

Supplementary files

S-1: Optimized method for Intact Mass Analysis

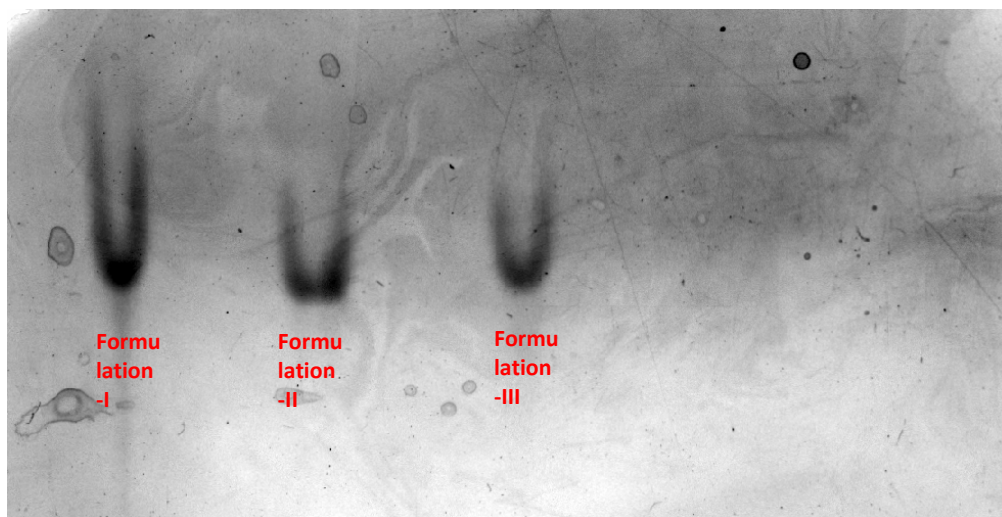
Source Parameters	VCap	3800
	Ion Source Type	H-ESI
	Sheath Gas	40
	Aux Gas	10
	Sweep Gas	2
	Ion Transfer Tube Temp	320°C
	Vaporizer Temp	310°C
	Default Charge State	6
Method Parameters	Diverter Setting	0-1.5- Waste; 1.5-5.8-MS; 5.8-6.0-Waste
	Acquisition Range	500-4000m/z
	Orbitrap Resolution	120000
LC-Parameters	AGC Target	4X10 ⁵
	Column	BioZen XB C18 (150 X 4.6mm, 2.6µm)
	Mobile Phase	(A) Water: 0.1%(%v/v) Formic acid, (B) ACN: 0.1%(%v/v) Formic acid
	Flow Rate	400µl/min
	Injection Volume	5µL
LC-Parameters	Gradient Program	Tmin/A:B- T _{0.2} /70:30; T _{1.5} /50:50; T ₃ /10:90; T ₄ /10:90; T _{4.7} /70:30; T ₆ /70:30

