Supporting Information

Revealing deamidation and isoaspartate formation during peptide analysis, purification and storage by tandem mass spectrometry

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Table of Contents

Peptide Synthesis

Chemicals

Fmoc and side chain protected natural amino acid building blocks, the rink amide and wang resins and 2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) were purchased from Gyros Protein Technologies (Uppsala, Sweden). L-Fmoc-aspartic acid alpha-t-butyl ester (isoaspartate building block), was obtained from fluorochem (Hadfield, UK). Dimethylformamide (DMF; EMPLURA Supelco) and diethyl ether (Emsure Supelco, Ph. Eur.) were obtained from Merck (Darmstadt, Germany). Pyrrolidine (99+%) and N-methylmorpholine (NMM; 99+%) were purchased from Thermo Scientific (MA, USA). Trifluoroacetic acid (TFA; 99.5%) was purchased from Apollo Scientific (Stockport, UK). Acetic anhydride (Ac_2O; 99% puriss.), dichloromethane (DCM; >99.8%), triisopropylsilane (TIS), formic acid (FA; 98.0-100%), dimethyl sulfoxide (DMSO) and acetonitrile (ACN; HPLC gradient grade, ≥ 99.9%) were purchased from Sigma Aldrich (Buchs, CH). ACN for LC-MS analysis (OPTIMA, LC-MS grade) was obtained from Fisher Chemicals (Loughborough, UK). Nanopure water was used from an in-house ELGA Purelab water purification system (VWS, Celle, Germany).

Automated solid phase peptide synthesis

Automated peptide synthesis was conducted on a PurePep Chorus peptide synthesizer (Gyros Protein Technologies, Uppsala, Sweden). Rink amide (0.78 mmol/g) or wang resin (0.57 mmol/g) equivalent to 0.1-0.2 mmol was swelled in 3 mL DMF at RT for 15 min. Fmoc deprotection was conducted twice with 2.5 mL 20% pyrrolidine in DMF for 5 min.[1] Protected amino acids (5 eq.) were activated with NMM (10 eq.) and HCTU (5 eq.) in 7 mL DMF.[2] The coupling reaction was performed for 10 min at 60 °C.[3] All steps were performed while shaking. After each deprotection and coupling step, the resin was rinsed 3 times with DMF. Deprotection and coupling cycles were repeated until the desired sequence was synthesized. The subsequent steps were conducted manually. N-terminal acetylation was performed after the last deprotection step on resin two times in 3 mL 20% Ac_2O in DMF at RT for 15 min. Before cleavage, the resin was washed three times with DCM. Peptide cleavage off resin and of side chain protecting groups was performed in 3 mL of a mixture of TFA, TIS and H_2O with a ratio of 95/2.5/2.5 (v/v) for 2 h at RT. The peptide dissolved in the cleavage mixture was washed three times in ice cold diethyl ether and precipitated by 10 min centrifugation at 10'000 rpm and -10 °C.

LC-ESI-MS analysis

After each peptide synthesis, the presence of the expected peptide was determined by LC-ESI-MS analysis. LC analysis was conducted on a Waters Acquity UPLC with the Zorbax Eclipse Plus C18 analytical column (2.1 x 5.0 mm, 1.8 µm) at 25 °C. Gradient elution with a flow of 0.5 mL/min was performed with 0.1% FA in H2O (solvent A) and 0.1% FA in ACN LC-MS grade (solvent B) as follows: starting conditions and 2 min hold at 95% A, at 12 min to 10% A and hold until 15 min with subsequent reequilibration at 95% A for 5 min. ESI ionization was performed with a heated electronspray ionization probe (HESI-II, Thermo Scientific, USA). The settings were the following: heater temperature at 0 °C (corresponds to approx. 50 °C operating temperature), sheath gas flow rate at 34 arbitraty units (arb), auxiliary gas flow rate at 11 arb, sweep gas flow rate at 0, spray voltage at 5 kV, capillary temperature at 275 °C, capillary voltage at 31 V and tube lens at 80 V. MS detection was conducted with an LTQ XL ion trap mass spectrometer (Thermo Scientific, USA) at a mass range from 100 to 2000 m/z (scan type 'Full'). Data was acquired with normal scan rate, positive polarity and data type profile. For analysis 5 µL of a 0.1 mM solution of crude product were injected onto the HPLC-ESI-MS system. Filtering of obtained chromatograms for expected masses was conducted using the X Calibur software (Thermo Scientific, Version 4.4.16.14).

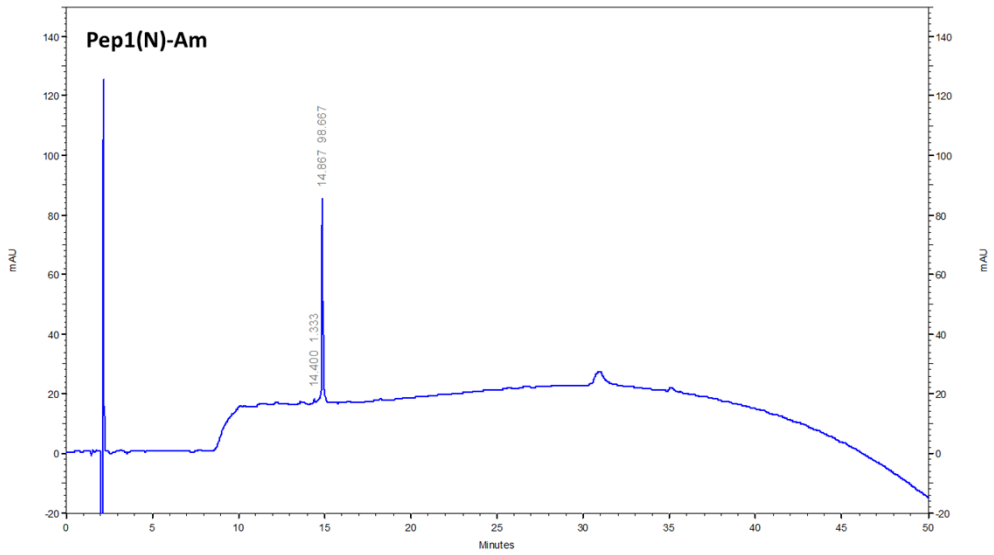
Peptide purification by flash chromatography

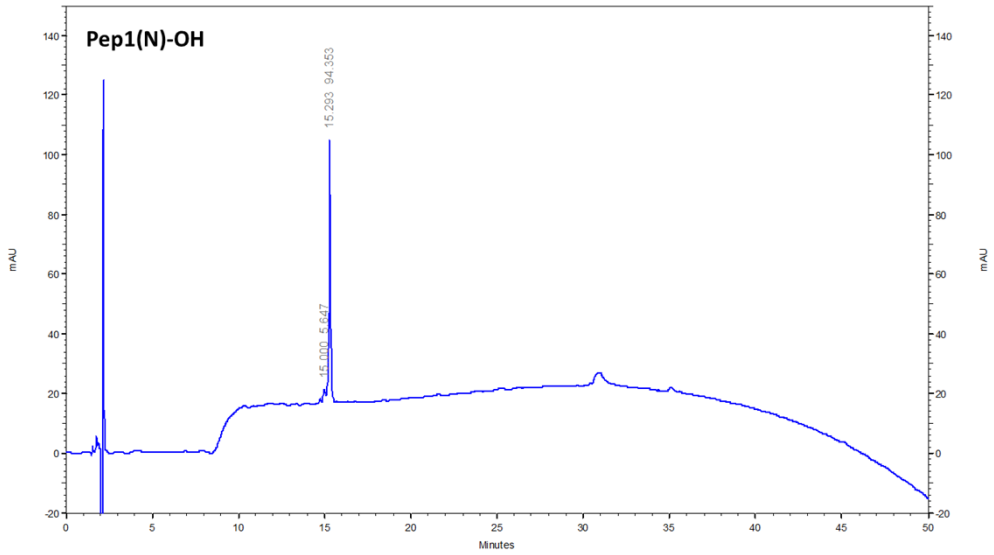
Purification of the peptides was conducted by flash chromatography with a puriFlash XS 520Plus (Advion Interchim, USA).[4] For purification C18 flash chromatography columns (Interchim) of various sizes were used. The column size was chosen so the amount of peptide crude to purify did not exceed 1% of the column weight according to supplier guidelines. The peptide crude was dissolved in the least possible amount of DMSO before loading on the column. Gradient elution starting at 0.1% TFA in 5/95 ACN/H_2O gradually going to 0.1% TFA in 40/60 ACN/H2O within 10 to 30 column volumes depending on the peptide polarity. Elution was performed at the flow rate automatically adjusted to the column size. After each purification the column was flushed with 100% ACN. Fraction collection was conducted based on UV detection at a wavelength of 214 nm. Collected fractions were frozen at -80 °C and freeze dried by lyophilization. Collected Fractions and lyophilized products were reanalyzed by LC-ESI-MS as described above. Found masses are listed in Table S1-1.

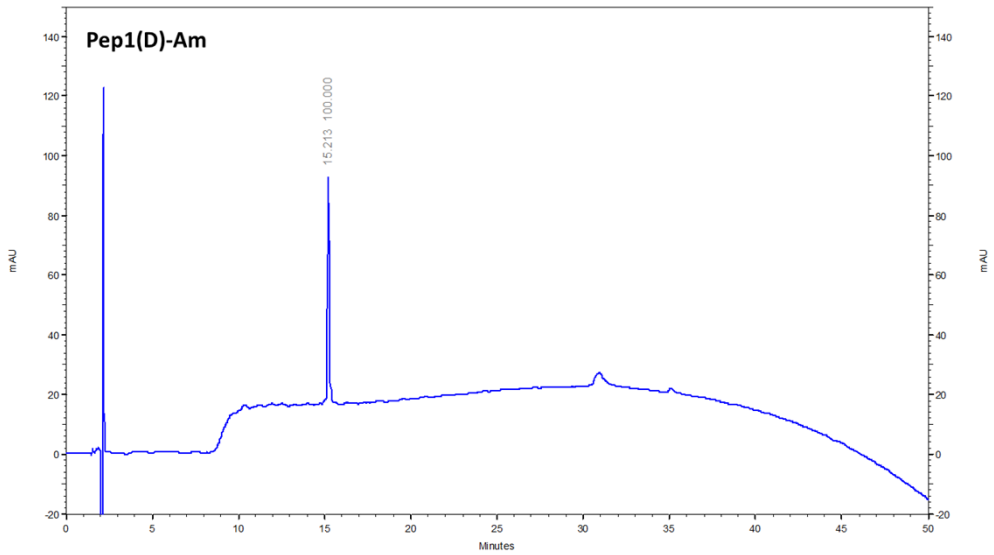
Peptide purity determination by LC-UV

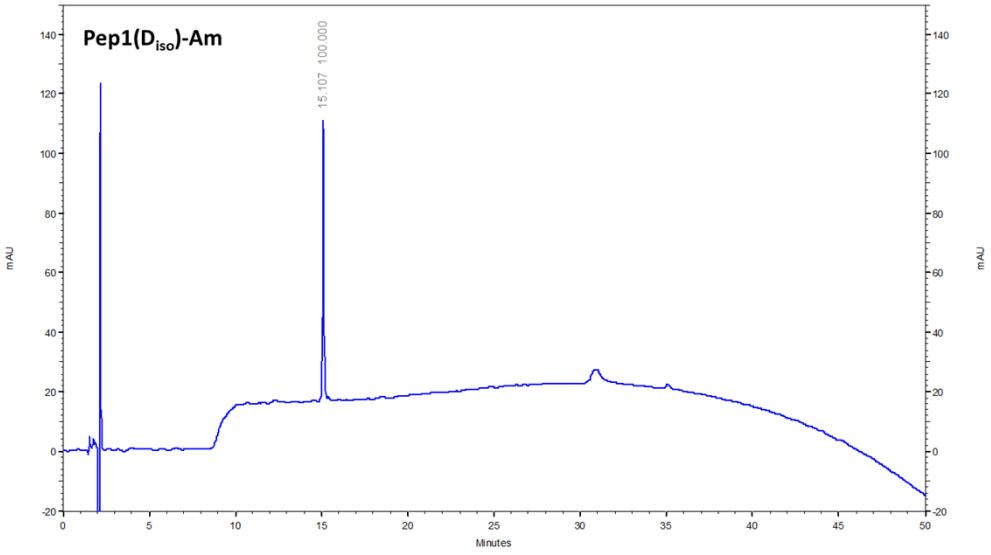
To determine the purity of the purified peptides LC-UV analysis was conducted. For LC analysis an ELITE La Chrom Hitachi HPLC device equipped with an L-2130 pump, an L-2200 autosampler, an L-2350 column oven and an L-2455 DAD detector was used (Tokyo, Japan). 20 µL of a 0.1 mM peptide solution were analyzed on a Waters X Bridge C18 column (3.5 µm, 4.6 x 150 mm) by gradient elution with the solvents (A) 0.1% TFA in ACN and (B) 0.1% TFA in H_2O. The gradient program was as follows: 5 min hold at 5% A, increase to 95% A at 50 min, hold at 95% A until 55 min and 10 min reequilibration at starting conditions. UV absorbance was monitored at 214 nm. Data acquisition and analysis were conducted using Agilent OpenLab CDS software (Version A.04.08). Resulting purities are listed in Table S1-1. According chromatograms are shown below.

