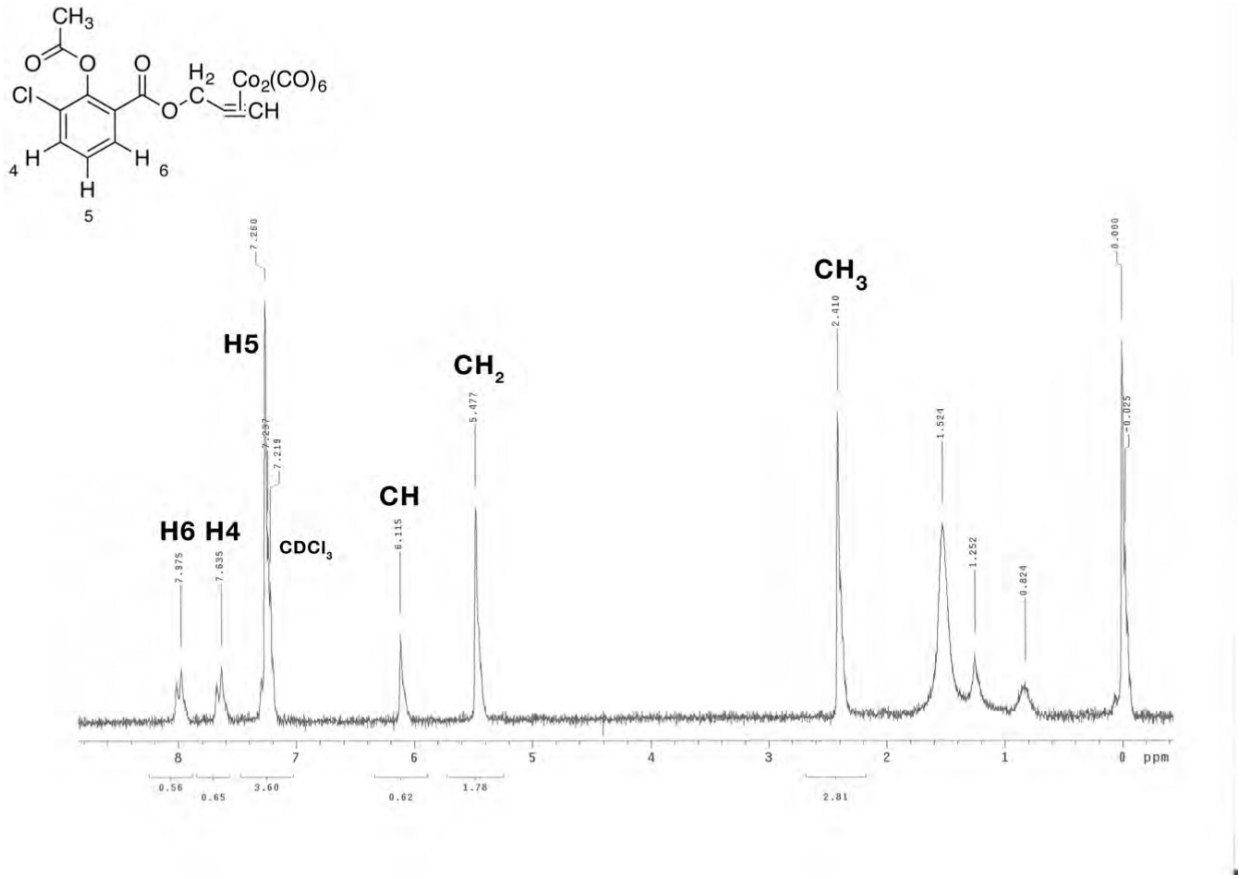
72 Stunden



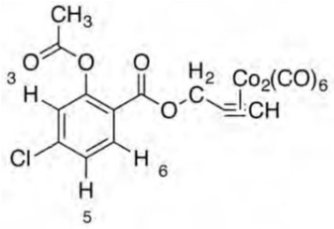
96 Stunden

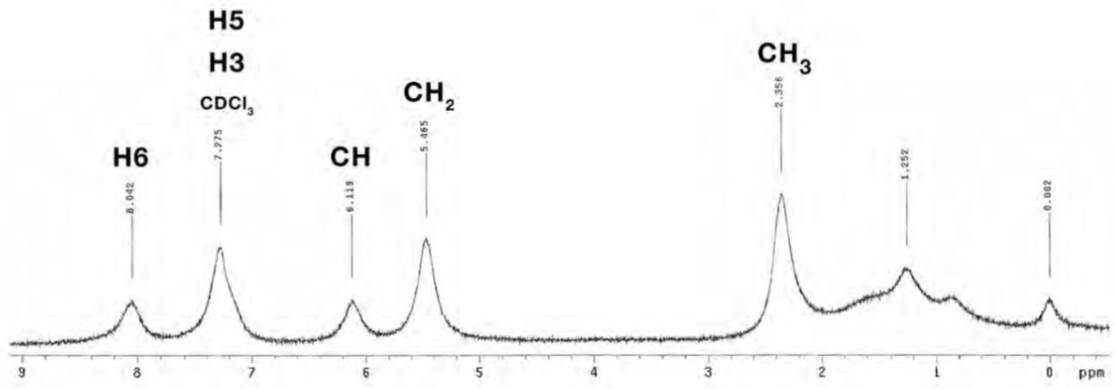


