Supporting Information

Development of Macrocyclic PRMT5 Adaptor Protein Interaction Inhibitors

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Table S1. Sequences and HRMS of linear peptides with amino acid modifications.

Name	Sequence	HRMS	
		m/z calculated	m/z found
S2	Ac-**Tyr**-PGQFDDADK(Fitc)-NH_2	1585.55764 $[M+H]^+$	1585.56081
S3	Ac-**Phe**-PGQFDDADK(Fitc)-NH_2	1569.56272 $[M+H]^+$	1569.56604
S4	Ac-**hPhe**-PGQFDDADK(Fitc)-NH_2	1583.57837 $[M+H]^+$	1583.58125
S5	Ac-**Trp**-PGQFDDADK(Fitc)-NH_2	1608.57362 $[M+H]^+$	1608.57625
S6	Ac-**Asp**-PGQFDDADK(Fitc)-NH_2	1537.52125 $[M+H]^+$	1537.52441
S7	Ac-**Glu**-PGQFDDADK(Fitc)-NH_2	1551.53690 $[M+H]^+$	1551.54015
S8	Ac-VPG-**Asn**-FDDADK(Fitc)-NH_2	1507.54707 $[M+H]^+$	1507.55099
S9	Ac-VPG-**Dab**-FDDADK(Fitc)-NH_2	1493.56781 $[M+H]^+$	1493.56975
S10	Ac-VPG-**Dab(Alloc)**-FDDADK(Fitc)-NH_2	1577.58894 $[M+H]^+$	1577.59177
S11	Ac-VPG-**Cit**-FDDADK(Fitc)-NH_2	1550.58927 $[M+H]^+$	1550.59317
32	Ac-VPGQ-**Phe(3-F)**-DDADK(Fitc)-NH_2	1539.55330 $[M+H]^+$	1539.55557
34	Ac-VPGQ-**Bip**-DDADK(Fitc)-NH_2	1597.59402 $[M+H]^+$	1597.59731
35	Ac-VPGQ-**Phe(3,4-F_2)**-DDADK(Fitc)-NH_2	1557.54388 $[M+H]^+$	1557.54775
36	Ac-VPGQ-**Phe(4-F)**-DDADK(Fitc)-NH_2	1539.55330 $[M+H]^+$	1539.55660
37	Ac-VPGQ-**Phe(4-Br)**-DDADK(Fitc)-NH_2	1599.47323 $[M+H]^+$	1599.47609
38	Ac-VPGQ-**Phe(4-I)**-DDADK(Fitc)-NH_2	1647.45936 $[M+H]^+$	1647.46158
39	Ac-VPGQ-**Phe(4-Cl)**-DDADK(Fitc)-NH_2	1555.52375 $[M+H]^+$	1555.52694
41	Ac-VPGQ-**Phe(4-NO_2)**-DDADK(Fitc)-NH_2	1566.54780 $[M+H]^+$	1566.55130
S12	Ac-VPGQ-**Phe(2-F)**-DDADK(Fitc)-NH_2	1539.55330 $[M+H]^+$	1539.55666
S13	Ac-VPGQ-**Phe(F_5)**-DDADK(Fitc)-NH_2	1611.51561 $[M+H]^+$	1611.51901
S14	Ac-VPGQ-**Phe(2-I)**-DDADK(Fitc)-NH_2	1647.45936 $[M+H]^+$	1647.46197
S15	Ac-VPGQ-**Phe(3-Cl)**-DDADK(Fitc)-NH_2	1555.52375 $[M+H]^+$	1555.52705
S16	Ac-VPGQ-**Phe(3-CF_3)**-DDADK(Fitc)-NH_2	1589.55011 $[M+H]^+$	1589.55440
S17	Ac-VPGQ-**2-Pal**-DDADK(Fitc)-NH_2	1522.55797 $[M+H]^+$	1522.56122
S18	Ac-VPGQ-**Tyr**-DDADK(Fitc)-NH_2	1537.55764 $[M+H]^+$	1537.56120
S19	Ac-VPGQ-**Phe(4-COOH)**-DDADK(Fitc)-NH_2	1565.55255 $[M+H]^+$	1565.55556
S20	Ac-VPGQ-**Phe(4-guanidino)**-DDADK(Fitc)-NH_2	1578.59542 $[M+H]^+$	1578.59706
S21	Ac-VPGQ-**hPhe**-DDADK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58161
S22	Ac-VPGQ-**3-(2-biphenylyl)-Ala**-DDADK(Fitc)-NH_2	1597.59402 $[M+H]^+$	1597.59787
S23	Ac-VPGQ-**Bpa**-DDADK(Fitc)-NH_2	1625.58894 $[M+H]^+$	1625.59170
S24	Ac-VPGQ-**2-Nal**-DDADK(Fitc)-NH_2	1571.57837 $[M+H]^+$	1571.58183
S25	Ac-VPGQ-**1-Nal**-DDADK(Fitc)-NH_2	1571.57837 $[M+H]^+$	1571.58167
S26	Ac-VPGQFD-**Gla**-ADK(Fitc)-NH_2	1579.56820 $[M+H]^+$	1579.57063
S27	Ac-VPGQFDD-**Abu**-DK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58199
S28	Ac-VPGQFDD-**Nva**-DK(Fitc)-NH_2	1549.59402 $[M+H]^+$	1549.59737
S29	Ac-VPGQFDD-**Nle**-DK(Fitc)-NH_2	1563.60967 $[M+H]^+$	1563.61310
S30	Ac-VPGQFDD-**Cha**-DK(Fitc)-NH_2	1603.64097 $[M+H]^+$	1603.64485
S31	Ac-VPGQFDD-**Ser**-DK(Fitc)-NH_2	1537.55764 $[M+H]^+$	1537.56164
33	Ac-VPGQFDDA-**Glu**-K(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58145
40	Ac-VPGQFDDA-**Gla**-K(Fitc)-NH_2	1579.56820 $[M+H]^+$	1579.57062
S32	Ac-VPGQFDDA-**Tyr**-K(Fitc)-NH_2	1569.59911 $[M+H]^+$	1569.60176
S33	Ac-VPGQFDDA-**Trp**-K(Fitc)-NH_2	1592.61509 $[M+H]^+$	1592.61751
S34	Ac-VPGQFDDA-**hPhe**-K(Fitc)-NH_2	1567.61984 $[M+H]^+$	1567.62296
42	Ac-VPGQ-**Phe(4-Cl)**-DDA-**Gla**-K(Fitc)-NH_2	1613.52923 $[M+H]^+$	1613.53080
43	Ac-VPGQ-**Phe(4-NO_2)**-DDA-**Gla**-K(Fitc)-NH_2	1624.55328 $[M+H]^+$	1624.55454

Table S2. Direct binding FP results for *N*-methylated linear peptides **44**-**49**.

Peptide	Sequence	K_D (μM)
44	Ac-VPGQFDDA-**(*N*-Me)D**-K(Fitc)-NH_2	0.6 ± 0.1
45	Ac-VPGQFDD-**(*N*-Me)A**-DK(Fitc)-NH_2	>2
46	Ac-VPGQFD-**(*N*-Me)D**-ADK(Fitc)-NH_2	>2
47	Ac-VPGQ-**(*N*-Me)F**-DDADK(Fitc)-NH_2	>2
48	Ac-VP-**(*N*-Me)G**-QFDDADK(Fitc)-NH_2	>2
49	Ac-**(*N*-Me)V**-PGQFDDADK(Fitc)-NH_2	>2

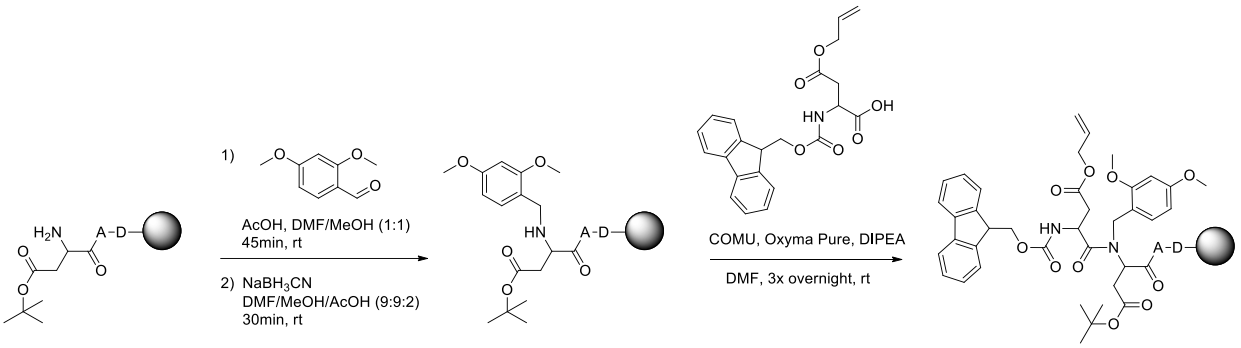
Table S3. Sequences and HRMS data of linear RioK1-derived peptides.

Name	Sequence	HRMS	
		m/z calculated	m/z found
1	Ac-SRVVPaQFDDAD-NH_2	1360.64917 $[M+H]^+$	1360.65083
2	Ac-SRVVPGQFaDAD-NH_2	1302.64369 $[M+H]^+$	1302.64518
3	Ac-SRVVPaQFaDAD-NH_2	1316.65934 $[M+H]^+$	1316.66100
4	Ac-SRVVPGQFDDAD-NH_2	1346.63352 $[M+H]^+$	1346.63623
28	Fitc-O2Oc-VPGQFDDAD-NH_2	1496.53109 $[M+H]^+$	1496.53270
29	Ac-VPGQFDDADK(Fitc)-NH_2	1521.56272 $[M+H]^+$	1521.56580
31	Ac-VPGQFDDAD-NH_2	1004.43197 $[M+H]^+$	1004.42952
44	Ac-VPGQFDDA-**(*N*-Me)D**-K(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58195
45	Ac-VPGQFDD-**(*N*-Me)A**-DK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58169
46	Ac-VPGQFD-**(*N*-Me)D**-ADK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58178
47	Ac-VPGQ-**(*N*-Me)F**-DDADK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58196
48	Ac-VP-**(*N*-Me)G**-QFDDADK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58188
49	Ac-**(*N*-Me)V**-PGQFDDADK(Fitc)-NH_2	1535.57837 $[M+H]^+$	1535.58070
S1	Fitc-O2Oc-SRVVPGQFDDADSSD-NH_2	1064.41546 $[M+2H]^{2+}$	1064.41816
H4 peptide	Ac-SGRGKGGKGLGKGGAKRHRKV-NH2	1066.64103 $[M+2H]^{2+}$	1066.64198

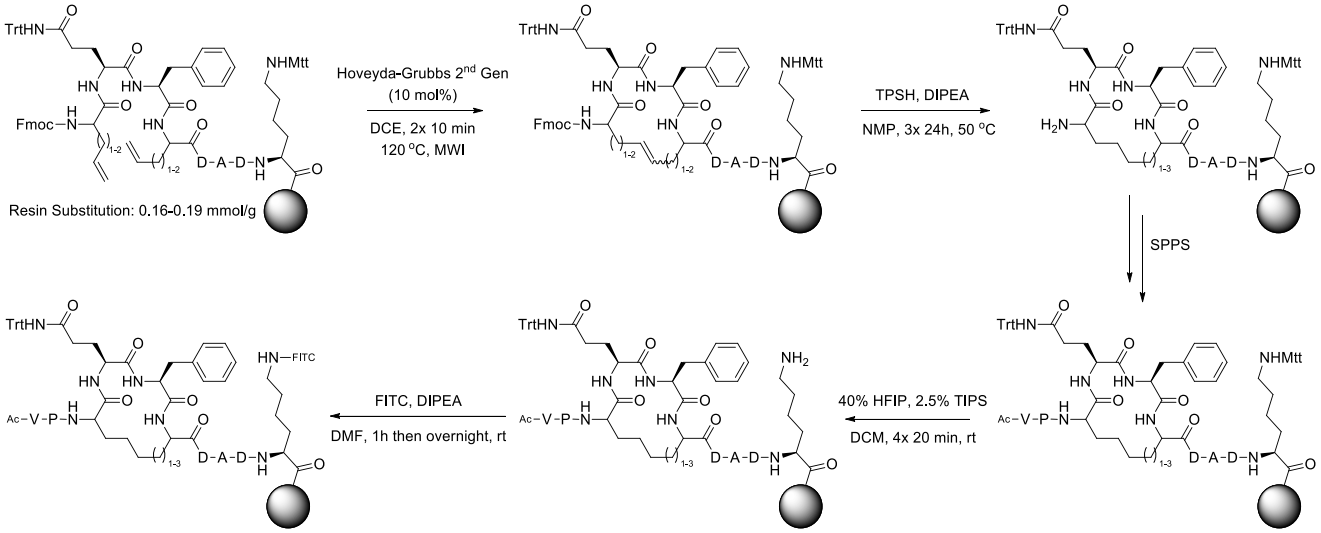
Table S4. HRMS of cyclic peptides.

Name	HRMS	
	m/z calculated	m/z found
5	925.37795 $[M+2H]^{2+}$	925.37895
6	932.38578 $[M+2H]^{2+}$	932.38677
7	932.38578 $[M+2H]^{2+}$	932.38673
8	939.39360 $[M+2H]^{2+}$	939.39476
9	925.37795 $[M+2H]^{2+}$	925.37888
10	932.38578 $[M+2H]^{2+}$	932.38677
11	932.38578 $[M+2H]^{2+}$	932.38673
12	939.39360 $[M+2H]^{2+}$	939.39480
13	916.88287 $[M+2H]^{2+}$	916.88398
14	917.89069 $[M+2H]^{2+}$	917.89179
15 isomer 1	916.88287 $[M+2H]^{2+}$	916.88396
15 isomer 2	916.88287 $[M+2H]^{2+}$	916.88397
16	917.89069 $[M+2H]^{2+}$	917.89172
17	758.29791 $[M+2H]^{2+}$	758.29659
18 isomer 1	765.30573 $[M+2H]^{2+}$	765.30446
18 isomer 2	765.30573 $[M+2H]^{2+}$	765.30464
19	765.30573 $[M+2H]^{2+}$	765.30440
20	772.31356 $[M+2H]^{2+}$	772.31227
21	766.31356 $[M+2H]^{2+}$	766.31240
22	773.32138 $[M+2H]^{2+}$	773.32047
23	765.30573 $[M+2H]^{2+}$	765.30441
24	765.30573 $[M+2H]^{2+}$	765.30475
25	772.31356 $[M+2H]^{2+}$	772.31239
26	766.31356 $[M+2H]^{2+}$	766.31275
27	773.32138 $[M+2H]^{2+}$	773.32047
30	1014.48909 $[M+H]^{+}$	1014.48752
50	1634.61040 $[M+H]^{+}$	1634.61054
51	1438.48922 $[M+H]^{+}$	1438.48948
52	1634.61040 $[M+H]^{+}$	1634.61064
53	1117.47964 $[M+H]^{+}$	1117.48050
54	1117.47964 $[M+H]^{+}$	1117.48031
55	1460.66521 $[M+H]^{+}$	1460.66729
56	1460.66521 $[M+H]^{+}$	1460.66757

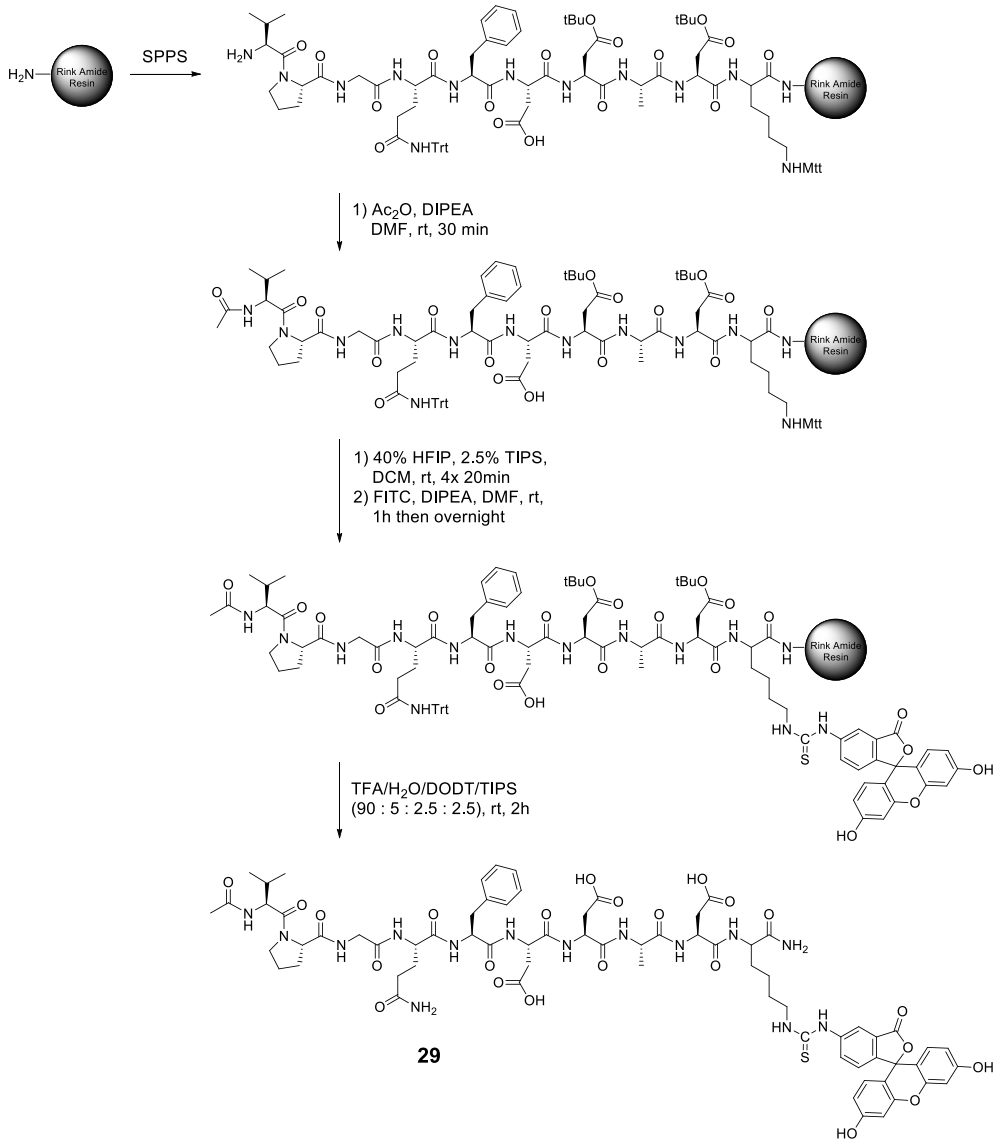
Supporting Synthetic Schemes



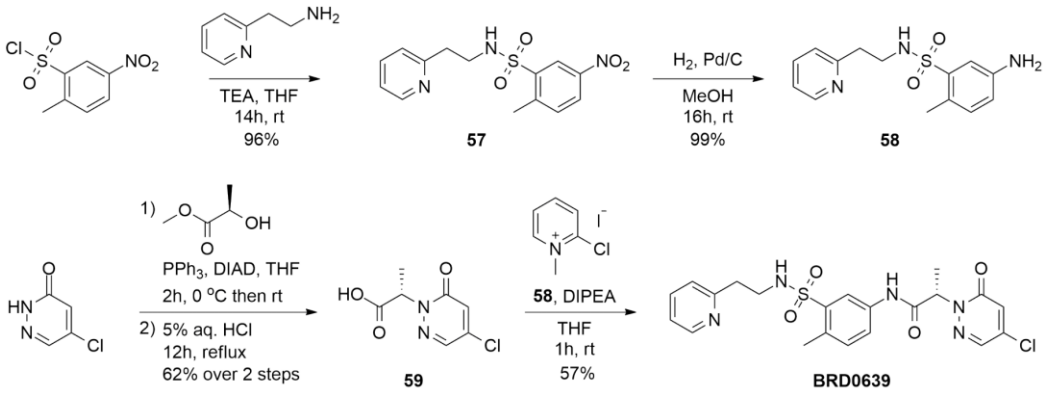
Scheme S1. On-resin protection with Dmb and coupling of the resulting Dmb-DAD sequence to Fmoc-Asp(OAll), en route to peptides **5**, **7**, **9** and **11**.



Scheme S2. Cyclisation through RCM and reduction of the resulting double bond, followed by Mtt removal and labelling with FITC for C-terminally labelled macrocycles. For peptides with a double bond the TPSH reduction step was omitted.



Scheme S3. An exemplary synthetic scheme leading to N-terminally acetylated and C-terminally FITC-labelled linear peptide **29**.



Scheme S4. Synthesis of the covalent PRMT5 PPI inhibitor BRD0639.

Supporting Figures

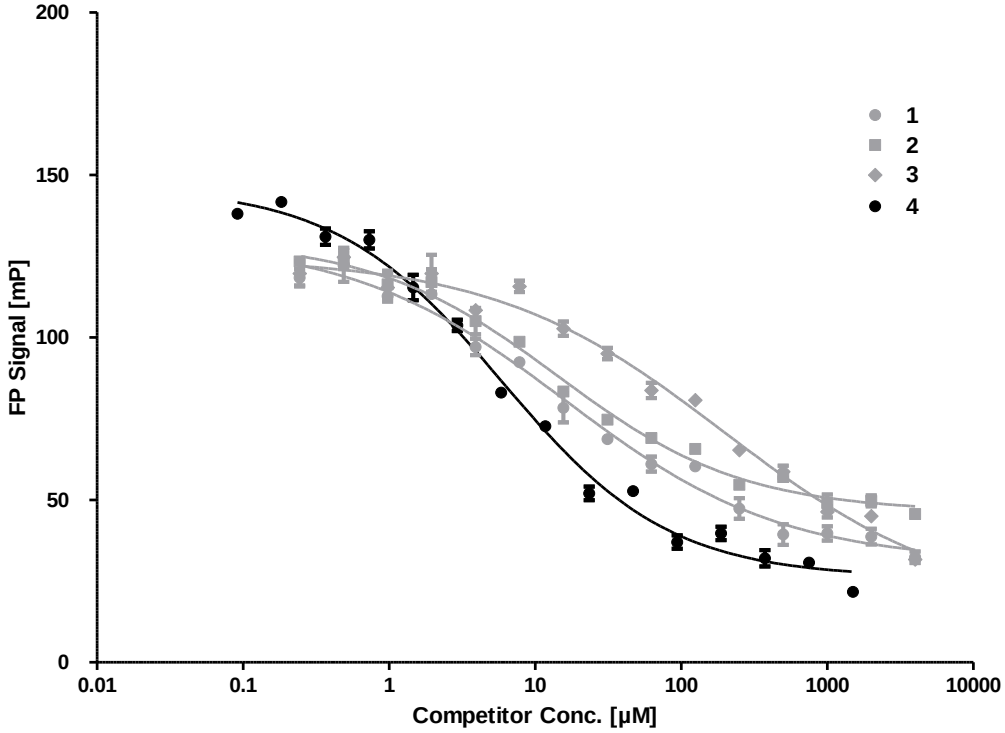


Figure S1. Competitive binding FP with PRMT5-MEP50 and peptide **S1** (Table S1) used as a fluorescent tracer, for compounds **1-4**.

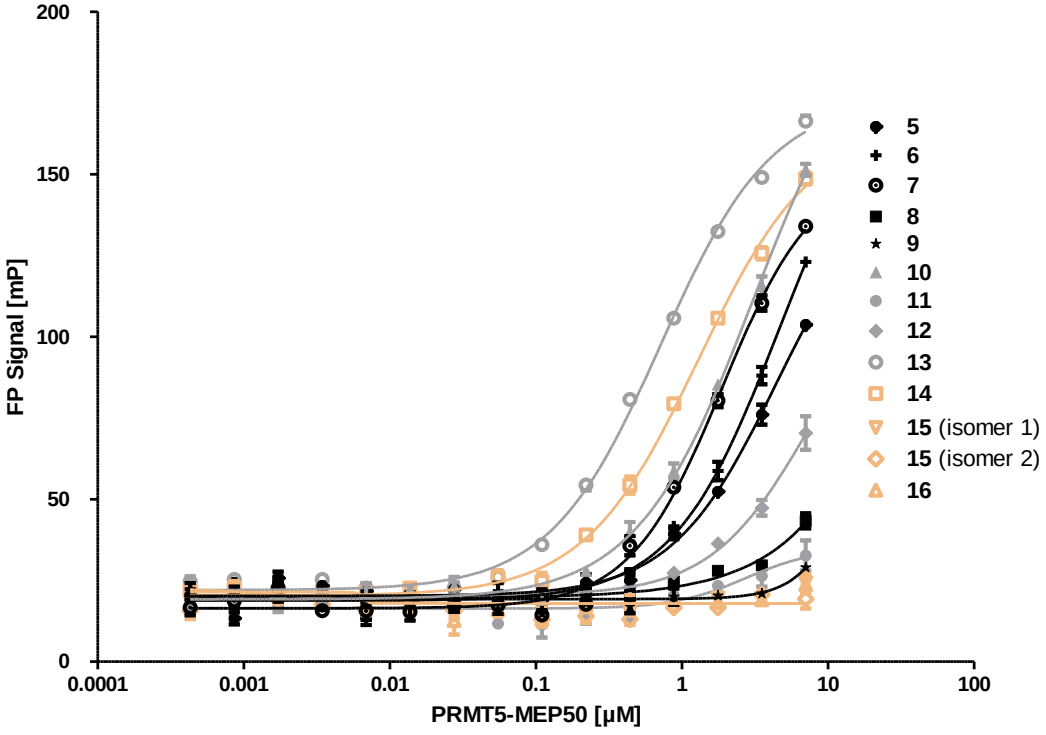


Figure S2. Direct binding FP with PRMT5-MEP50 for compounds **5-16**.

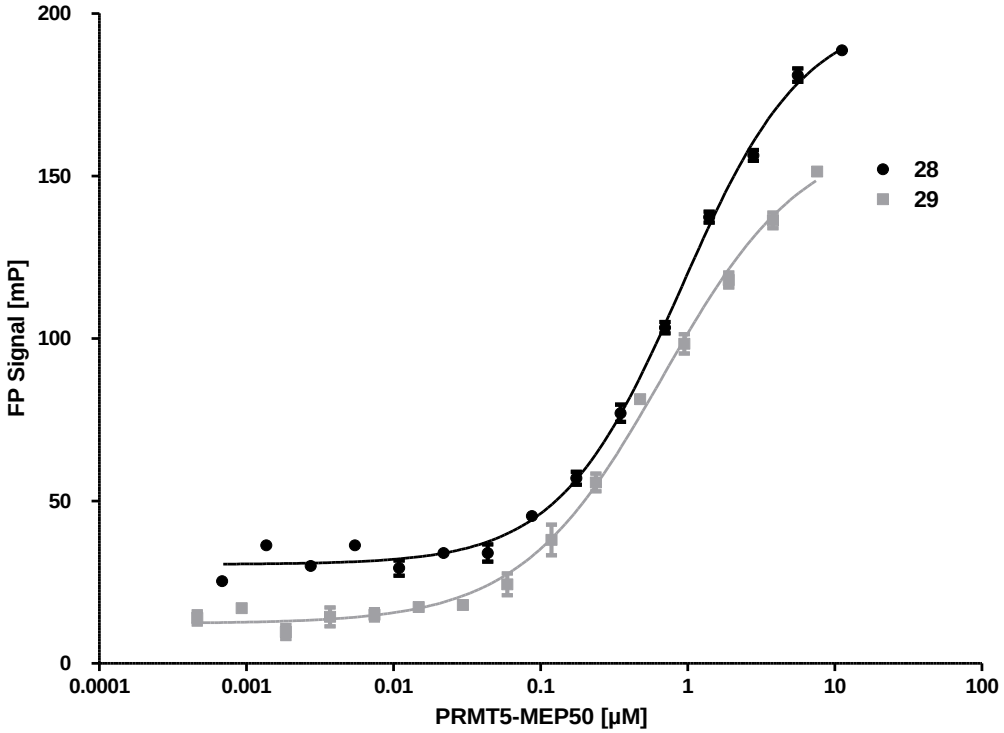


Figure S3. Direct binding FP with PRMT5-MEP50 for compound **28** and **29**.

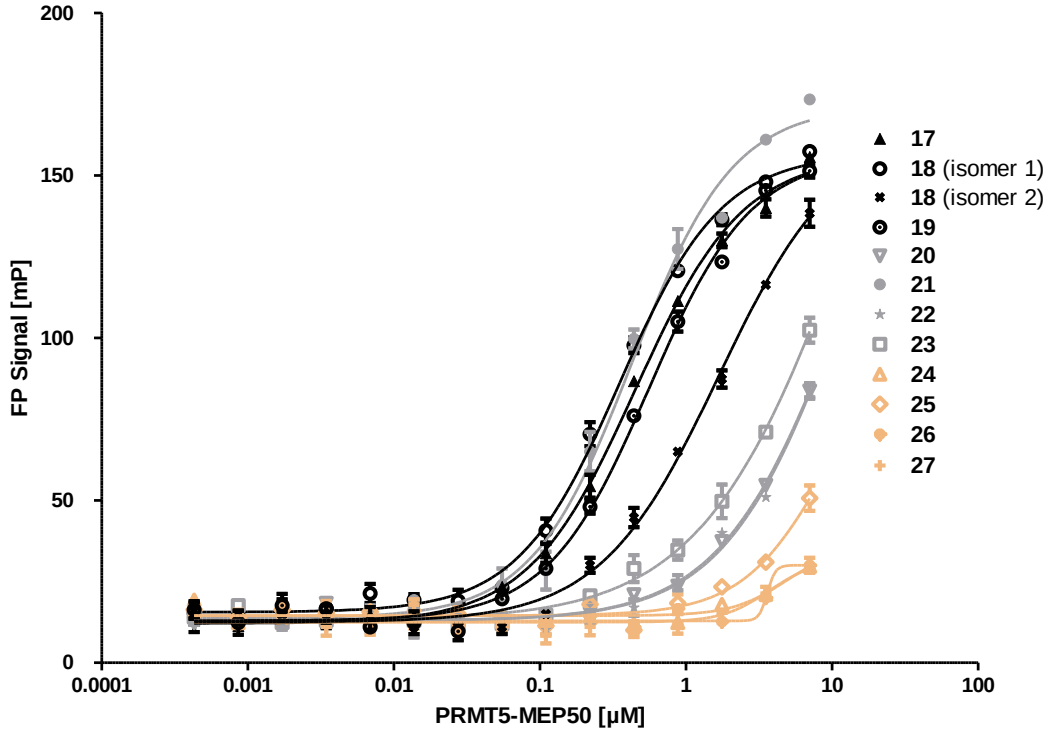


Figure S4. Direct binding FP with PRMT5-MEP50 for compounds **17-27**.