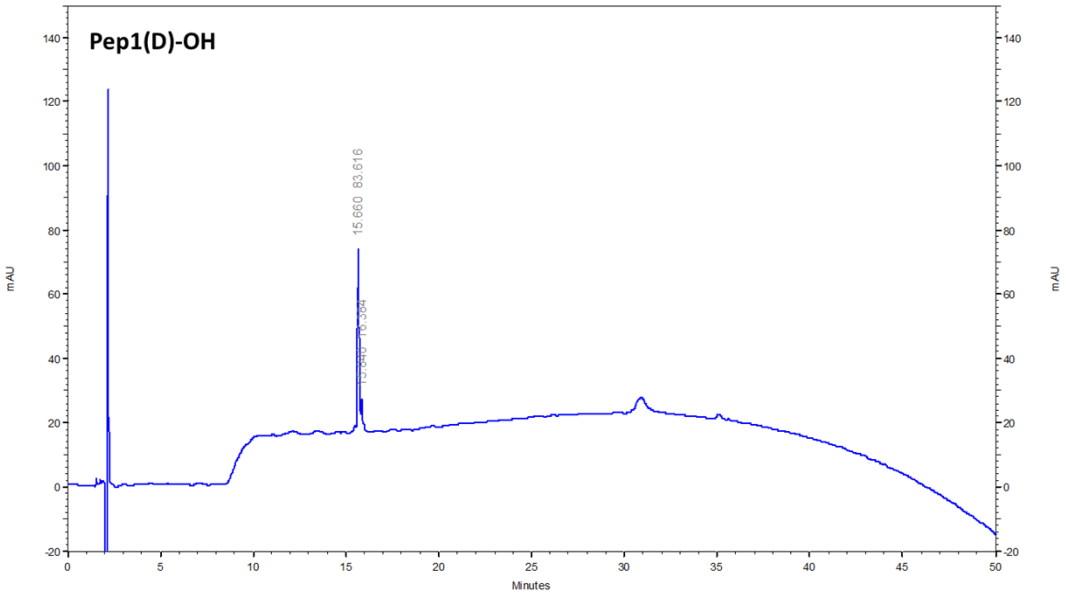
Figure S1: LC-UV Data

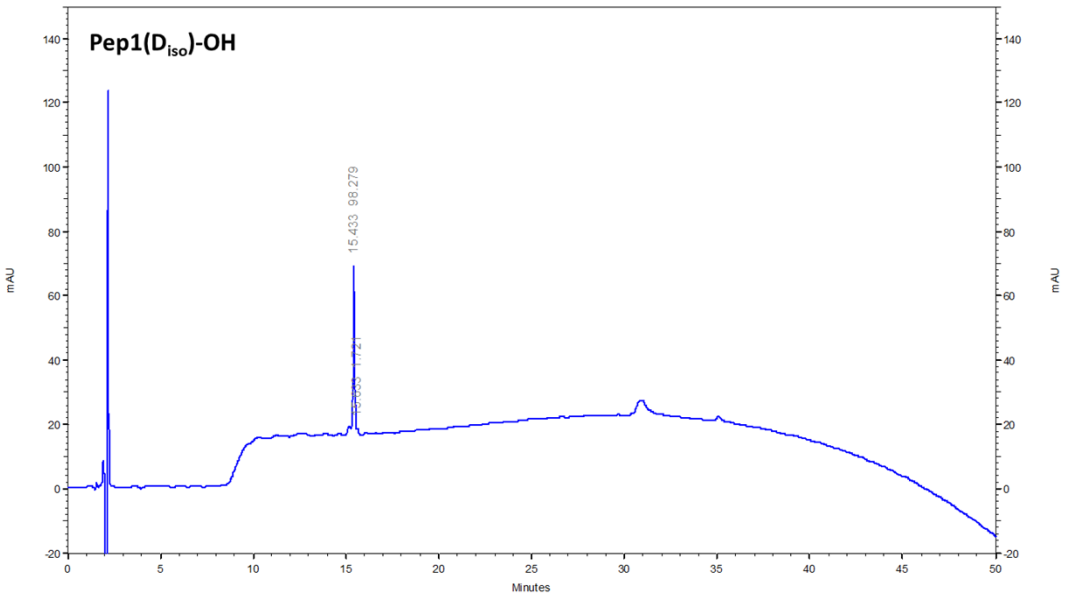







Pep1(D$_{iso}$)-Am
15.107 100.000
mAU
Minutes


Pep1(D)-OH
15.660 83.616
mAU
Minutes


Pep1(D$_{iso}$)-OH
15.433 98.279
mAU
Minutes

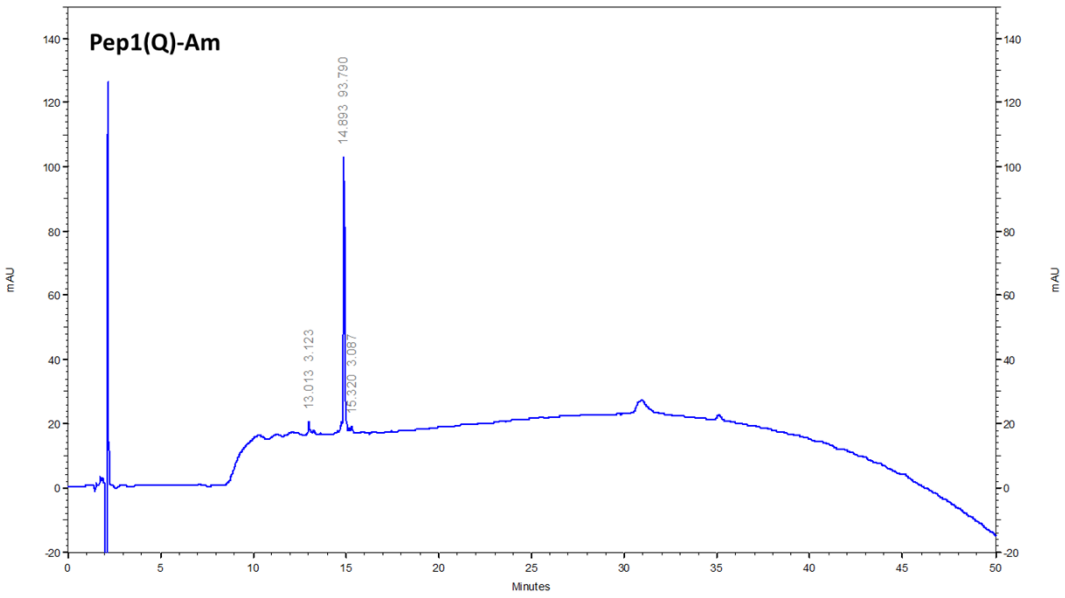
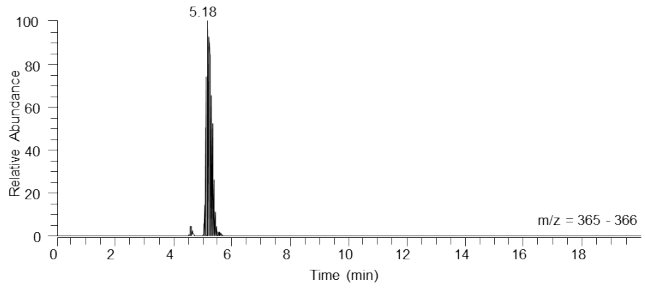
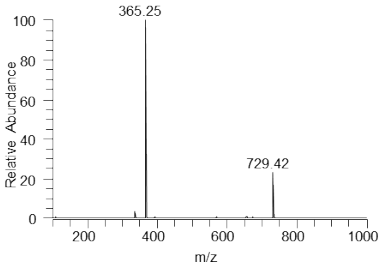