Supporting Figure 15: ^{1}H-NMR spectrum of 3-Cl-Co-ASS

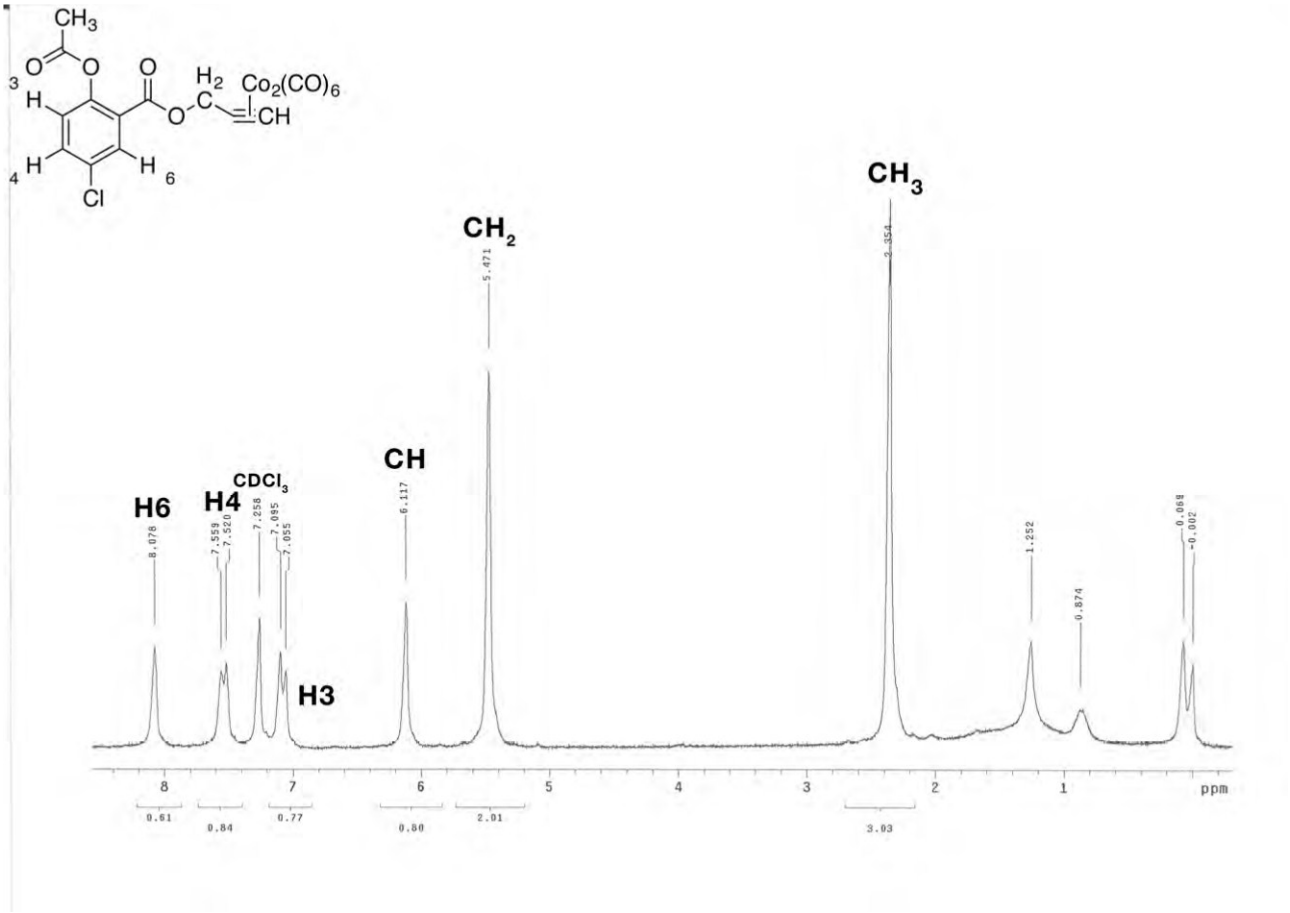


Supporting Figure 16: ^{1}H-NMR spectrum of 4-Cl-Co-ASS