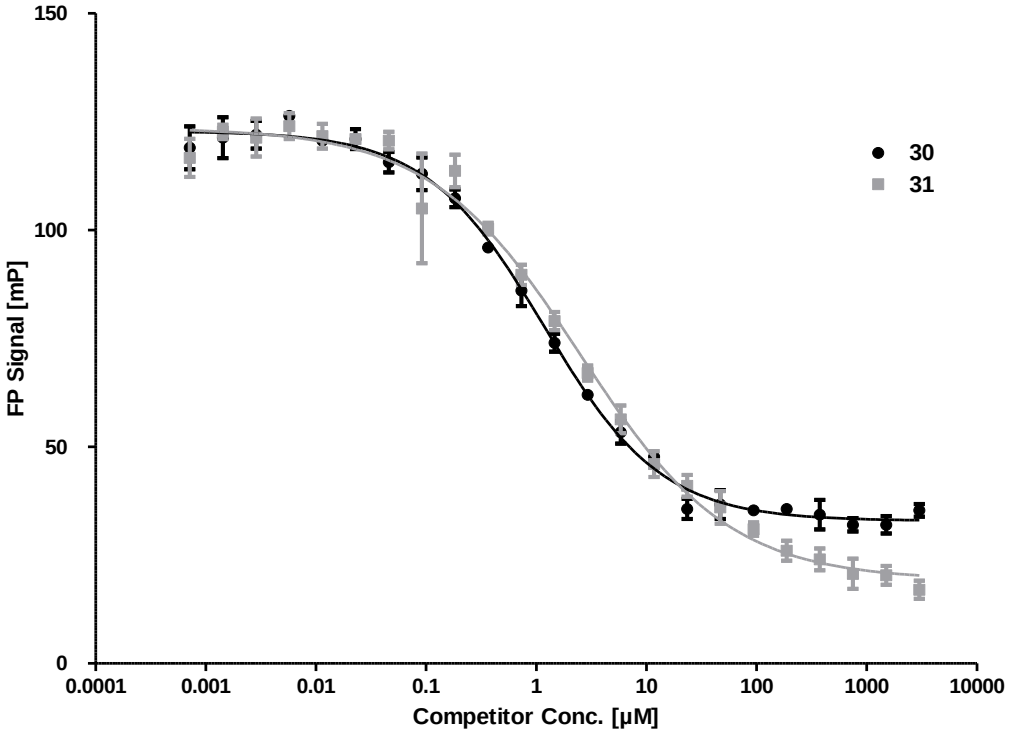


Figure S5. Competitive binding FP with PRMT5-MEP50 and fluorescent **21** used as a tracer, for compounds **30** and **31**.

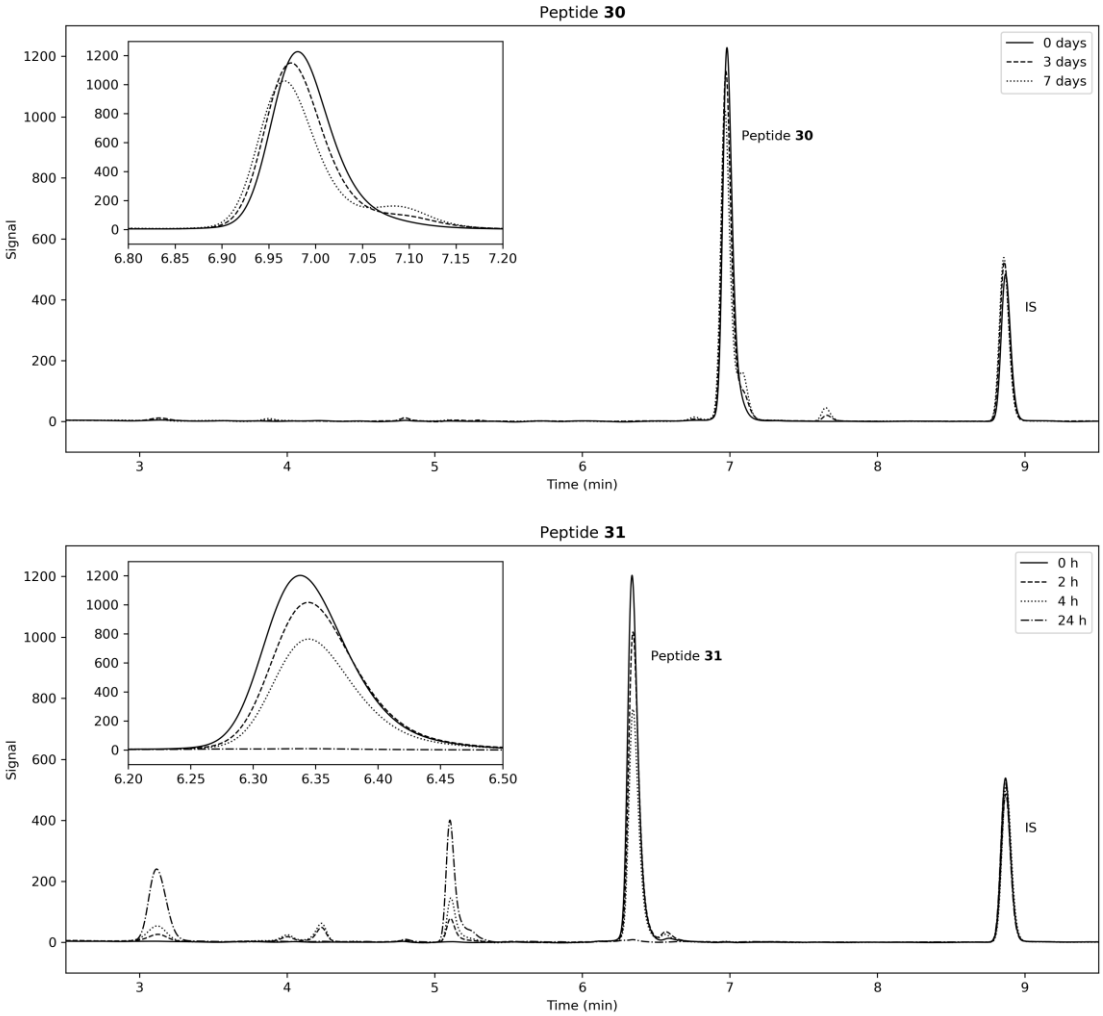


Figure S6. HPLC analysis of cyclic **30** and linear **31** after incubation in the U2OS cell lysate. Presented timepoints: between 0-7 days for **30** and between 0-24 h for **31**. IS = Internal Standard.

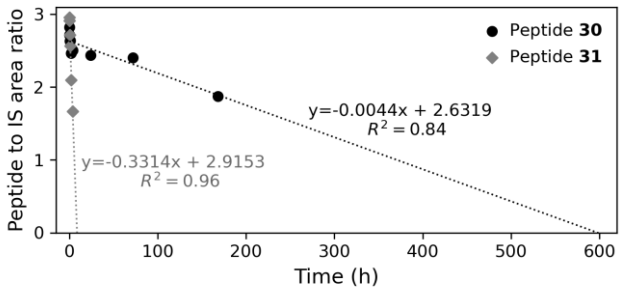


Figure S7. Linear regression models based on the obtained stability data for **30** and **31**. T½ = 299 h or 12.5 days for **30** and t½ = 4.4 h for **31** (n=2).

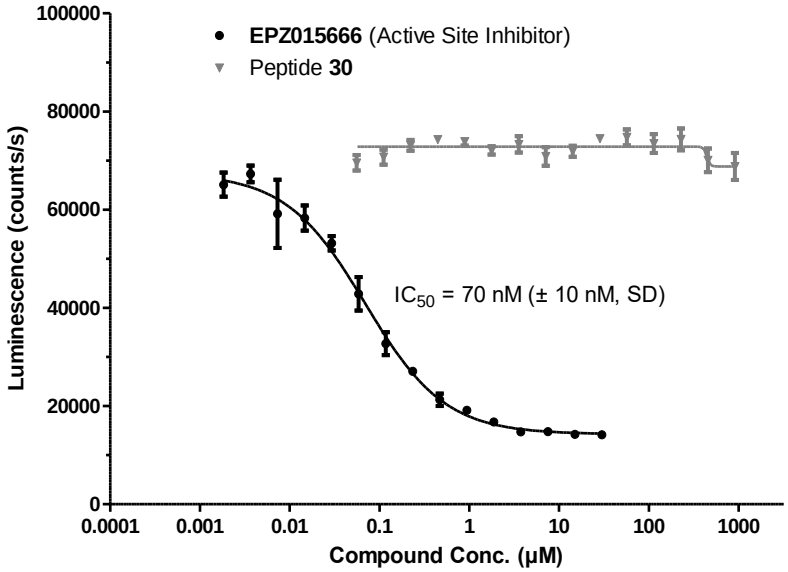


Figure S8. Mtase-Glo™ Methyltransfarase Assay performed on the isolated PRMT-MEP50 complex with **30** and the reference active-site methyltransferase inhibitor **EPZ015666**. Compound **EPZ015666** is able to inhibit the direct methylation of the H4 histone tail peptide by PRMT5, whereas **30** has no effect (n=3).

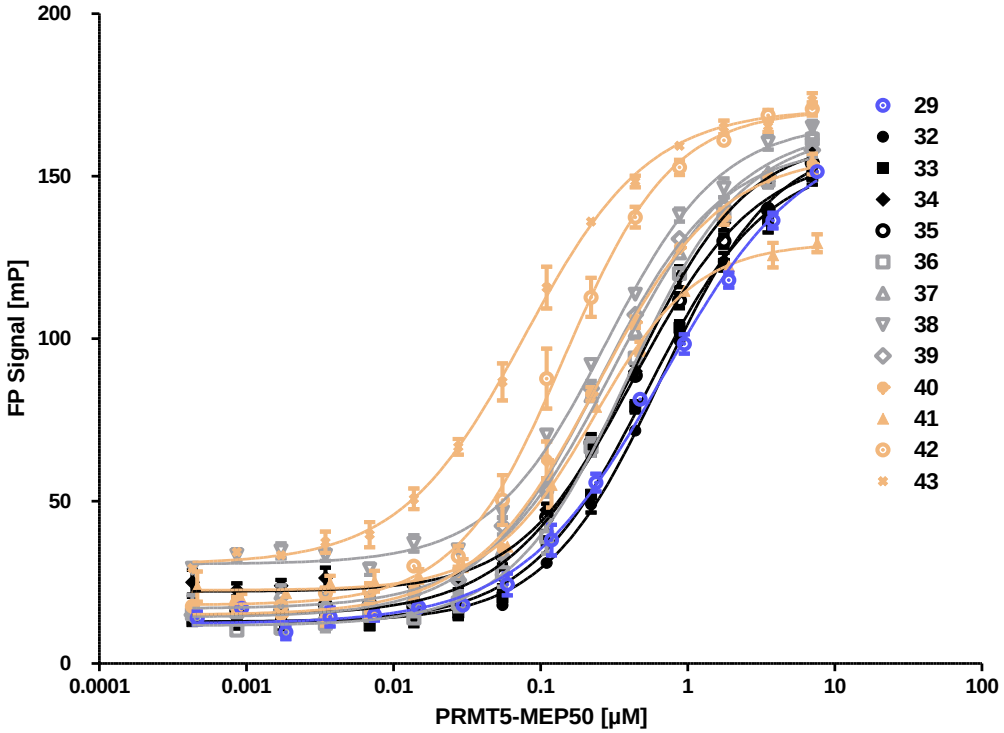


Figure S9. Direct binding FP with PRMT5-MEP50 for compounds **29** and **32-43**.

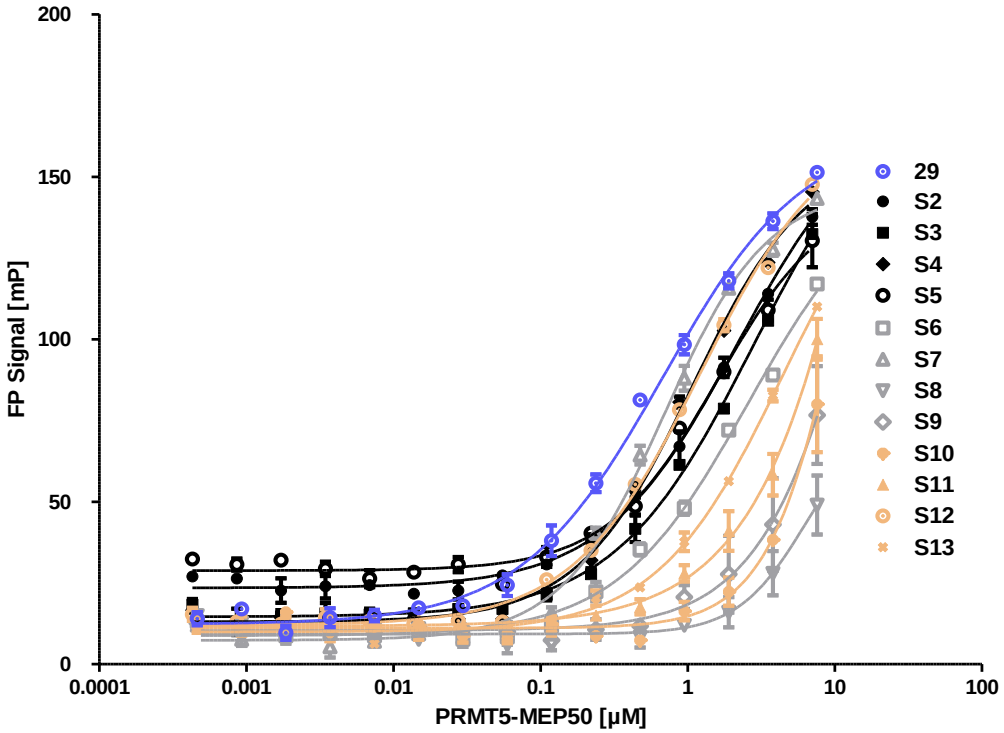


Figure S10. Direct binding FP with PRMT5-MEP50 for compounds **29** and **S3-S14**.

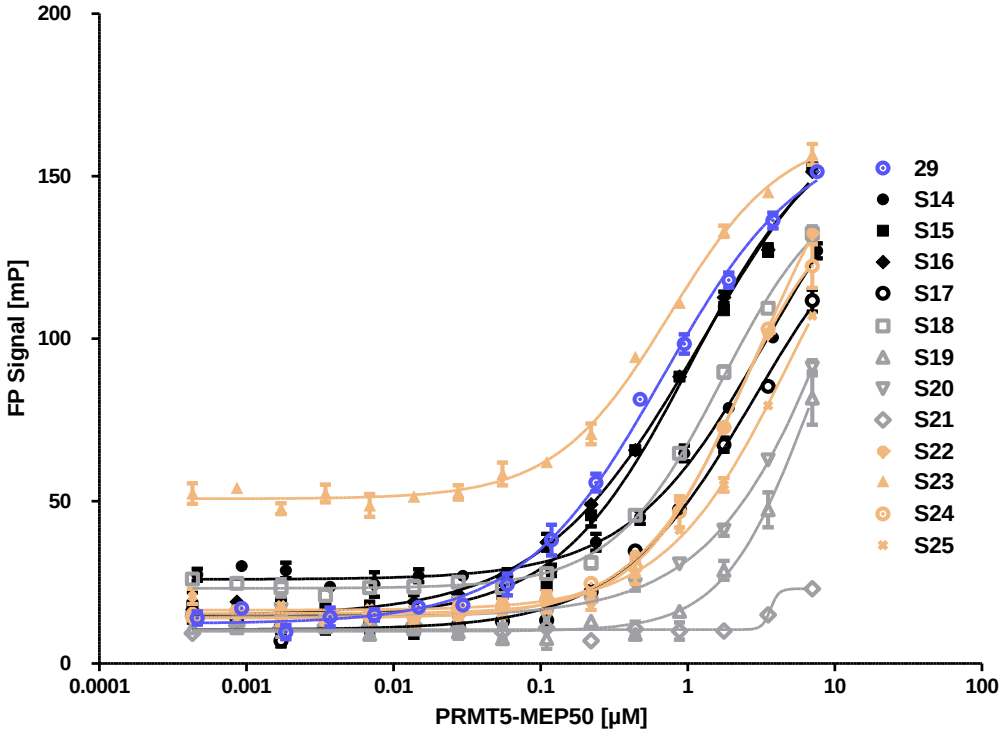


Figure S11. Direct binding FP with PRMT5-MEP50 for compounds **29** and **S14-S25**.

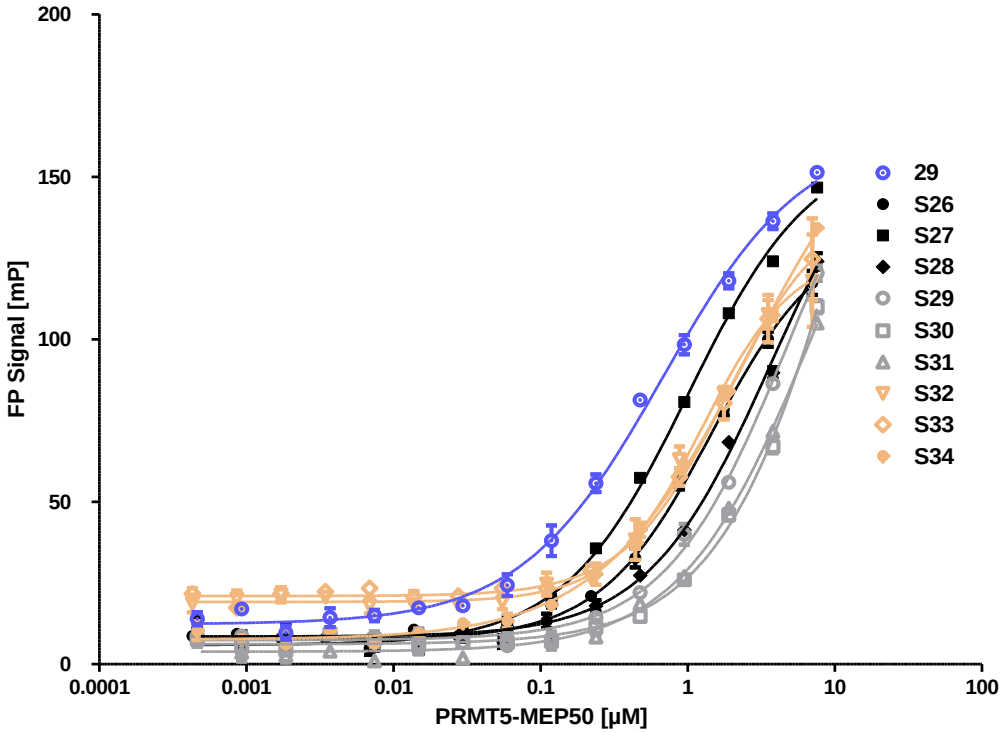


Figure S12. Direct binding FP with PRMT5-MEP50 for compounds **29** and **S26-S34**.

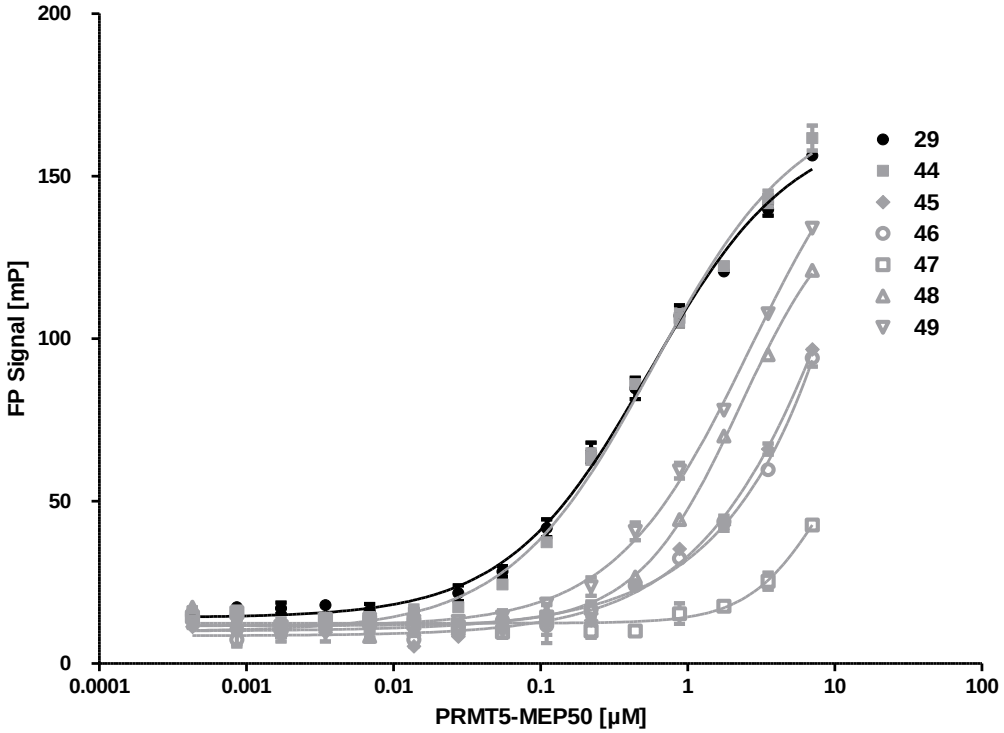


Figure S13. Direct binding FP with PRMT5-MEP50 for compounds **29** and **44**-**49**.

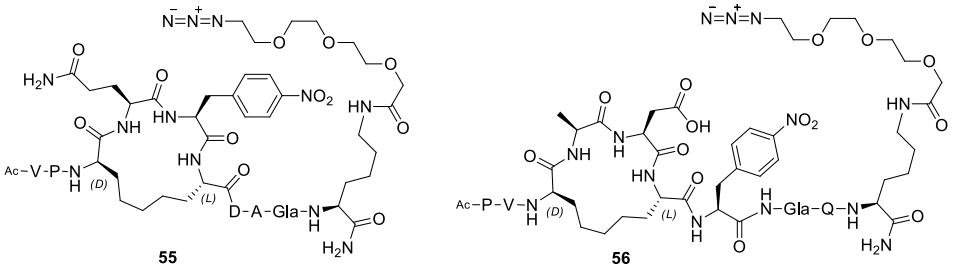


Figure S14. Structures of cyclic peptides **55** and **56**, equipped with azide group.

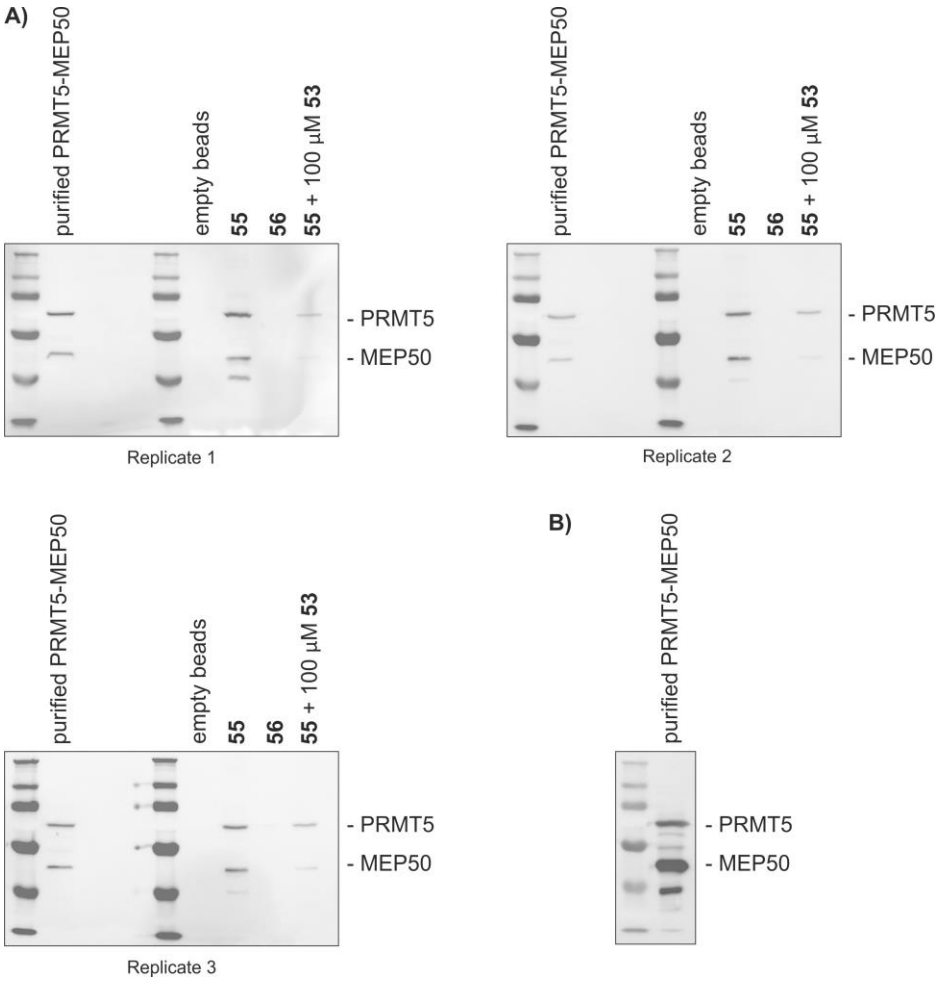


Figure S15. A) Results of the pull-down assay with peptide **55** and scrambled peptide **56** immobilised on the DBCO beads, using MCF7 cell lysate. **B)** Western blot of the purified PRMT5-MEP50 complex analysed at a higher concentration than in A).

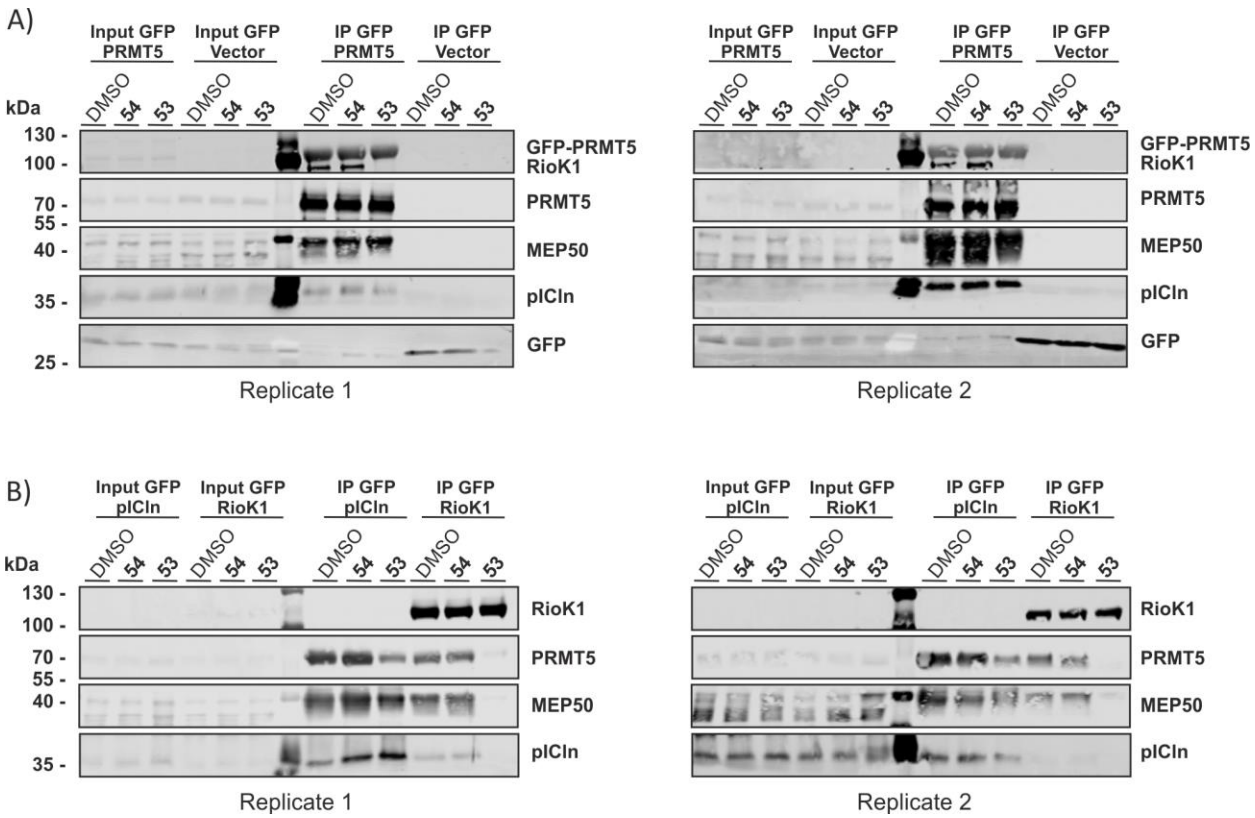
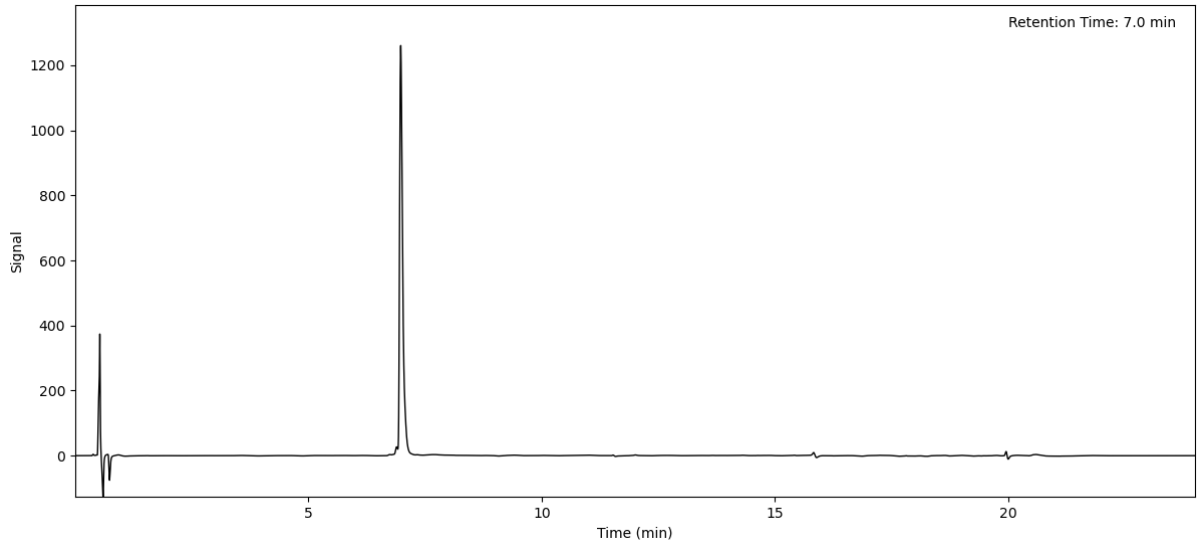


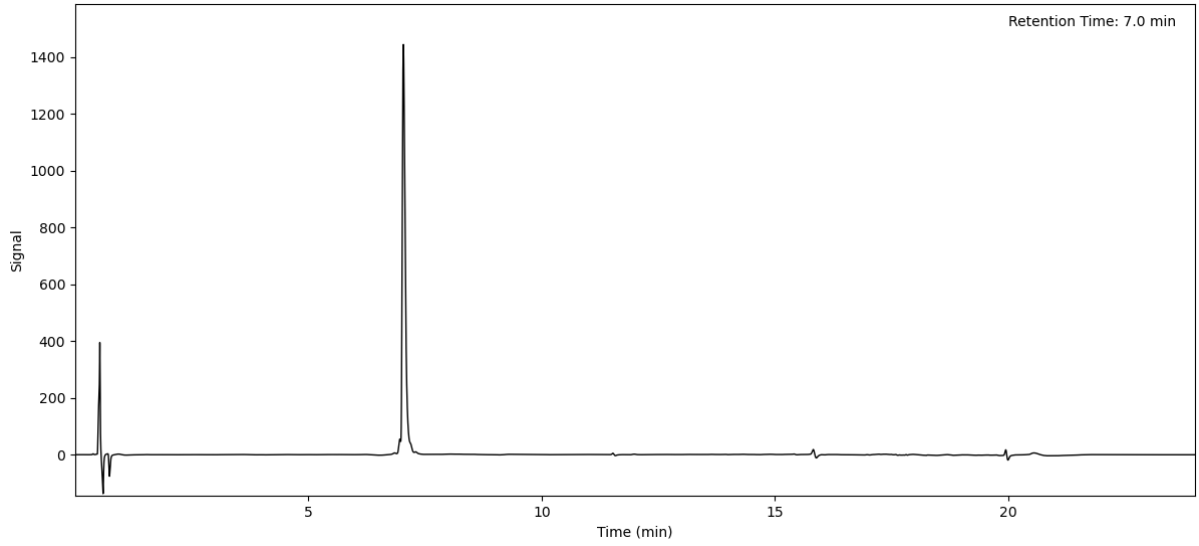
Figure S16. A) GFP-Immunopurification (GFP-IP) in Flp-In T-REx 293-GFP and Flp-In T-REx 293-GFP-PRMT5 overexpressing cells after testing active **53** and scrambled **54** at 50 µM and DMSO as a control. Therefore, cells were stimulated with 0.1 µg/ml doxycycline for 18 h before cytoplasm extraction (S100). GFP-IP was performed and analyzed by Tris/Glycine-SDS-PAGE and western blotting using antibodies against RioK1, PRMT5, MEP50, pICln and GFP. **B)** GFP-IP from Flp-In T-REx 293-GFP-pICln and Flp-In T-Rex 293-GFP-RioK1 cytoplasmic extract after testing active **53** and scrambled **54** at 50 µM and DMSO as a control. Induction of the overexpression, Tris/Glycine-SDS-PAGE and western blotting using antibodies against RioK1, PRMT5, MEP50 and pICln was performed as described in **A**.