Pep1(Q)-Am
14.893 93.790
13.013 3.123
15.320 3.087
mAU
Minutes

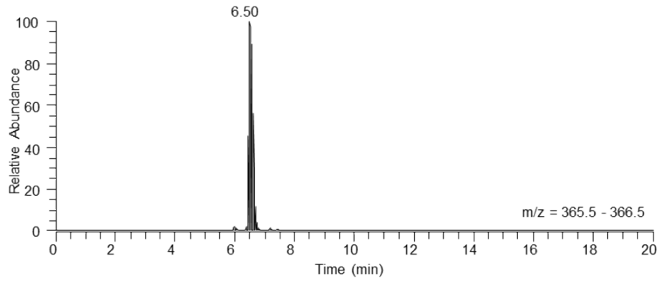
Figure S2: LC-ESI-MS Data

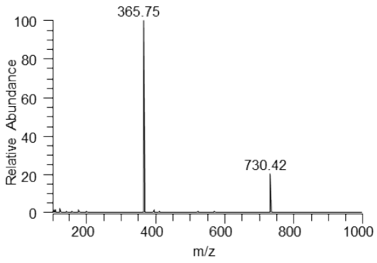
Pep1(N)-Am



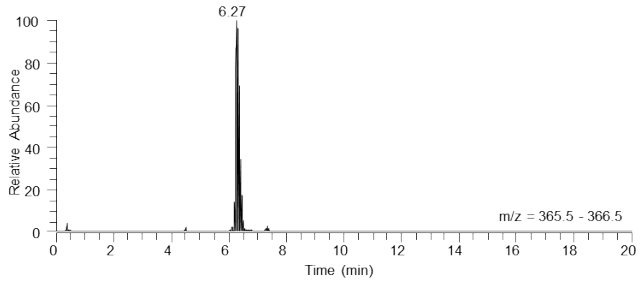


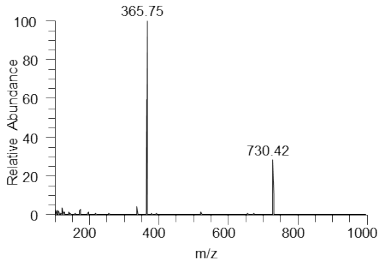
Pep1(N)-OH



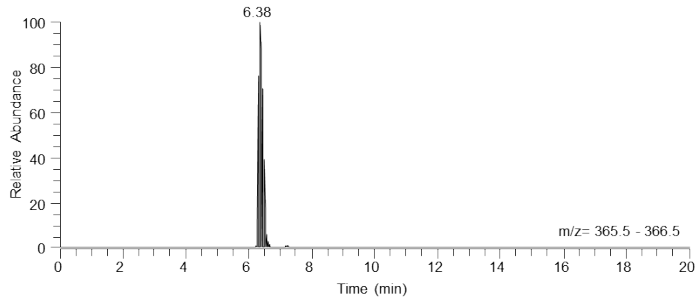


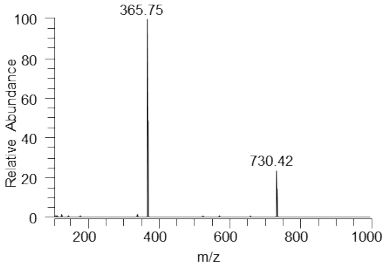
Pep1(D)-Am



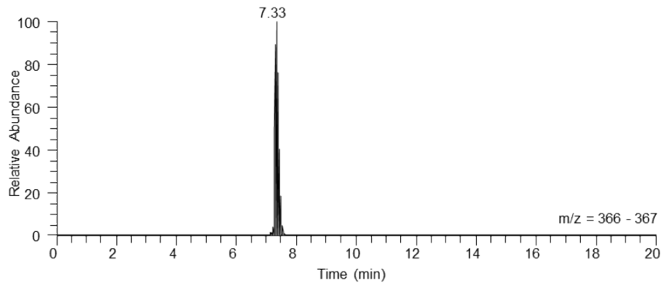


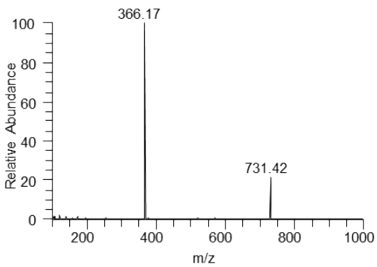
Pep1(D_{iso})-Am



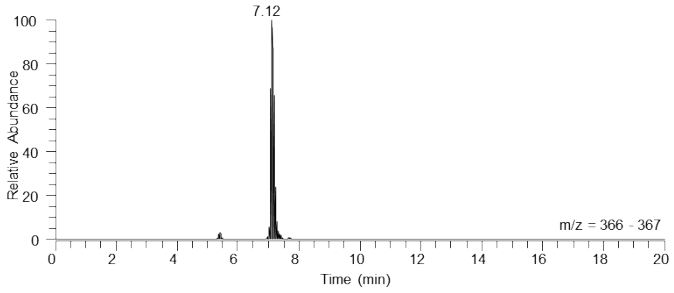


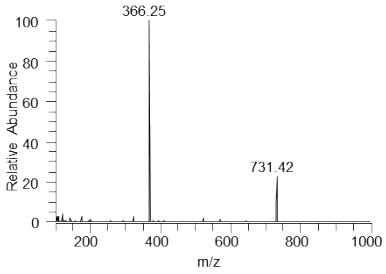
Pep1(D)-OH



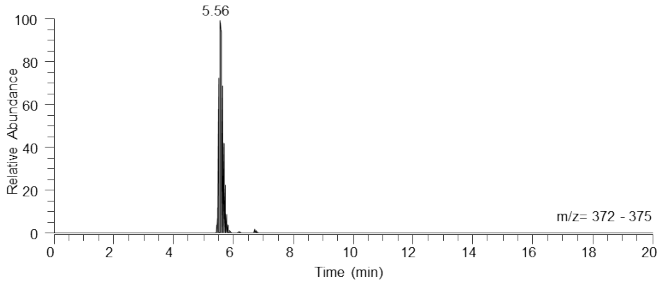


Pep1(D_{iso})-OH





Pep1(Q)-Am



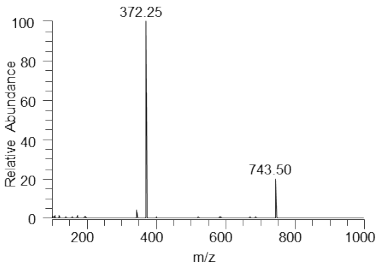
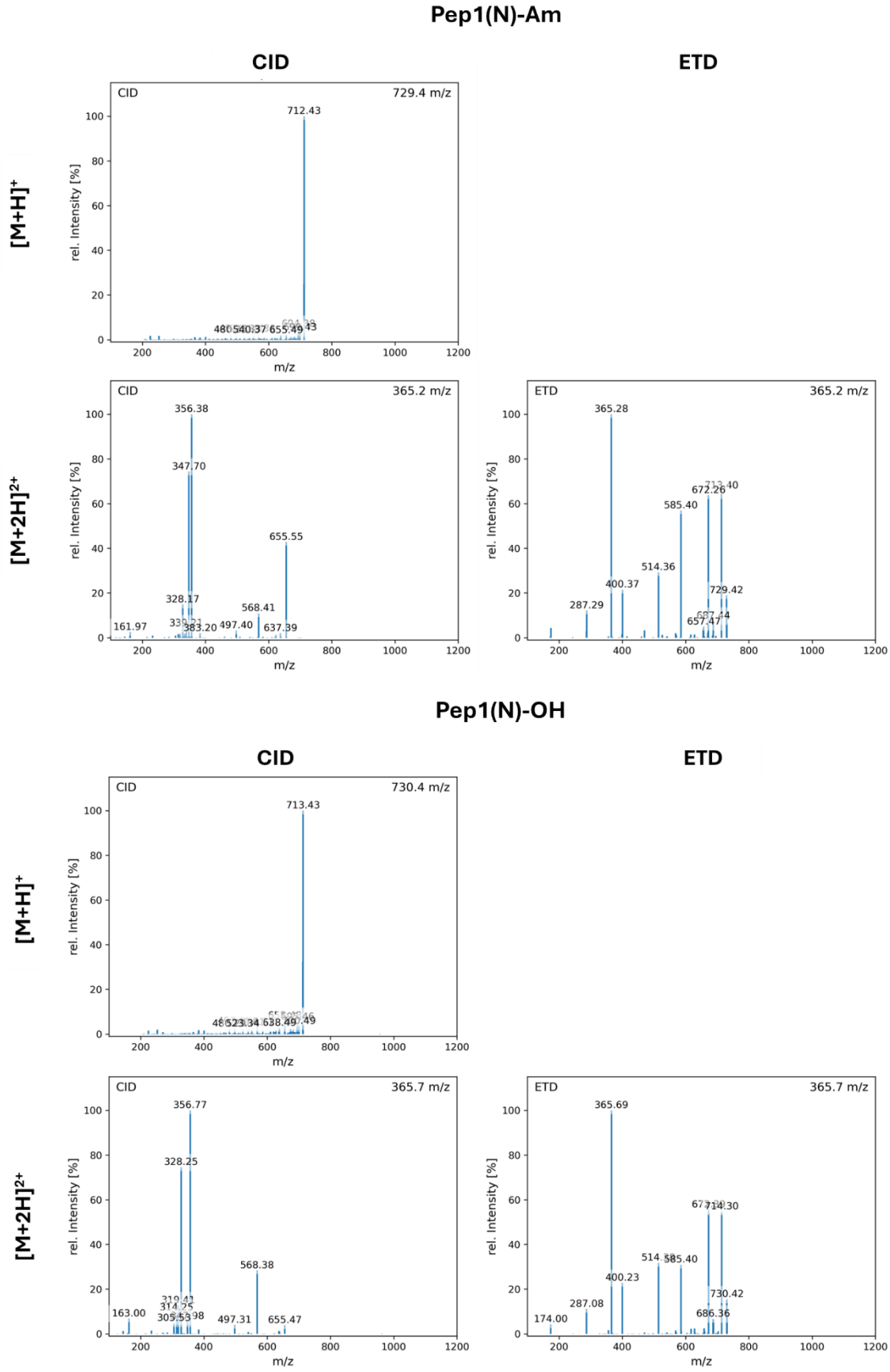
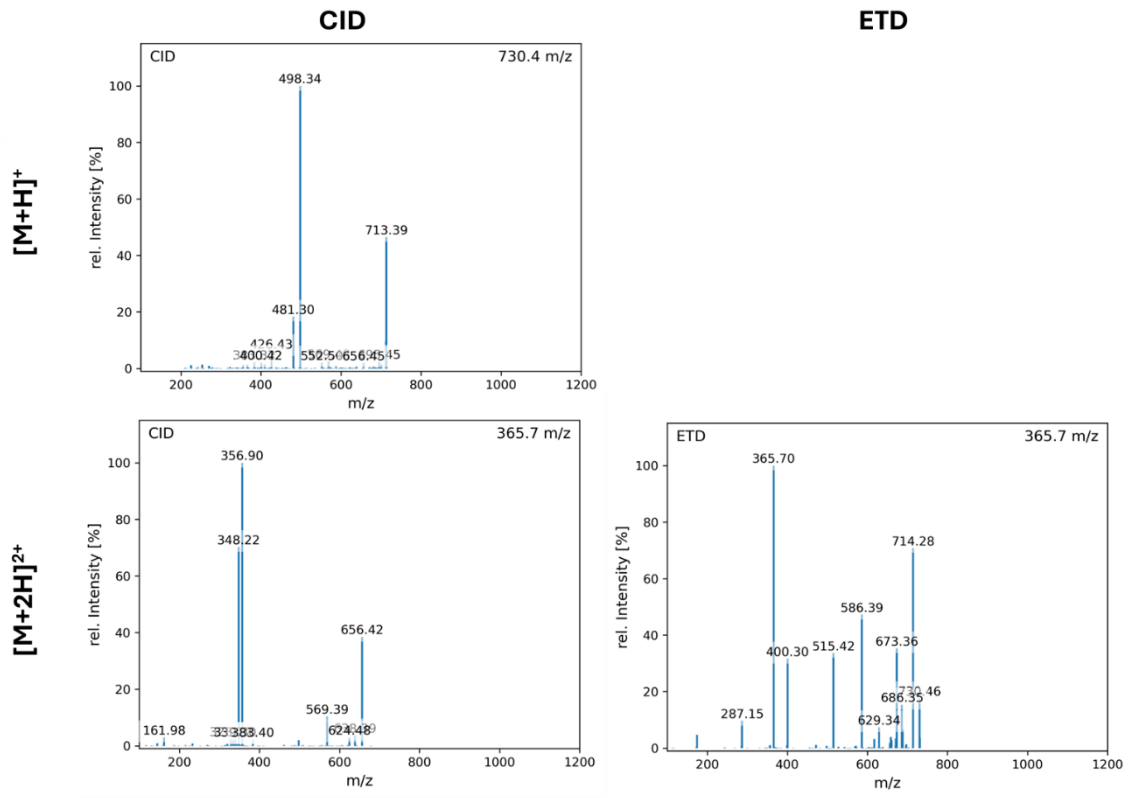