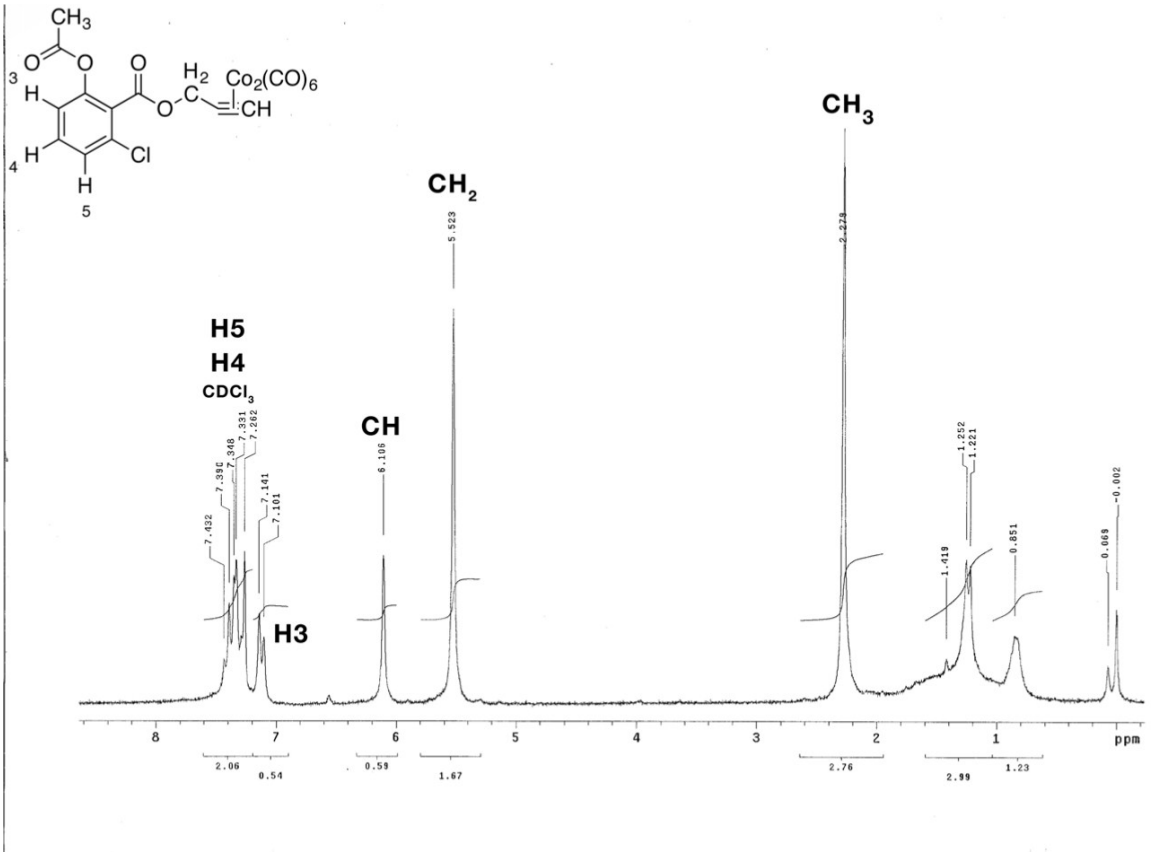




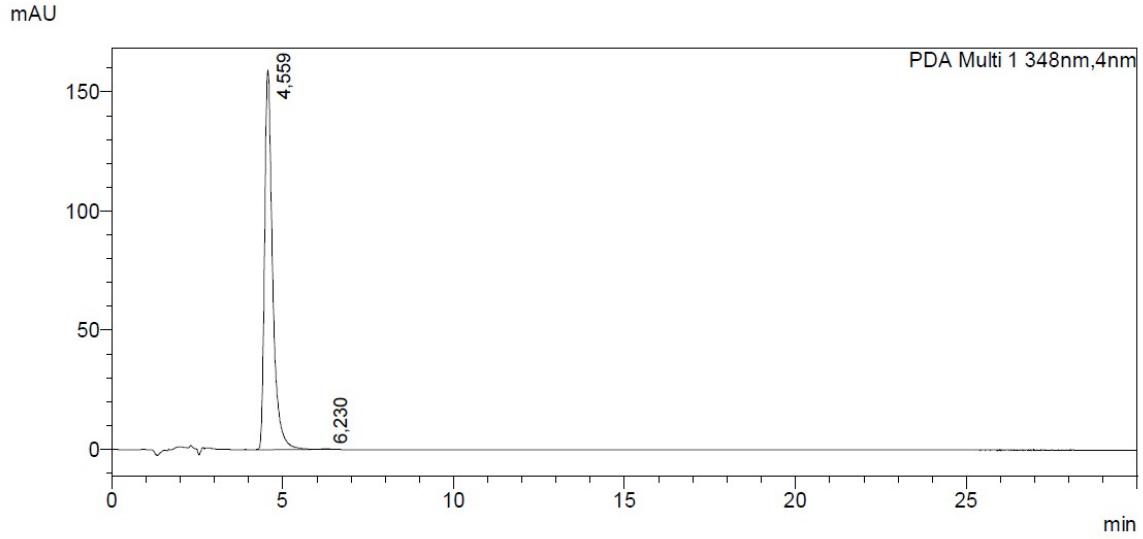
Supporting Figure 17: *^{1}H-NMR spectrum of 5-Cl-Co-ASS*