S-2: Optimized Parameters for Peptide Mapping

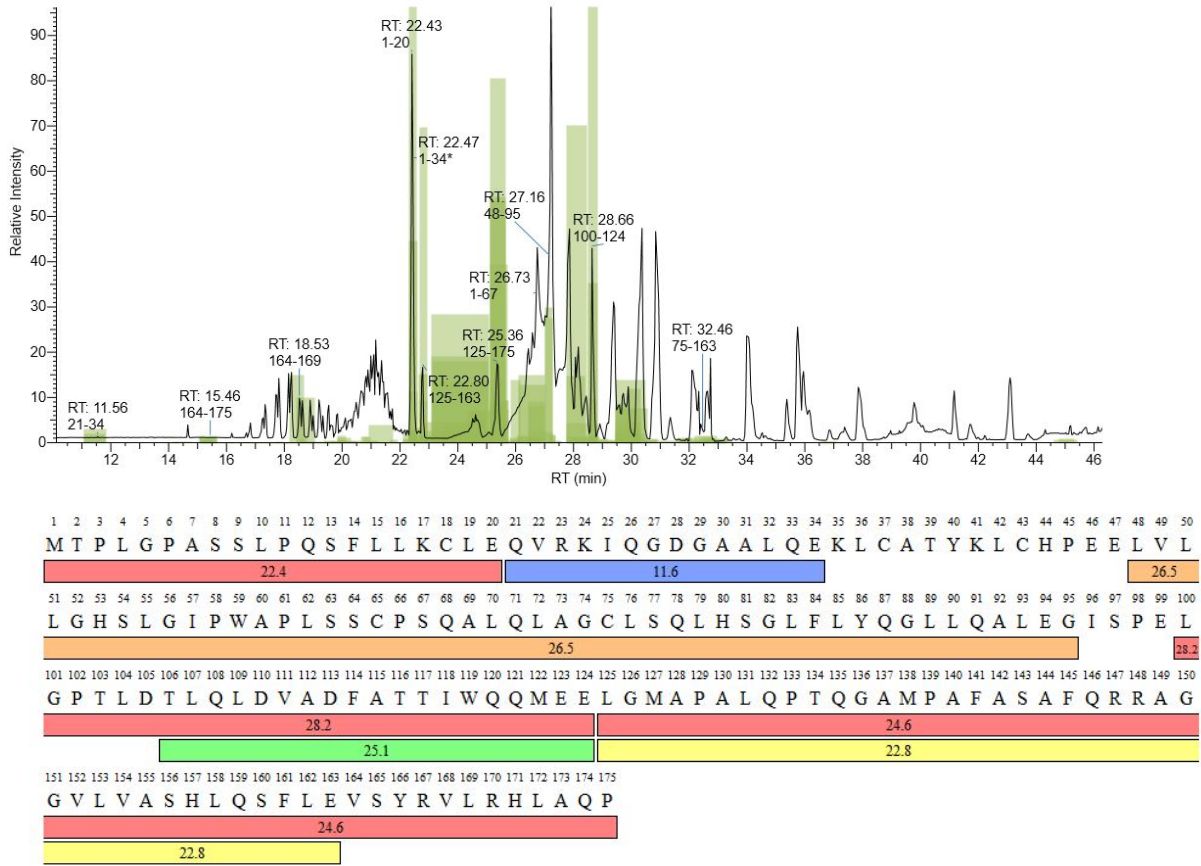
Source Parameters	VCap	3000
	Ion Source Type	H-ESI
	Sheath Gas	50
	Aux Gas	12
	Sweep Gas	1
	Ion Transfer Tube Temp	310°C
	Vaporizer Temp	340°C
	Default Charge State	2
Method Parameters	Diverter Setting	0-4: Waste; 4-62: MS; 62-65: Waste
	Acquisition Range	200-3000m/z
	Orbitrap Resolution	120000
	AGC Target	4X10 ⁵
DDA Parameters	Micro-Scans	5
	Charge state	2-8
	Isolation	Ion Trap (isolation width 2m/z)
	CID Assisted Collision Energies (%)	20,25,30
	MS/MS range	50-1500
LC-Parameters	Orbitrap Resolution	6000
	Column	BioZen XB C18 (150 X 4.6mm, 2.6µm)
	Mobile Phase	(A) Water: 0.1%(%v/v) Formic acid, (B) ACN: 0.1%(%v/v) Formic acid
	Flow Rate	400µl/min
	Injection Volume	5µL
LC-Parameters	Gradient Program	Tmin/A:B- T ₀ /95:5; T _{4.0} /95:5; T ₁₀ /90:10; T ₅₀ /5:95; T ₅₅ /5:95; T ₆₀ /95:5; T ₆₅ /95:5