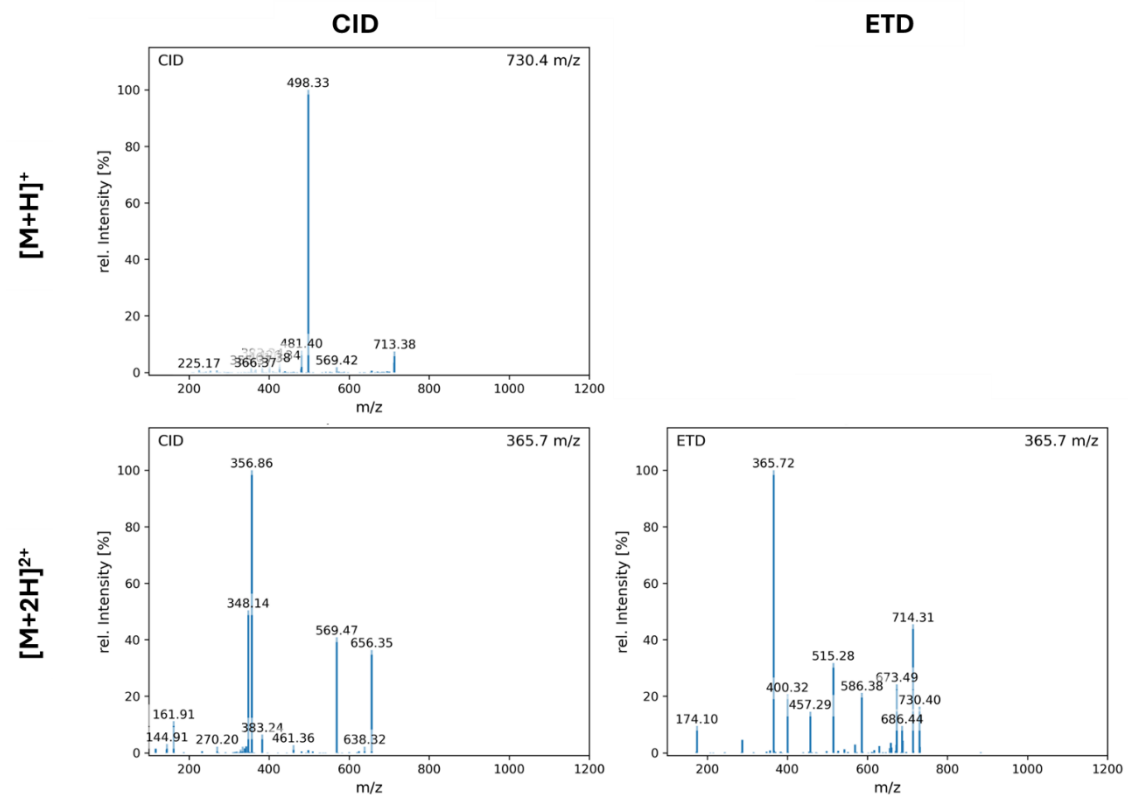
Figure S3: CID and ETD Spectra



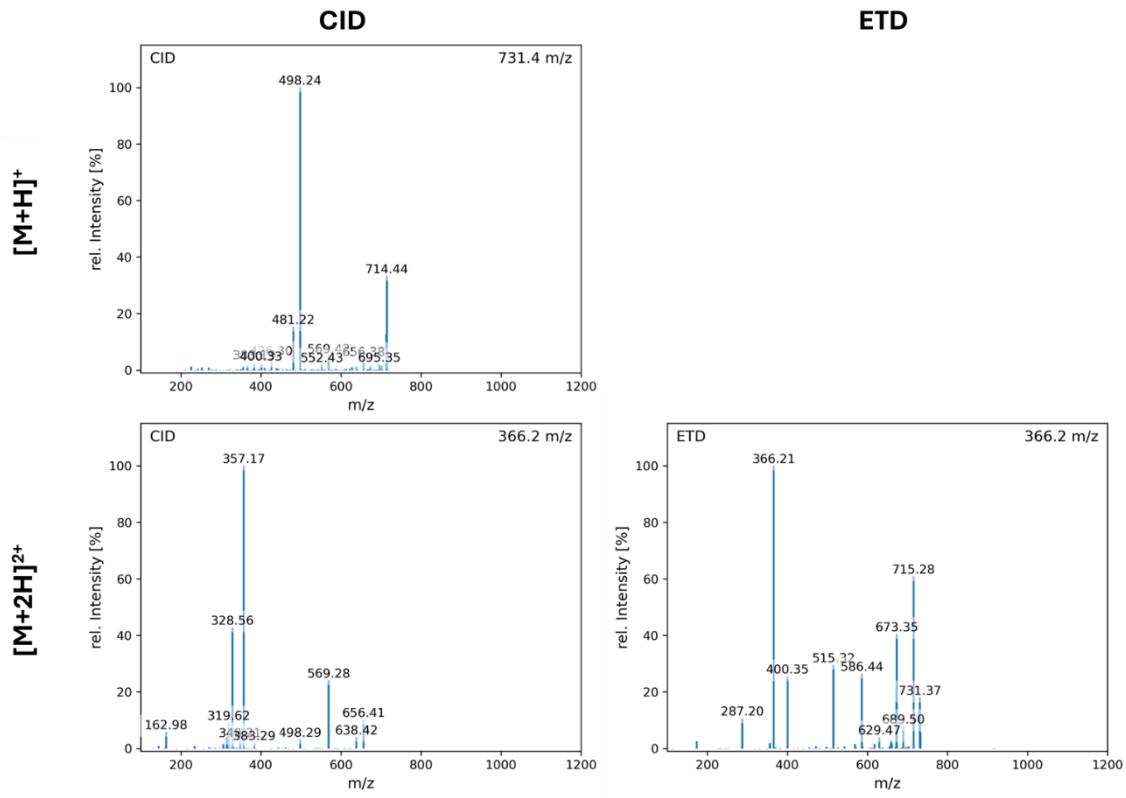
Pep1(D)-Am



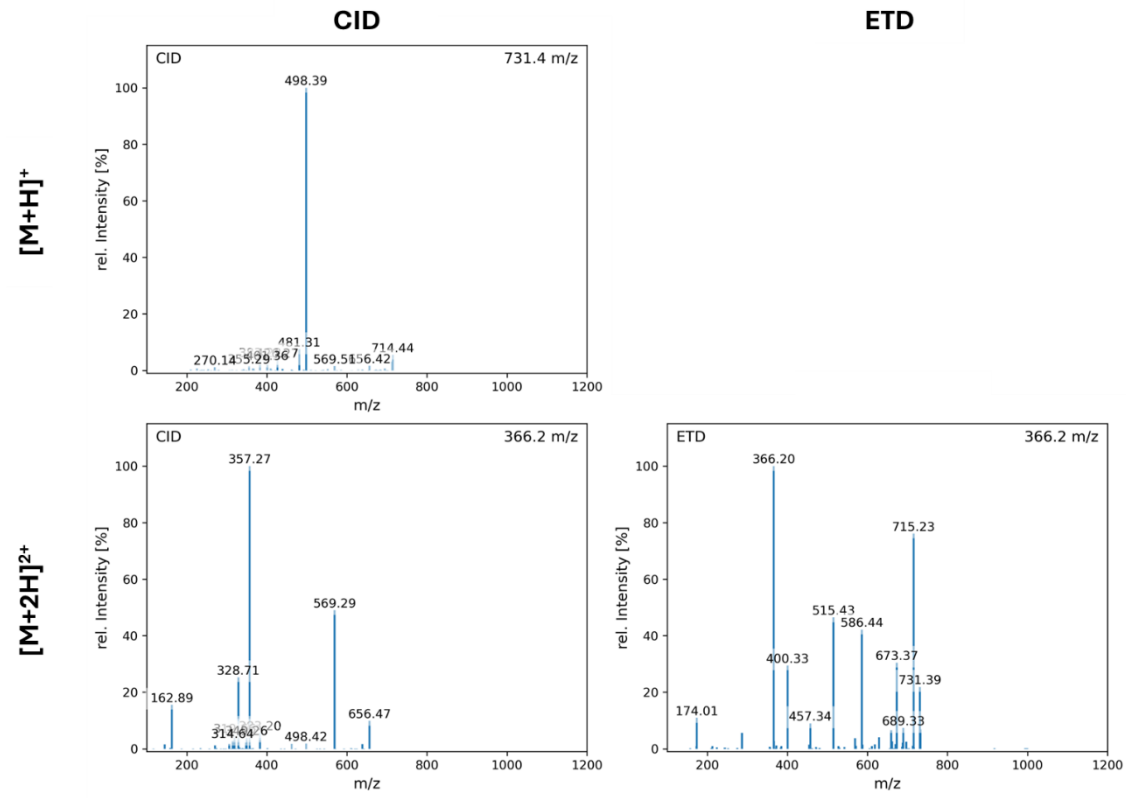
Pep1(D_{iso})-Am



Pep1(D)-OH



Pep1(D_{iso})-OH



Pep1(Q)-Am

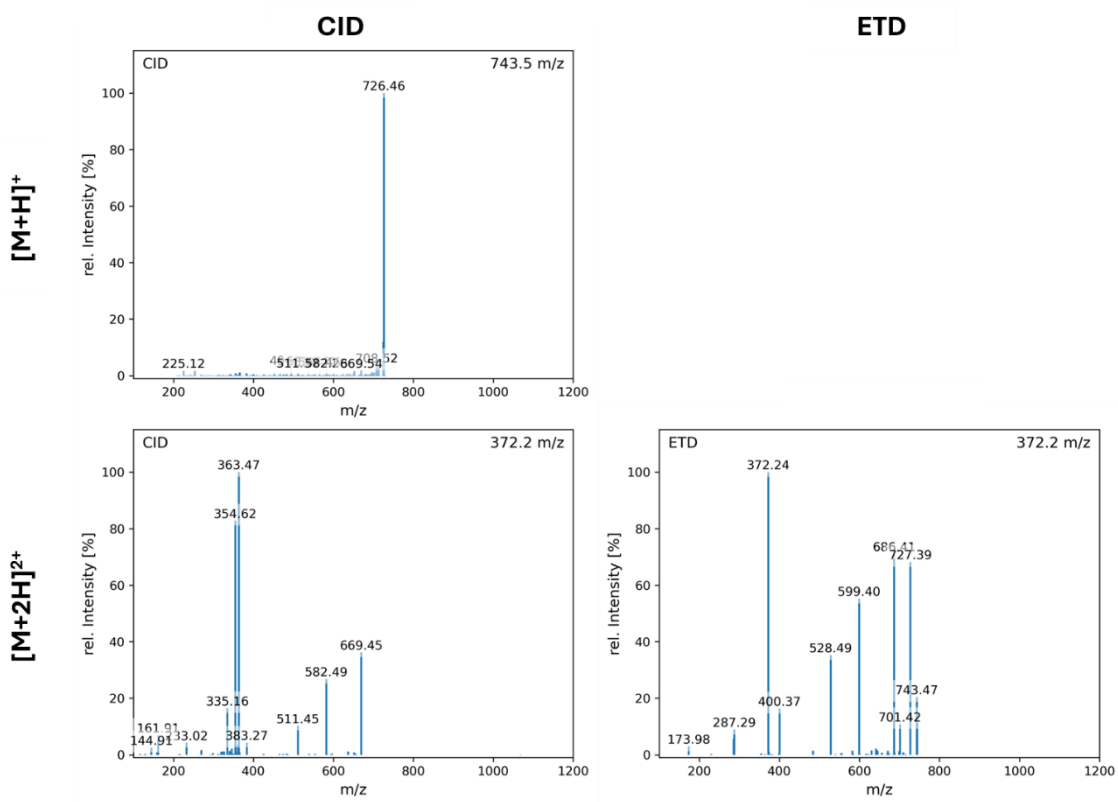

CID
ETD
[M+H]+
[M+2H]2+
CID
743.5 m/z
726.46
225.12
511.58
669.54
rel. Intensity [%]
m/z
CID
372.2 m/z
363.47
354.62
669.45
582.49
335.16
511.45
161.91
144.91
133.02
383.27
ETD
372.2 m/z
372.24
686.41
727.39
599.40
528.49
743.47
400.37
701.42
287.29
173.98

Figure S4: Additional Figures and Tables

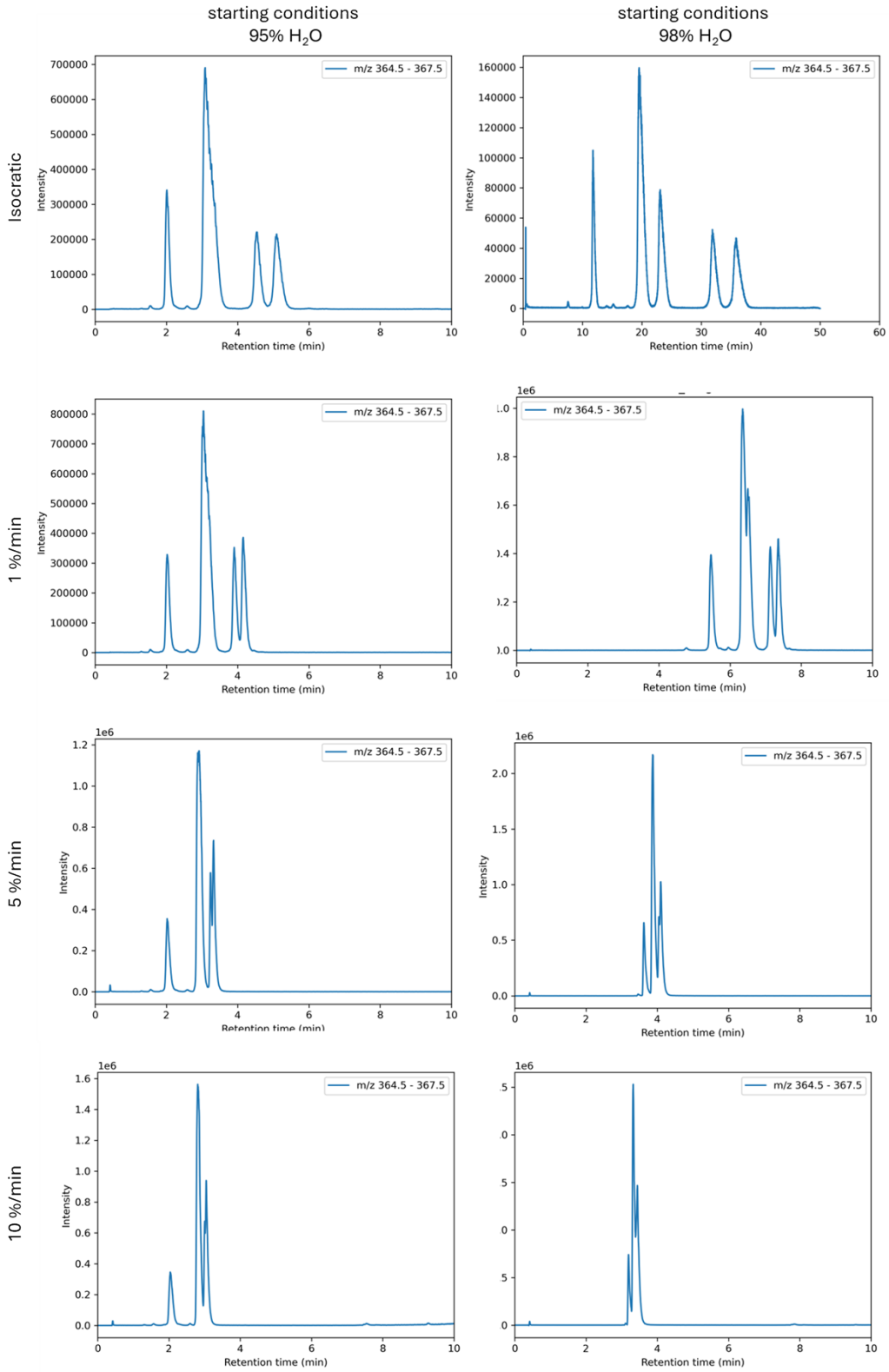


Figure S4-1: Chromatograms of different gradients and water content at starting conditions for the separation of Pep1(N)-Am, Pep1(D)-Am, Pep1(D_{iso})-Am, Pep1(N)-OH, Pep1(D_{iso})-OH and Pep1(D)-OH.

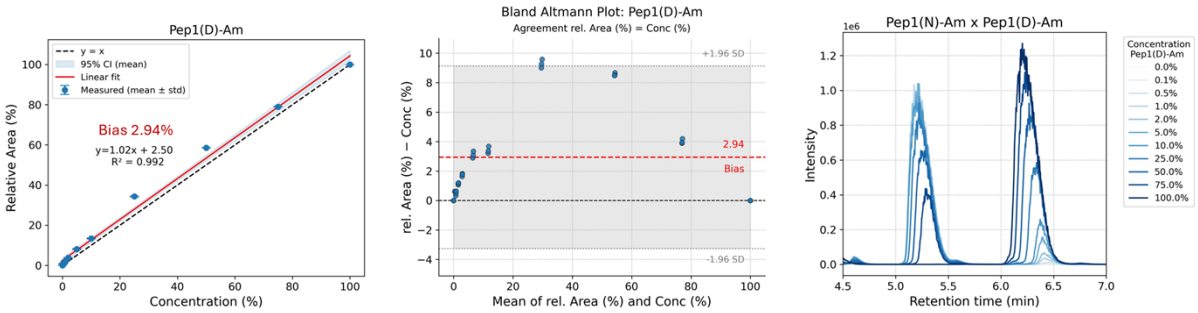


Figure S4-2: (Left) Linear correlation of relative peak area with relative concentration of Pep1(D)-Am in binary mixtures with Pep1(N)-Am (slope = 1.018, 95% CI 0.985–1.052; intercept = 2.495, 95% CI 1.110–3.881) (Middle) Bland Altmann plot of the correlation between relative area and relative concentration of Pep1(D)-Am in mixtures with Pep1(N)-Am. (Right) Overlap of chromatograms obtained from analysis of the Pep1(N)-Am / Pep1(D)-Am mixtures, filtered for m/z 364.5-367.5.

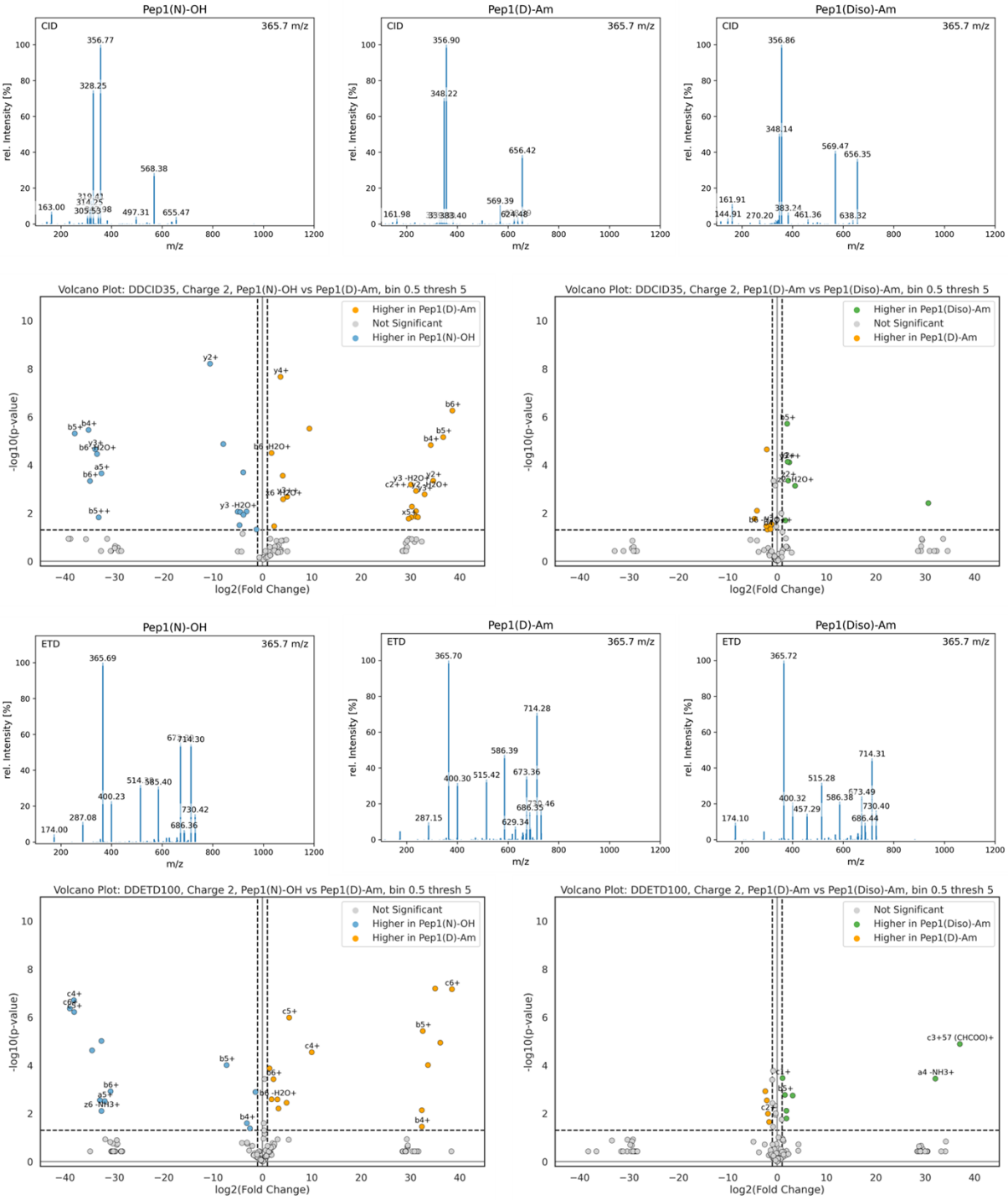


Figure S4-3: Volcano plot analysis to determine significantly differing signals between Pep1(N)-OH vs. Pep1(D)-Am (left) and Pep1(D)-Am vs. Pep1(D_{iso})-Am (right) for CID (top) and ETD (bottom) spectra. Theoretical fragments were assigned, if applicable.

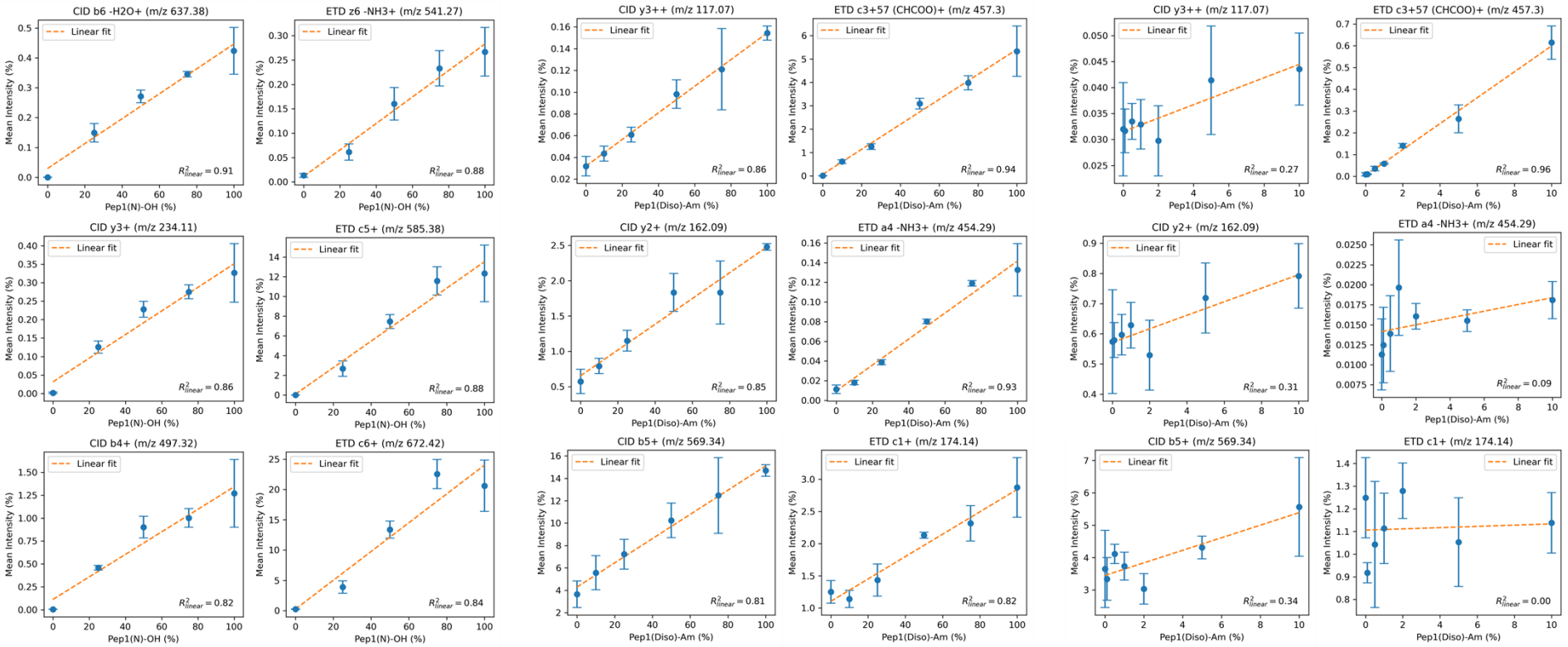


Figure S4-4: Correlation of signal intensity of diagnostic MS/MS fragment ions with Pep1(N)-OH content or Pep1(D_{iso})-Am content in mixtures with Pep1(D)-Am for each CID and ETD. For Pep1(D_{iso})-Am additionally a range of 0-10% is shown.