HPLC Traces

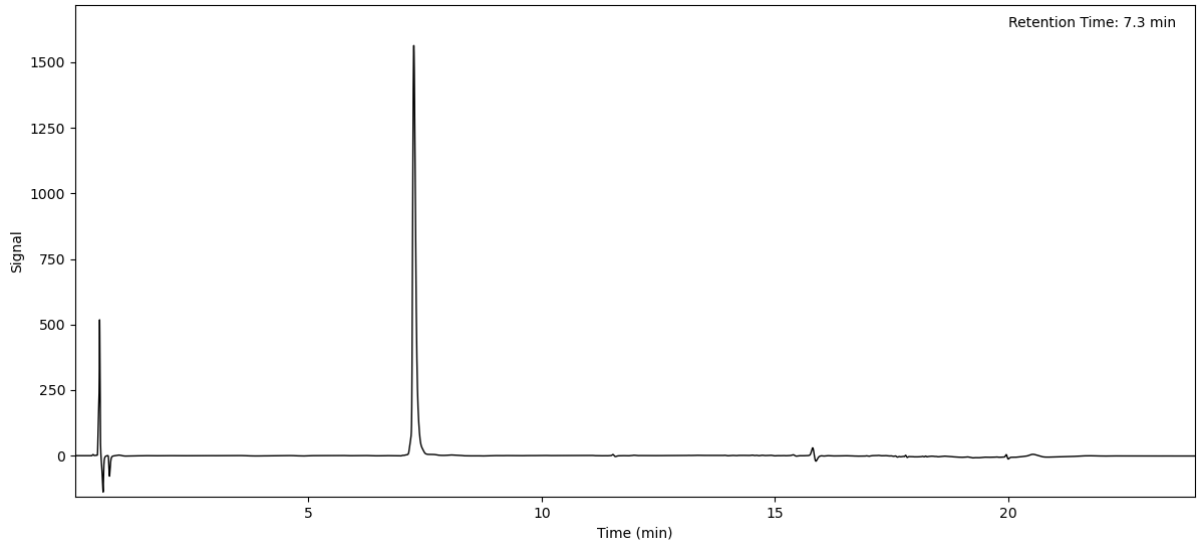
Peptide 1 (Method A)



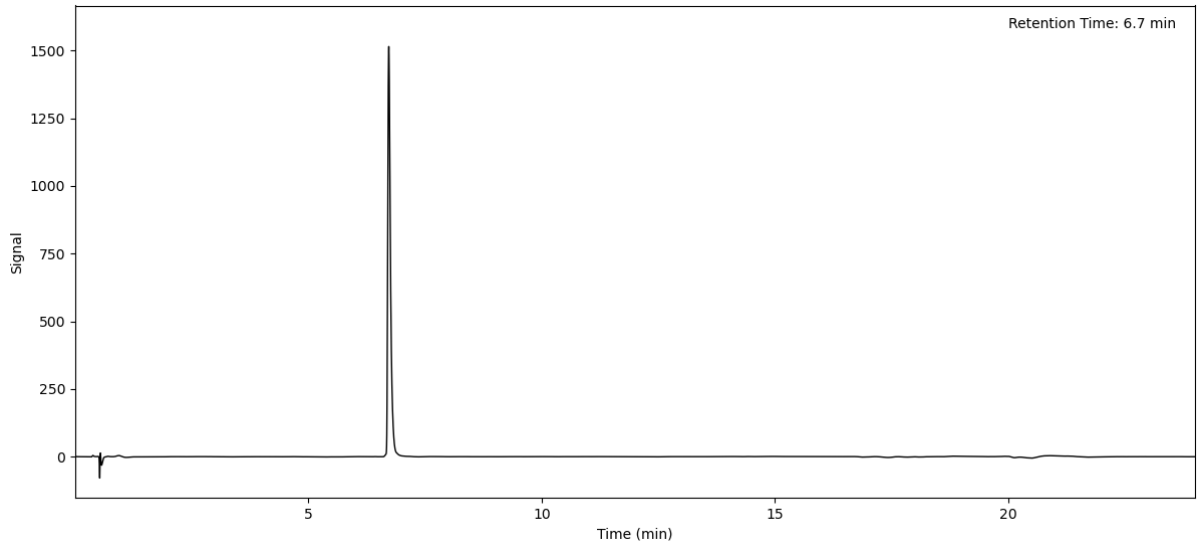
Peptide 2 (Method A)



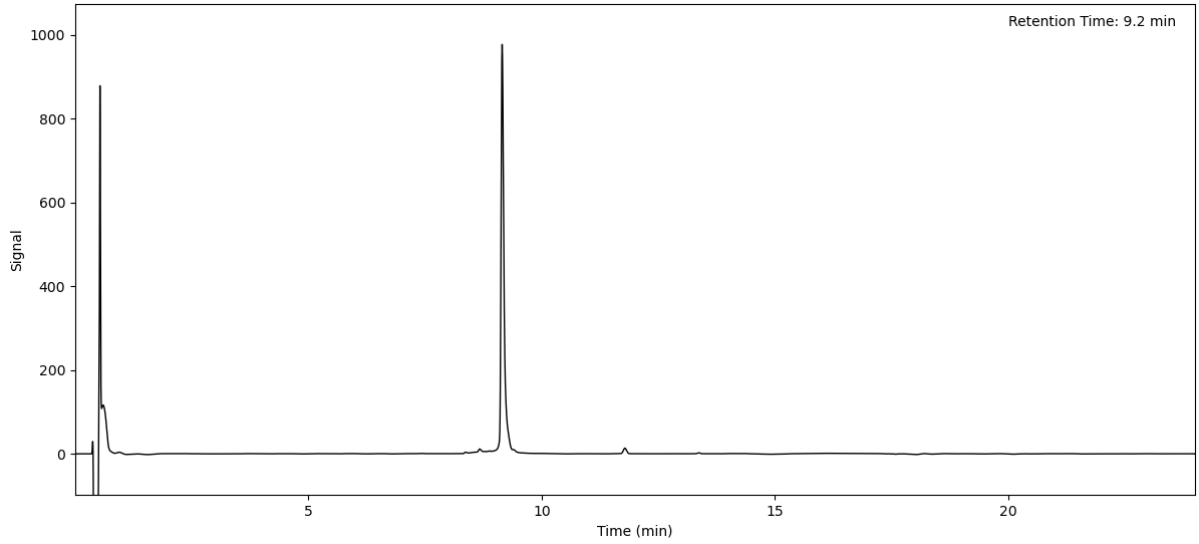
Peptide 3 (Method A)



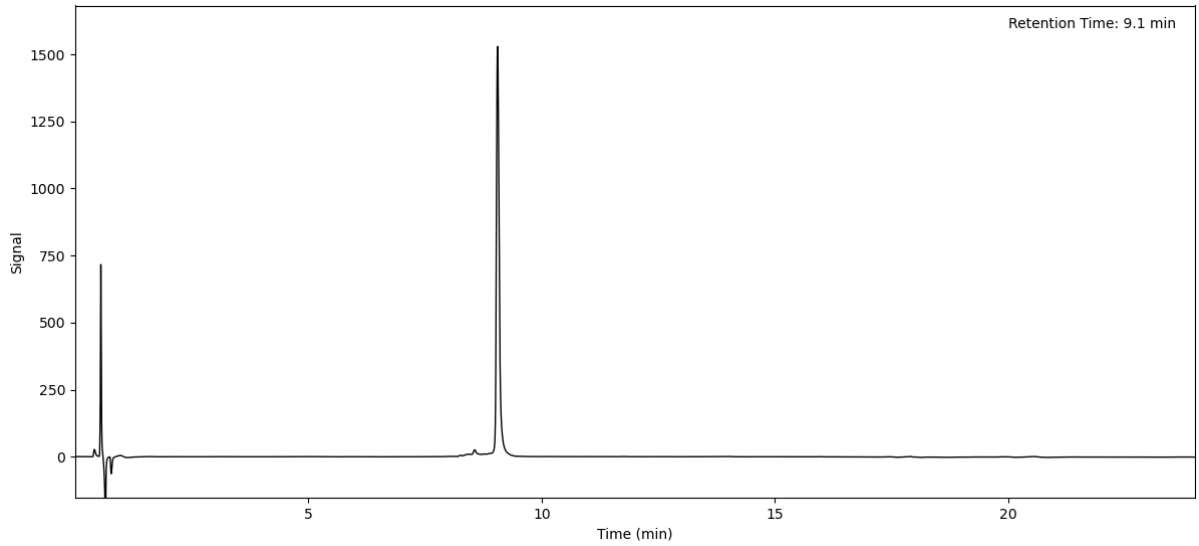
Peptide 4 (Method A)



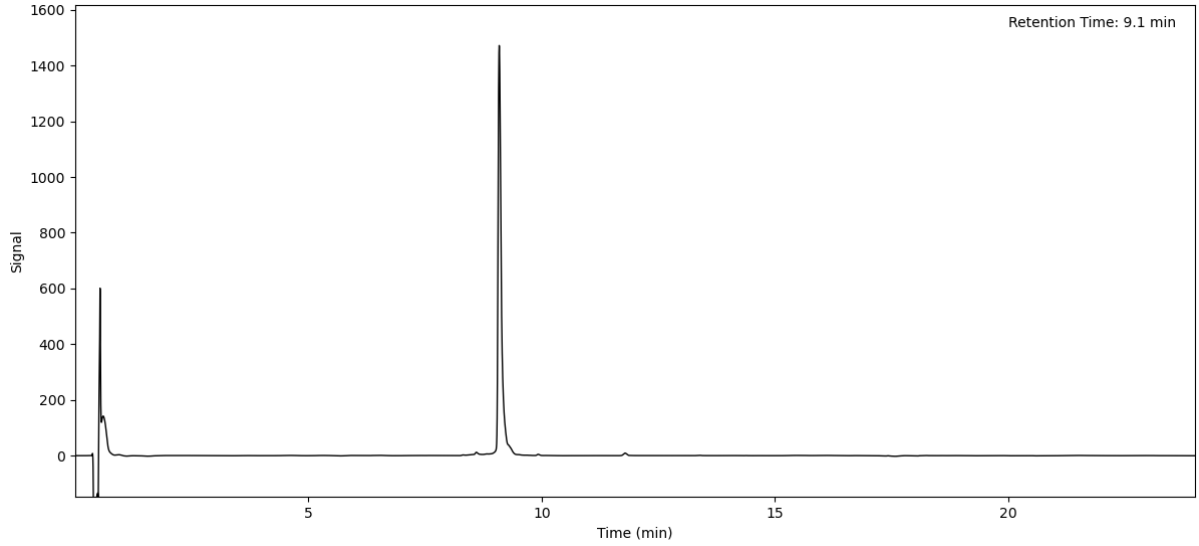
Peptide 5 (Method A)



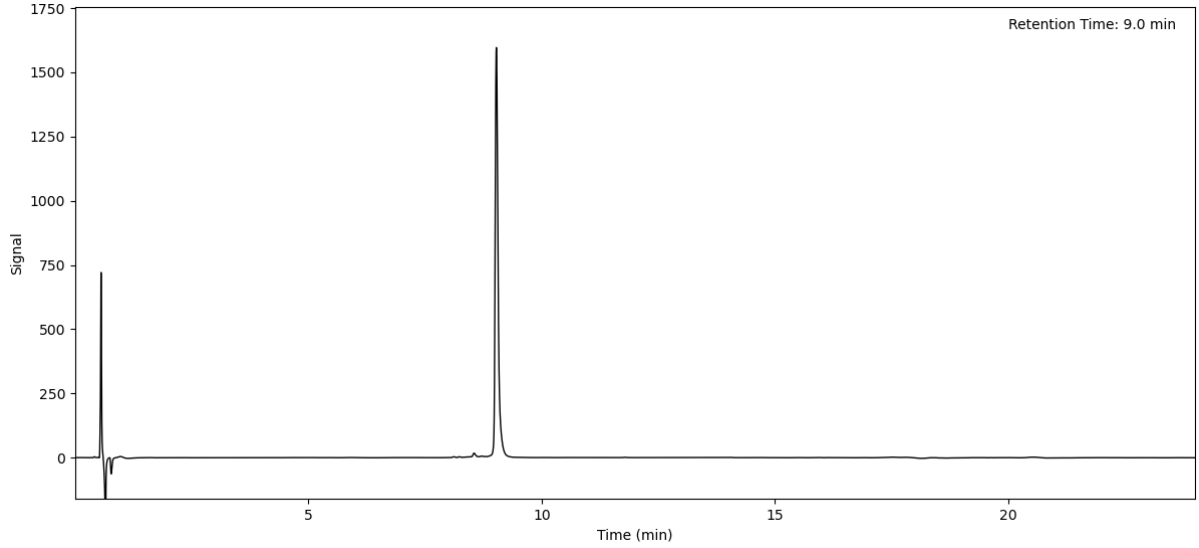
Peptide 6 (Method A)



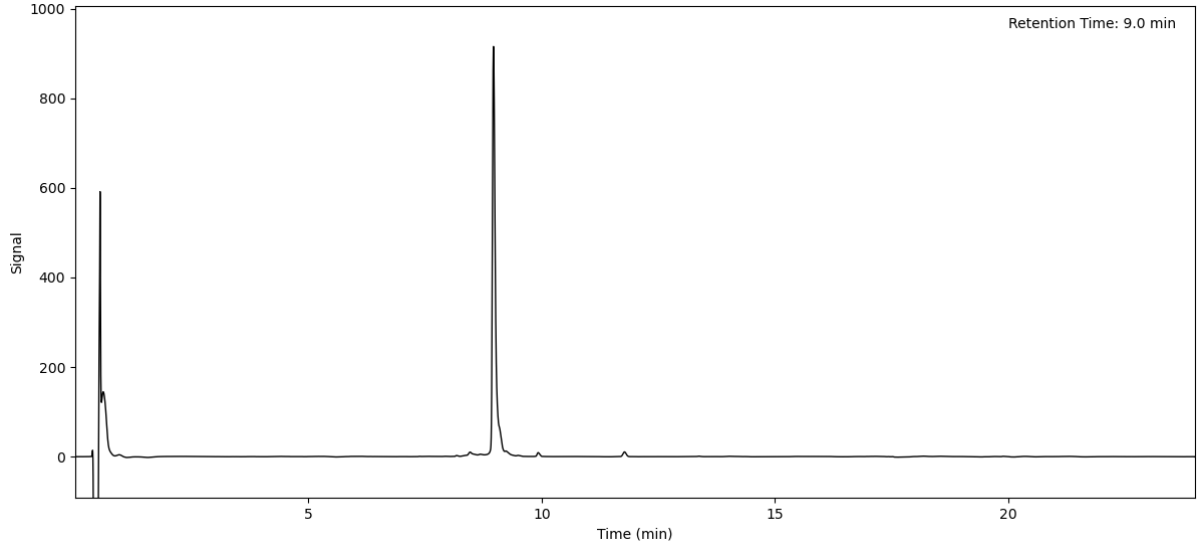
Peptide 7 (Method A)



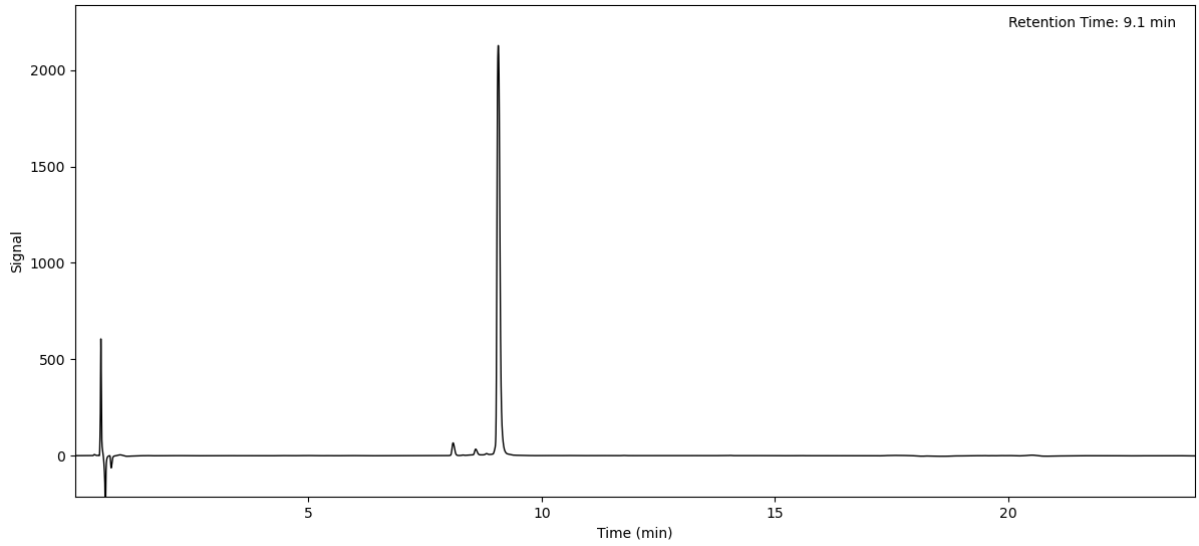
Peptide 8 (Method A)



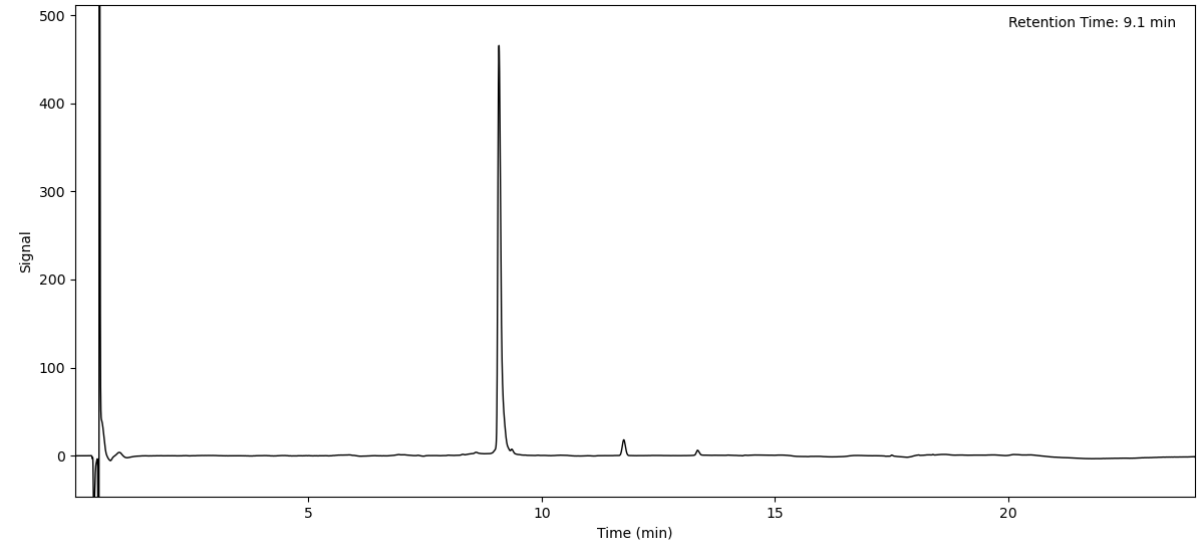
Peptide 9 (Method A)



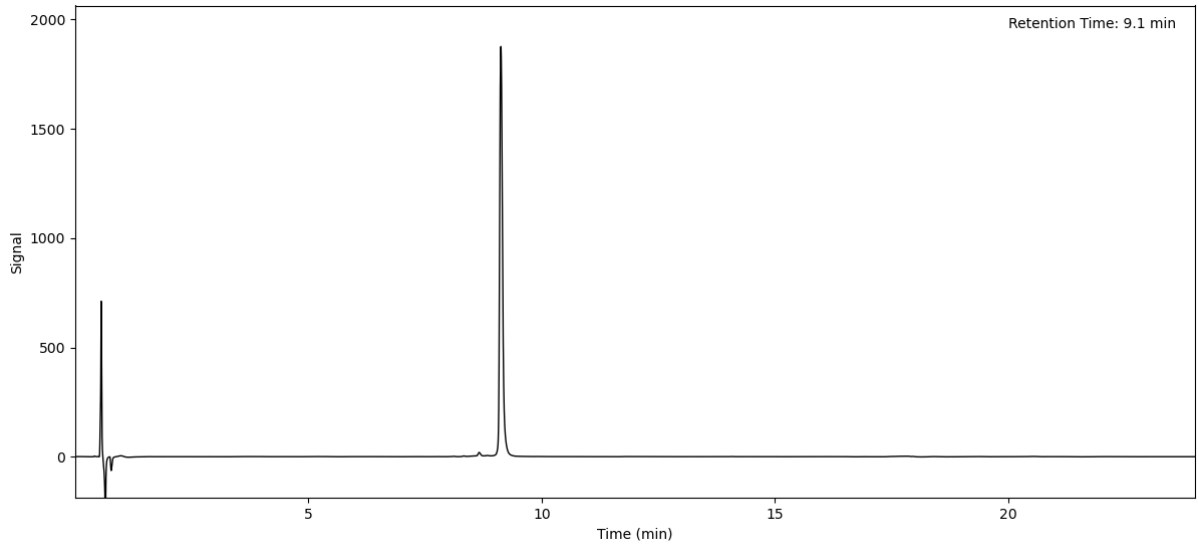
Peptide 10 (Method A)



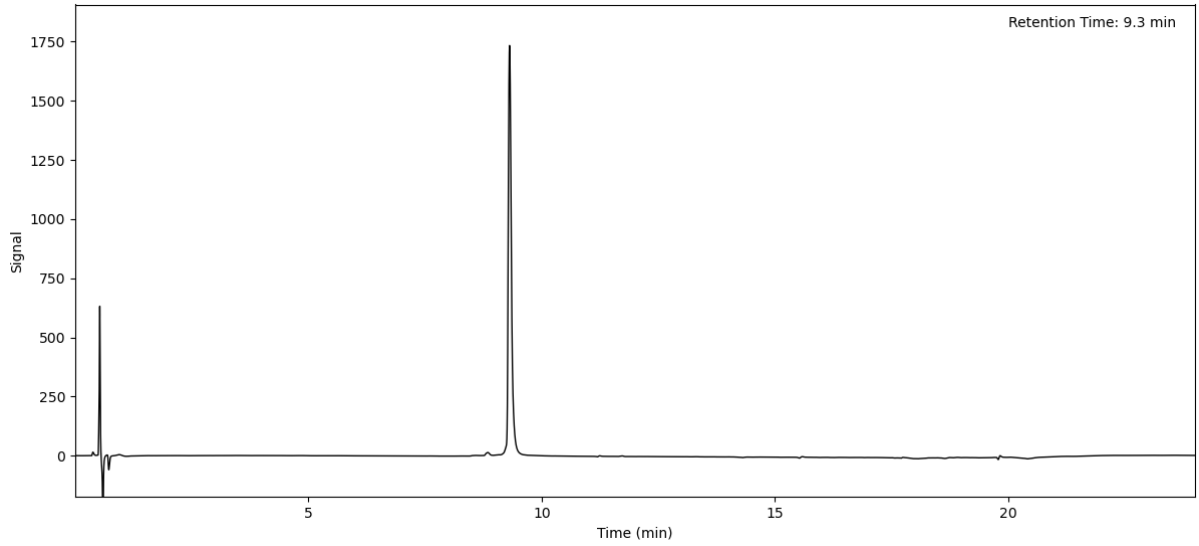
Peptide 11 (Method A)



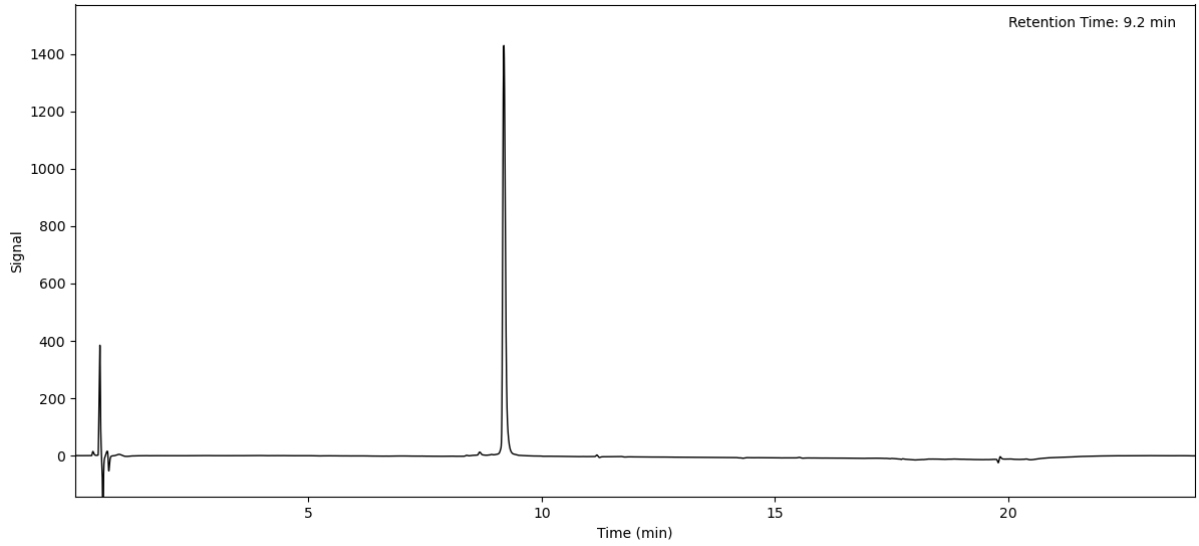
Peptide 12 (Method A)



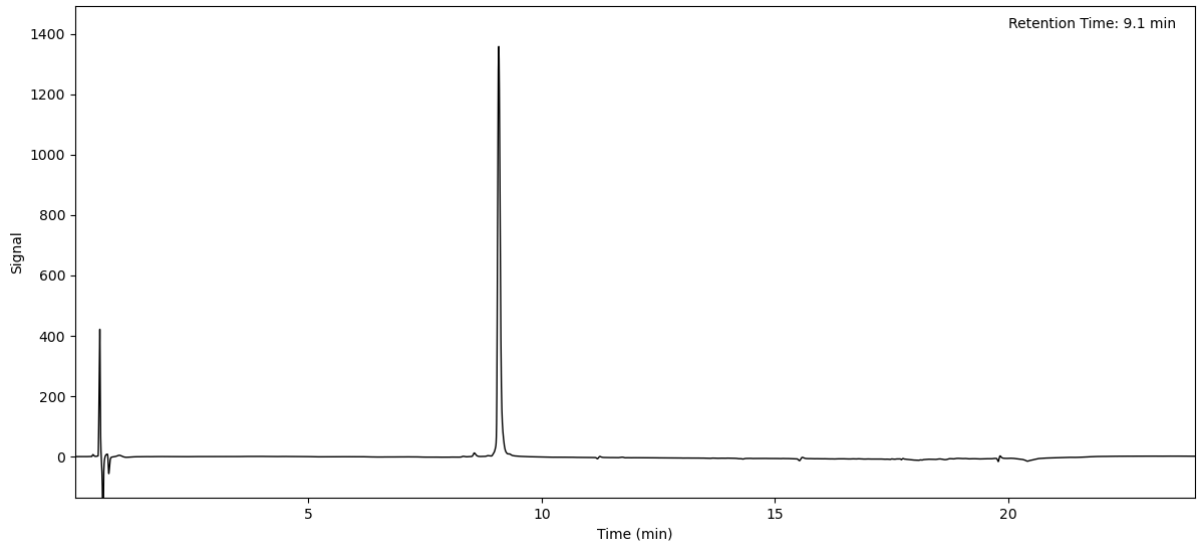
Peptide 13 (Method A)



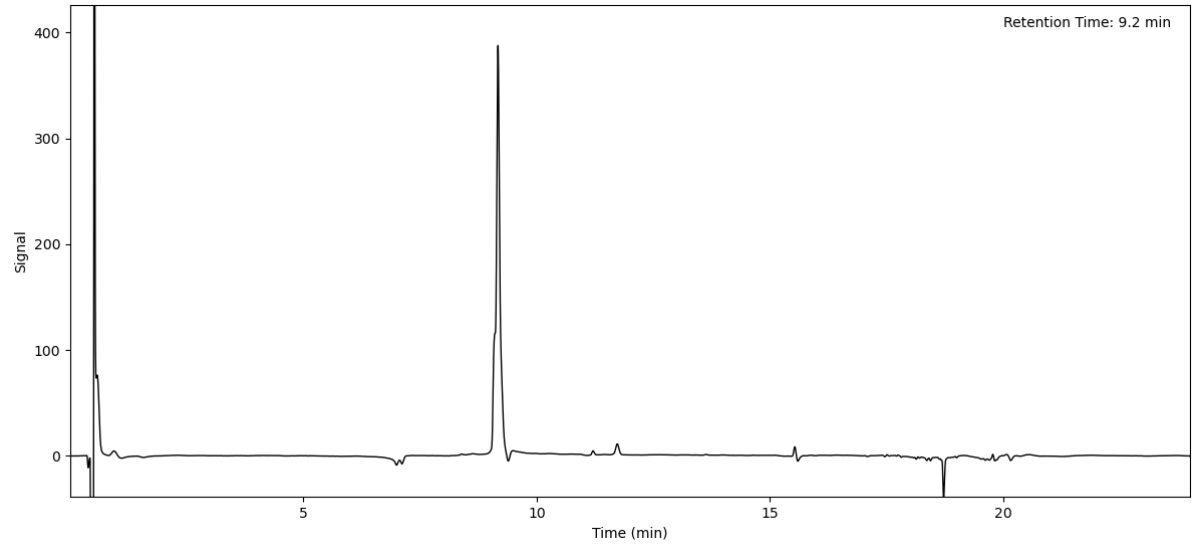
Peptide 14 (Method A)



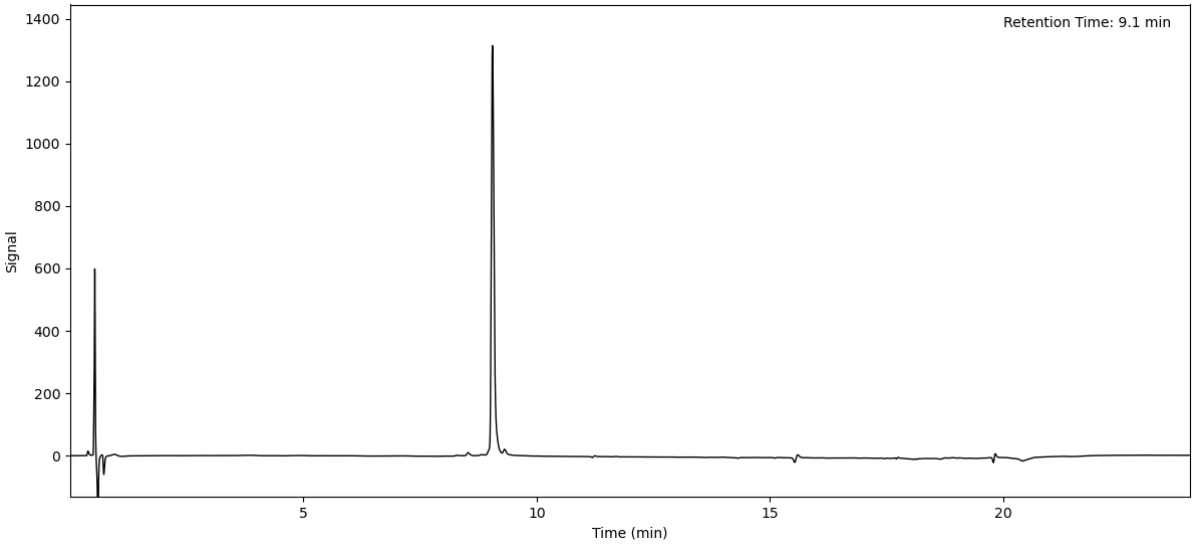
Peptide 15 isomer 1 (Method A)