S-3: Instrumental Parameters employed for HDX-MS setup

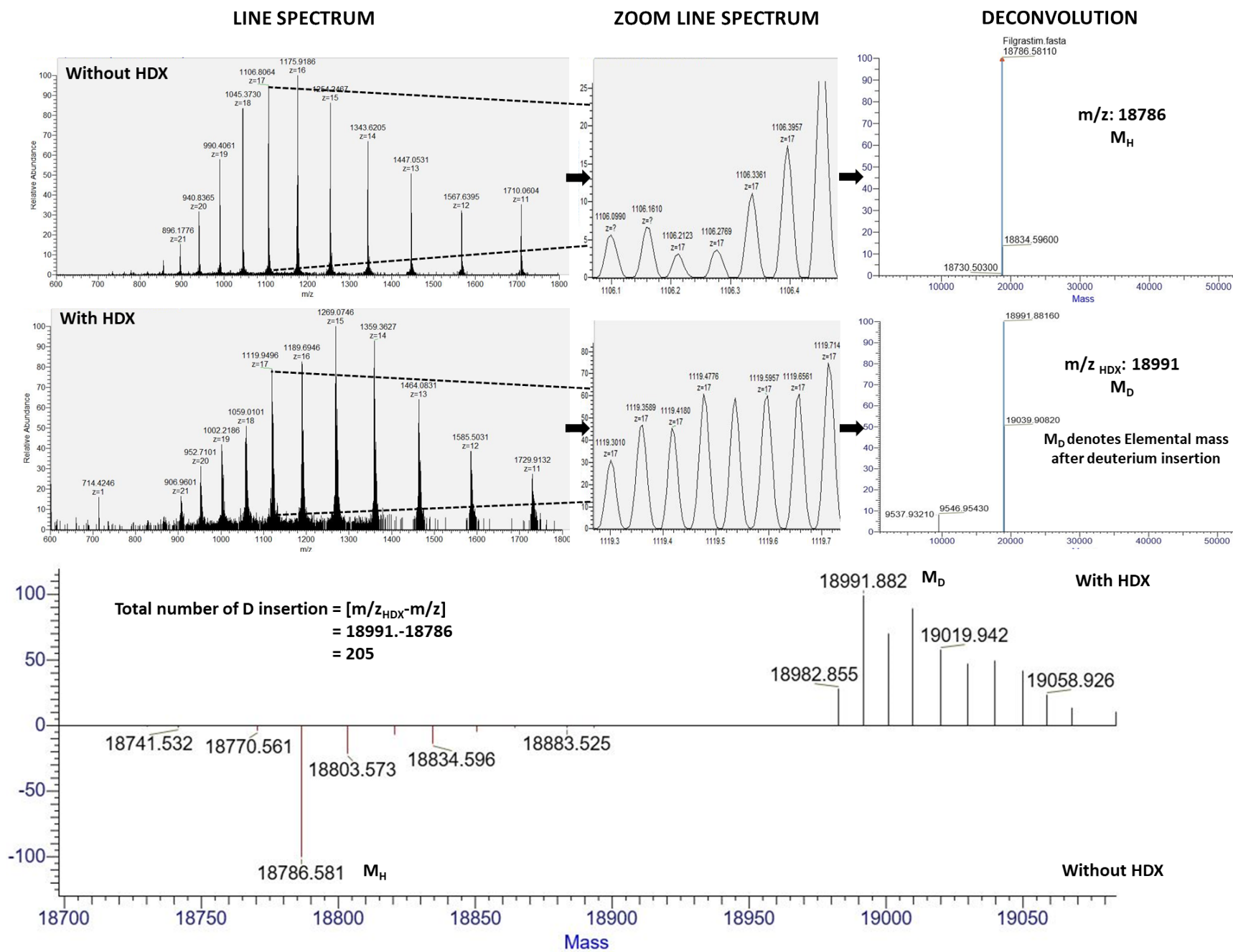
Source Parameters	VCap	3800
	Ion Source Type	H-ESI
	Sheath Gas	40
	Aux Gas	10
	Sweep Gas	2
	Ion Transfer Tube Temp	320°C
	Vaporizer Temp	310°C
	Default Charge State	6
	Diverter Setting	0-1.5- Waste; 1.5-5.8-MS; 5.8-6.0-Waste
Method Parameters	Acquisition Range	500-4000m/z
	Orbitrap Resolution	120000
	AGC Target	4X10 ⁵
LC-Parameters	Column	BioZen XB C18 (150 X 4.6mm, 2.6µm)
	Mobile Phase	(A) D ₂ O: 0.1%(%v/v) Formic acid, (B) ACN: 0.1%(%v/v) Formic acid
	Flow Rate	400µl/min
	Gradient Program	T _{min} /A:B- T _{0,2} /70:30; T _{1,5} /50:50; T ₃ /10:90; T ₄ /10:90; T _{4,7} /70:30; T ₆ /70:30
	Injection Volume	5µL