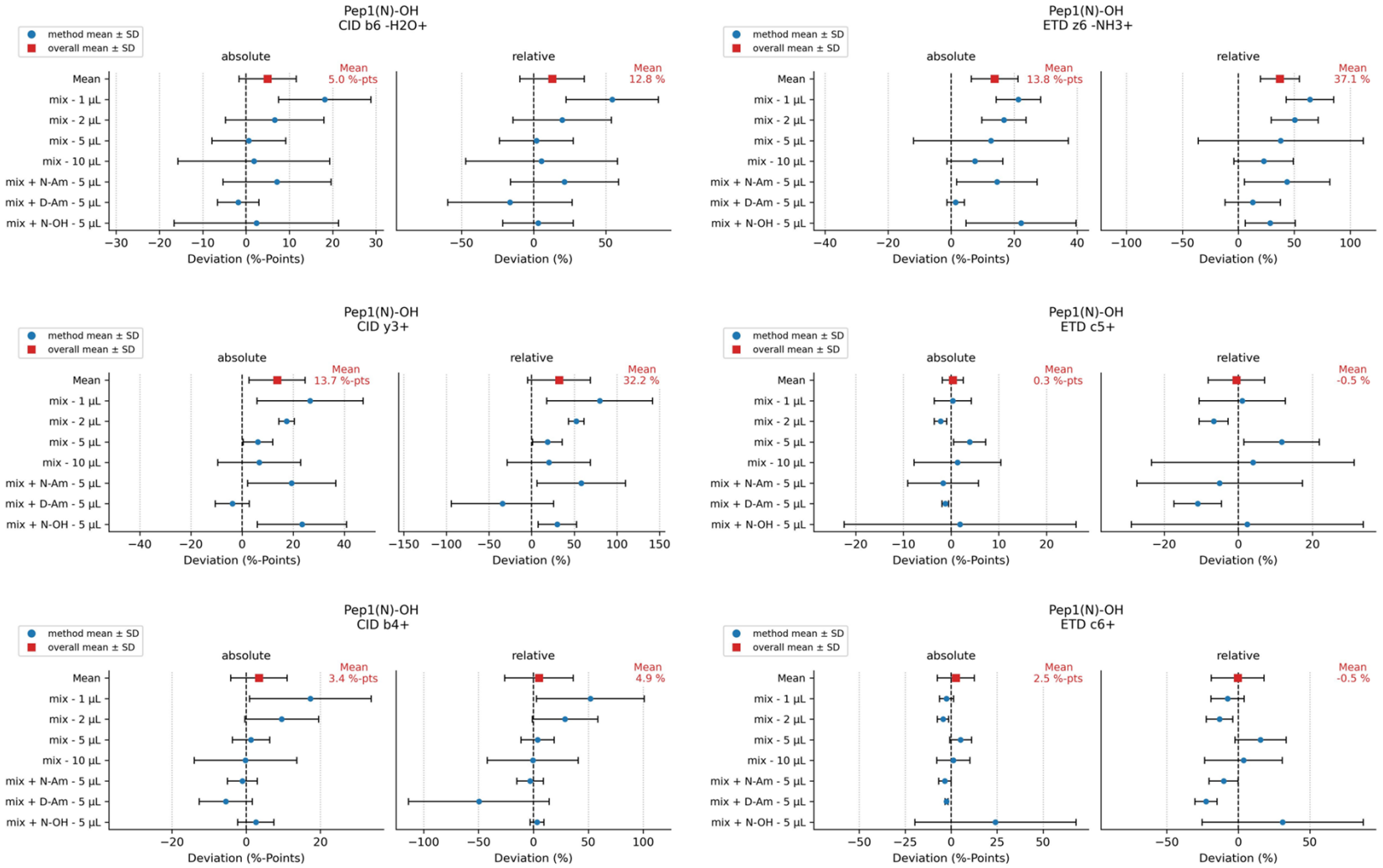


Figure S4-5: Absolute and relative accuracy of the content determination of Pep1(N)-OH in mixture analysis by peptide specific fragments ions.

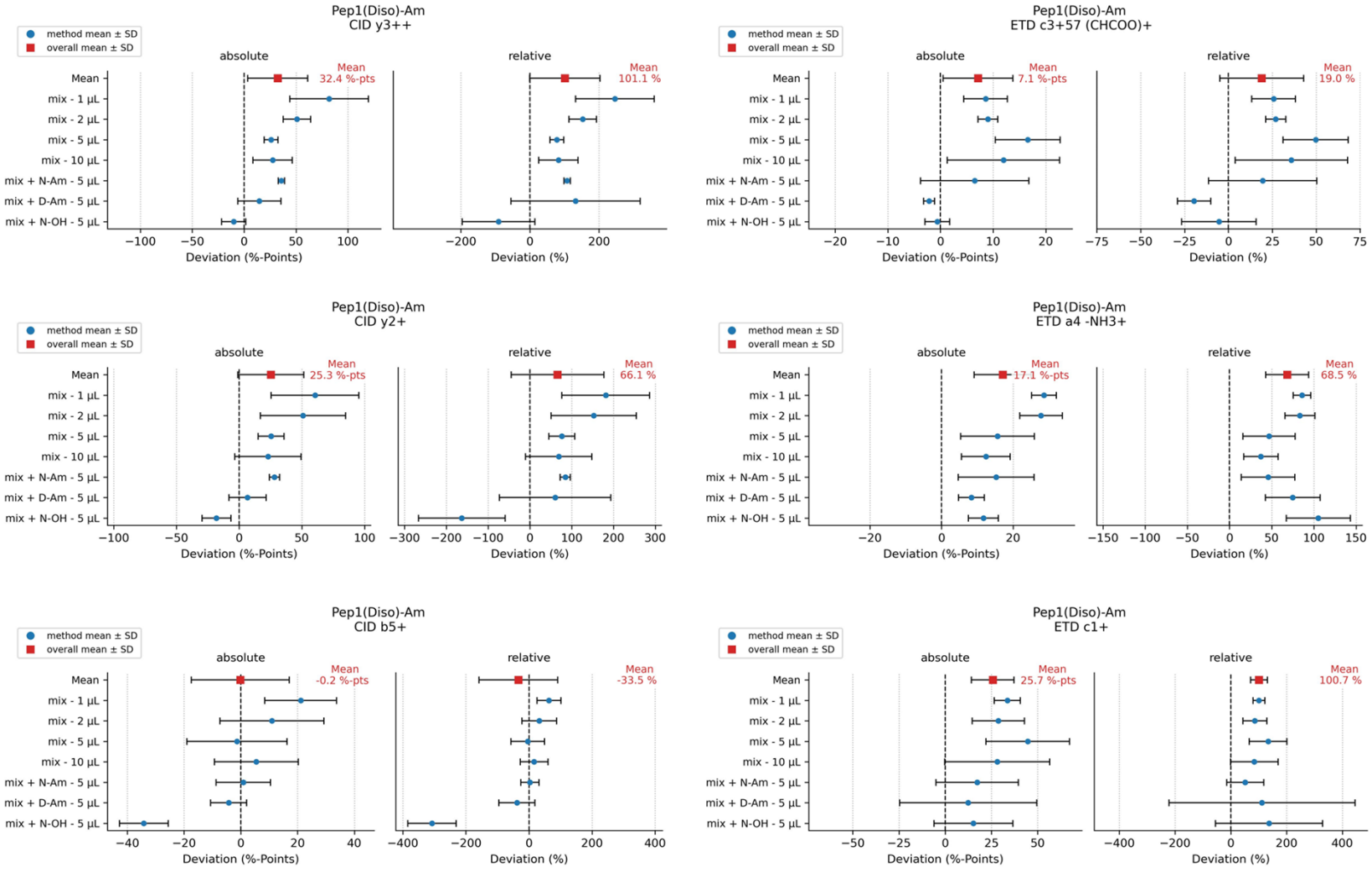


Figure S4-6: Absolute and relative accuracy of the content determination of Pep1(D_{iso})-Am in mixture analysis by peptide specific fragments ions.

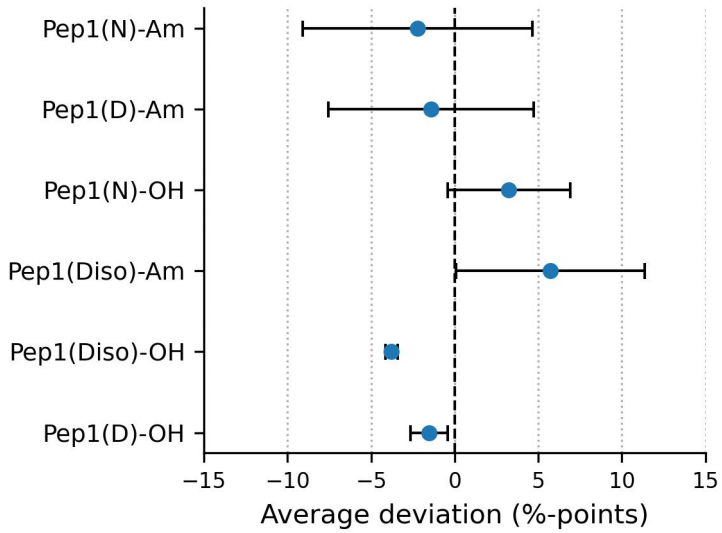


Figure S4-7: Average deviation of peptide content in %-points in mixture analyses (n = 7).

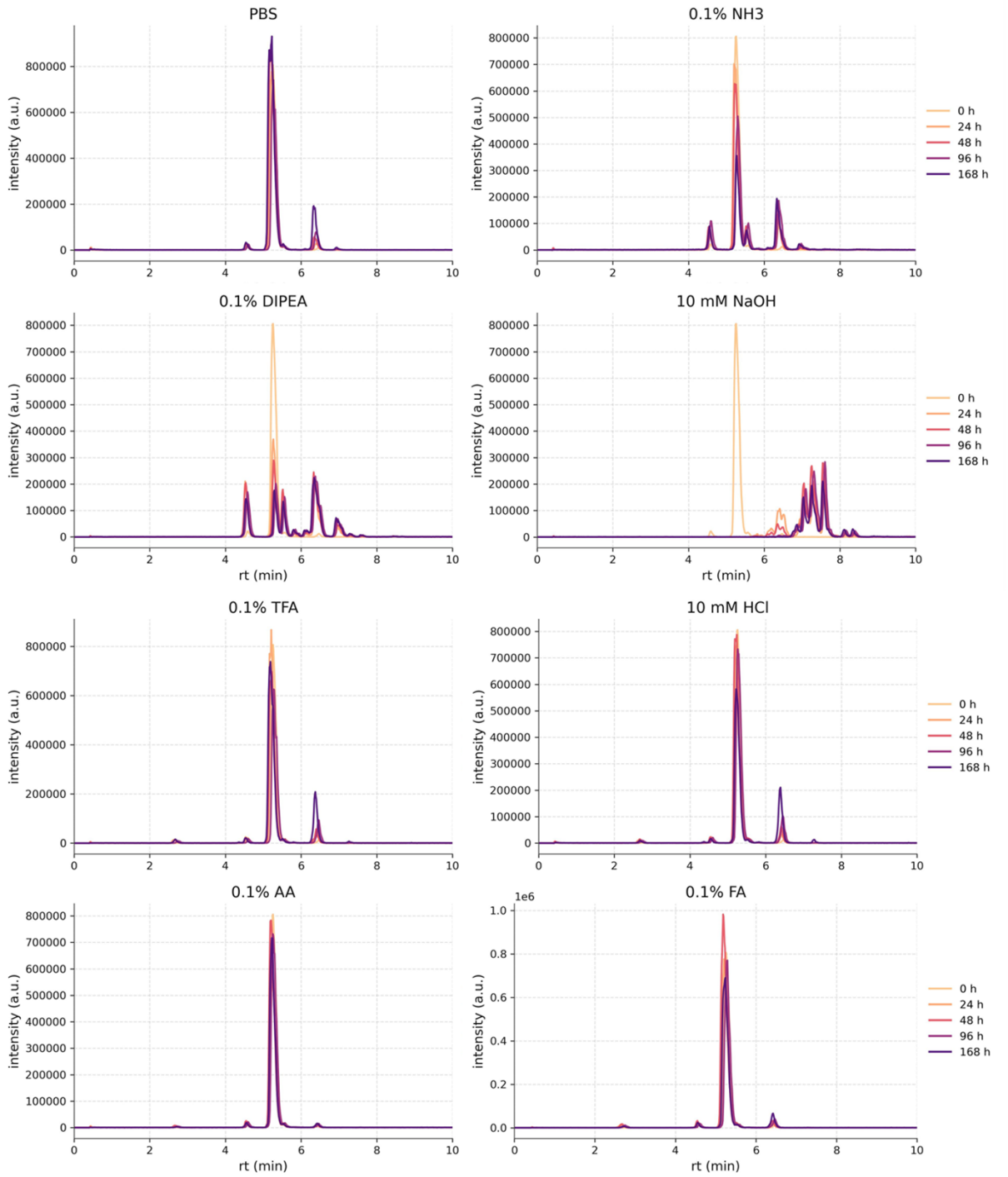


Figure S4-8: Obtained chromatograms from stability studies of Pep1(N)-Am in (top) PBS and under basic or (bottom) acidic conditions at 40 °C.