Supporting Figure 18: *^{1}H-NMR spectrum of 6-Cl-Co-ASS*

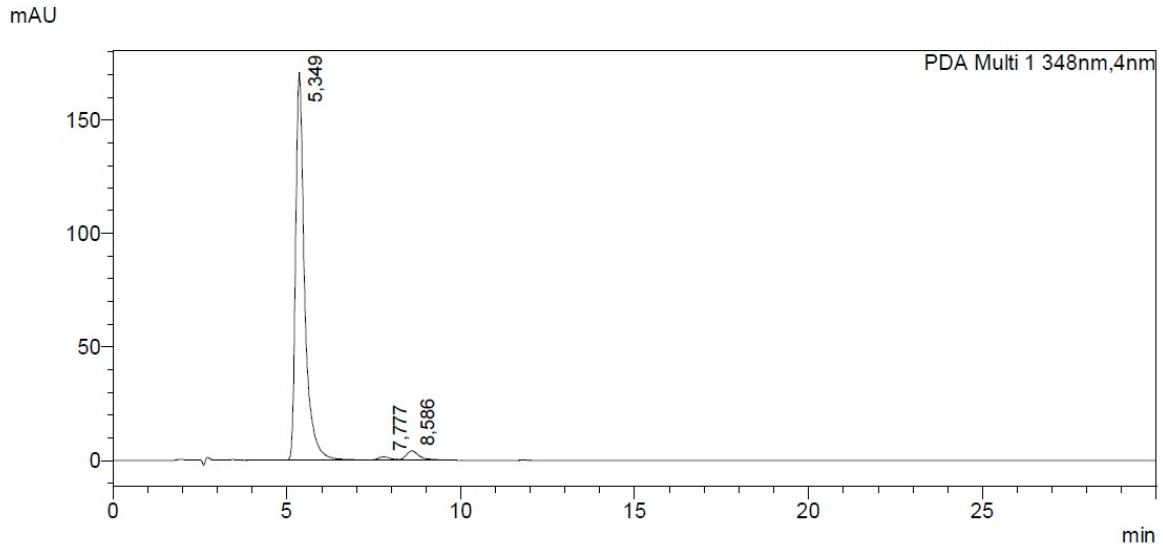


Supporting Figure 19: HPLC chromatogram of 3-Cl-Co-ASS



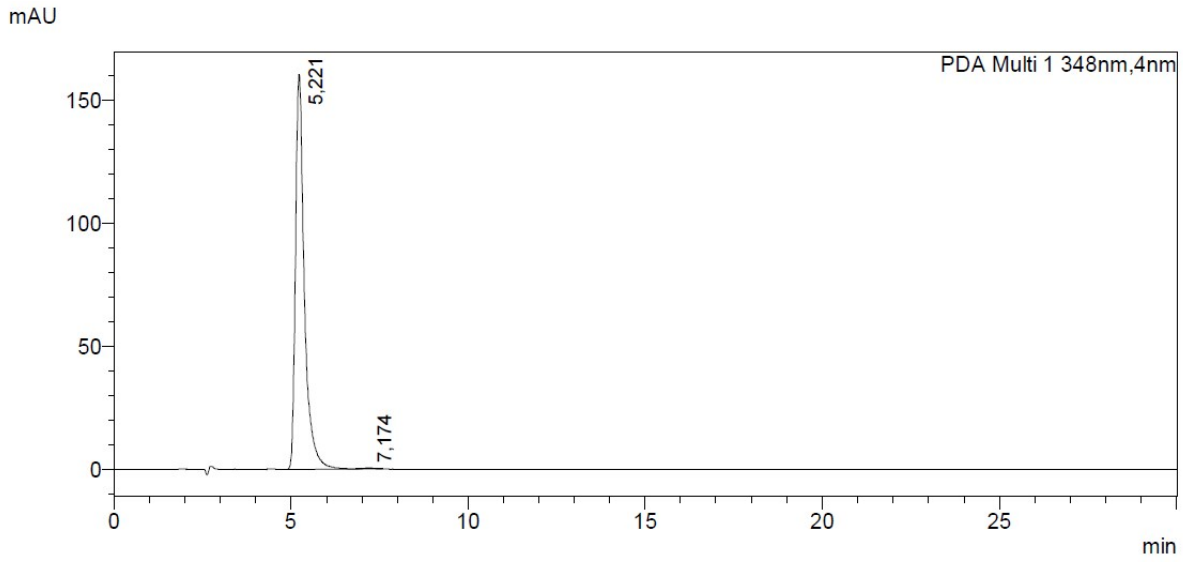
Wavelength	Retention Time	Area	Height	Concentration
348	4.559	2607625	159228	99.803