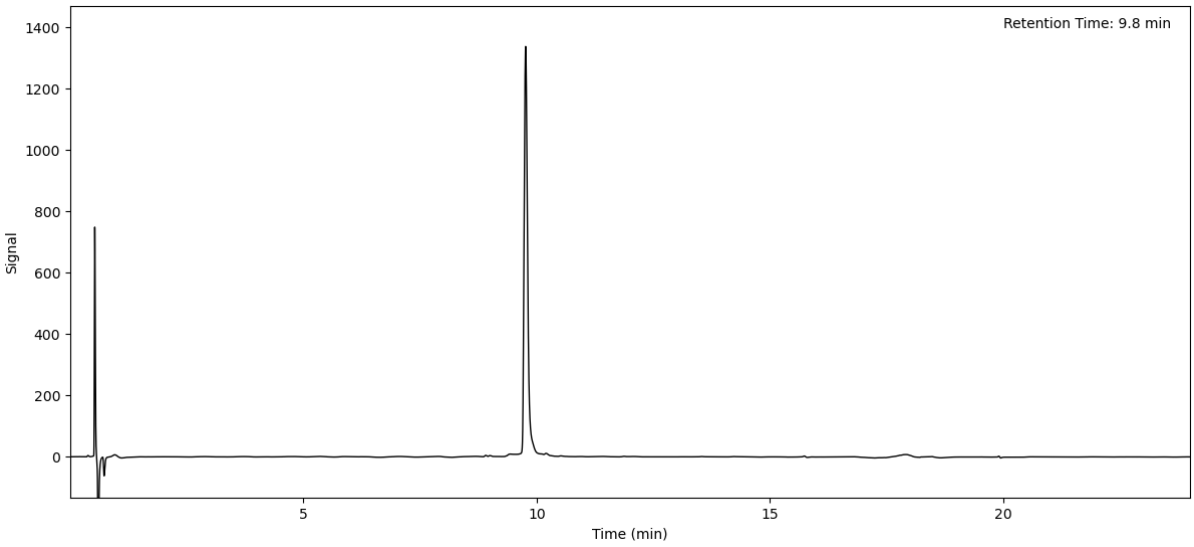
Peptide 15 isomer 2 (Method A)



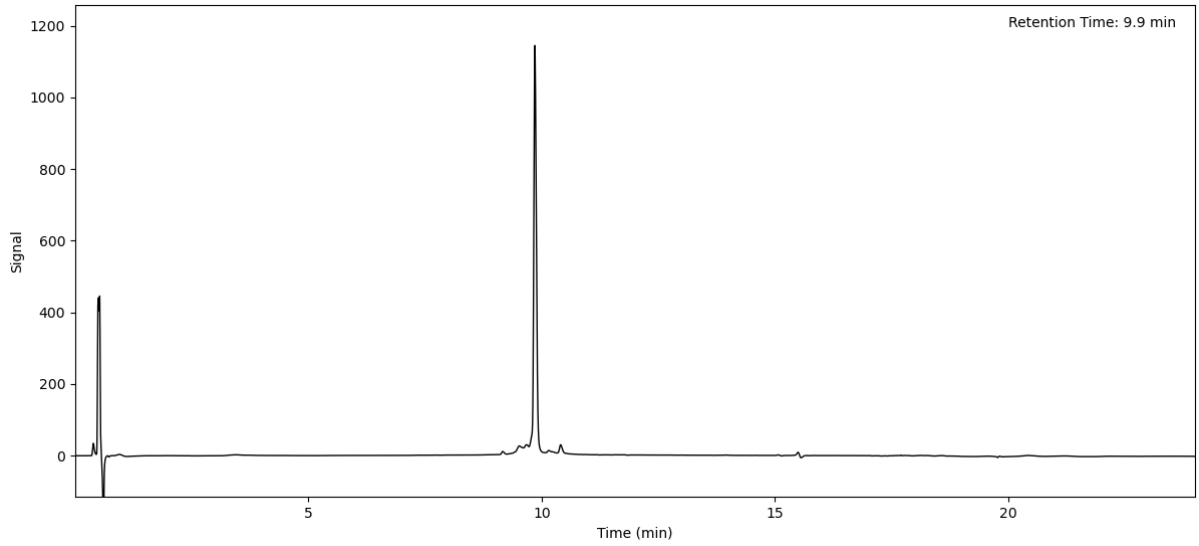
Peptide 16 (Method A)



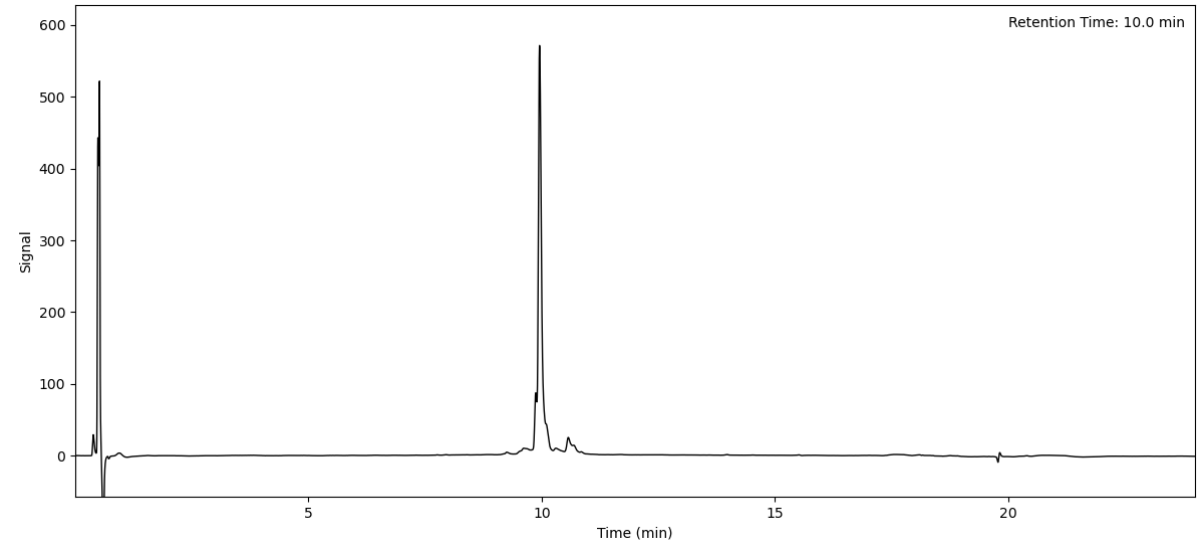
Peptide 17 (Method A)



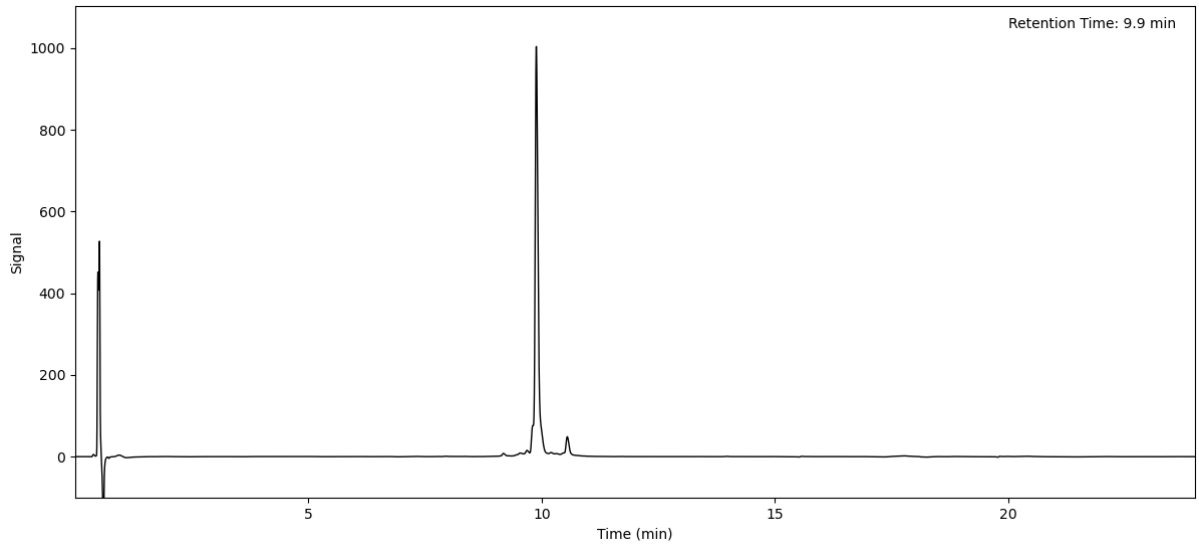
Peptide 18 isomer 1 (Method A)



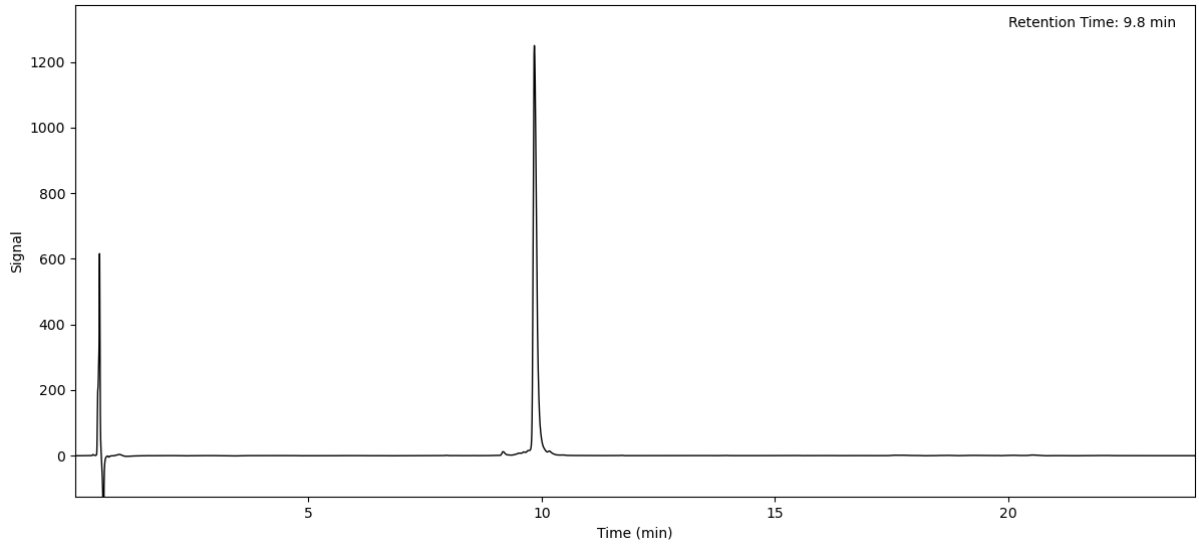
Peptide 18 isomer 2 (Method A)



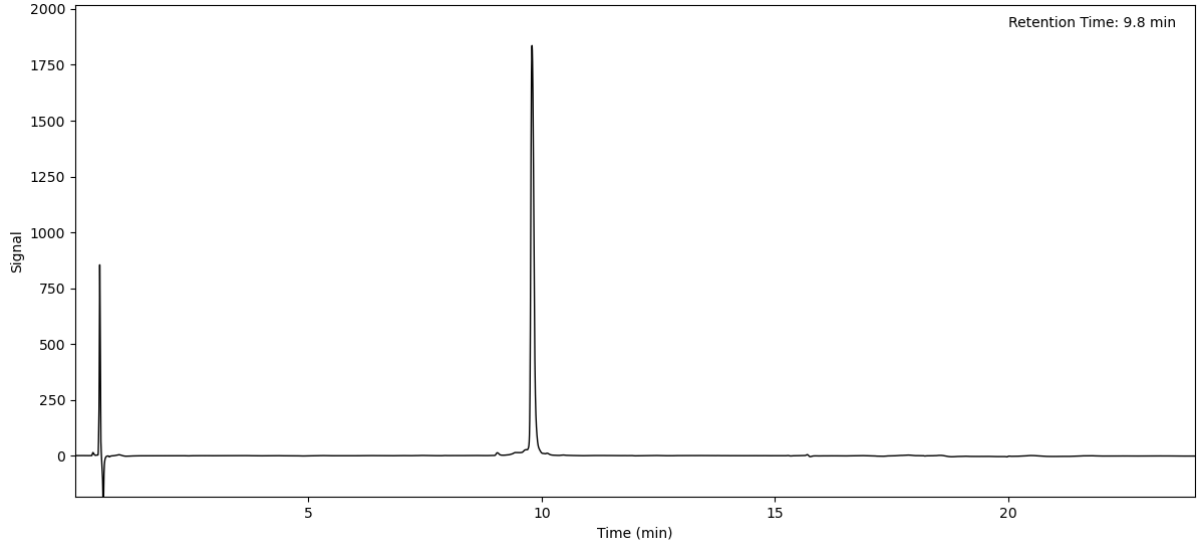
Peptide 19 (Method A)



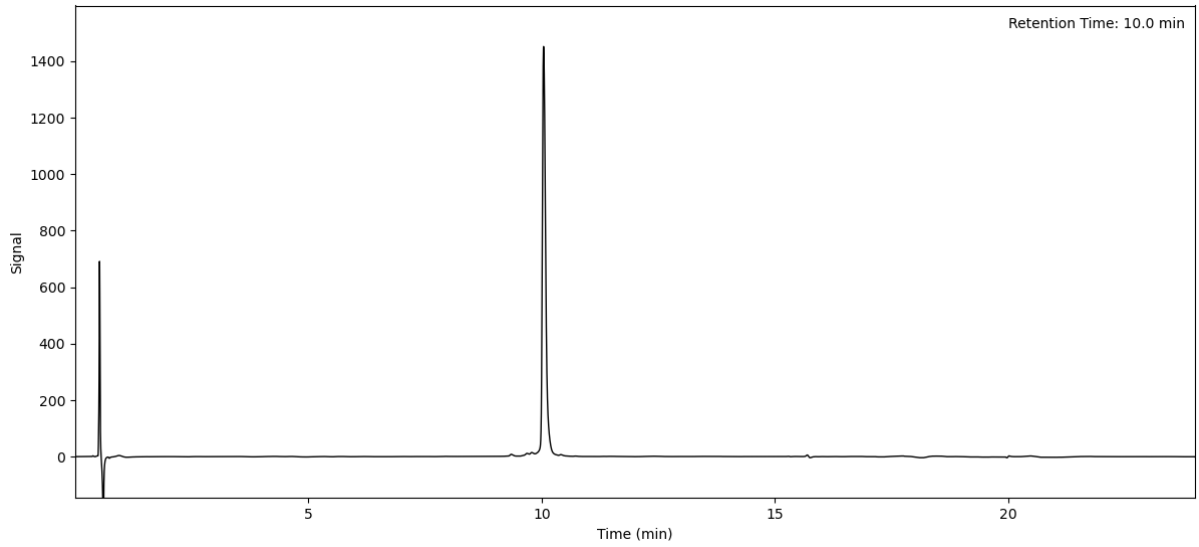
Peptide 20 (Method A)



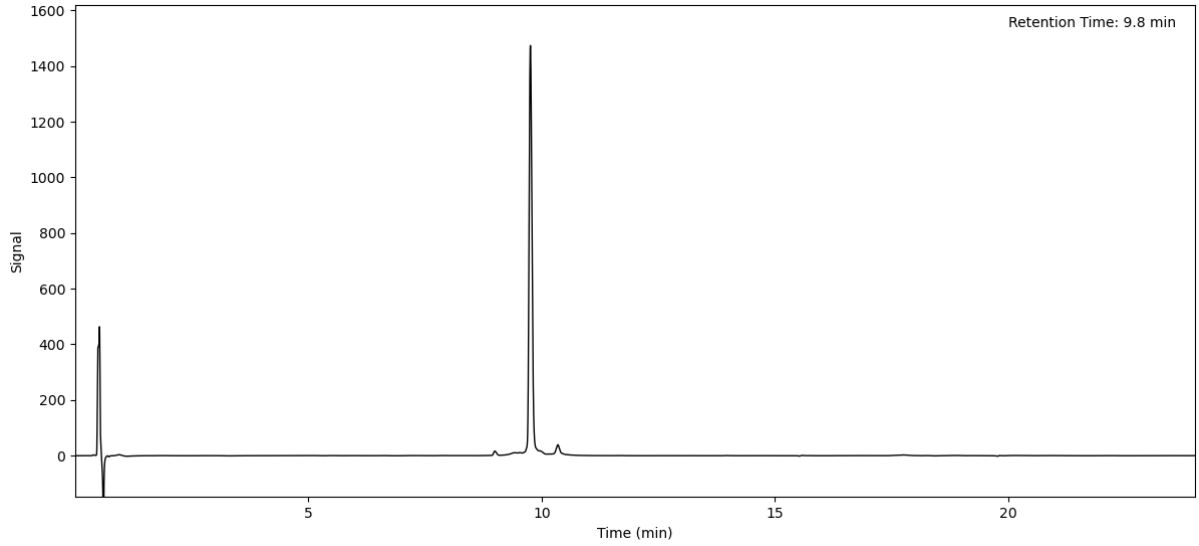
Peptide 21 (Method A)



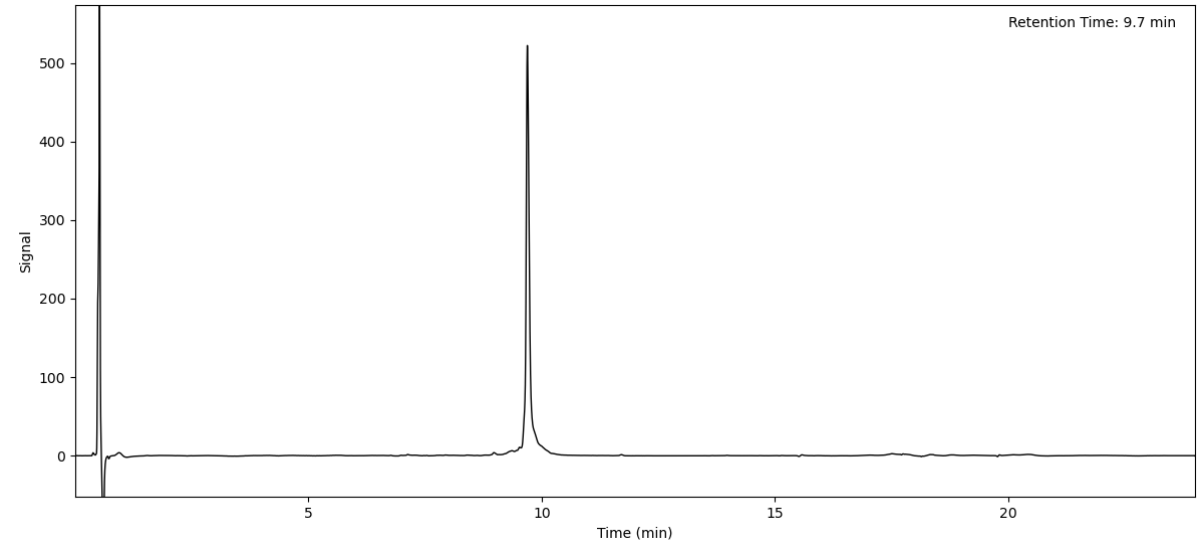
Peptide 22 (Method A)



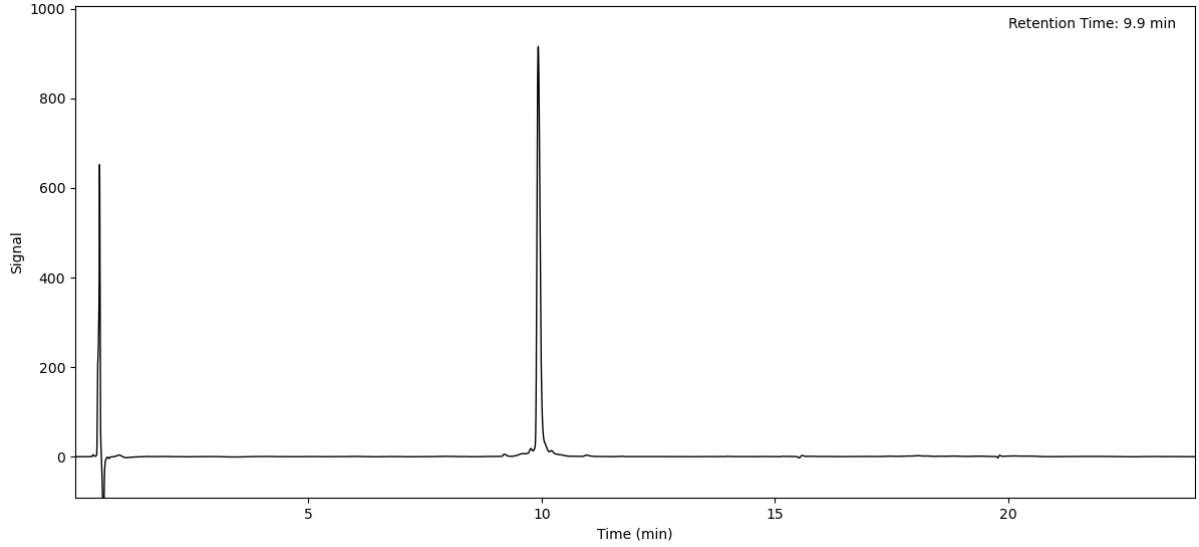
Peptide 23 (Method A)



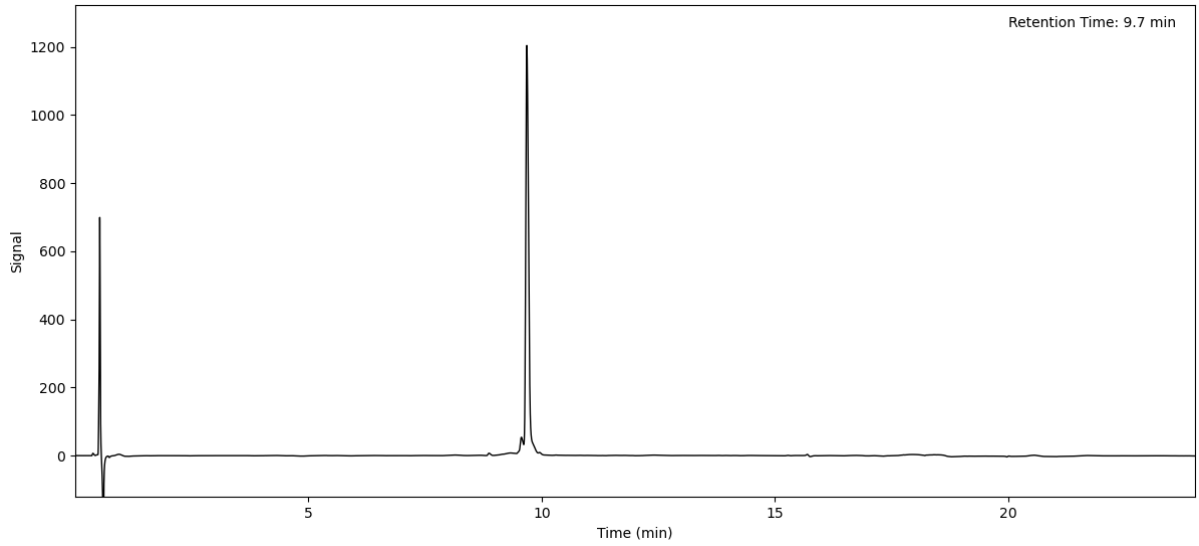
Peptide 24 (Method A)



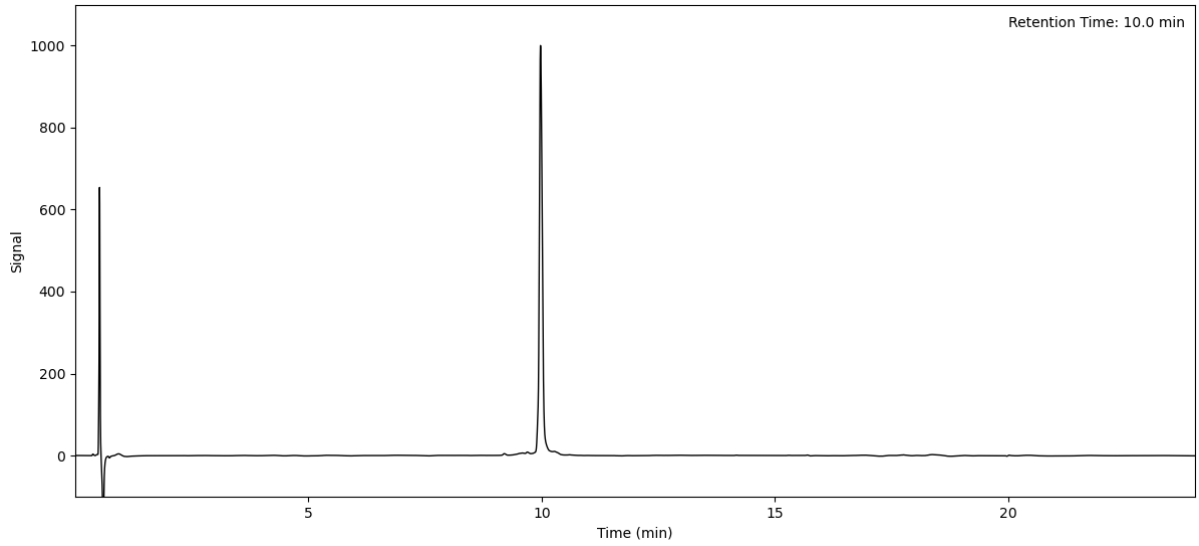
Peptide 25 (Method A)



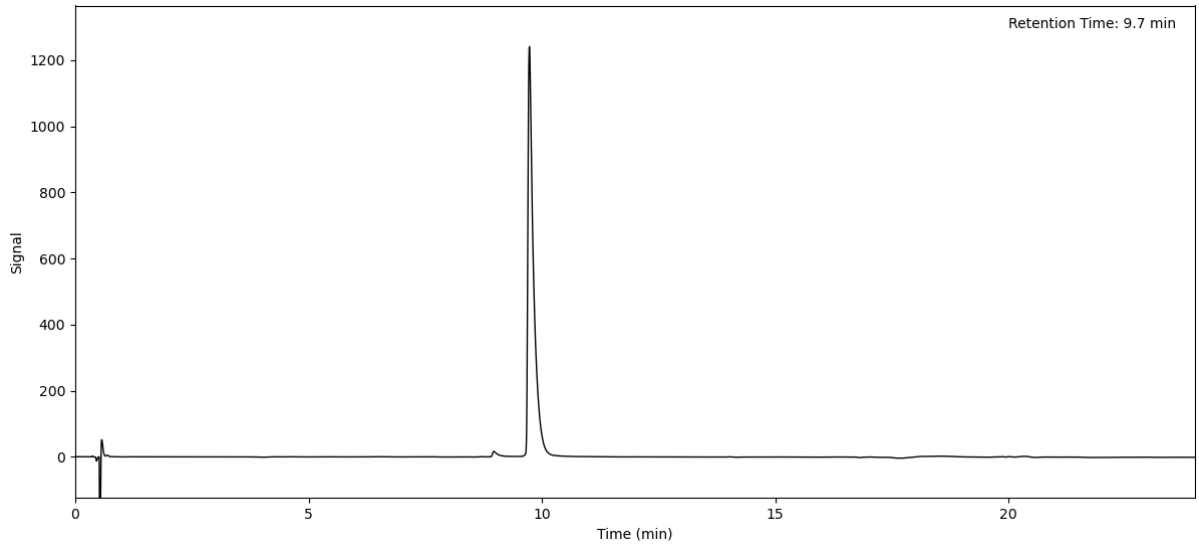
Peptide 26 (Method A)



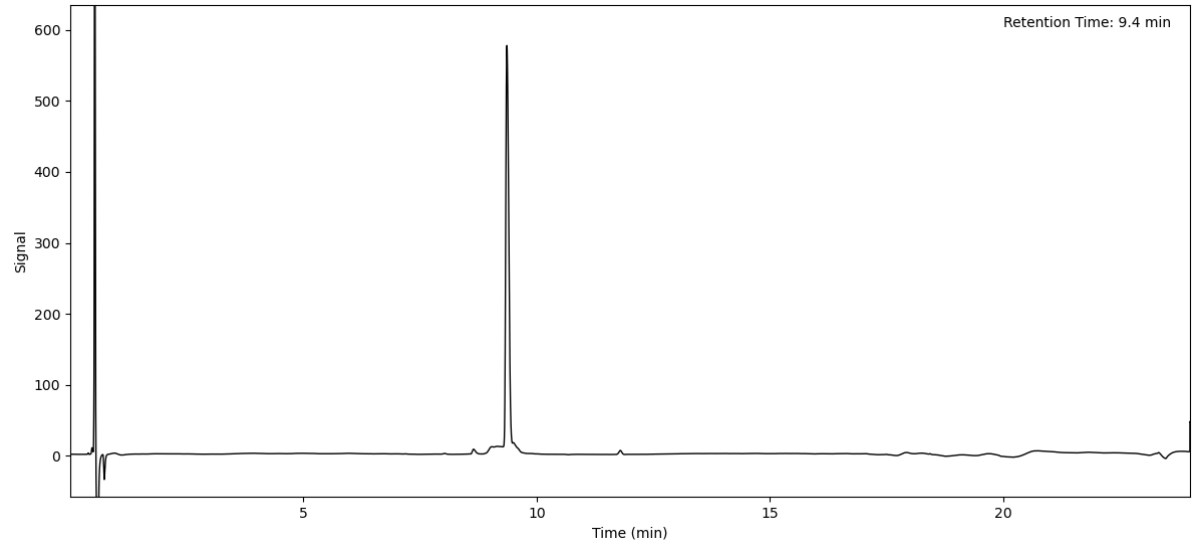
Peptide 27 (Method A)



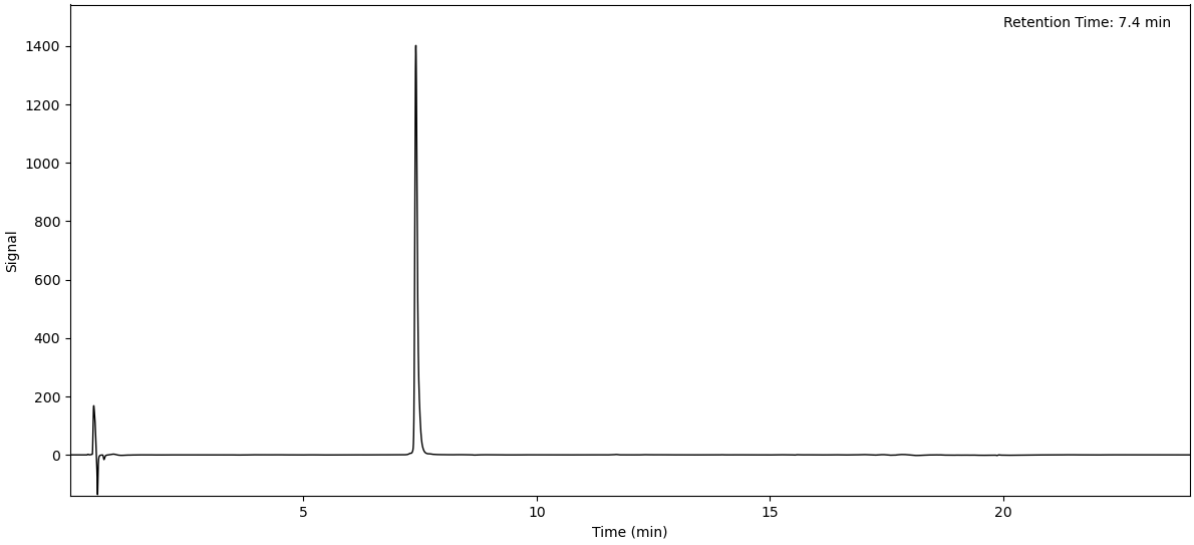
Peptide 28 (Method A)