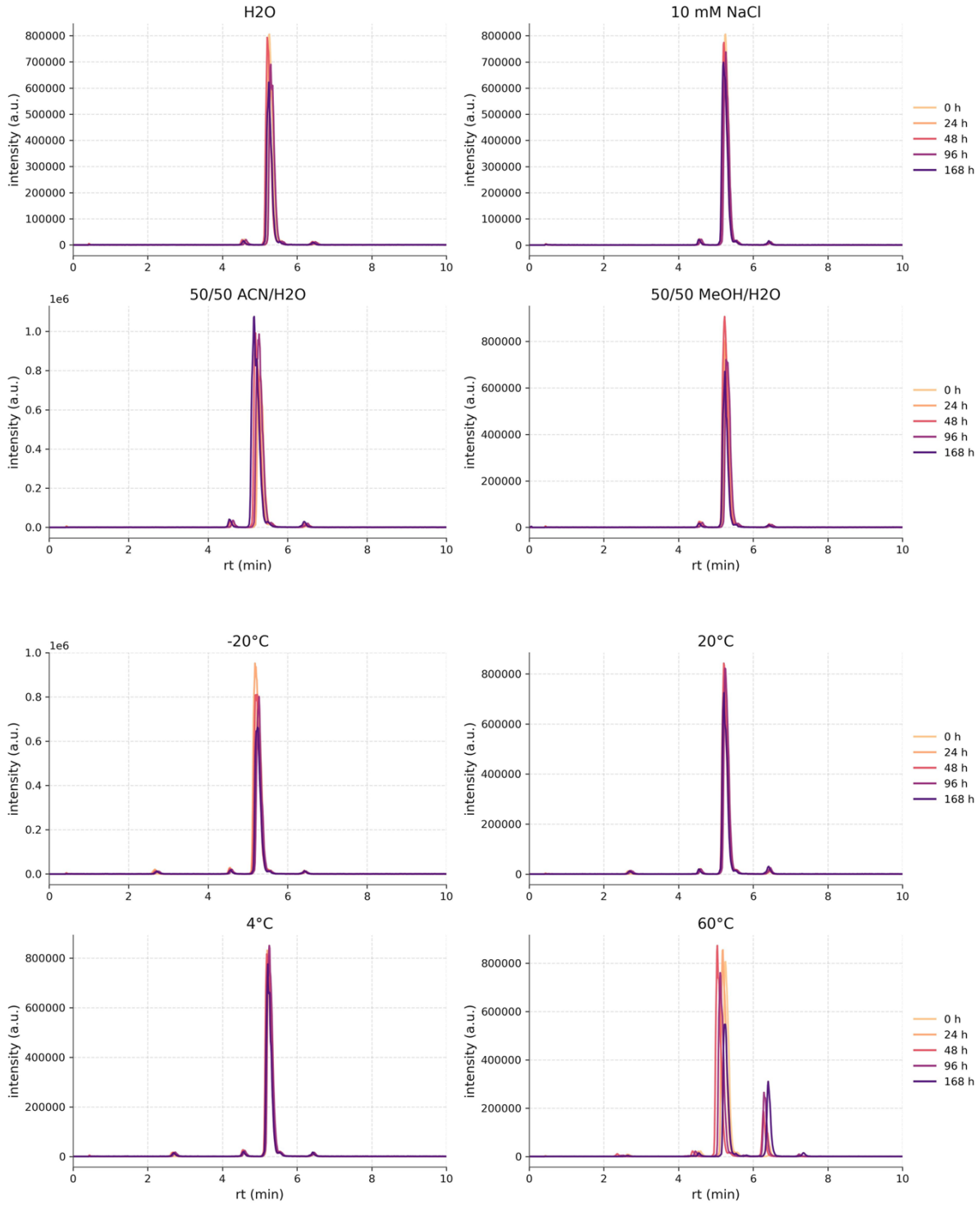


Figure S4-9: Obtained chromatograms from stability studies of Pep1(N)-Am in (top) neutral solution and solvent mixtures at 40 °C and (bottom) at different temperatures in 0.1% TFA.

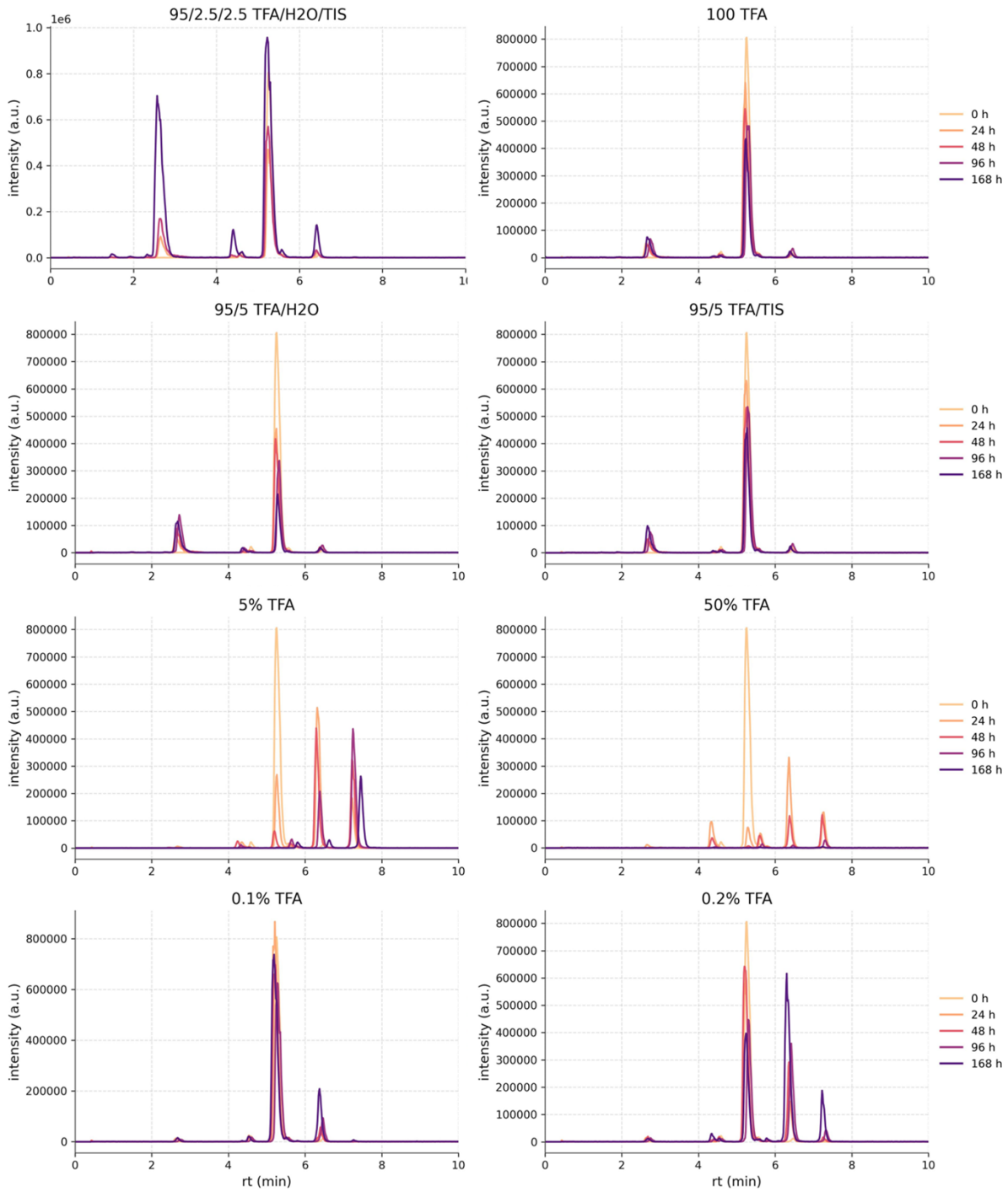


Figure S4-10: Obtained chromatograms from stability studies of Pep1(N)-Am in acidic TFA related solvents at 40 °C.

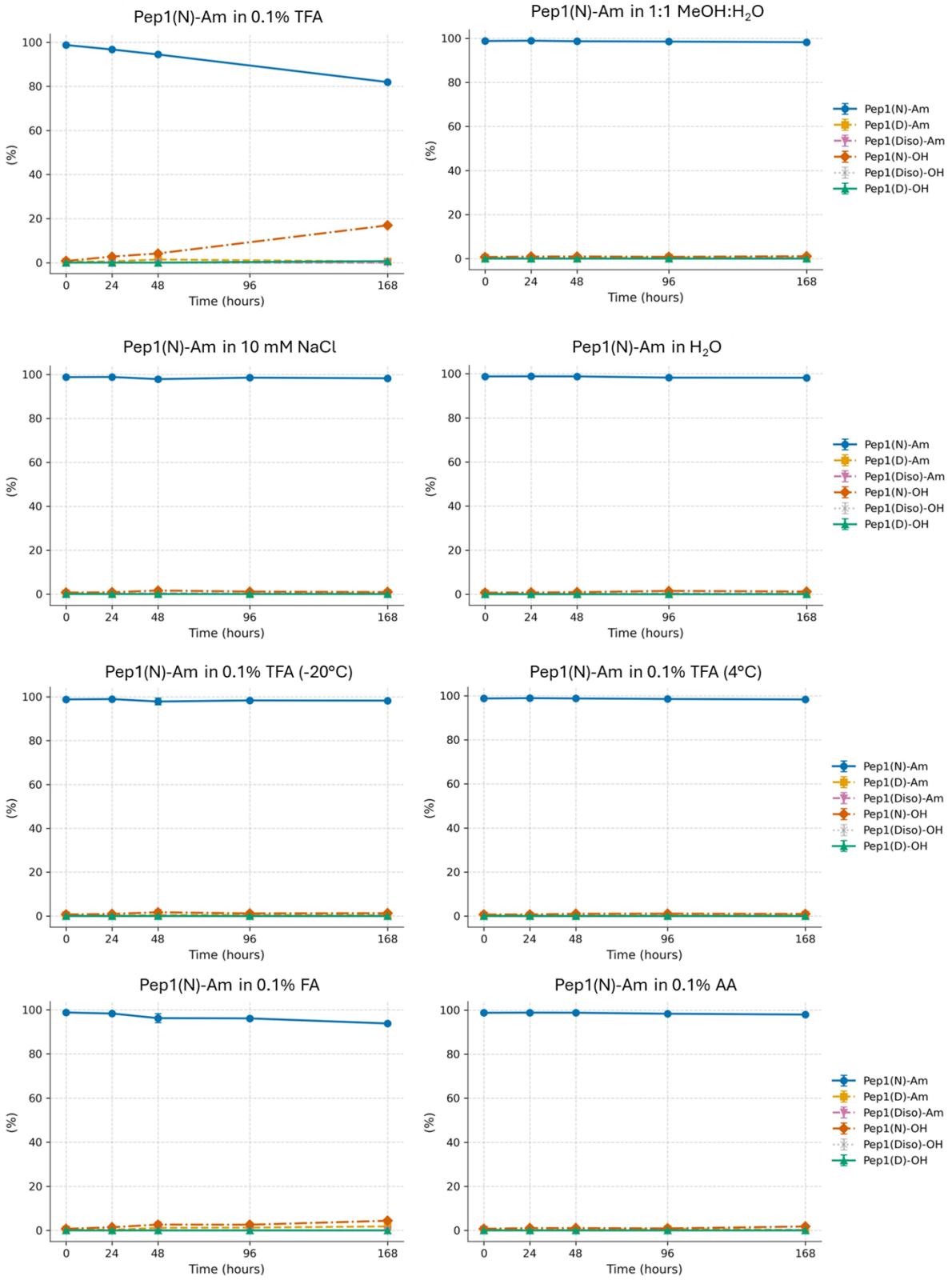


Figure S4-11: Relative content of deamidated species over time after incubation of Pep1(N)-Am in different solvents at 40°C if not stated otherwise.

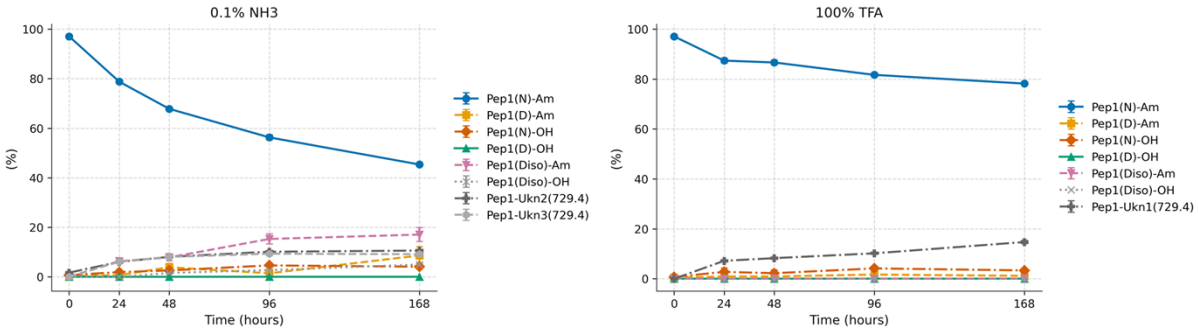


Figure S4-12: Relative content of deamidated and unknown species over time after incubation of Pep1(N)-Am in 0.1% NH_3 and 100% TFA at 40°C.

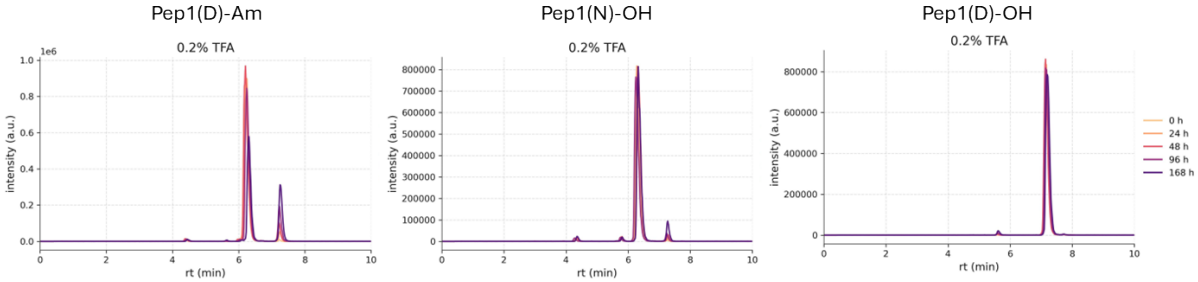


Figure S4-13: Obtained chromatograms from stability studies of Pep1(D)-Am, Pep1(N)-OH and Pep1(D)-OH in 0.2% TFA at 40 °C.