	6.230	5135	325	0.196

Supporting Figure 20: HPLC chromatogram of 4-Cl-Co-ASS



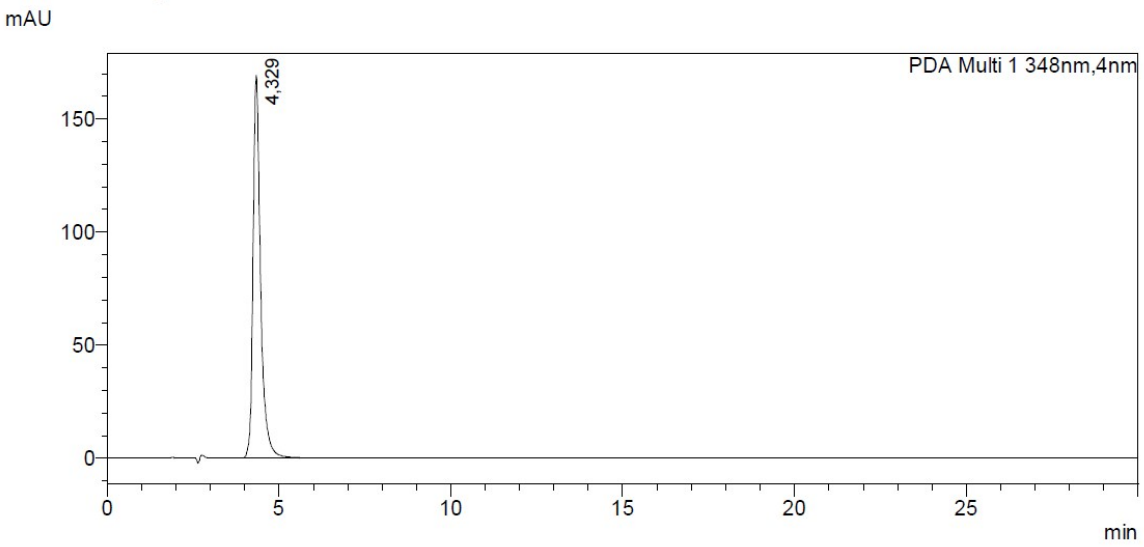
Wavelength	Retention Time	Area	Height	Concentration
348	5.349	3090301	170777	95.576
	7.777	34471	1448	1.066
	8.586	108547	4058	3.357