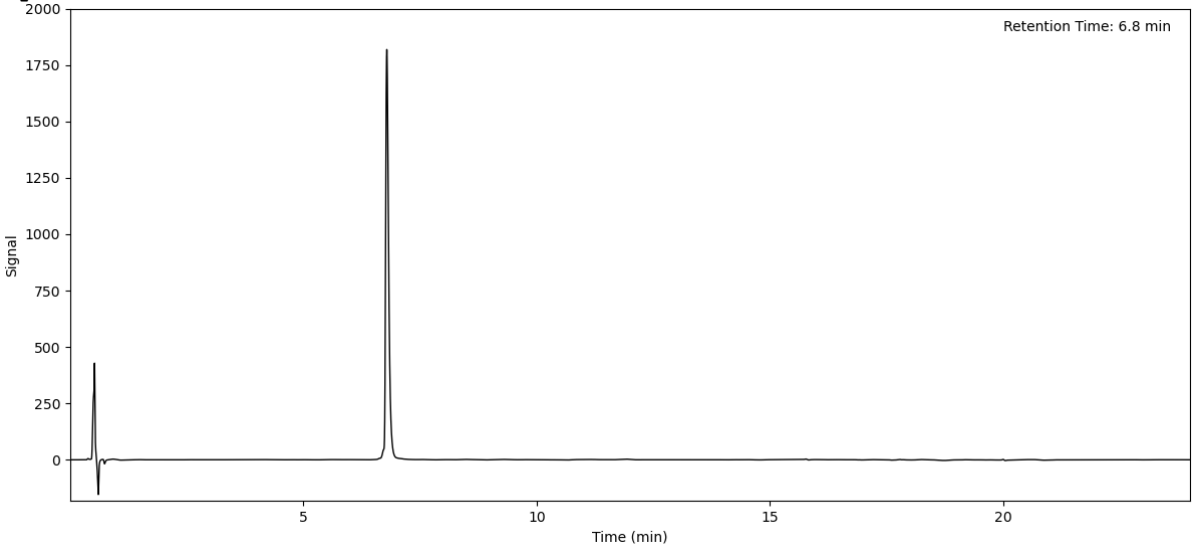
Peptide 29 (Method A)



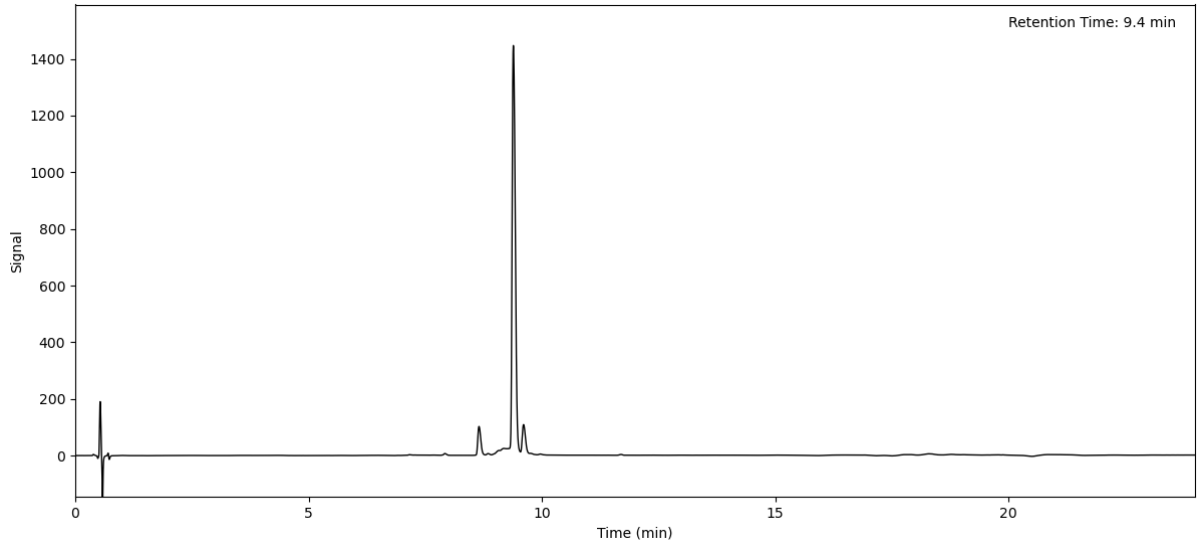
Peptide 30 (Method A)



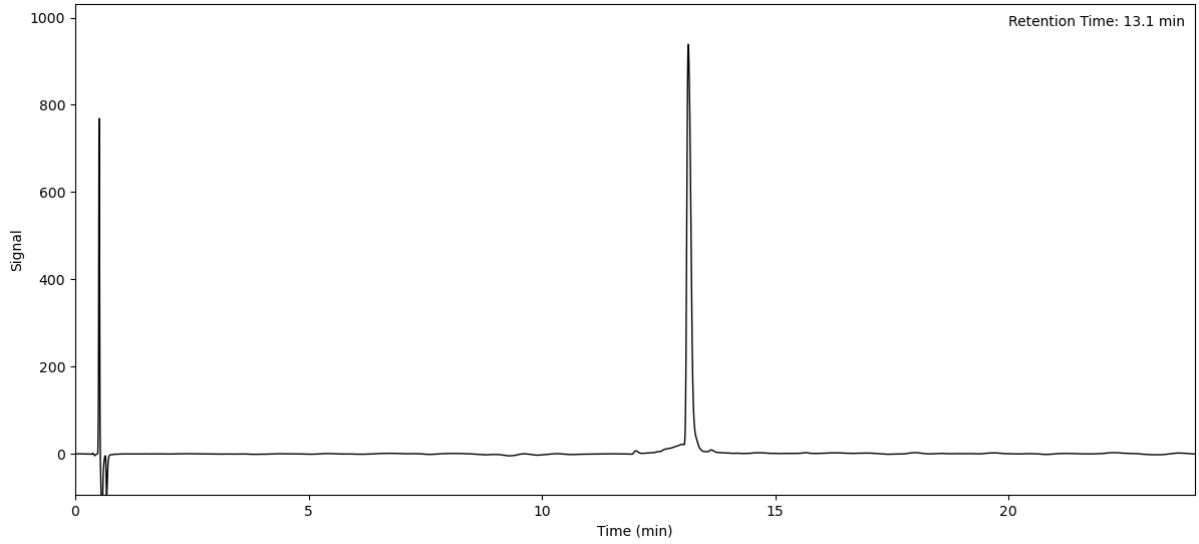
Peptide 31 (Method A)



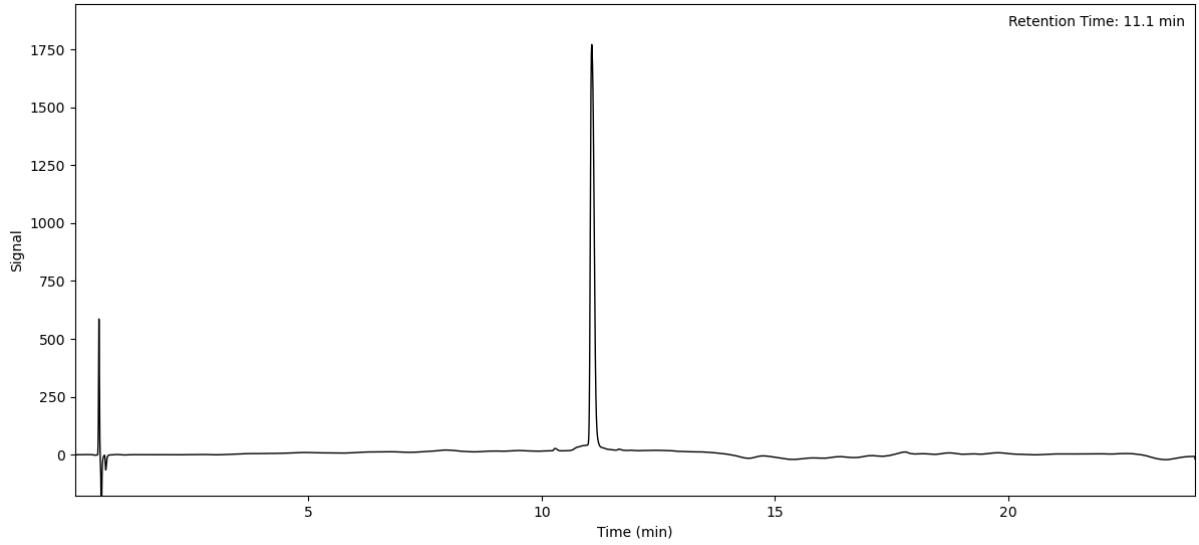
Peptide 32 (Method A)



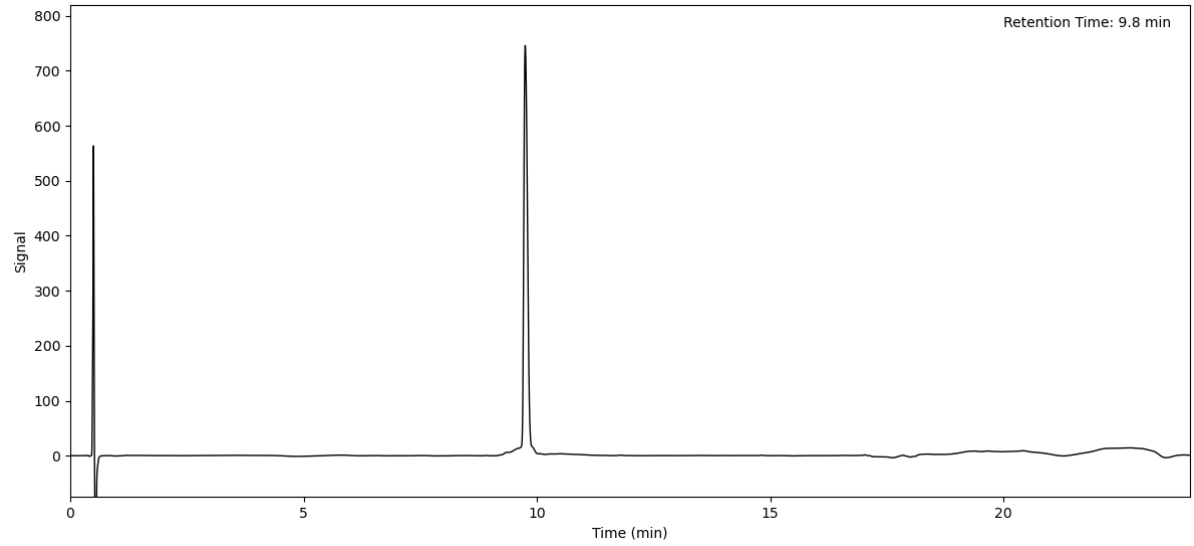
Peptide 33 (Method A)



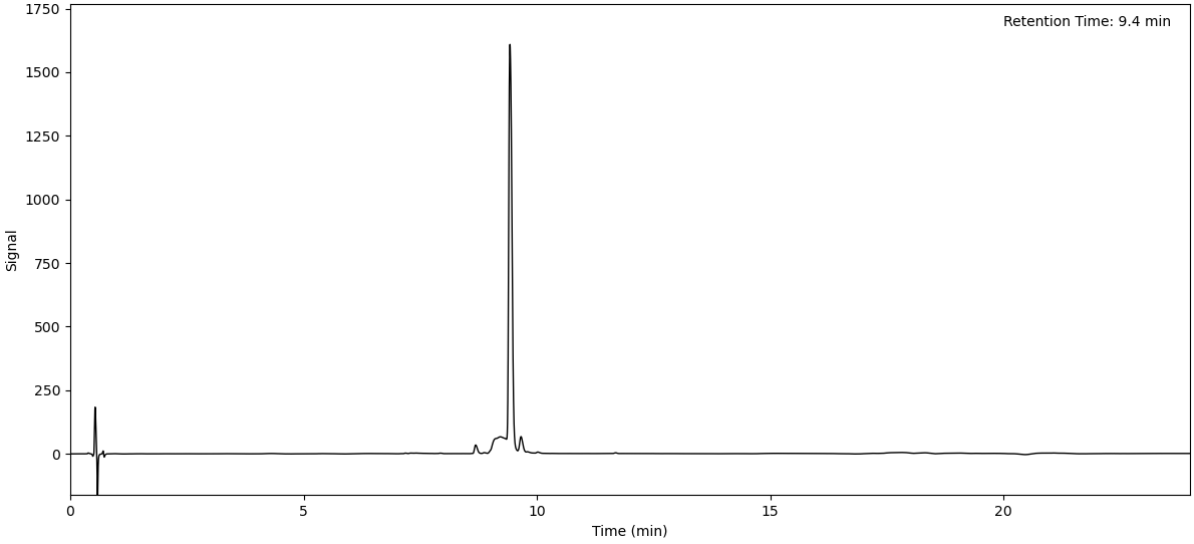
Peptide 34 (Method A)



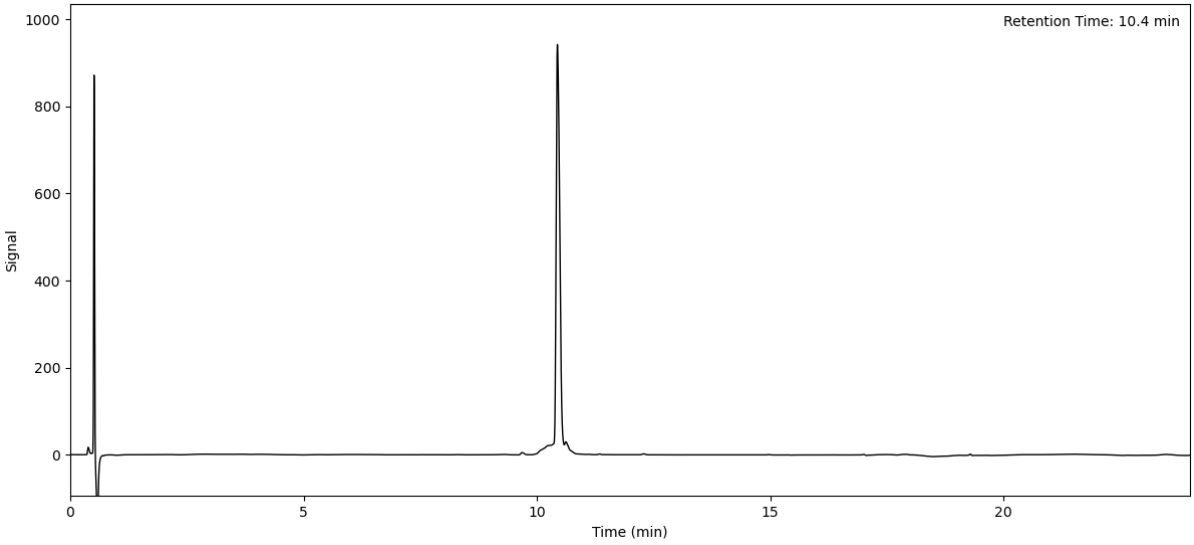
Peptide 35 (Method A)



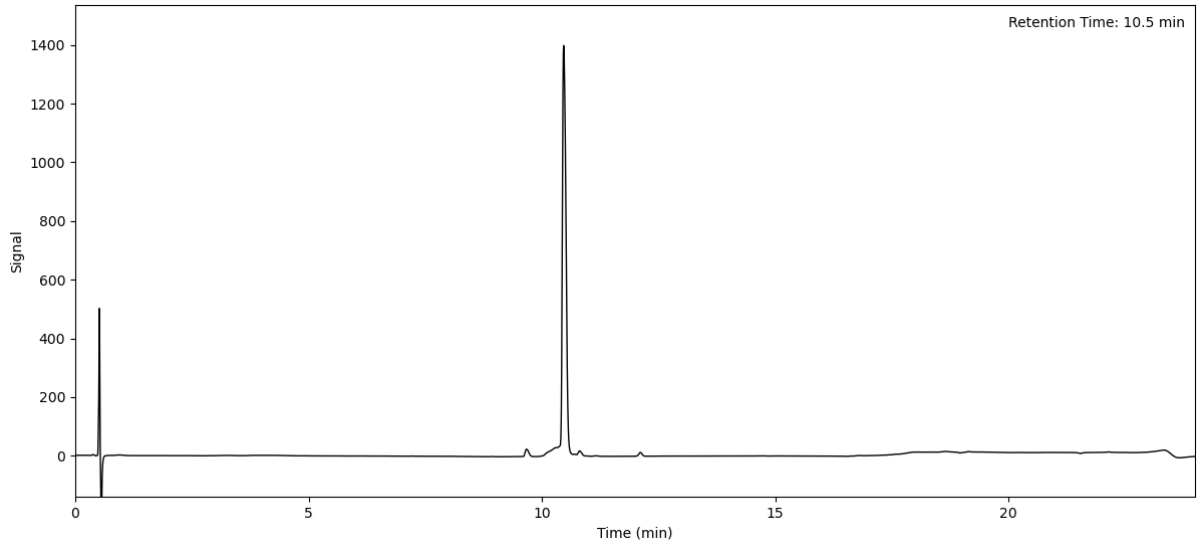
Peptide 36 (Method A)



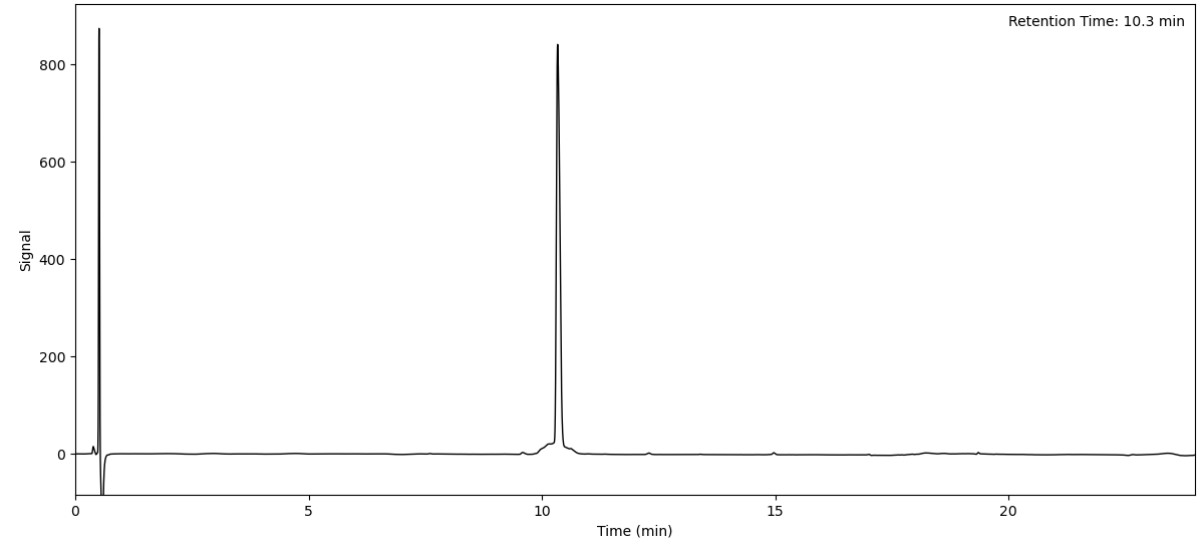
Peptide 37 (Method A)



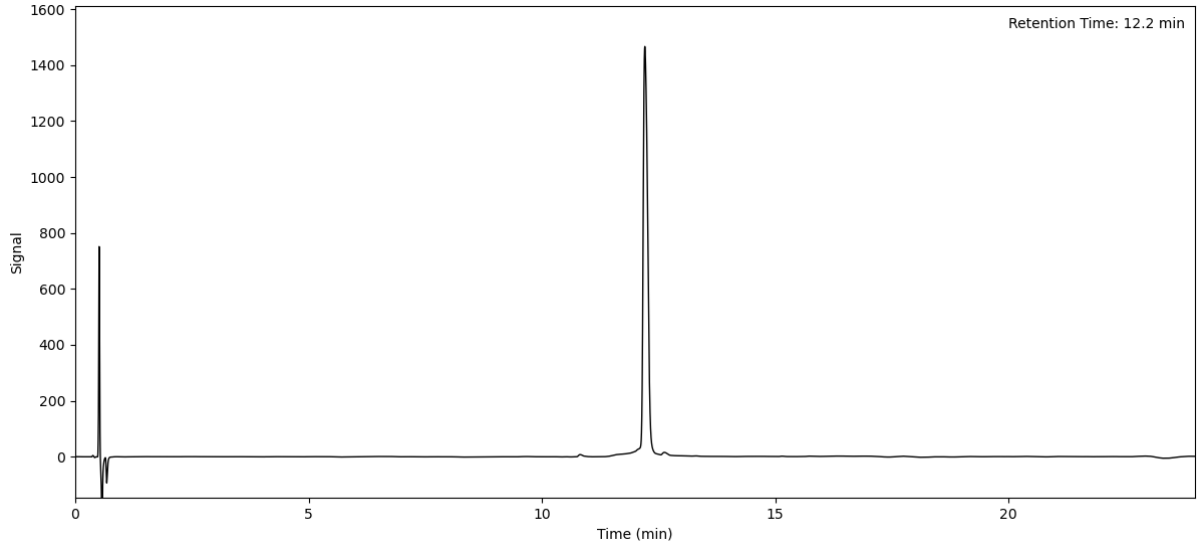
Peptide 38 (Method A)



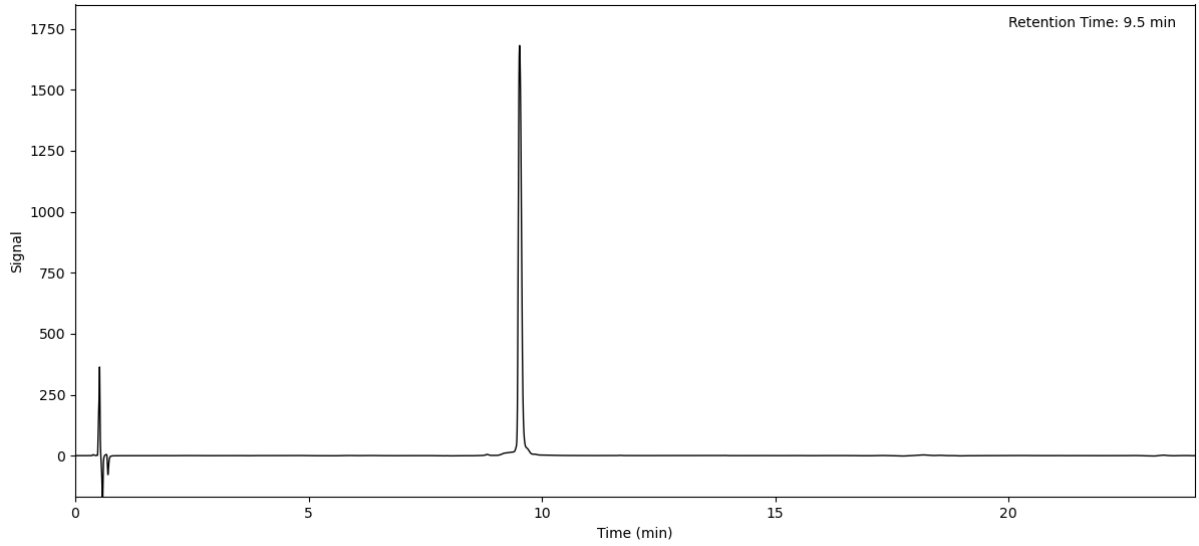
Peptide 39 (Method A)



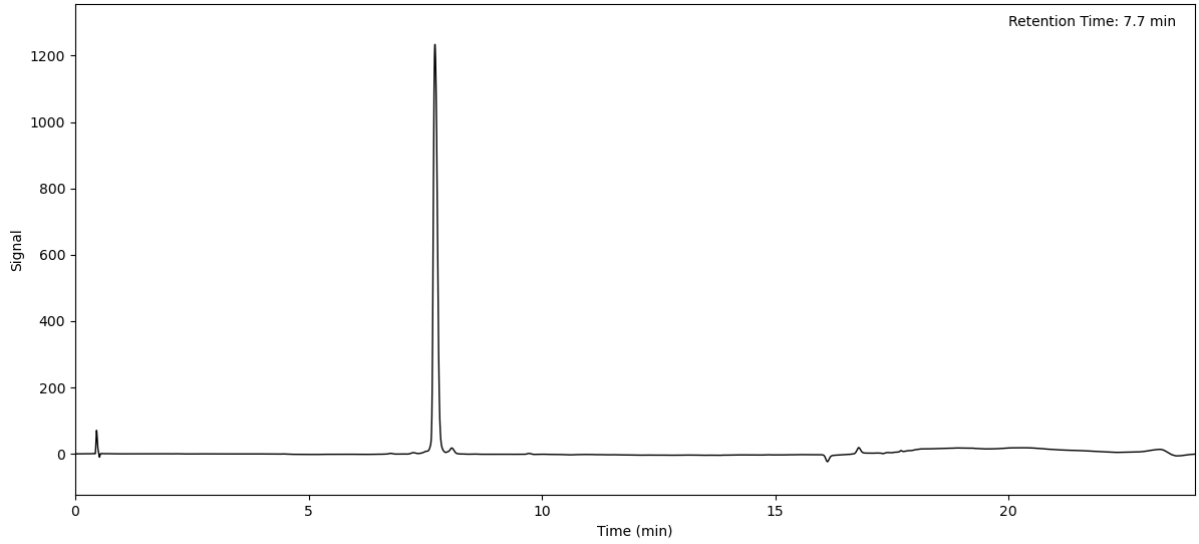
Peptide 40 (Method A)



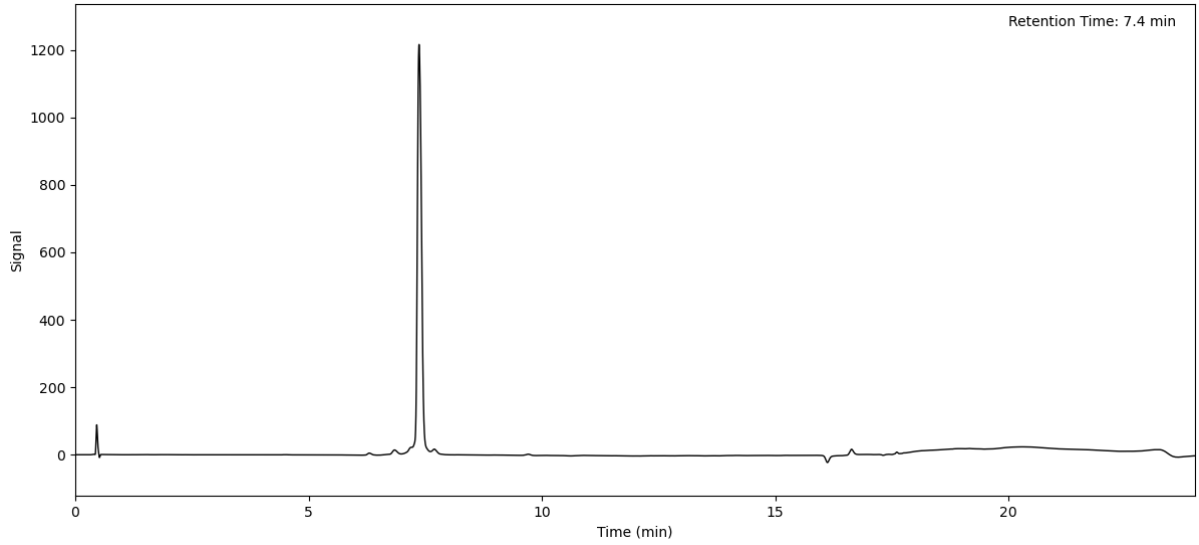
Peptide 41 (Method A)



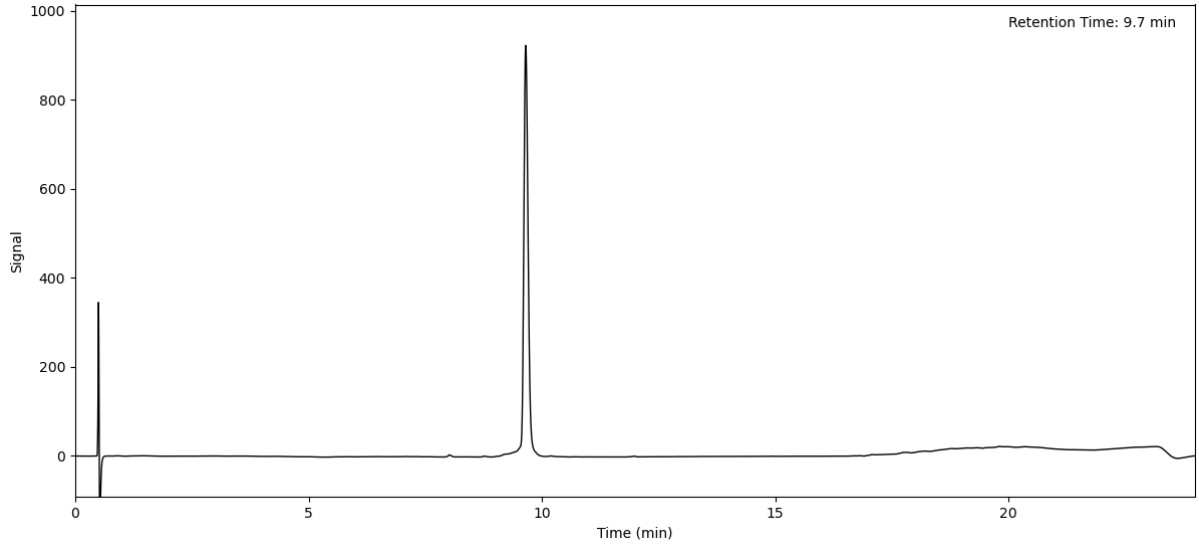
Peptide 42 (Method A)



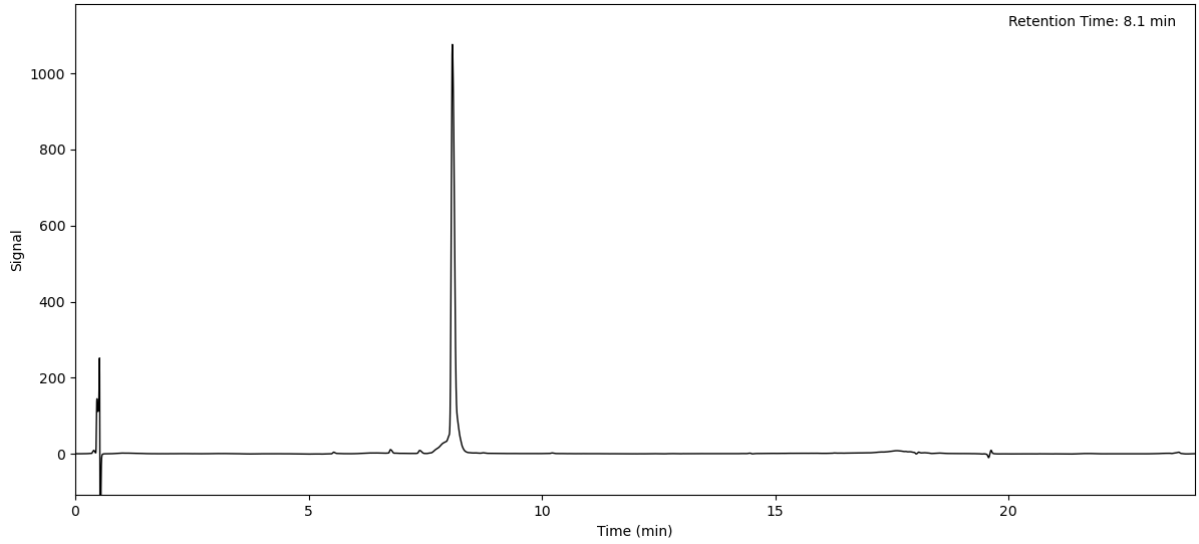
Peptide 43 (Method A)