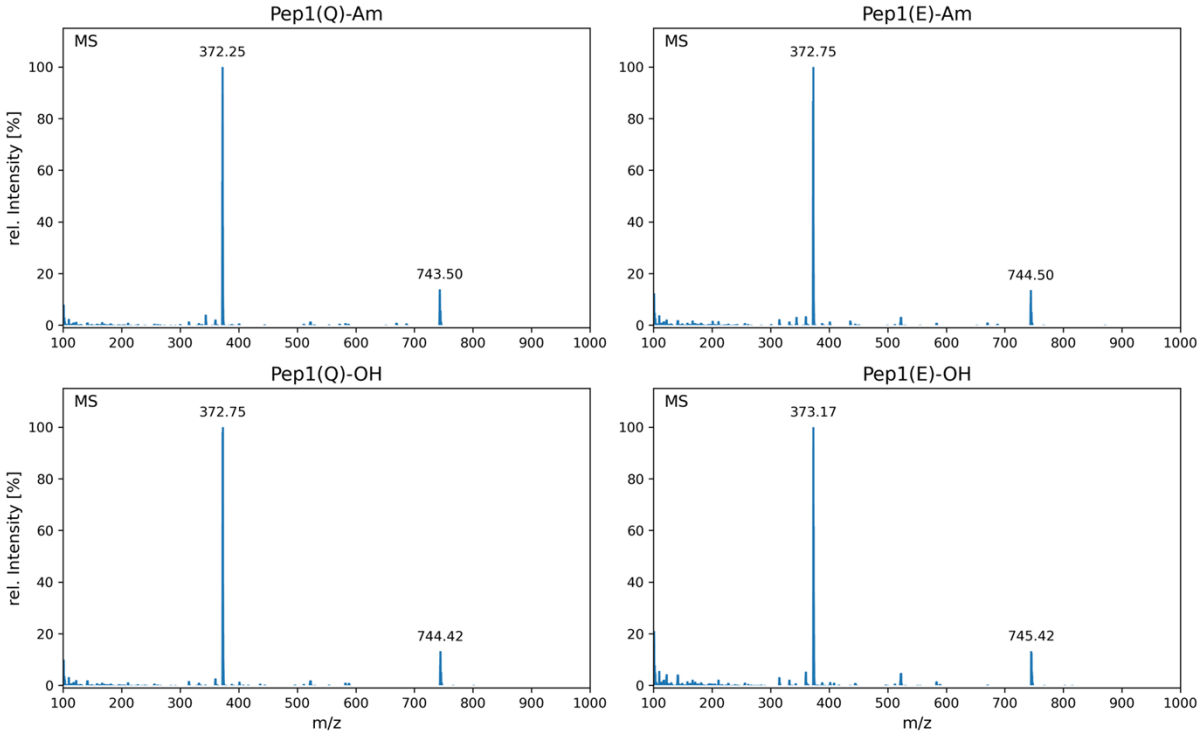


Figure S4-14: MS spectra of peaks in the Pep1(Q)-Am stability experiment after 168 hours.

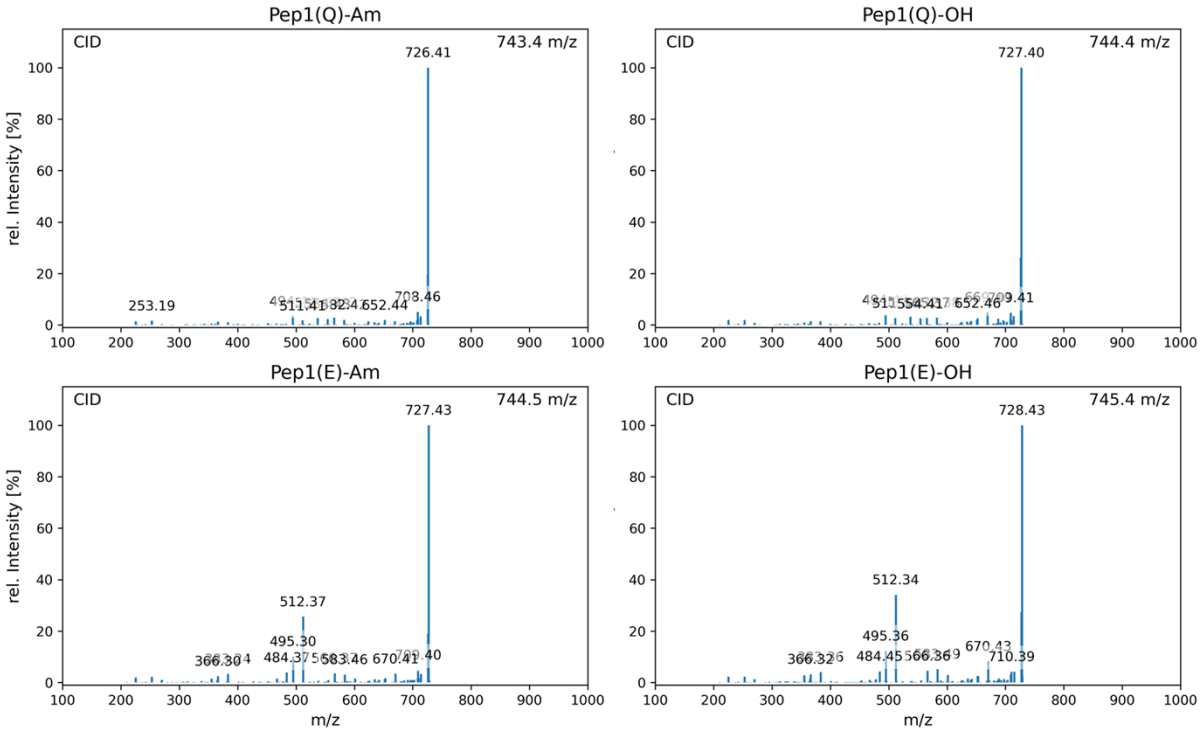


Figure S4-15: MS/MS spectra resulting from CID fragmentation of the [M+H]+ precursor of peaks in the Pep1(Q)-Am stability experiment after 168 hours.

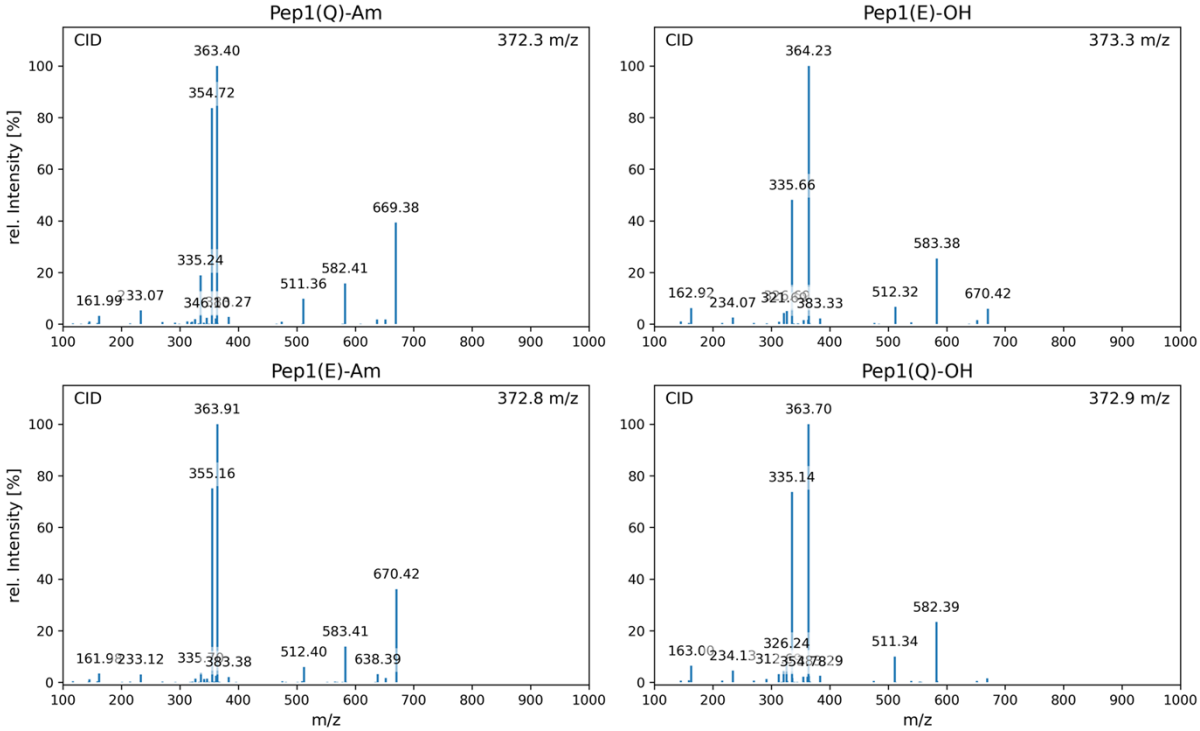


Figure S4-16: MS/MS spectra resulting from CID fragmentation of the [M+2H]+ precursor of peaks in the Pep1(Q)-Am stability experiment after 168 hours.

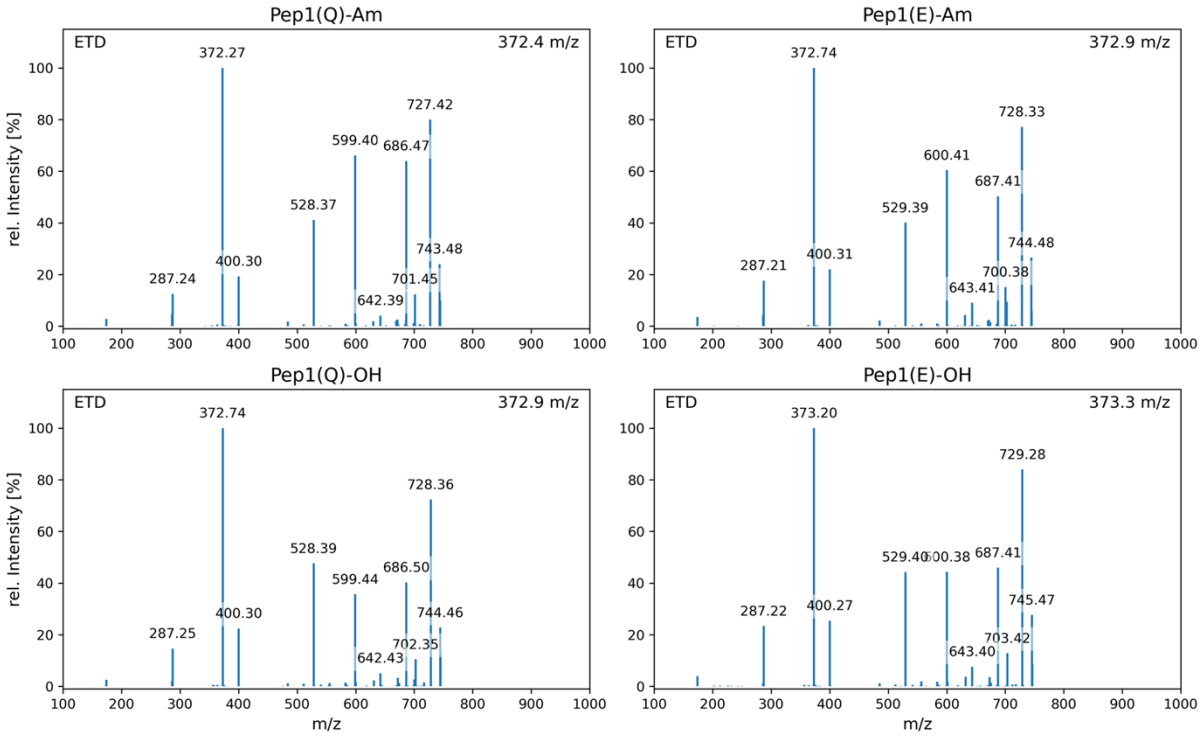


Figure S4-17: MS/MS spectra resulting from ETD fragmentation of the [M+2H]+ precursor of peaks in the Pep1(Q)-Am stability experiment after 168 hours.

Table S1: Overview of peptides with purity and calculated masses found (± 0.05 m/z) highlighted.

Name	Peptide			Purity (%)	Masses found in mass spectrometry			
	N-term	Sequence	C-term		$[M+H]^+$	$[M+2H]^{2+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$
Pep1(N)-Am	H_2N -	RLL**N**ASG	**- Am**	98.67	729.4371	365.2225	243.8176	183.1152
Pep1(N)-OH	H_2N -	RLL**N**ASG	- OH	94.35	730.4212	365.7145	244.1456	183.3612
Pep1(D)-Am	H_2N -	RLL*D*ASG	**- Am**	100.00	730.4212	365.7145	244.1456	183.3612
Pep1(D_{iso})-Am	H_2N -	RLLD_{iso}ASG	**- Am**	100.00	730.4212	365.7145	244.1456	183.3612
Pep1(D)-OH	H_2N -	RLL*D*ASG	- OH	83.62	731.4052	366.2065	244.4736	183.6072
Pep1(D_{iso})-OH	H_2N -	RLLD_{iso}ASG	- OH	98.28	731.4052	366.2065	244.4736	183.6072
Pep1(Q)-Am	H_2N -	RLL**Q**ASG	**- Am**	93.79	743.4528	372.2303	248.4895	186.6191

Table S2-1: Calculated fragments of Pep1(D)-Am and Pep1(D_{iso})-Am.