Supporting Figure 21: HPLC chromatogram of 5-Cl-Co-ASS



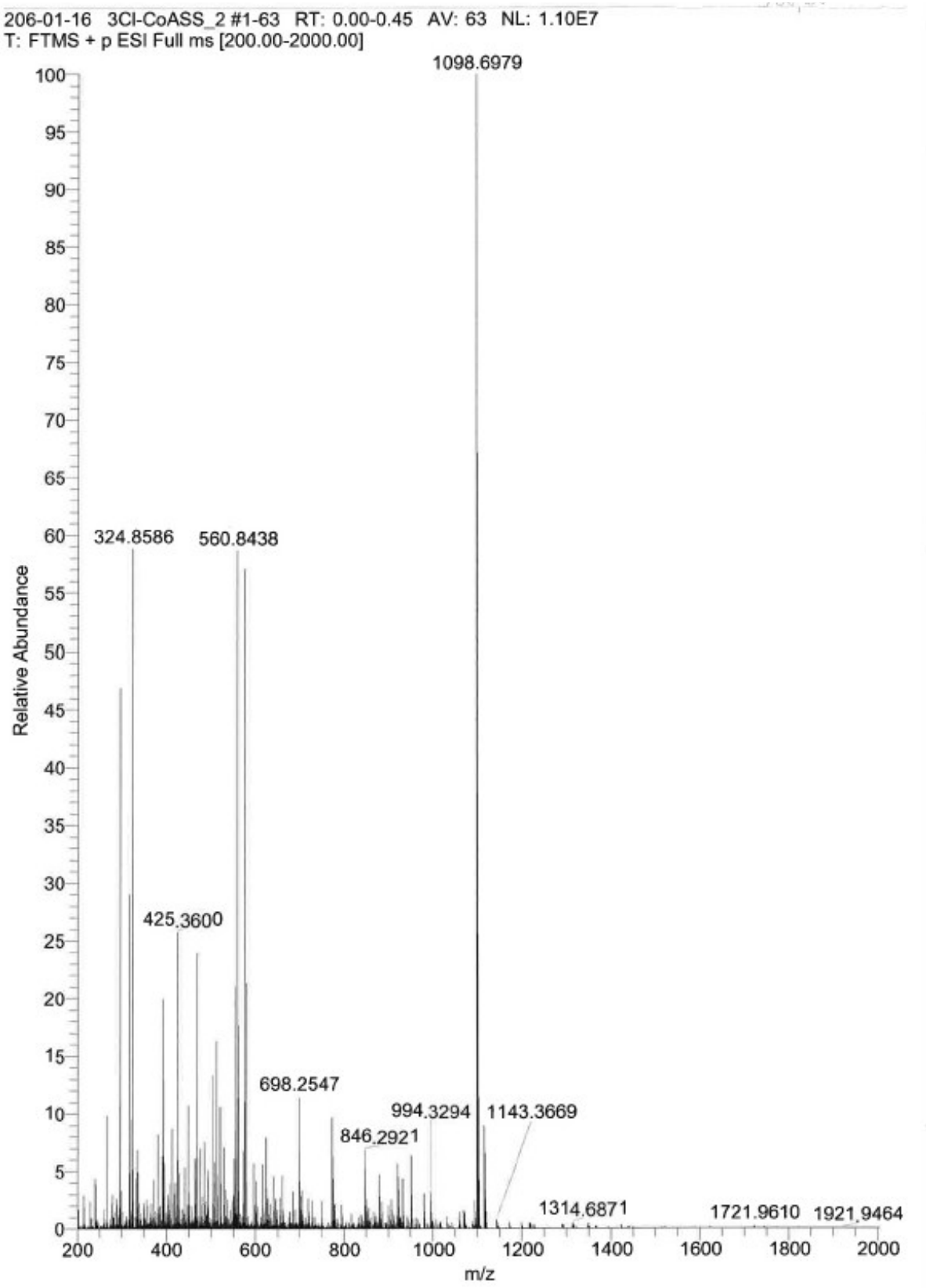
Wavelength	Retention Time	Area	Height	Concentration
348	5.221	2812359	160382	99.638
	7.174	10215	545	0.361