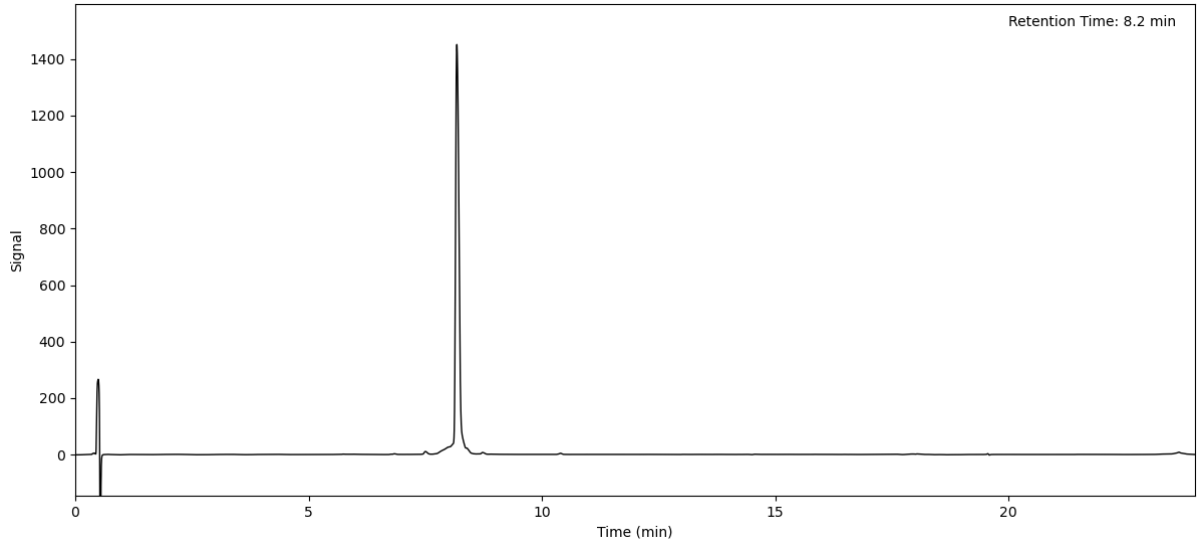
Peptide 44 (Method A)



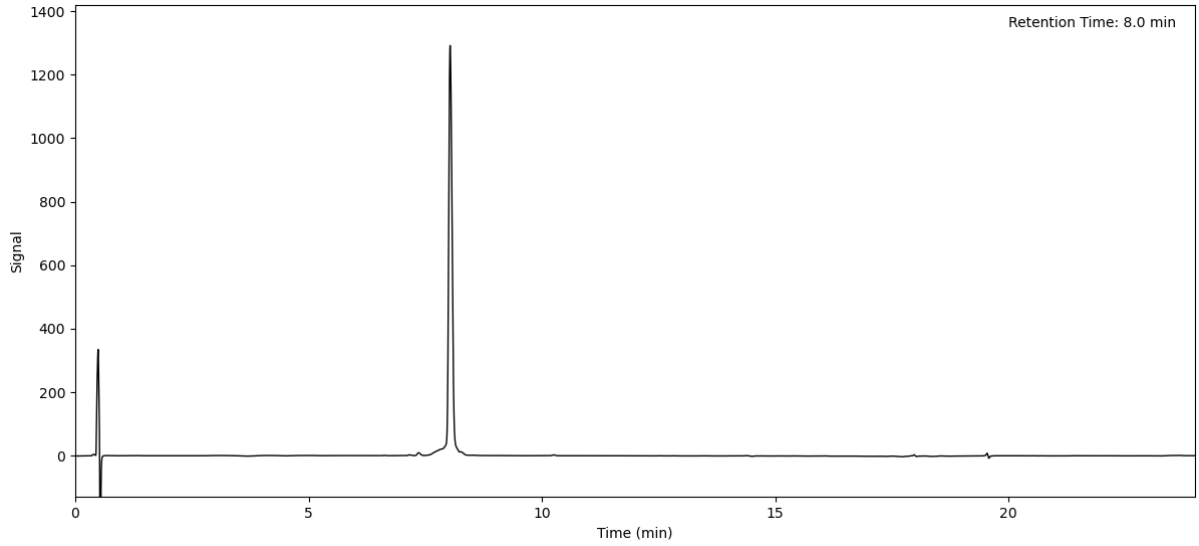
Peptide 45 (Method A)



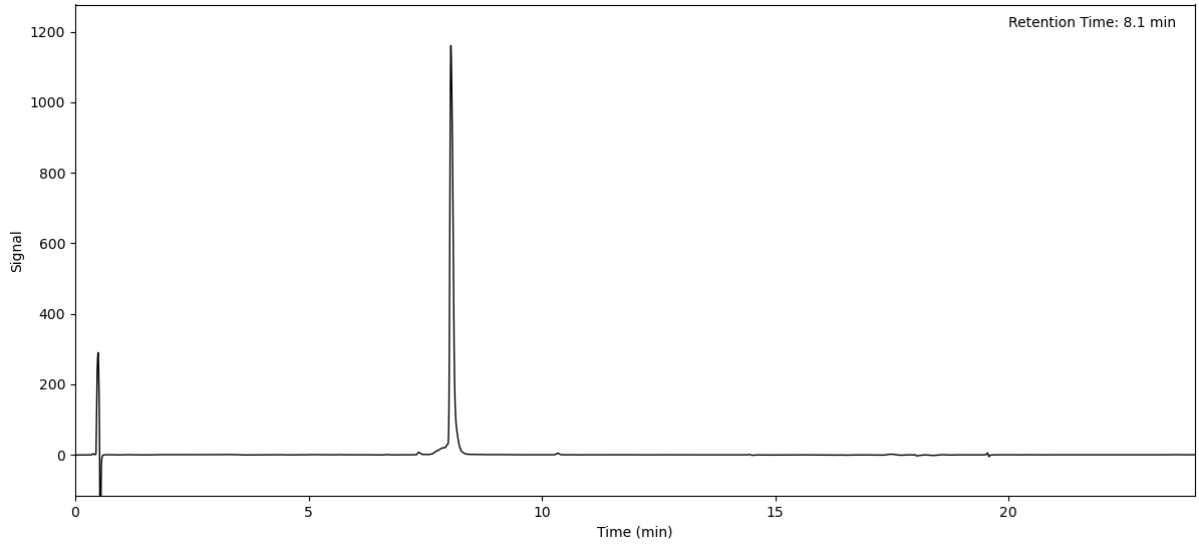
Peptide 46 (Method A)



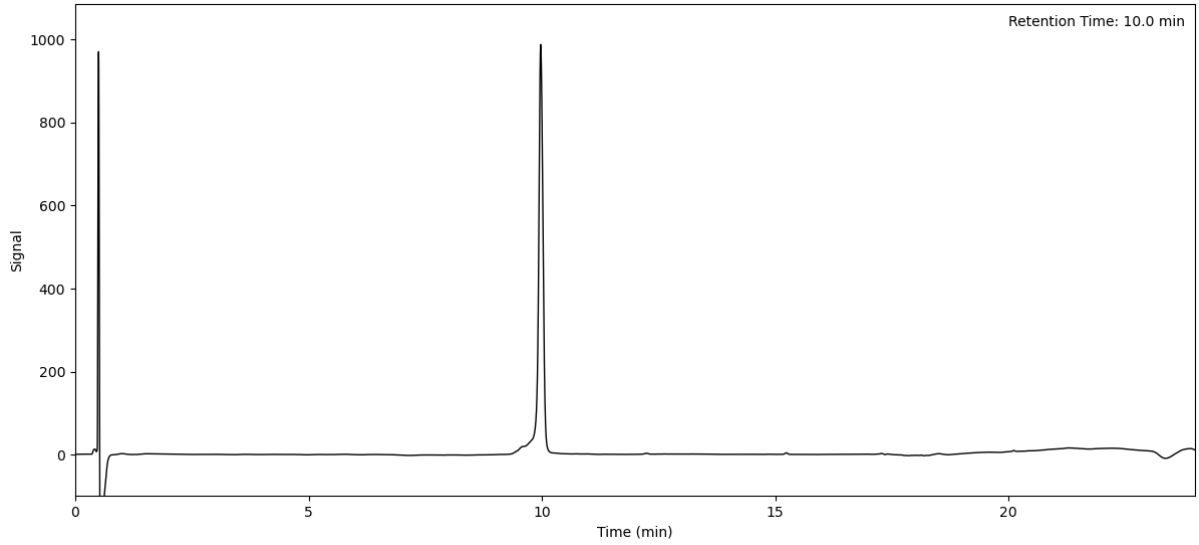
Peptide 47 (Method A)



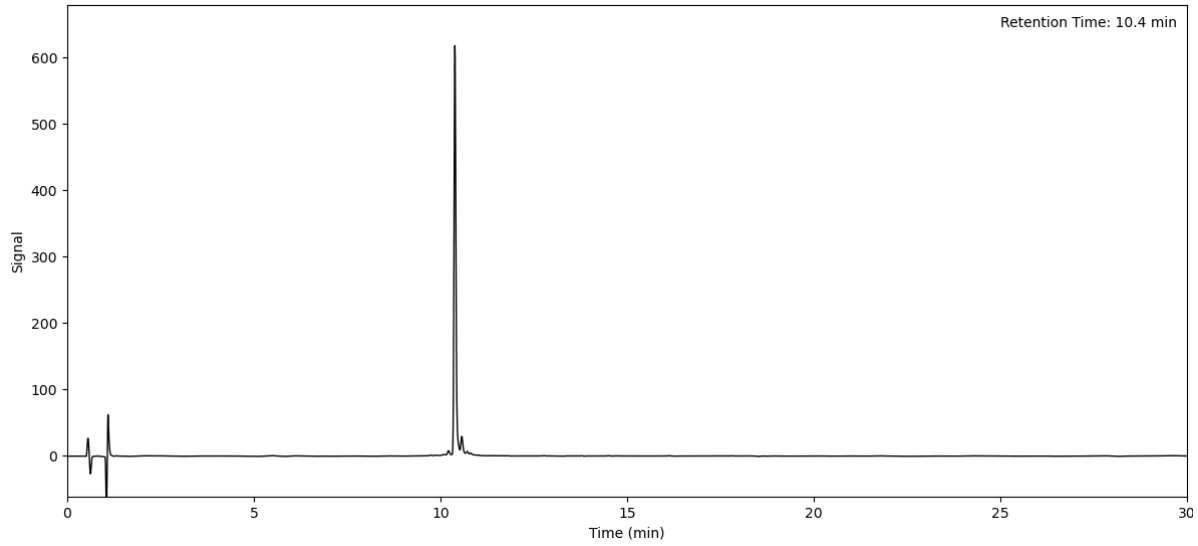
Peptide 48 (Method A)



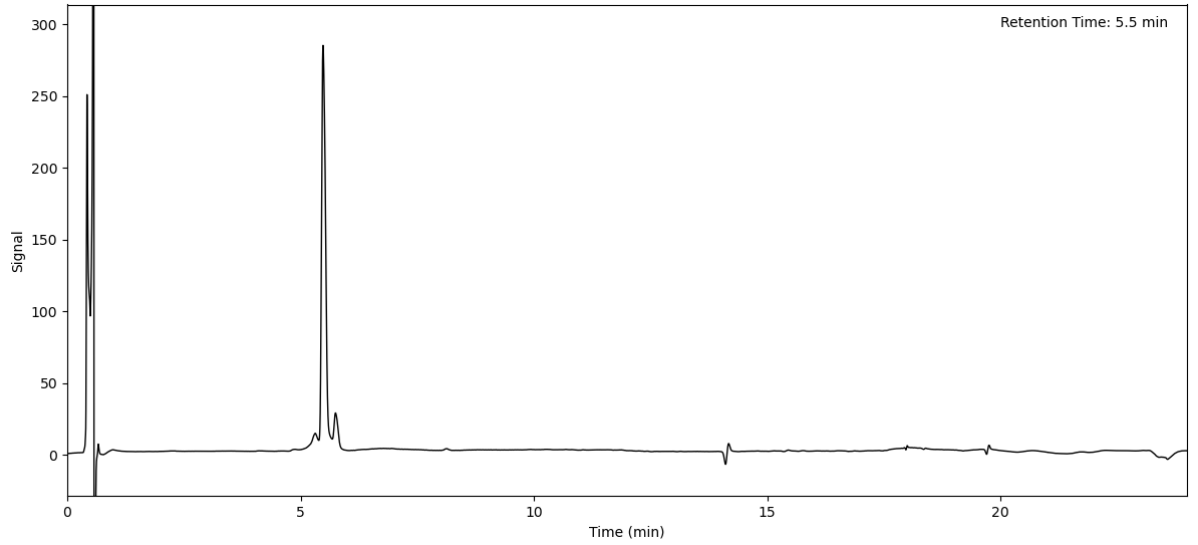
Peptide 49 (Method A)



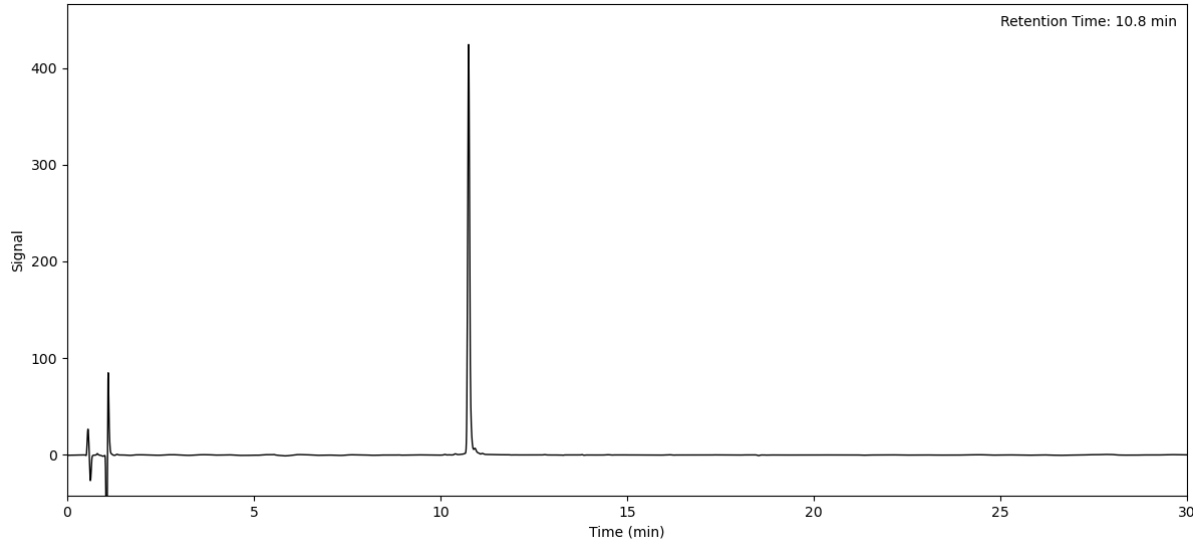
Peptide 50 (Method B)



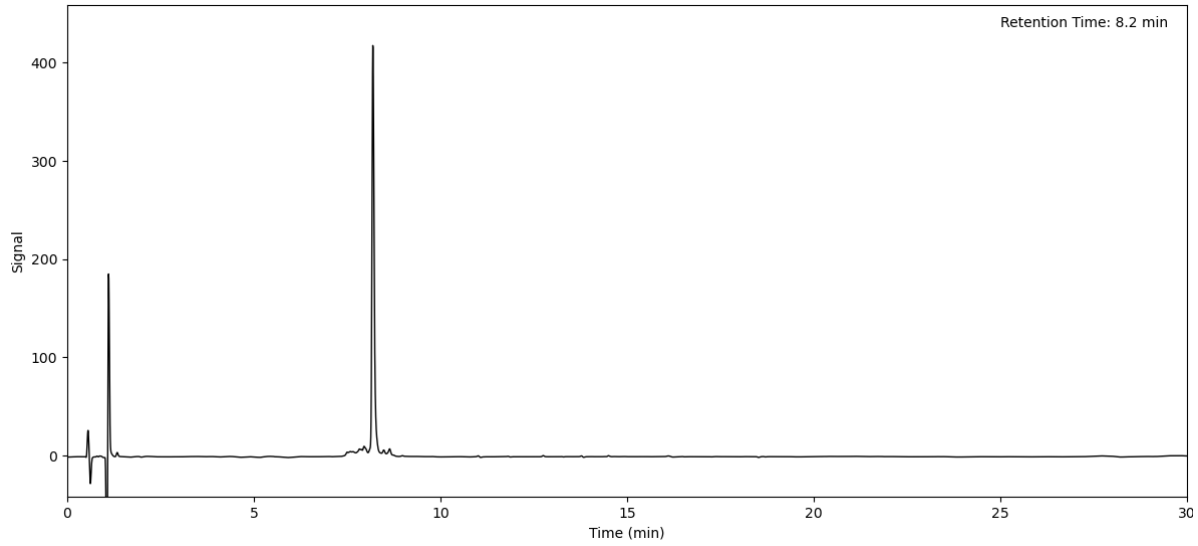
Peptide 51 (Method A)



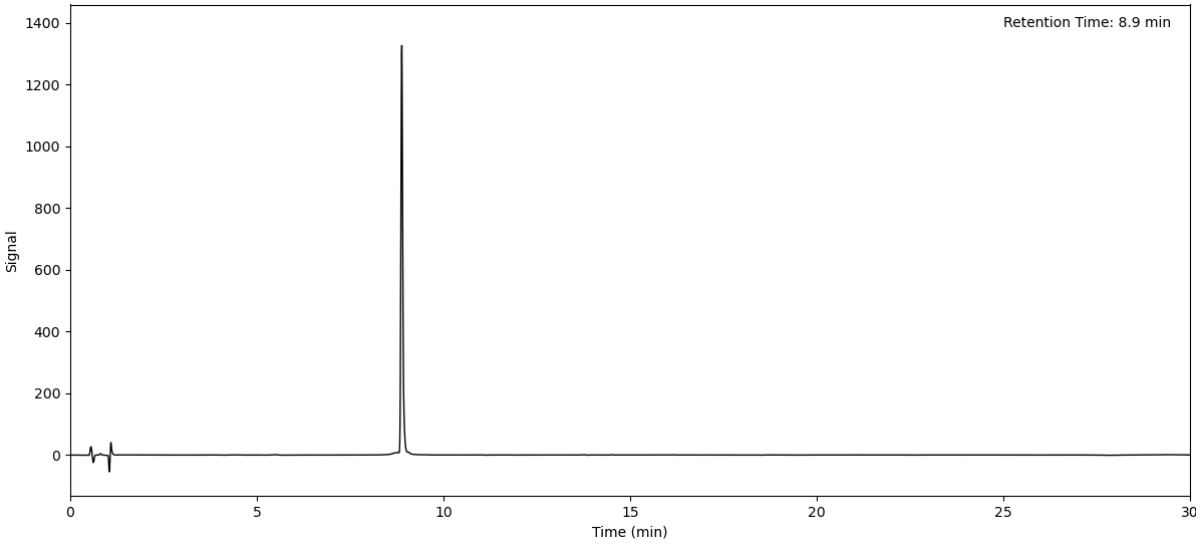
Peptide 52 (Method B)



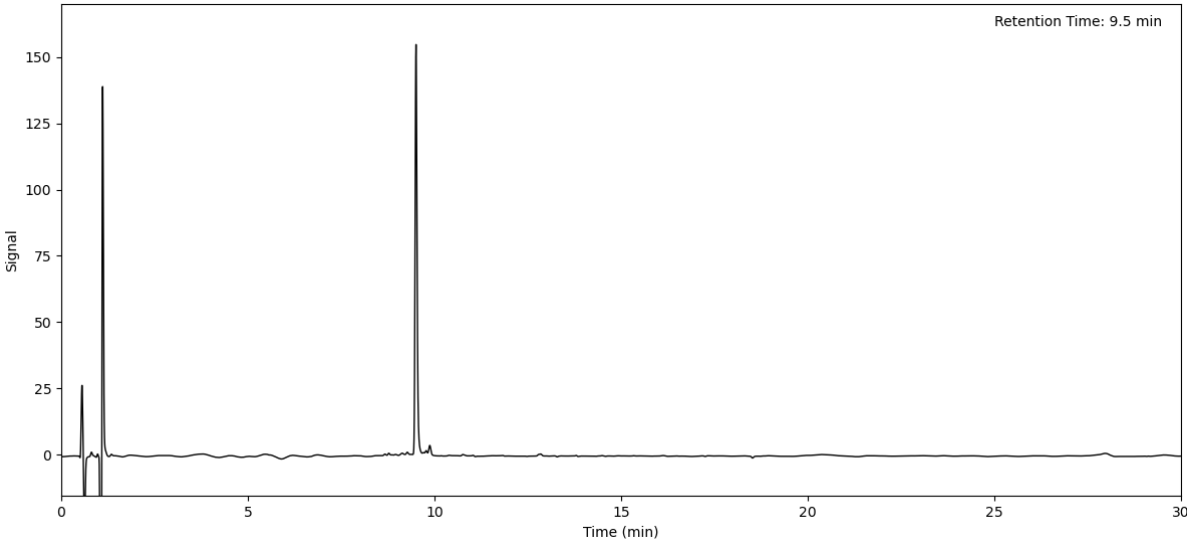
Peptide 53 (Method B)



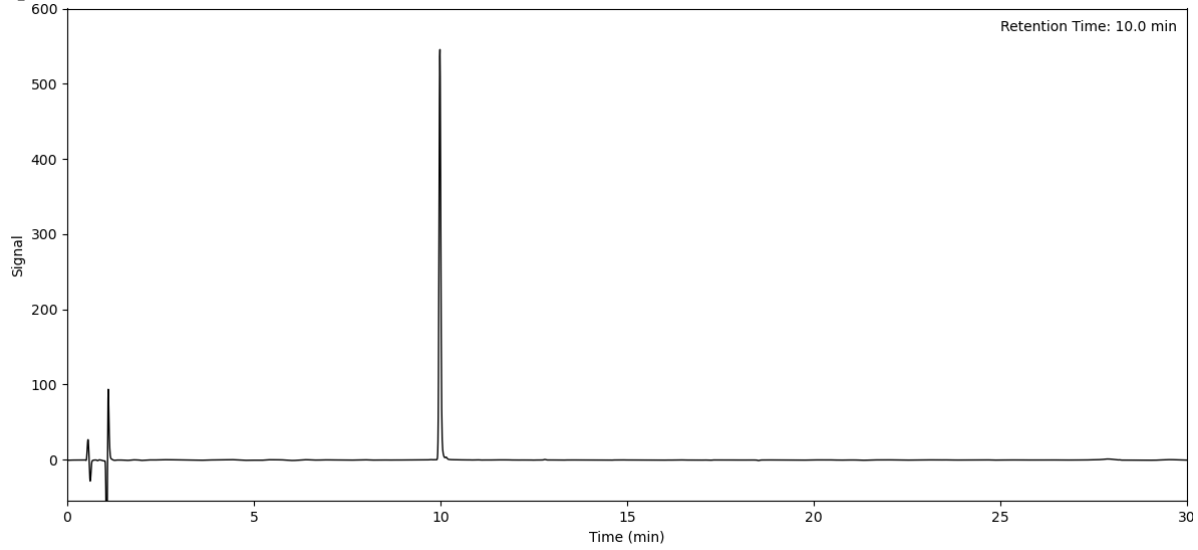
Peptide 54 (Method B)



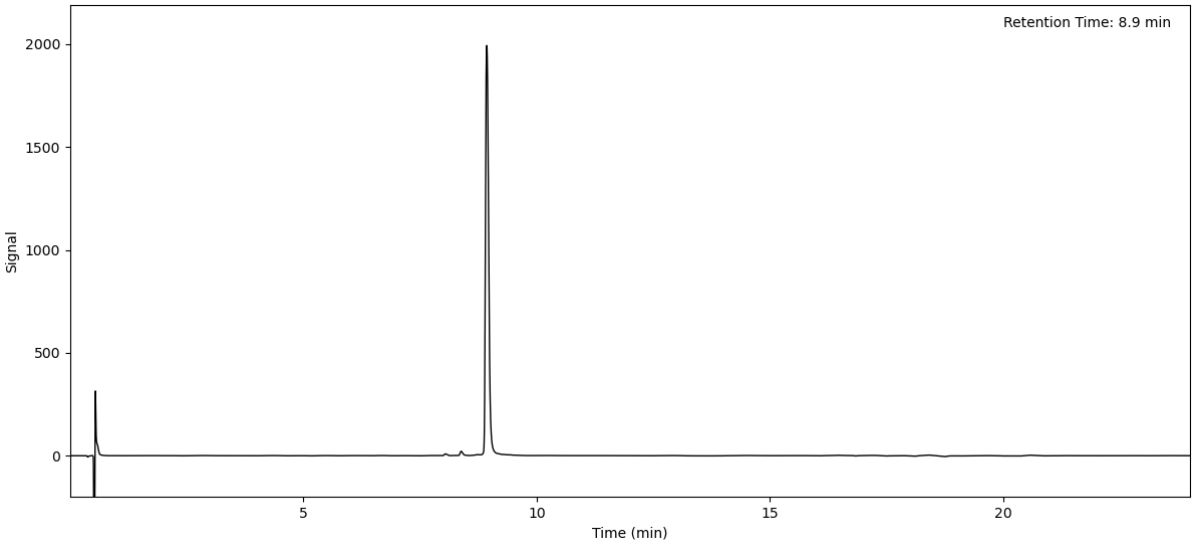
Peptide 55 (Method B)



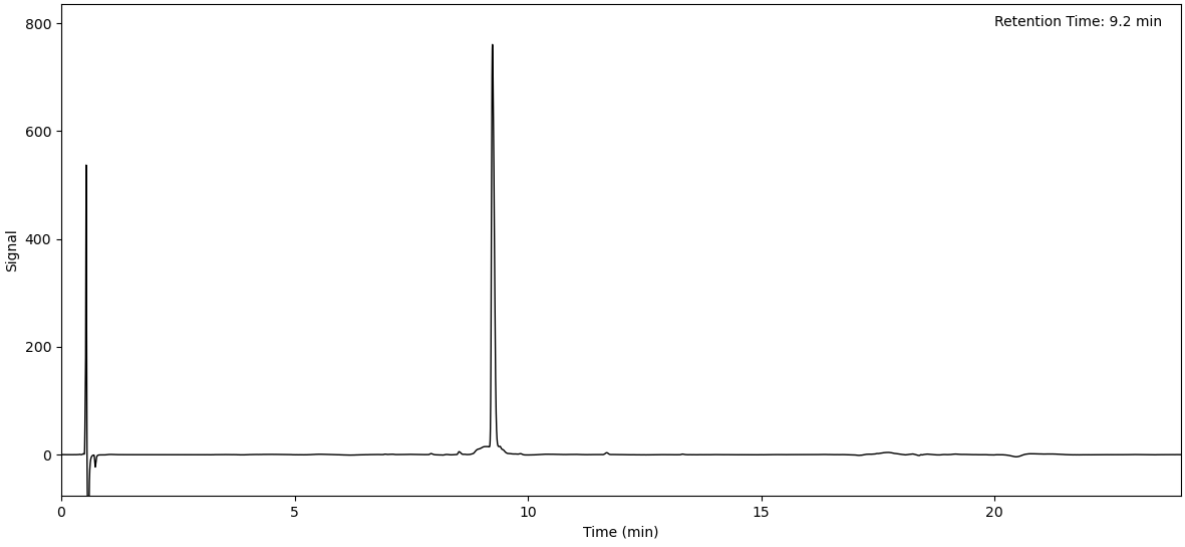
Peptide 56 (Method B)



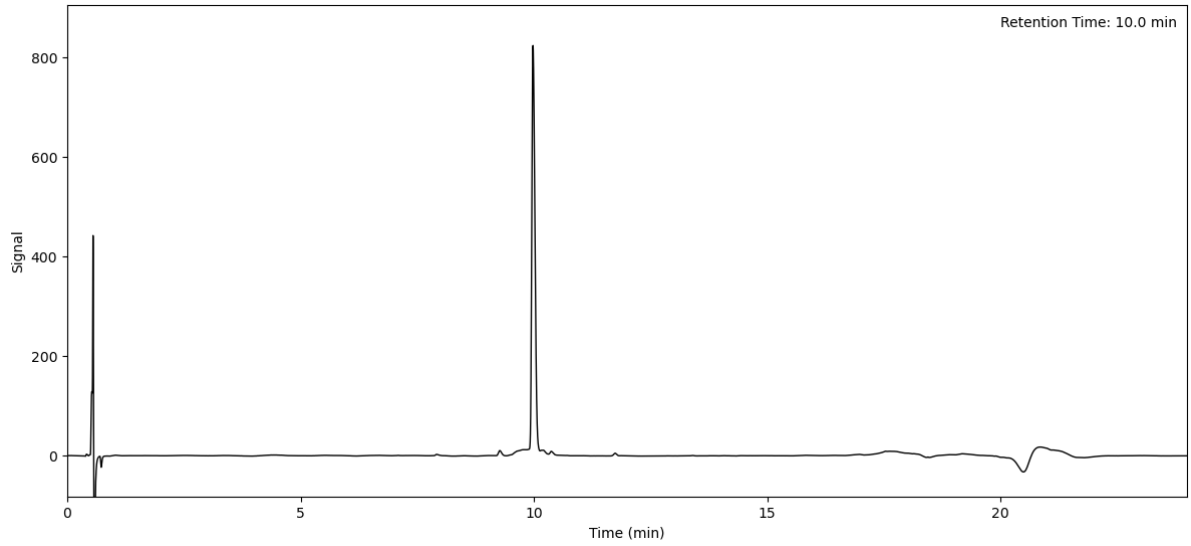
Peptide S1 (Method A)



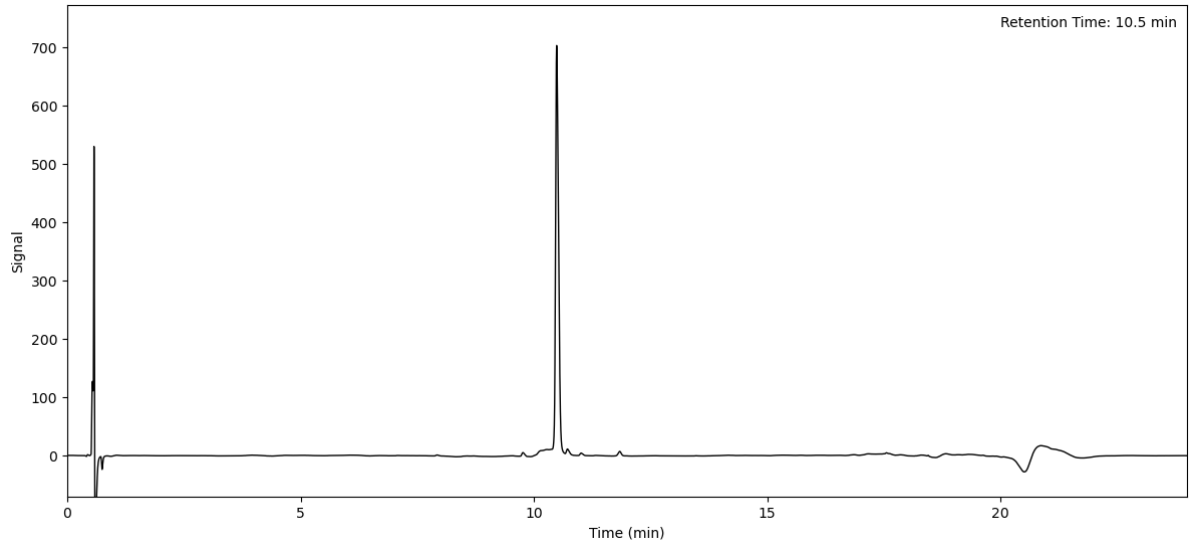
Peptide S2 (Method A)