S-4: Native PAGE for three formulations



S-5: Base Peak Chromatogram (BPC) showing elution pattern of peptides from digested Formulation along with Peptide Mapping showing coverage



S-6: Representative Line spectrum (a) Deconvolution of both samples with and without HDX and (b) Mirror plot of the obtained deconvoluted spectrum.

S-8 Continue....

9	III-9	87.5	15.3	5.94	8.0	4.09	4.00	204	209	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
10	III-10	86.3	14.5	5.92	8.0	4.07	3.95	203	194	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
11	III-11	84.9	14.6	5.51	8.0	4.06	3.88	203	204	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
12	III-12	84.1	15.0	7.41	8.0	4.05	3.97	206	193	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
13	III-13	84.3	14.6	4.75	8.0	4.08	3.92	208	195	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
14	III-14	83.9	13.9	2.46	8.0	4.12	3.91	207	197	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
15	III-15	84.8	12.9	6.97	8.0	4.10	4.00	206	202	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
16	III-16	85.3	14.2	5.36	8.0	4.07	3.92	203	204	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
17	III-17	84.5	15.7	7.64	8.0	4.07	3.87	207	199	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
18	III-18	86.1	17.2	5.48	8.0	4.08	3.92	202	197	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
19	III-19	84.3	13.8	6.26	8.0	4.08	3.89	209	193	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
20	III-20	84.8	15.6	7.28	8.0	4.07	3.91	205	194	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
21	III-21	85.9	14.0	6.31	8.0	4.09	3.91	205	199	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
22	III-22	85.6	14.7	6.61	8.0	4.10	3.89	204	198	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
23	III-23	84.5	14.2	6.00	8.0	4.10	3.87	210	195	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
24	III-24	85.1	13.7	5.96	8.0	4.10	3.93	201	194	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
25	III-25	85.5	15.5	4.69	8.0	4.09	3.88	203	199	83	8	0.71	0.23	0	1	0	0	1	1	1	1	1	1	1	0
26	I-26	84.4	14.5	6.79	8.0	4.10	3.86	207	196	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
27	I-27	84.5	15.0	6.85	8.0	4.14	3.74	209	201	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
28	I-28	85.1	15.1	6.83	8.0	4.06	3.84	209	193	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
29	I-29	84.9	15.6	6.37	8.0	4.08	3.95	207	202	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
30	I-30	84.8	15.2	6.38	8.0	4.12	3.83	209	203	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
31	I-31	84.8	15.1	6.47	8.0	4.06	3.86	208	196	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
32	I-32	84.9	15.0	6.54	8.0	4.15	3.89	208	197	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
33	I-33	85.1	15.3	6.35	8.0	4.13	3.92	209	196	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
34	I-34	84.3	15.1	6.36	8.0	4.10	3.86	206	199	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
35	I-35	84.9	14.7	6.84	8.0	4.09	3.81	207	202	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
36	I-36	84.8	15.4	6.95	8.0	4.11	3.89	210	201	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
37	I-37	85.5	15.0	6.33	8.0	4.22	3.97	211	197	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
38	I-38	84.3	15.3	6.88	8.0	4.14	3.93	208	201	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
39	I-39	85.3	15.9	6.79	8.0	4.16	3.87	208	202	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
40	I-40	84.6	15.1	6.81	8.0	4.10	3.90	208	205	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
41	I-41	85.5	15.3	6.31	8.0	4.07	3.87	207	200	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
42	I-42	84.6	14.8	6.48	8.0	4.15	3.80	208	204	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
43	I-43	85.1	15.0	6.38	8.0	4.22	3.85	207	203	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
44	I-44	84.5	15.0	6.77	8.0	4.12	3.89	206	206	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
45	I-45	84.5	14.9	6.51	8.0	4.12	3.85	208	204	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
46	I-46	85.8	15.6	6.53	8.0	4.12	4.00	206	197	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
47	I-47	84.3	14.5	6.81	8.0	4.10	3.96	210	199	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
48	I-48	85.2	15.6	6.62	8.0	4.12	3.86	208	199	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
49	I-49	85.1	15.3	6.69	8.0	4.09	3.94	205	199	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
50	I-50	84.8	15.2	6.57	8.0	4.11	3.93	208	204	83	8	1.49	0.52	0	0	0	0	1	1	1	1	1	1	1	1
51	II-51	82.0	19.4	12.66	3.0	4.03	3.98	203	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
52	II-52	82.1	17.5	15.57	3.0	4.04	3.98	210	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0