Supporting Figure 22: HPLC chromatogram of 6-Cl-Co-ASS

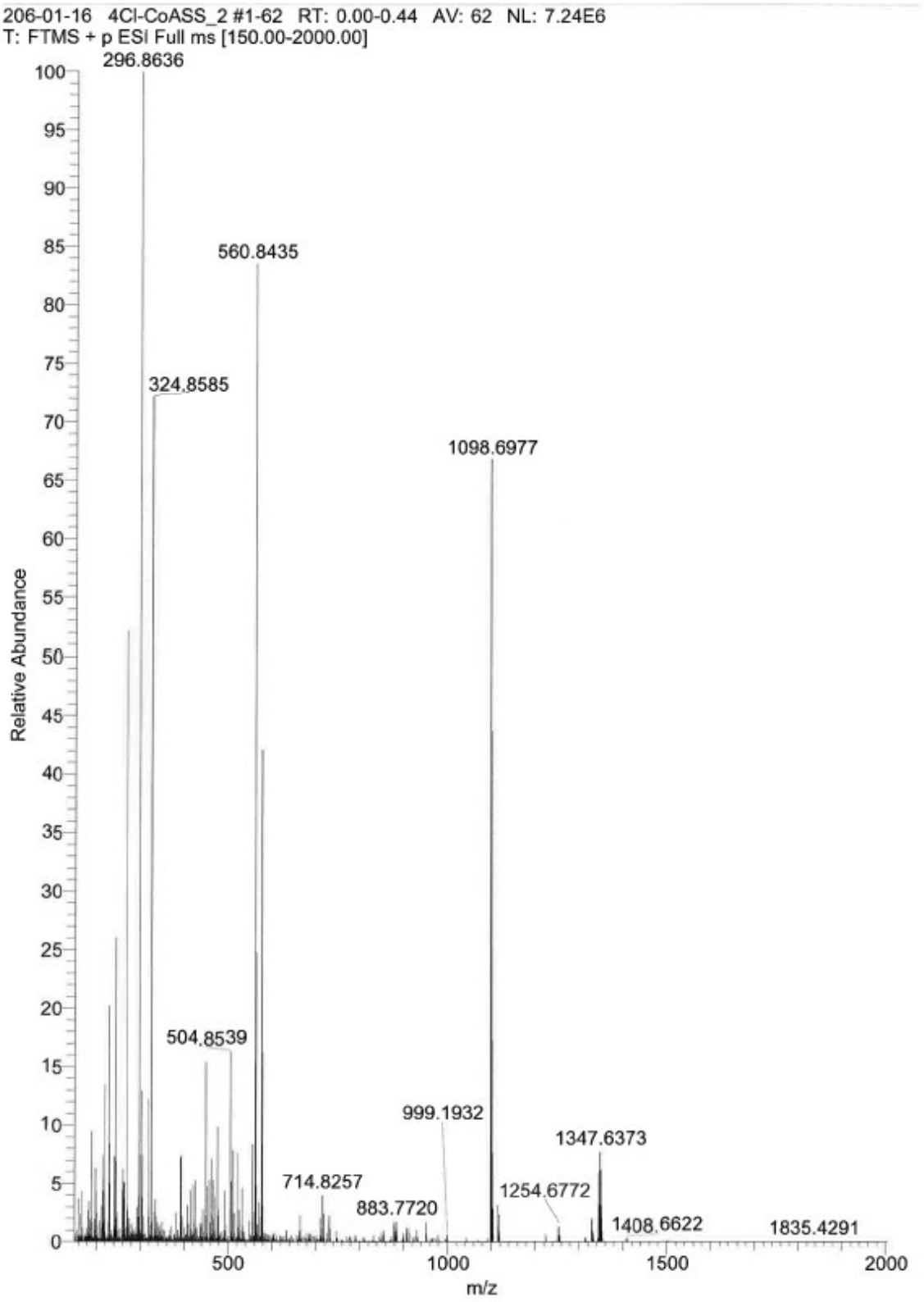


Wavelength	Retention Time	Area	Height	Concentration
348	4.329	2578456	169291	100.0