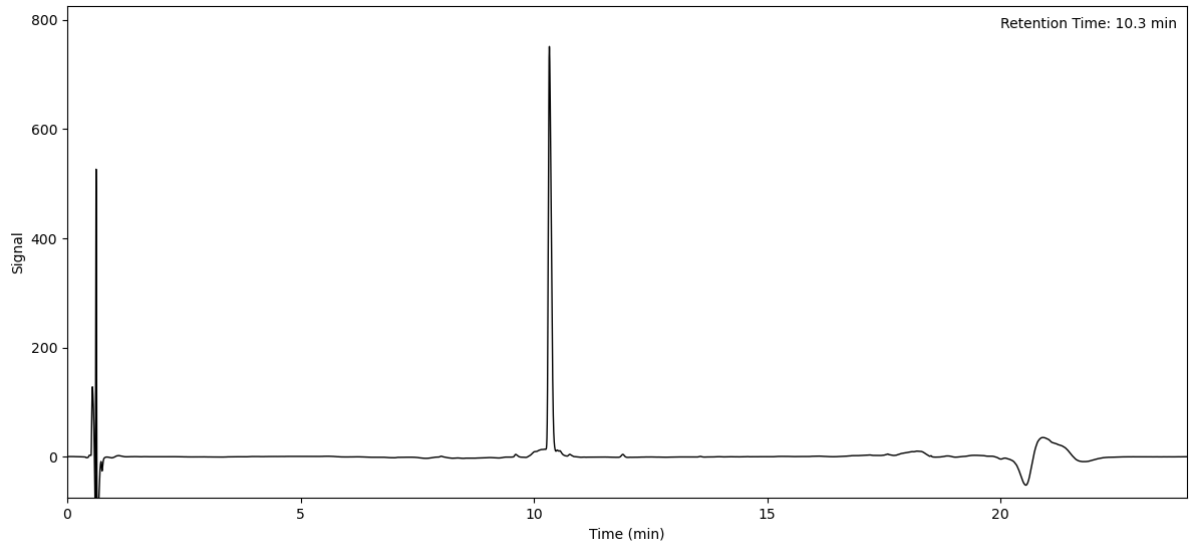
Peptide S3 (Method A)



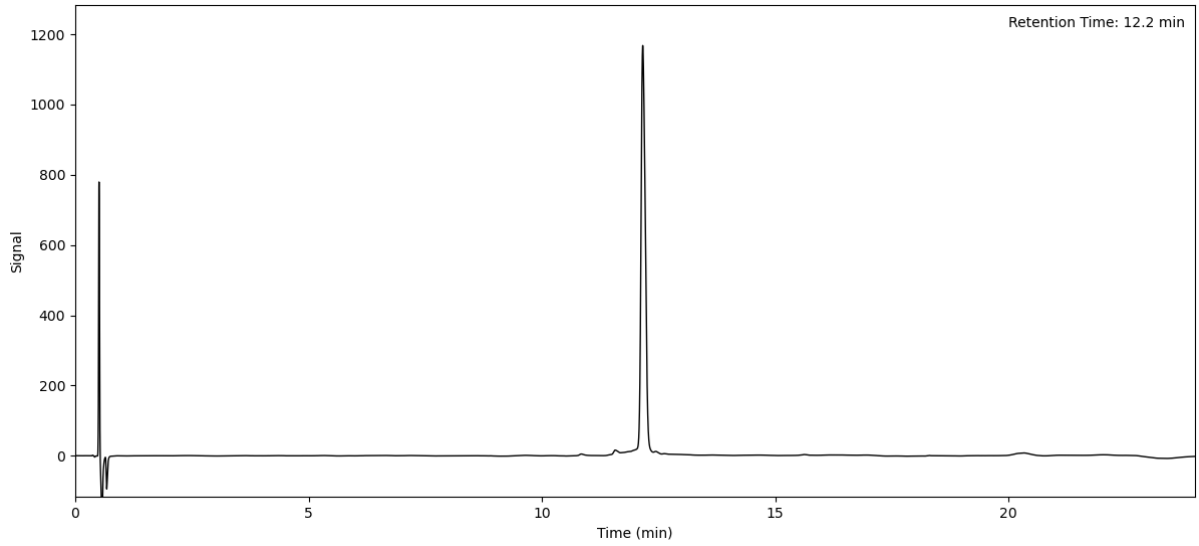
Peptide S4 (Method A)



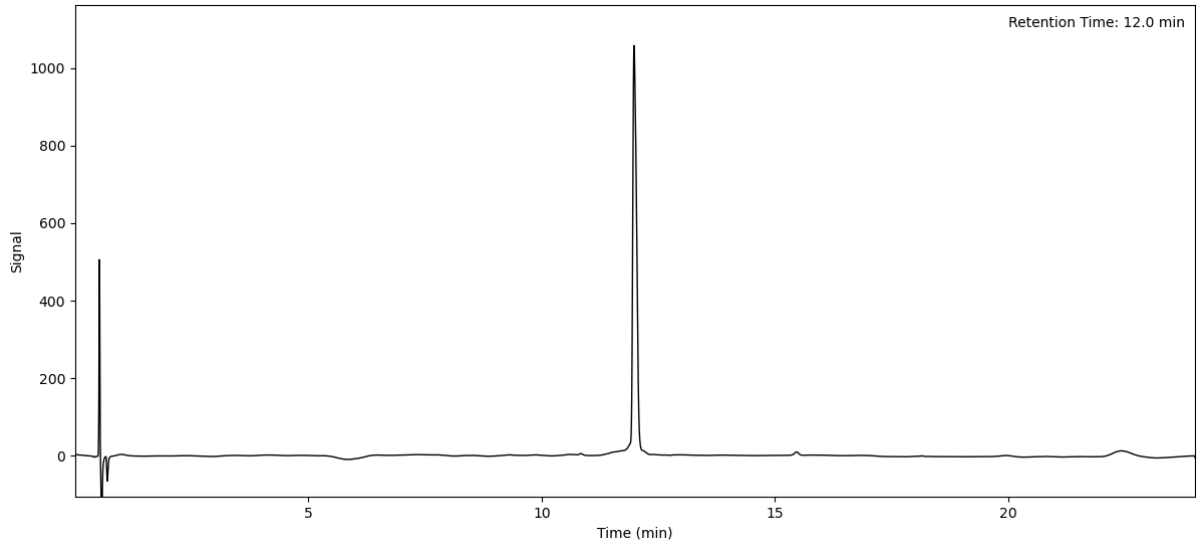
Peptide S5 (Method A)



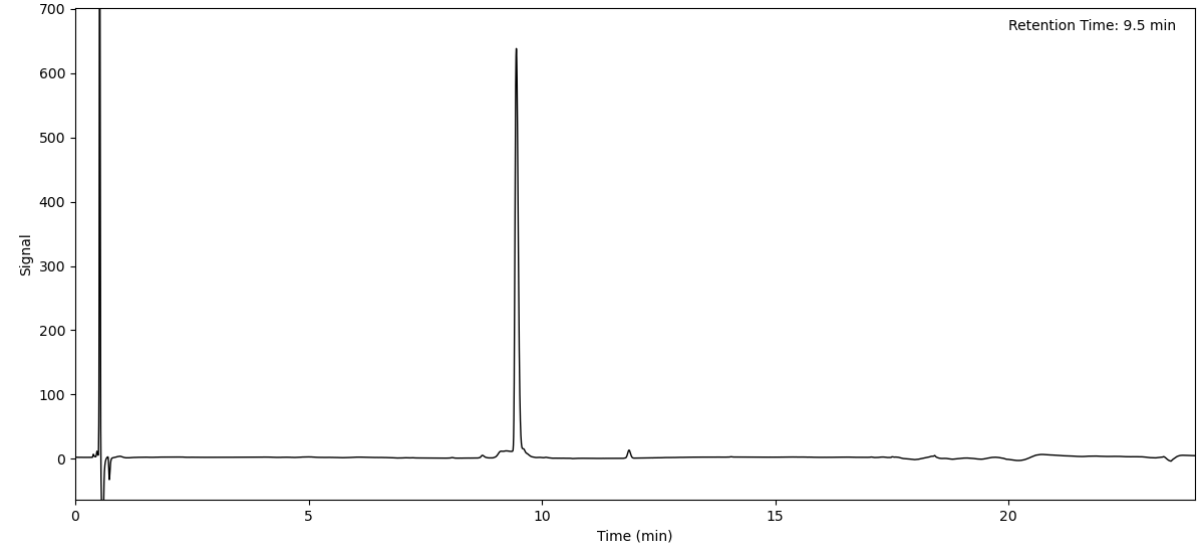
Peptide S6 (Method A)



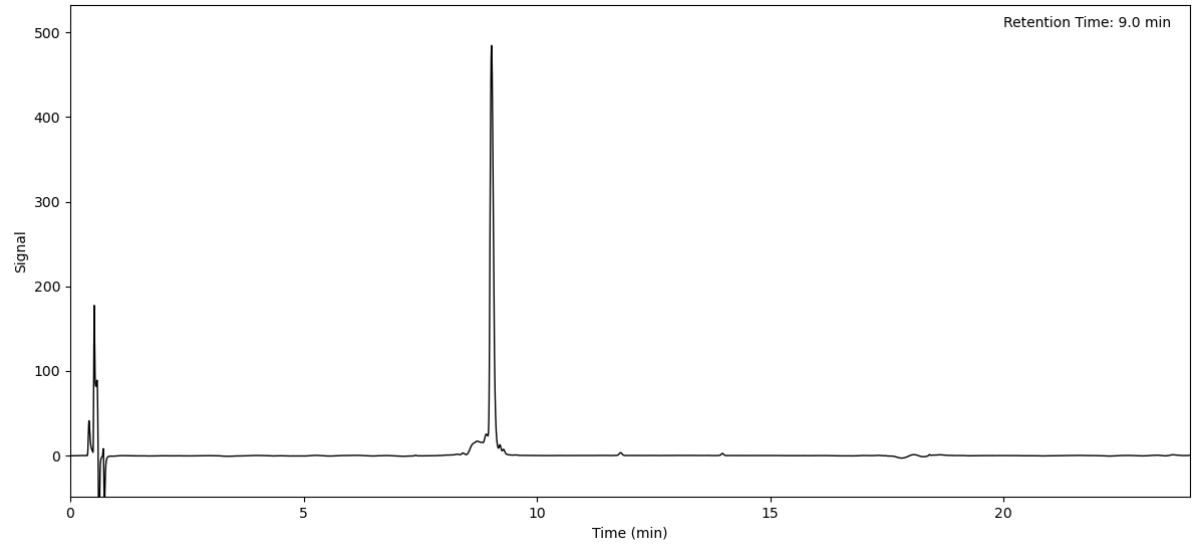
Peptide S7 (Method A)



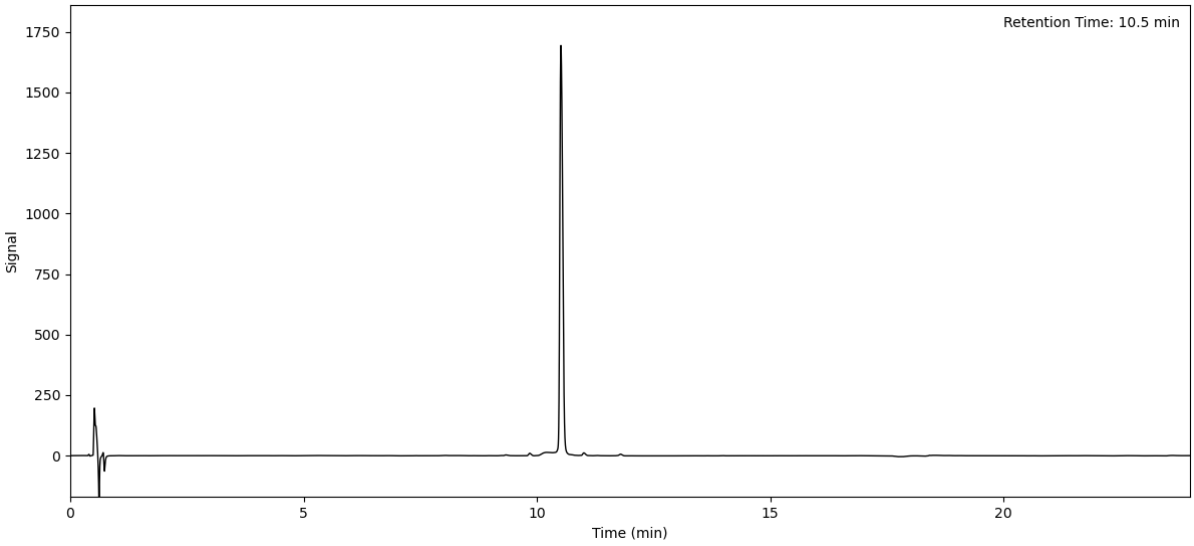
Peptide S8 (Method A)



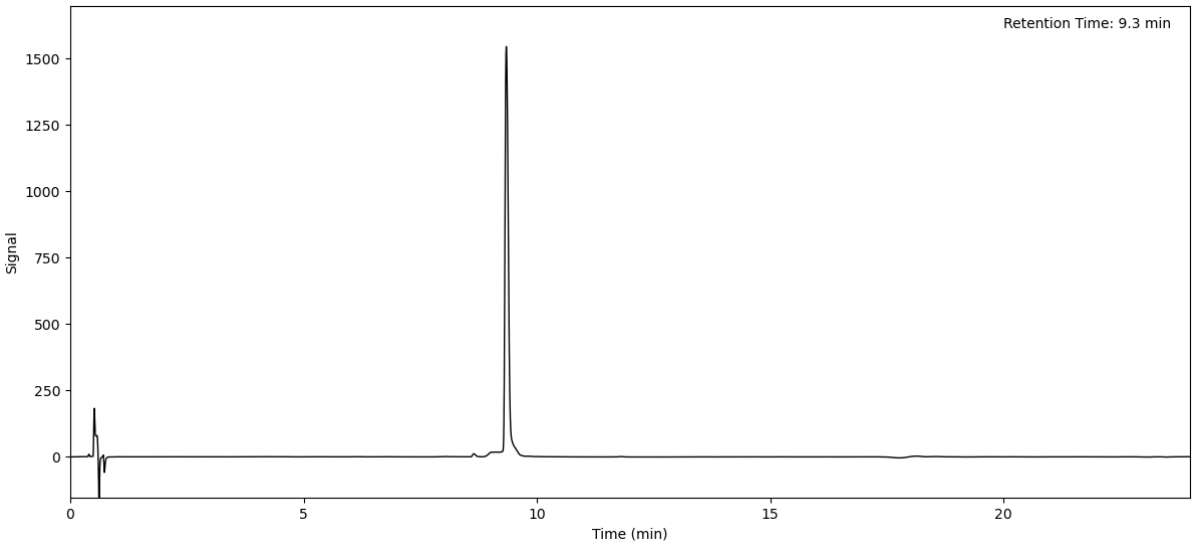
Peptide S9 (Method A)



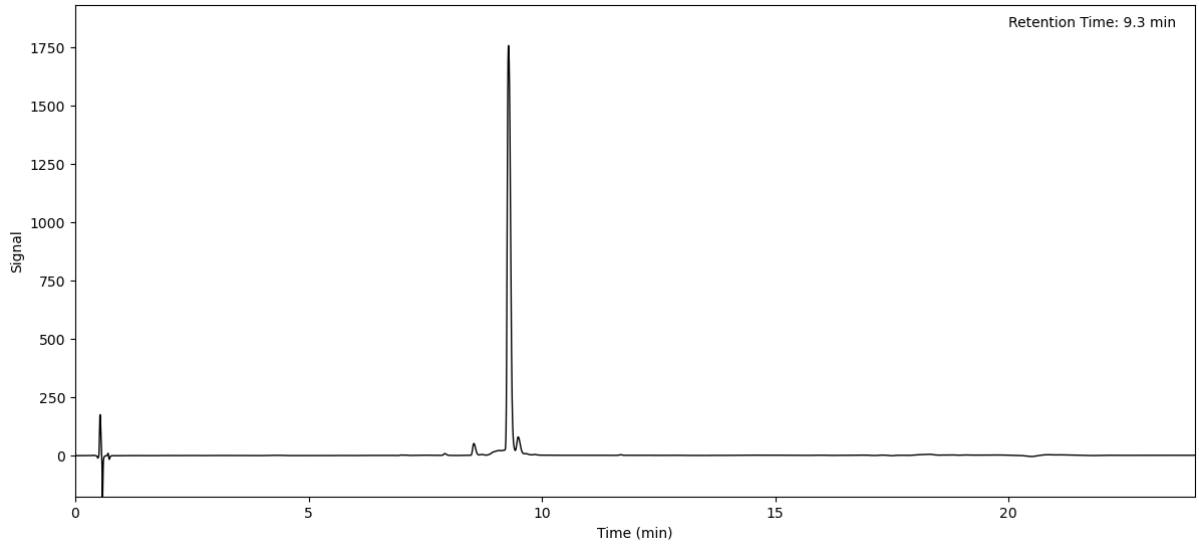
Peptide S10 (Method A)



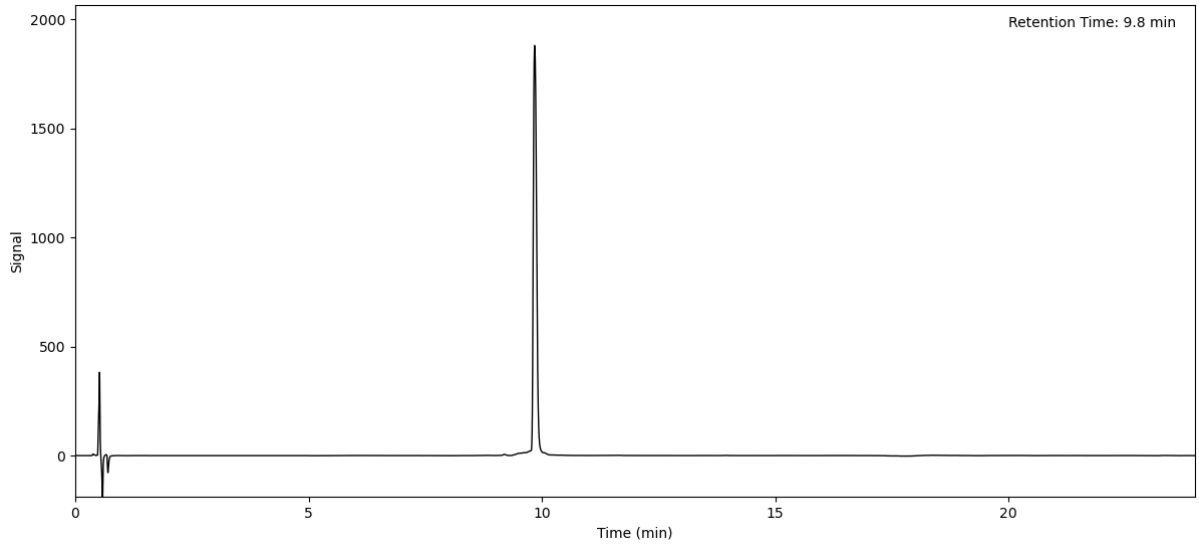
Peptide S11 (Method A)



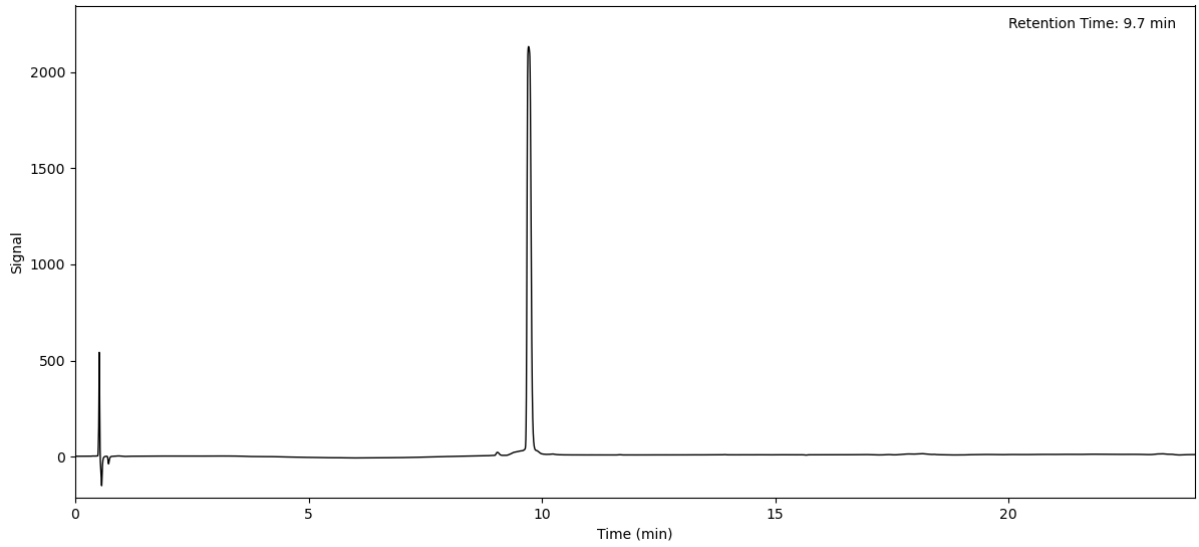
Peptide S12 (Method A)



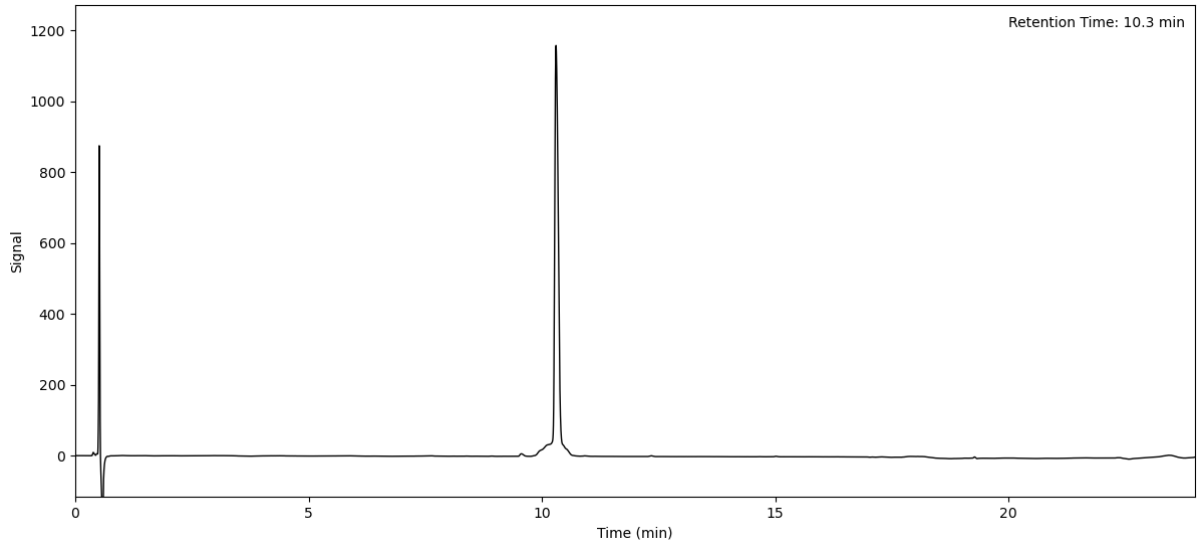
Peptide S13 (Method A)



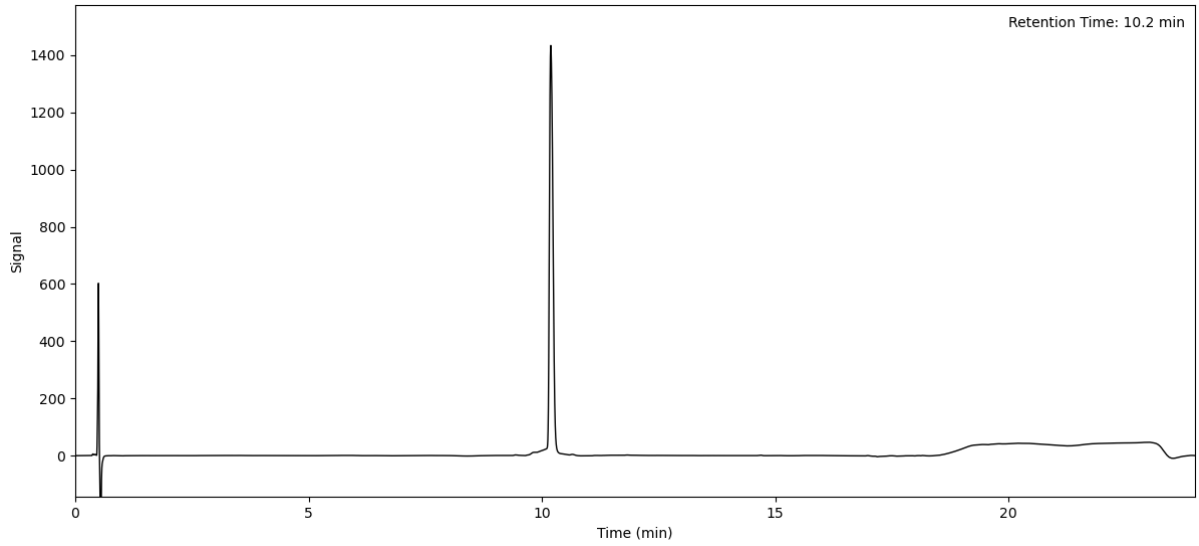
Peptide S14 (Method A)



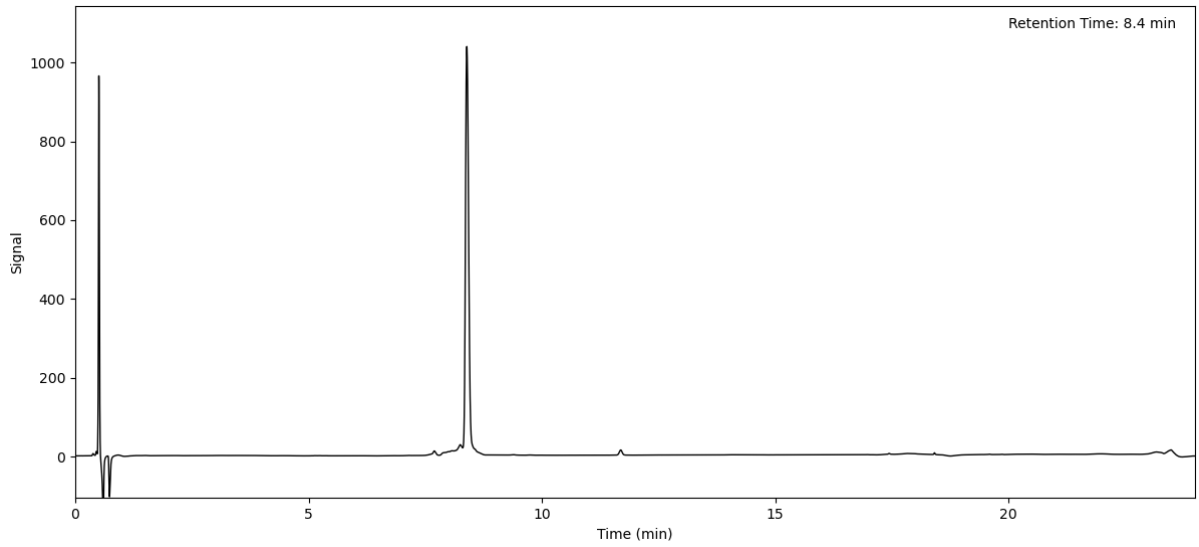
Peptide S15 (Method A)



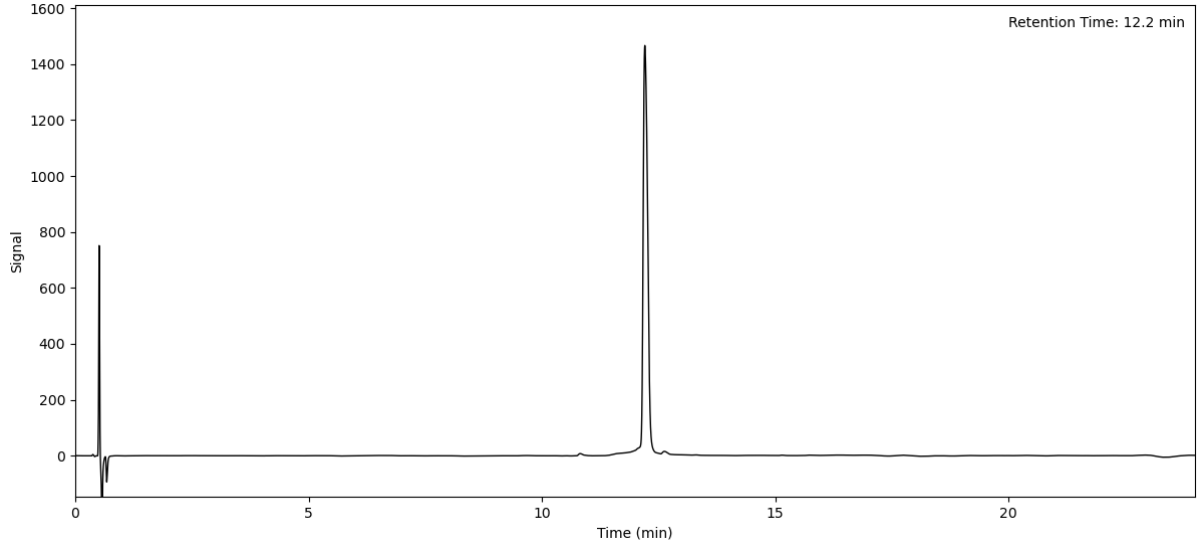
Peptide S16 (Method A)



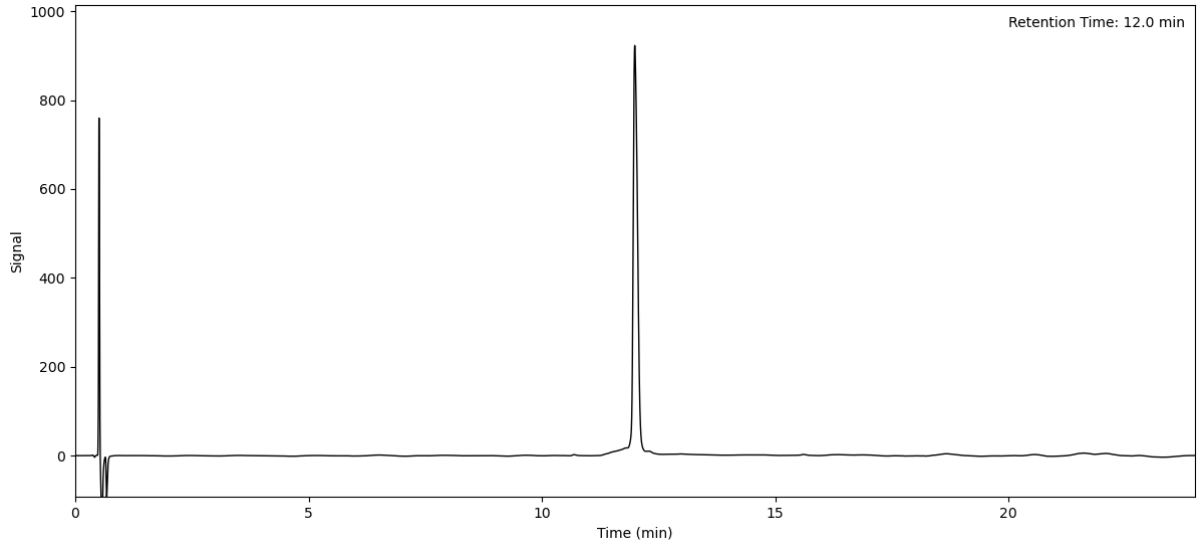
Peptide S17 (Method A)