Pep1(D)-Am and Pep1(Diso)-Am								
Fragments								
Charge		a	b	c	x	y	z	
1	1	129.11	157.11	174.14	600.30	574.32	557.29	6
1	2	242.20	270.19	287.22	487.22	461.24	444.21	5
1	3	355.28	383.28	400.30	374.13	348.15	331.13	4
1	4	470.31	498.30	515.33	259.10	233.13	216.10	3
1	5	541.35	569.34	586.37	188.07	162.09	145.06	2
1	6	628.38	656.37	673.40	101.04	75.06	58.03	1
2	1	65.06	79.06	87.57	300.65	287.66	279.15	6
2	2	121.60	135.60	144.11	244.11	231.12	222.61	5
2	3	178.15	192.14	200.66	187.57	174.58	166.07	4
2	4	235.66	249.66	258.17	130.06	117.07	108.55	3
2	5	271.18	285.17	293.69	94.54	81.55	73.03	2
2	6	314.69	328.69	337.20	51.02	38.03	29.52	1
Fragments water loss (-H2O)								
Charge		a	b	c	x	y	z	
1	1	111.10	139.10	156.12	582.29	556.31	539.28	6
1	2	224.19	252.18	269.21	469.20	443.23	426.20	5
1	3	337.27	365.27	382.29	356.12	330.14	313.11	4
1	4	452.30	480.29	497.32	241.09	215.11	198.09	3
1	5	523.34	551.33	568.36	170.06	144.08	127.05	2
1	6	610.37	638.36	655.39	83.02	57.05	40.02	1
2	1	56.06	70.05	78.57	291.65	278.66	270.15	6
2	2	112.60	126.60	135.11	235.11	222.12	213.60	5
2	3	169.14	183.14	191.65	178.56	165.57	157.06	4
2	4	226.65	240.65	249.16	121.05	108.06	99.55	3
2	5	262.17	276.17	284.68	85.53	72.54	64.03	2
2	6	305.69	319.69	328.20	42.02	29.03	20.51	1
Fragments ammonia loss (-NH3)								
Charge		a	b	c	x	y	z	
1	1	113.10	141.09	158.12	584.28	558.30	541.27	6
1	2	226.18	254.17	271.20	471.20	445.22	428.19	5
1	3	339.26	367.26	384.28	358.11	332.13	315.11	4
1	4	454.29	482.29	499.31	243.09	217.11	200.08	3
1	5	525.33	553.32	570.35	172.05	146.07	129.04	2
1	6	612.36	640.35	657.38	85.02	59.04	42.01	1
2	1	57.05	71.05	79.56	292.64	279.65	271.14	6
2	2	113.59	127.59	136.10	236.10	223.11	214.60	5
2	3	170.14	184.13	192.65	179.56	166.57	158.06	4
2	4	227.65	241.65	250.16	122.05	109.06	100.54	3
2	5	263.17	277.17	285.68	86.53	73.54	65.03	2
2	6	306.68	320.68	329.19	43.01	30.02	21.51	1
Additional fragments Pep1(Diso)-Am								
Charge		a	b	c	x	y	z	
1	b3+18 (H2O)		401.2877			302.1464		y4-46 (COOH+H+)
1	c3+57 (CHCOO)			457.3013			274.1277	z4-57
2	b3+18 (H2O)		201.1478			151.5771		y4-46 (COOH+H+)
2	c3+57 (CHCOO)			229.1545			137.5678	z4-57

Table S2-2: Calculated fragments of Pep1(N)-OH.

Pep1(N)-OH								
Fragments								
Charge		a	b	c	x	y	z	
1	1	129.11	157.11	174.14	600.30	574.32	557.29	6
1	2	242.20	270.19	287.22	487.22	461.24	444.21	5
1	3	355.28	383.28	400.30	374.13	348.15	331.13	4
1	4	469.33	497.32	514.35	260.09	234.11	217.08	3
1	5	540.36	568.36	585.38	189.05	163.07	146.05	2
1	6	627.39	655.39	672.42	102.02	76.04	59.01	1
2	1	65.06	79.06	87.57	300.65	287.66	279.15	6
2	2	121.60	135.60	144.11	244.11	231.12	222.61	5
2	3	178.15	192.14	200.66	187.57	174.58	166.07	4
2	4	235.17	249.16	257.68	130.55	117.56	109.05	3
2	5	270.69	284.68	293.20	95.03	82.04	73.53	2
2	6	314.20	328.20	336.71	51.51	38.52	30.01	1
Fragments water loss (-H2O)								
Charge		a	b	c	x	y	z	
1	1	111.10	139.10	156.12	582.29	556.31	539.28	6
1	2	224.19	252.18	269.21	469.20	443.23	426.20	5
1	3	337.27	365.27	382.29	356.12	330.14	313.11	4
1	4	451.31	479.31	496.34	242.08	216.10	199.07	3
1	5	522.35	550.35	567.37	171.04	145.06	128.03	2
1	6	609.38	637.38	654.41	84.01	58.03	41.00	1
2	1	56.06	70.05	78.57	291.65	278.66	270.15	6
2	2	112.60	126.60	135.11	235.11	222.12	213.60	5
2	3	169.14	183.14	191.65	178.56	165.57	157.06	4
2	4	226.16	240.16	248.67	121.54	108.55	100.04	3
2	5	261.68	275.68	284.19	86.02	73.03	64.52	2
2	6	305.20	319.19	327.71	42.51	29.52	21.01	1
Fragments ammonia loss (-NH3)								
Charge		a	b	c	x	y	z	
1	1	113.10	141.09	158.12	584.28	558.30	541.27	6
1	2	226.18	254.17	271.20	471.20	445.22	428.19	5
1	3	339.26	367.26	384.28	358.11	332.13	315.11	4
1	4	453.31	481.30	498.33	244.07	218.09	201.06	3
1	5	524.34	552.34	569.36	173.03	147.05	130.03	2
1	6	611.38	639.37	656.40	86.00	60.02	42.99	1
2	1	57.05	71.05	79.56	292.64	279.65	271.14	6
2	2	113.59	127.59	136.10	236.10	223.11	214.60	5
2	3	170.14	184.13	192.65	179.56	166.57	158.06	4
2	4	227.16	241.15	249.67	122.54	109.55	101.04	3
2	5	262.68	276.67	285.19	87.02	74.03	65.52	2
2	6	306.19	320.19	328.70	43.50	30.51	22.00	1

Table S2-3: Comparison between data dependent and targeted MS/MS data acquisition for the content determination of Pep1(D_{iso})-Am in Pep1(D_{iso})-Am/Pep1(D)-Am mixtures for detected fragments significantly differing between these two peptides.

± m/z window for signal detection	Mean standard deviation detected signal (%)		R^2 for linear correlations	
	Data dependent	Targeted	Data dependent	Targeted
0.1	0.459	0.192	0.405 ± 0.305	0.565 ± 0.296
0.15	0.470	0.175	0.470 ± 0.326	0.583 ± 0.298
0.2	0.270	0.170	0.528 ± 0.320	0.591 ± 0.298
0.25	0.254	0.169	0.570 ± 0.312	0.592 ± 0.300
0.3	0.259	0.168	0.578 ± 0.309	0.593 ± 0.299

Table S2-4: Overview of MS/MS fragments screened for correlation of Pep1(N)-OH and Pep1(D_{iso})-Am content in mixtures with Pep1(D)-Am for their semiquantitative detection.

Type of fragmentation	Peptide	Fragment	Mass	Range (%)	Linear model				Quadratic model			
					Intercept	Slope	R	R^2	Quad a	Quad b	Quad c	R^2 Quad
CID	Pep1(N)-OH	y3+	234.109	0-100	3.17E-04	3.19E-05	0.93	0.86	-2.29E-07	5.49E-05	3.08E-05	0.90
CID	Pep1(N)-OH	b4+	497.3200	0-100	1.12E-03	1.23E-04	0.90	0.82	-8.14E-07	2.04E-04	1.04E-04	0.85
CID	Pep1(N)-OH	b6 -H2O+	637.3786	0-100	2.96E-04	4.17E-05	0.96	0.91	-2.17E-07	6.34E-05	2.43E-05	0.93
ETD	Pep1(N)-OH	z6 -NH3+	541.2748	0-100	1.11E-04	2.72E-05	0.94	0.88	-6.20E-08	3.34E-05	3.30E-05	0.88
ETD	Pep1(N)-OH	c5+	585.3837	0-100	9.55E-04	1.34E-03	0.94	0.88	-5.11E-06	1.85E-03	-5.44E-03	0.89
ETD	Pep1(N)-OH	c6+	672.4157	0-100	2.60E-03	2.38E-03	0.92	0.84	-1.31E-05	3.68E-03	-1.38E-02	0.87
CID	Pep1(D_{iso})-Am	y3++	117.0664	0-100	3.20E-04	1.22E-05	0.93	0.86	-7.14E-09	1.29E-05	3.12E-04	0.86
CID	Pep1(D_{iso})-Am	y3++	117.0664	0-10	3.15E-04	1.29E-05	0.52	0.27	-4.20E-07	1.70E-05	3.12E-04	0.27
CID	Pep1(D_{iso})-Am	y2+	162.0879	0-100	6.53E-03	1.82E-04	0.92	0.85	-6.43E-07	2.46E-04	5.81E-03	0.86
CID	Pep1(D_{iso})-Am	y2+	162.0879	0-10	5.71E-03	2.24E-04	0.55	0.31	2.58E-06	1.99E-04	5.73E-03	0.31
CID	Pep1(D_{iso})-Am	b5+	569.3411	0-100	4.29E-02	1.08E-03	0.90	0.81	-3.78E-06	1.45E-03	3.86E-02	0.82
CID	Pep1(D_{iso})-Am	b5+	569.3411	0-10	3.45E-02	1.94E-03	0.58	0.34	2.25E-04	-2.57E-04	3.61E-02	0.37
ETD	Pep1(D_{iso})-Am	c1+	174.1355	0-100	1.10E-02	1.74E-04	0.91	0.82	1.84E-07	1.56E-04	1.12E-02	0.82
ETD	Pep1(D_{iso})-Am	c1+	174.1355	0-10	1.11E-02	2.74E-05	0.05	0.00	-1.06E-05	1.30E-04	1.10E-02	0.00
ETD	Pep1(D_{iso})-Am	a4 -NH3+	454.2904	0-100	9.44E-05	1.32E-05	0.96	0.93	-3.71E-08	1.69E-05	5.27E-05	0.93
ETD	Pep1(D_{iso})-Am	a4 -NH3+	454.2904	0-10	1.42E-04	4.23E-06	0.30	0.09	-7.40E-07	1.14E-05	1.36E-04	0.11
ETD	Pep1(D_{iso})-Am	c3+57 +	457.3013	0-100	6.77E-04	5.34E-04	0.97	0.94	-8.27E-07	6.15E-04	-2.54E-04	0.94
ETD	Pep1(D_{iso})-Am	c3+57 +	457.3013	0-10	3.92E-05	5.96E-04	0.98	0.96	1.25E-05	4.74E-04	1.30E-04	0.96

Table S2-5: Calculated fragments of Pep1(Q)-Am, Pep1(E)-Am, Pep1(Q)-OH and Pep1(E)-OH.

Pep1(Q)-Am								
Charge		**a**	**b**	**c**	**x**	**y**	**z**	
1	1	129.114	157.1089	174.1355	613.331	587.3517	570.3251	6
1	2	242.1981	270.193	287.2195	500.2469	474.2676	457.2411	5
1	3	355.2821	383.2771	400.3036	387.1628	361.1836	344.157	4
1	4	483.3407	511.3356	528.3622	259.1042	233.125	216.0984	3
1	5	554.3778	582.3728	599.3993	188.0671	162.0879	145.0613	2
1	6	641.4099	669.4048	686.4313	101.0351	75.05584	58.02929	1
2	1	65.06092	79.05838	87.57166	307.1694	294.1798	285.6665	6
2	2	121.603	135.6004	144.1137	250.6274	237.6377	229.1244	5
2	3	178.145	192.1424	200.6557	194.0853	181.0957	172.5824	4
2	4	242.1743	256.1717	264.685	130.056	117.0664	108.5531	3
2	5	277.6928	291.6903	300.2036	94.53748	81.54785	73.03457	2
2	6	321.2088	335.2063	343.7196	51.02146	38.03183	29.51856	1
Pep1(E)-Am								
1	1	129.114	157.1089	174.1355	614.315	588.3357	571.3092	6
1	2	242.1981	270.193	287.2195	501.2309	475.2516	458.2251	5
1	3	355.2821	383.2771	400.3036	388.1468	362.1676	345.141	4
1	4	484.3247	512.3197	529.3462	259.1042	233.125	216.0984	3
1	5	555.3619	583.3568	600.3833	188.0671	162.0879	145.0613	2
1	6	642.3939	670.3888	687.4154	101.0351	75.05584	58.02929	1
2	1	65.06092	79.05838	87.57166	307.6614	294.6718	286.1585	6
2	2	121.603	135.6004	144.1137	251.1194	238.1297	229.6165	5
2	3	178.145	192.1424	200.6557	194.5773	181.5877	173.0744	4
2	4	242.6663	256.6637	265.177	130.056	117.0664	108.5531	3
2	5	278.1848	292.1823	300.6956	94.53748	81.54785	73.03457	2
2	6	321.7009	335.6983	344.2116	51.02146	38.03183	29.51856	1
Pep1(Q)-OH								
1	1	129.114	157.1089	174.1355	614.315	588.3357	571.3092	6
1	2	242.1981	270.193	287.2195	501.2309	475.2516	458.2251	5
1	3	355.2821	383.2771	400.3036	388.1468	362.1676	345.141	4
1	4	483.3407	511.3356	528.3622	260.0883	234.109	217.0824	3
1	5	554.3778	582.3728	599.3993	189.0511	163.0719	146.0453	2
1	6	641.4099	669.4048	686.4313	102.0191	76.03985	59.0133	1
2	1	65.06092	79.05838	87.57166	307.6614	294.6718	286.1585	6
2	2	121.603	135.6004	144.1137	251.1194	238.1297	229.6165	5
2	3	178.145	192.1424	200.6557	194.5773	181.5877	173.0744	4
2	4	242.1743	256.1717	264.685	130.548	117.5584	109.0451	3
2	5	277.6928	291.6903	300.2036	95.02949	82.03985	73.52658	2
2	6	321.2088	335.2063	343.7196	51.51347	38.52384	30.01056	1
Pep1(E)-OH								
1	1	129.114	157.1089	174.1355	615.299	589.3197	572.2932	6
1	2	242.1981	270.193	287.2195	502.2149	476.2357	459.2091	5
1	3	355.2821	383.2771	400.3036	389.1309	363.1516	346.125	4
1	4	484.3247	512.3197	529.3462	260.0883	234.109	217.0824	3
1	5	555.3619	583.3568	600.3833	189.0511	163.0719	146.0453	2
1	6	642.3939	670.3888	687.4154	102.0191	76.03985	59.0133	1
2	1	65.06092	79.05838	87.57166	308.1534	295.1638	286.6505	6
2	2	121.603	135.6004	144.1137	251.6114	238.6217	230.1085	5
2	3	178.145	192.1424	200.6557	195.0693	182.0797	173.5664	4
2	4	242.6663	256.6637	265.177	130.548	117.5584	109.0451	3
2	5	278.1848	292.1823	300.6956	95.02949	82.03985	73.52658	2
2	6	321.7009	335.6983	344.2116	51.51347	38.52384	30.01056	1

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