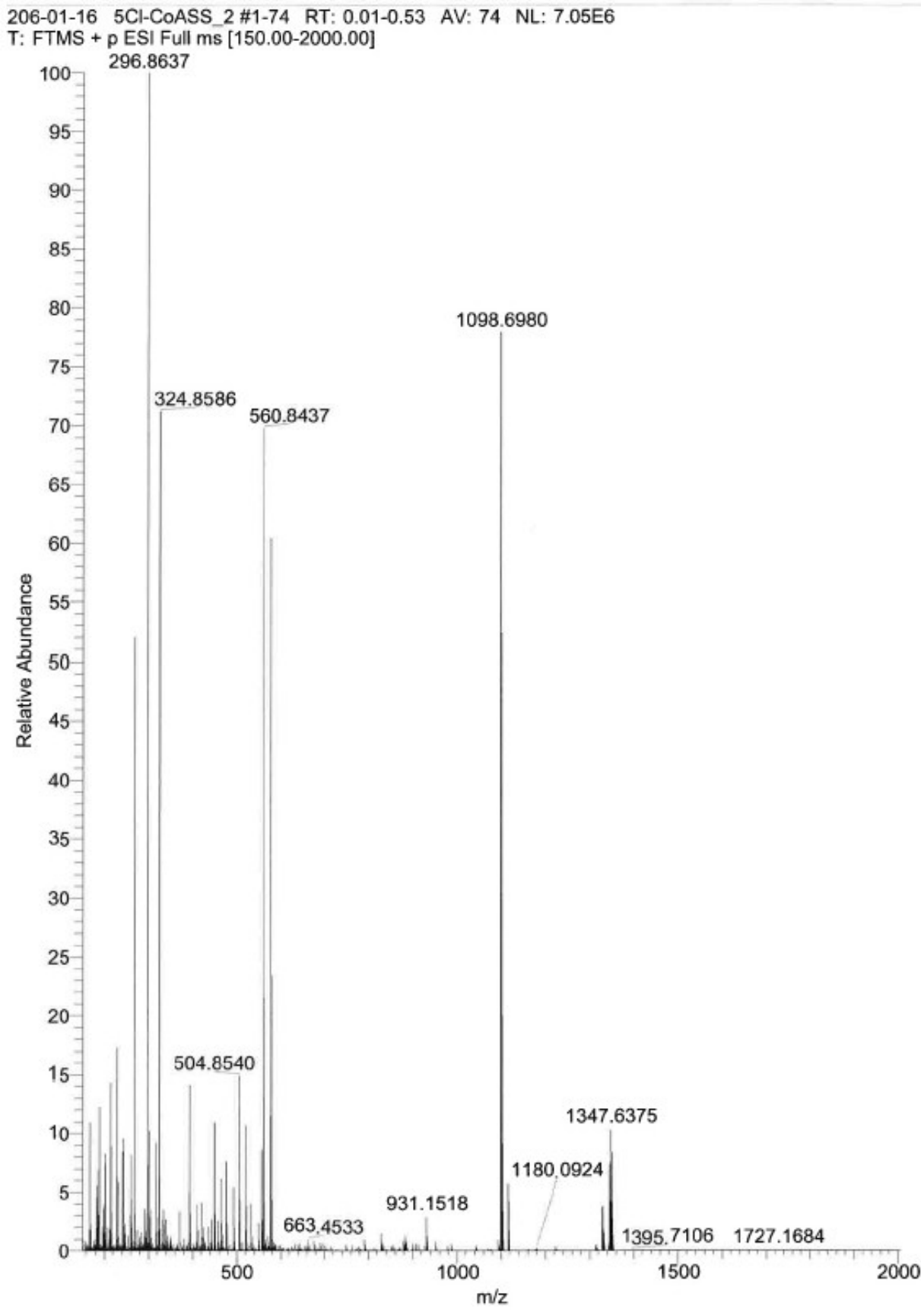
Supporting Figure 23: HR-MS spectrum of 3-Cl-Co-ASS



Supporting Figure 24: HR-MS spectrum of 4-Cl-Co-ASS



Supporting Figure 25: HR-MS spectrum of 5-Cl-Co-ASS



Supporting Figure 26: HR-MS spectrum of 6-Cl-Co-ASS