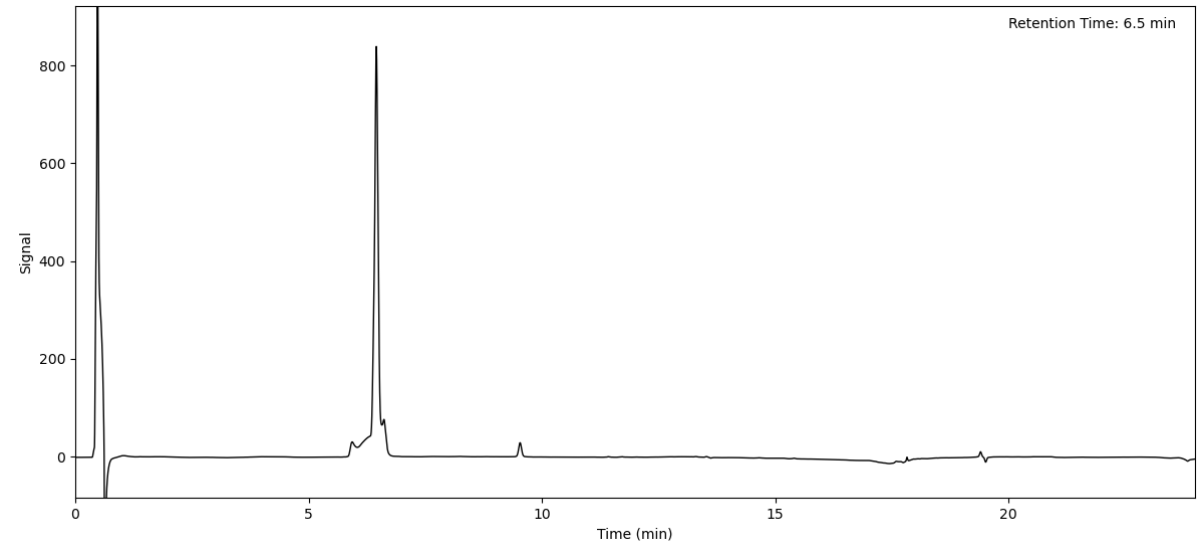
Peptide S18 (Method A)



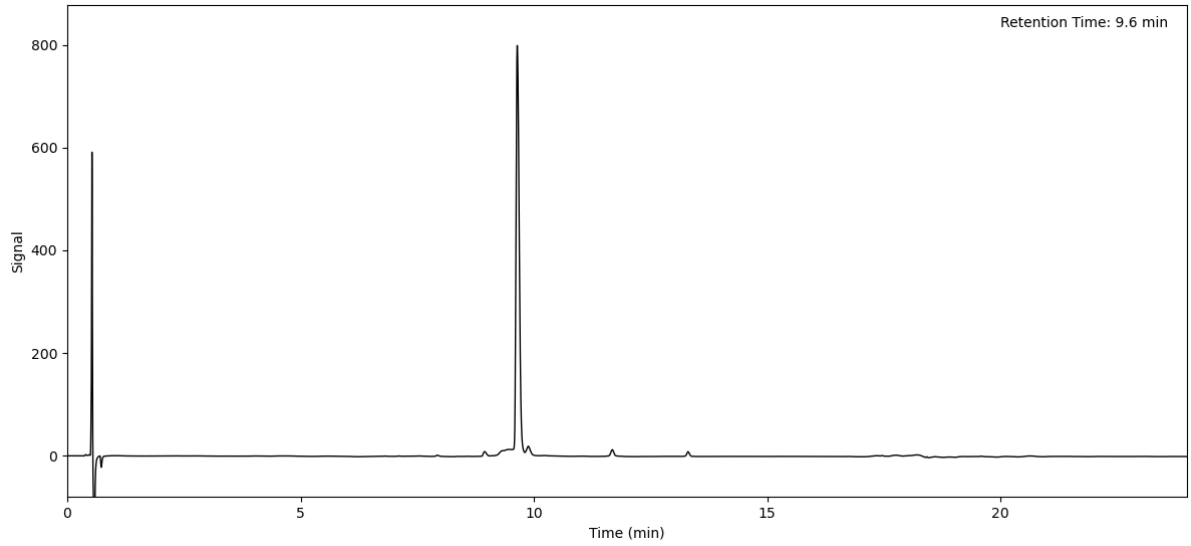
Peptide S19 (Method A)



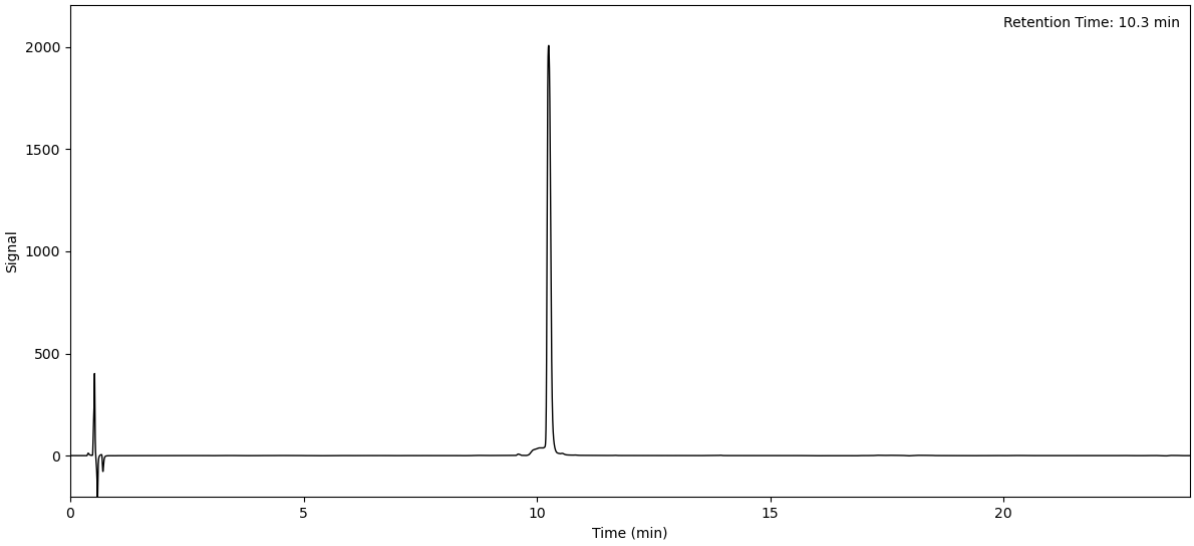
Peptide S20 (Method A)



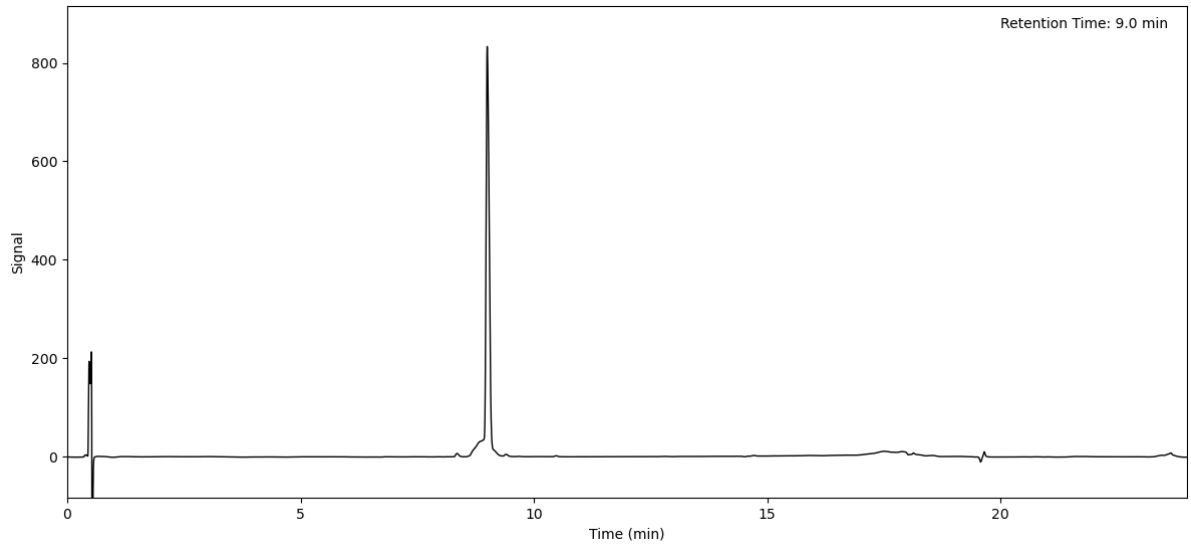
Peptide S21 (Method A)



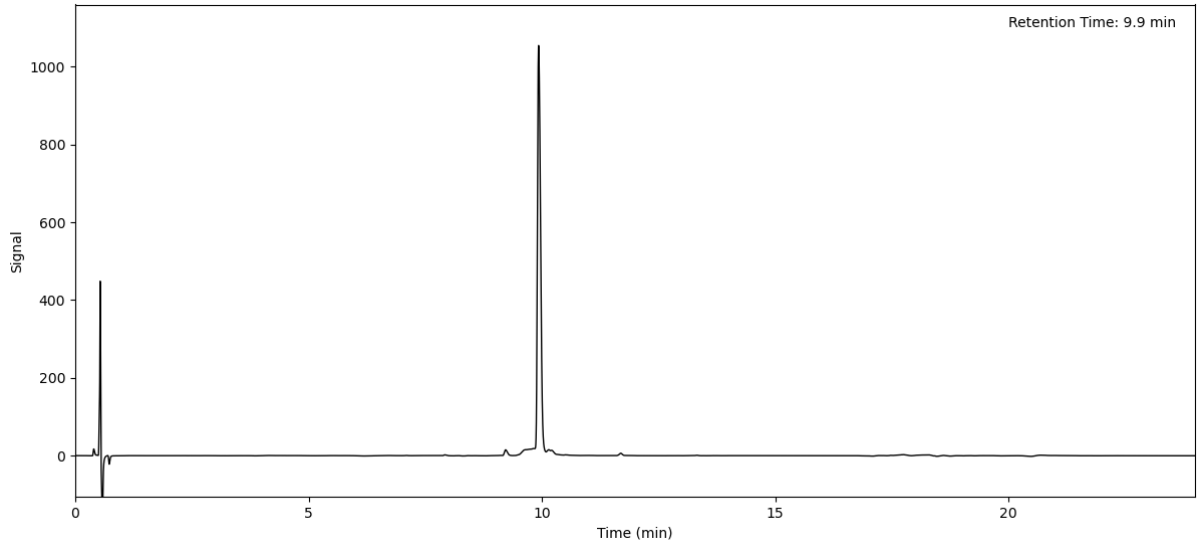
Peptide S22 (Method A)



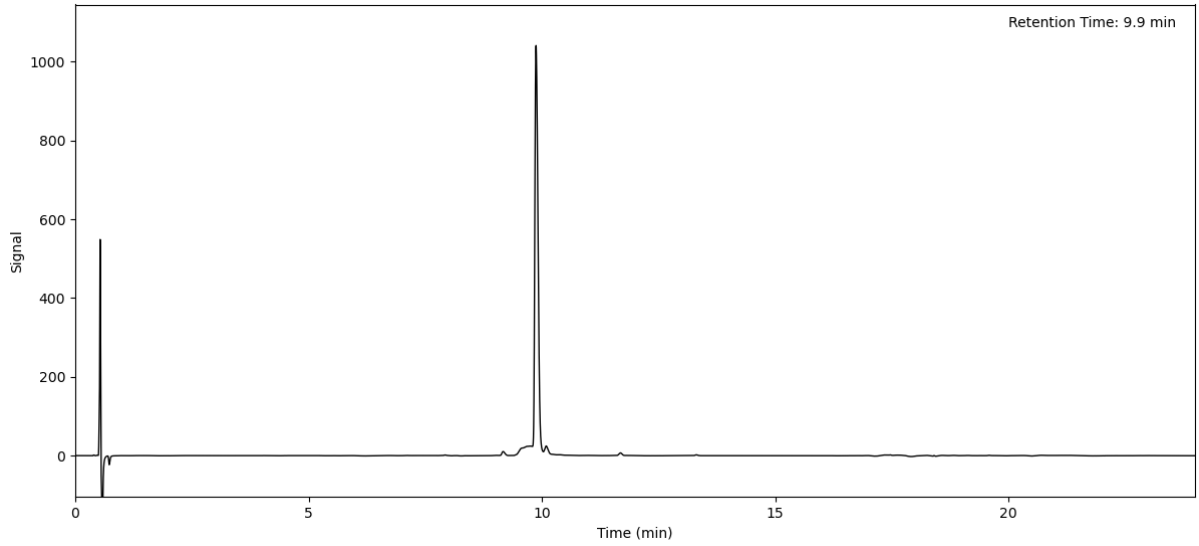
Peptide S23 (Method A)



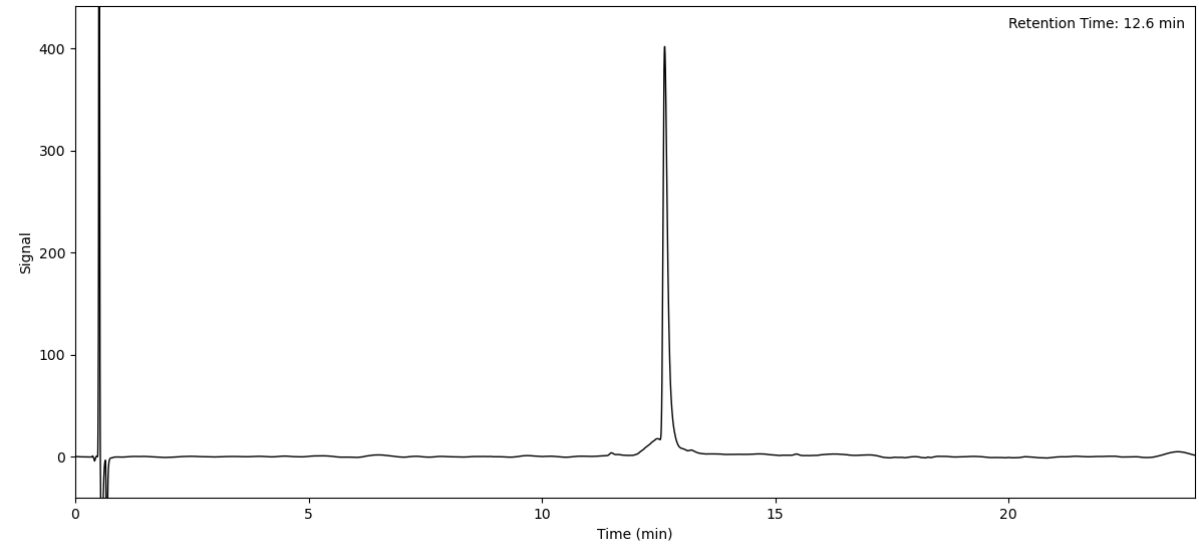
Peptide S24 (Method A)



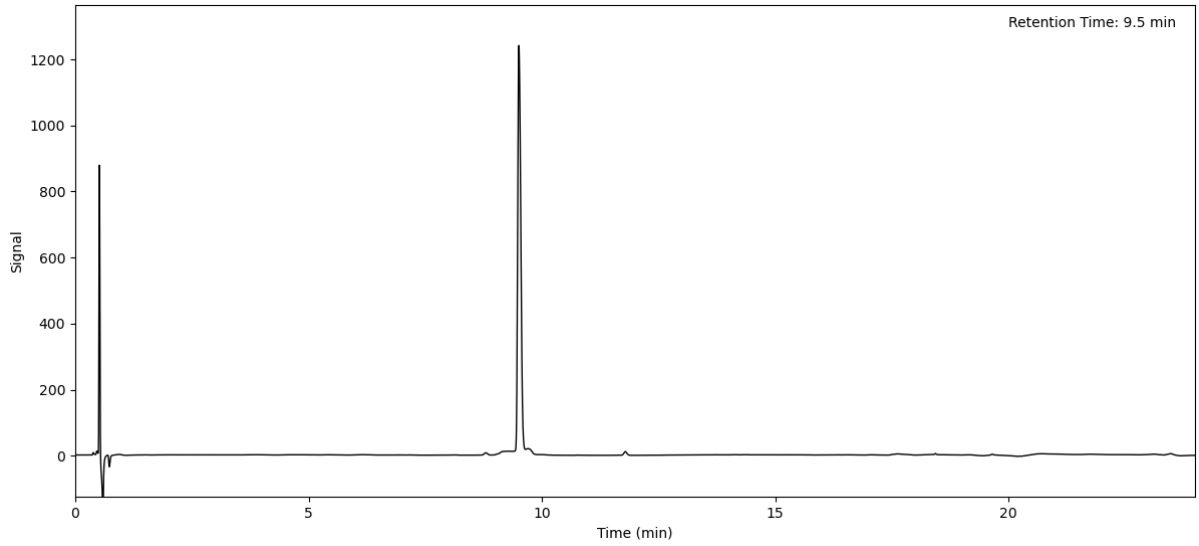
Peptide S25 (Method A)



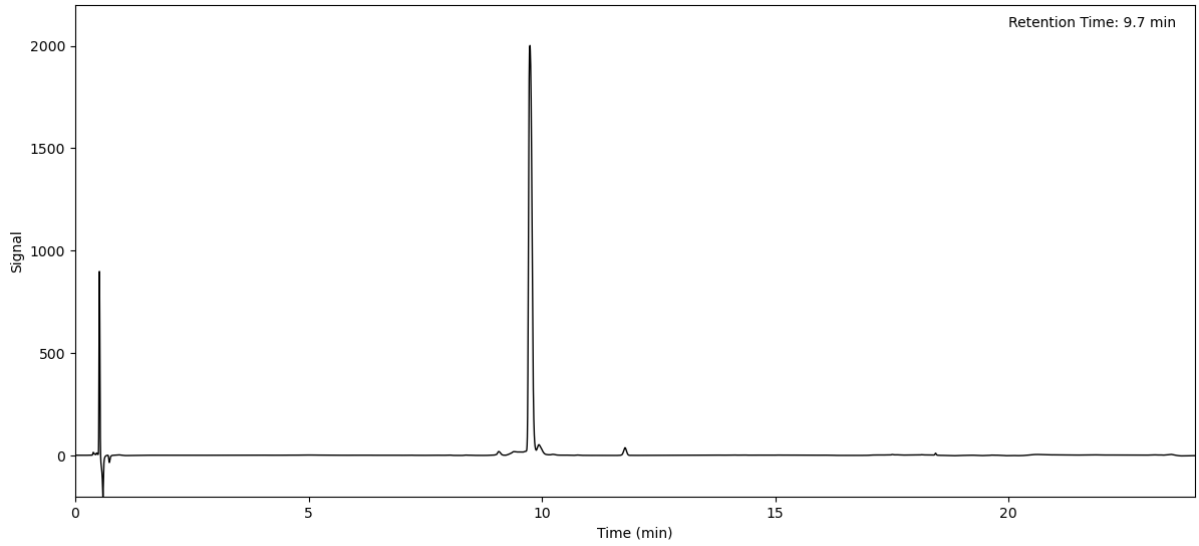
Peptide S26 (Method A)



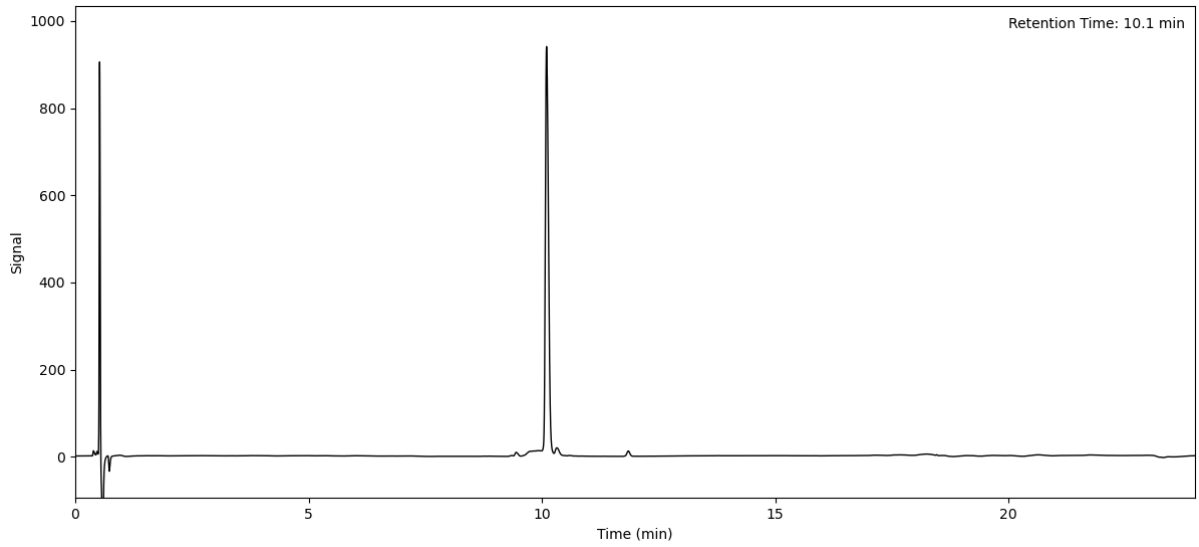
Peptide S27 (Method A)



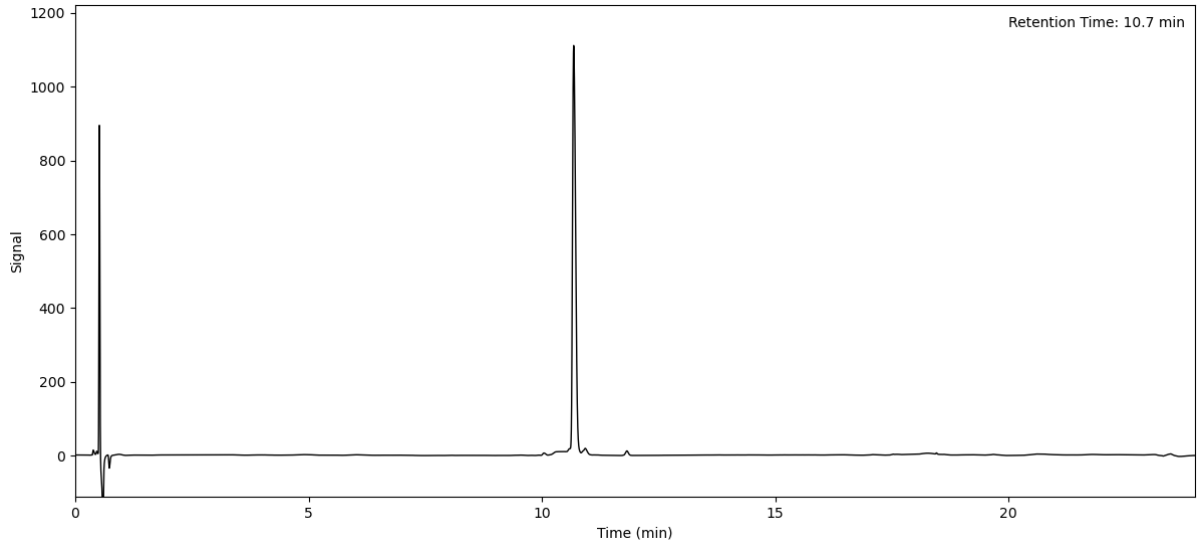
Peptide S28 (Method A)



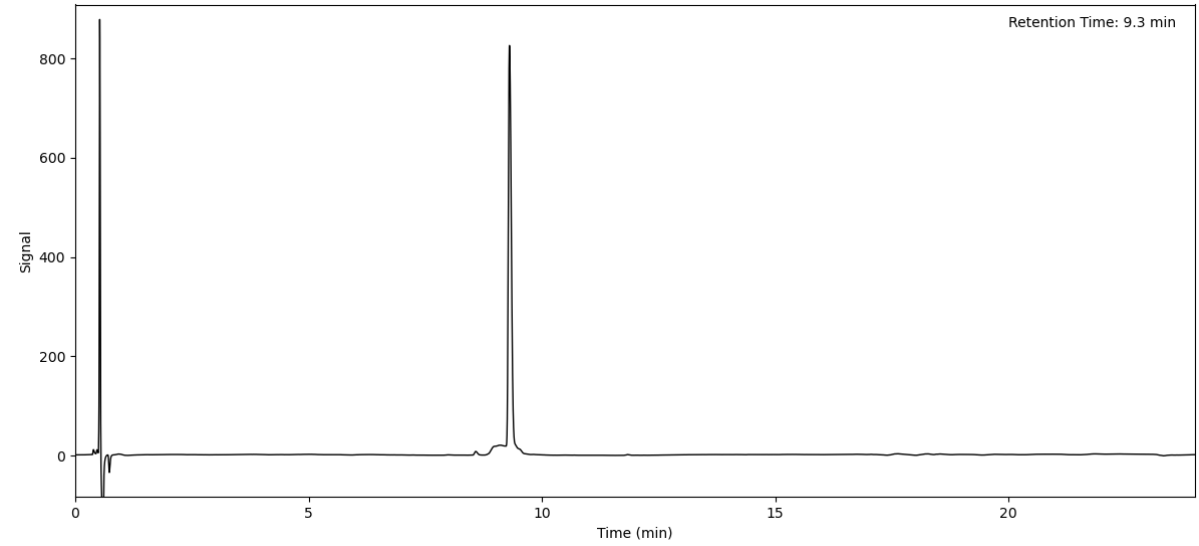
Peptide S29 (Method A)



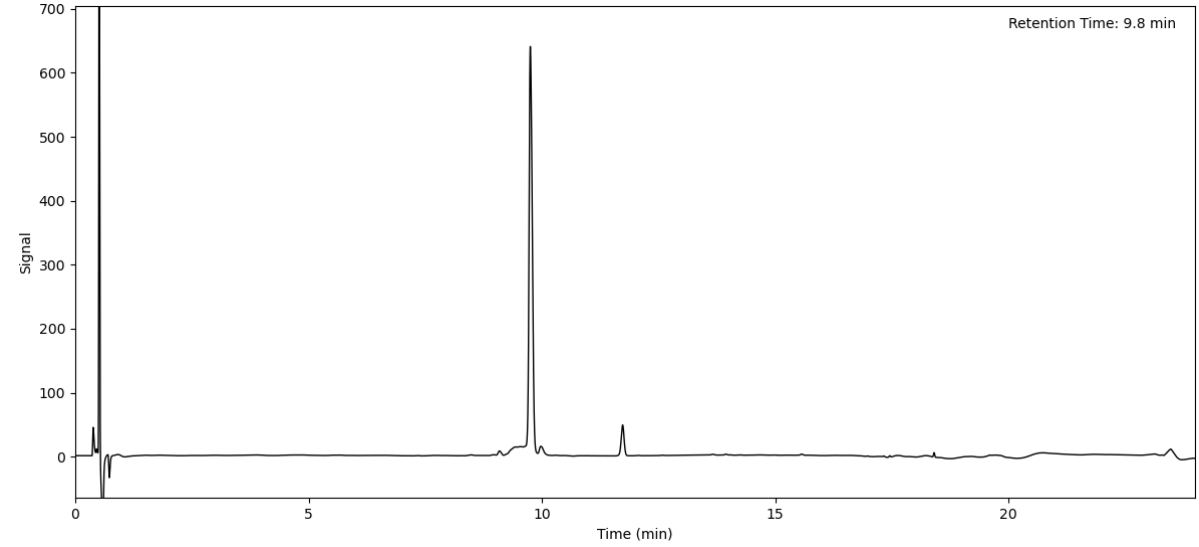
Peptide S30 (Method A)



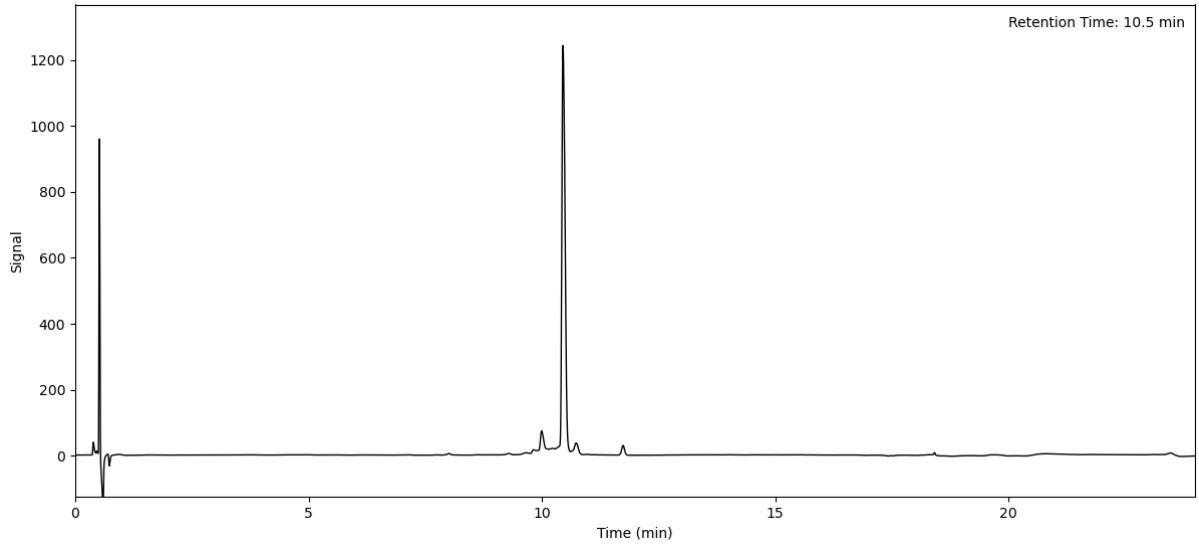
Peptide S31 (Method A)



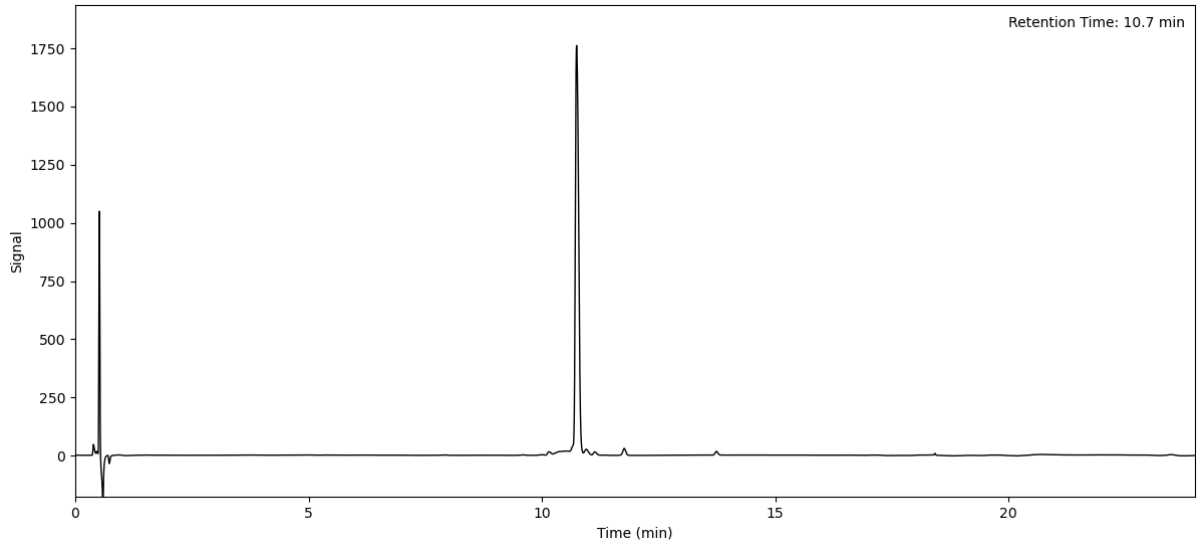
Peptide S32 (Method A)



Peptide S33 (Method A)



Peptide S34 (Method A)



H4 histone tail peptide(the MTase Glo substrate) (Method A)