53	II-53	81.0	18.5	14.55	3.0	4.04	3.98	206	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
54	II-54	82.4	18.0	12.81	3.0	4.05	3.98	207	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
55	II-55	80.8	17.3	13.32	3.0	4.03	3.99	210	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
56	II-56	81.0	18.4	12.63	3.0	4.04	3.98	209	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
57	II-57	81.8	17.8	15.23	3.0	4.04	3.99	206	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
58	II-58	81.6	17.7	15.30	3.0	4.03	3.98	208	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
59	II-59	82.4	17.9	14.91	3.0	4.04	3.98	207	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
60	II-60	81.5	18.1	15.20	3.0	4.04	3.98	212	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
61	II-61	80.5	19.2	13.16	3.0	4.04	3.97	204	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
62	II-62	81.4	18.6	14.45	3.0	4.04	3.98	208	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
63	II-63	82.9	18.7	13.97	3.0	4.05	3.98	206	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
64	II-64	81.7	17.5	16.00	3.0	4.03	3.99	212	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
65	II-65	82.4	18.5	14.89	3.0	4.04	3.99	211	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
66	II-66	82.2	18.6	15.91	3.0	4.04	3.98	209	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
67	II-67	81.2	18.4	14.84	3.0	4.03	3.98	210	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
68	II-68	82.0	19.4	13.67	3.0	4.04	3.98	208	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
69	II-69	82.1	16.8	16.19	3.0	4.04	3.98	209	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
70	II-70	82.3	18.9	13.14	3.0	4.05	3.99	207	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
71	II-71	82.9	19.4	13.69	3.0	4.05	3.99	208	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
72	II-72	81.9	19.1	13.81	3.0	4.03	4.00	208	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
73	II-73	80.4	18.6	13.21	3.0	4.05	3.98	211	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
74	II-74	82.0	16.7	15.31	3.0	4.03	3.98	209	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0
75	II-75	82.3	18.1	15.50	3.0	4.05	3.98	212	206	90	7	1.36	3.77	0	0	1	0	1	1	1	1	1	1	0	0

Note: * Have been excluded from PCA analysis as no discrimination was there between the formulations.

Description for the input:

A: Unique ID are created for easy identification where first letter stands for formulation followed by numerical number to separate 25 replicates of each formulation;

B: Relative intensity of major isoform-I based on Intact Mass Analysis;

C: Relative intensity of major isoform-II based on Intact Mass Analysis;

D: Relative ratio of oxidized protein in formulation

E: Number of charged species from Iso-Electric Focusing;

F: Log of relative H-D exchange contribution for major isoform-I from data of HDX-MS;

G: Log of relative H-D exchange contribution for major isoform-II from data of HDX-MS;

H: Difference in deconvoluted molecular mass of major isoform-I post H-D Exchange;

I: Difference in deconvoluted molecular mass of major isoform-II post H-D Exchange;

J: Sequence Coverage Score from peptide mapping;

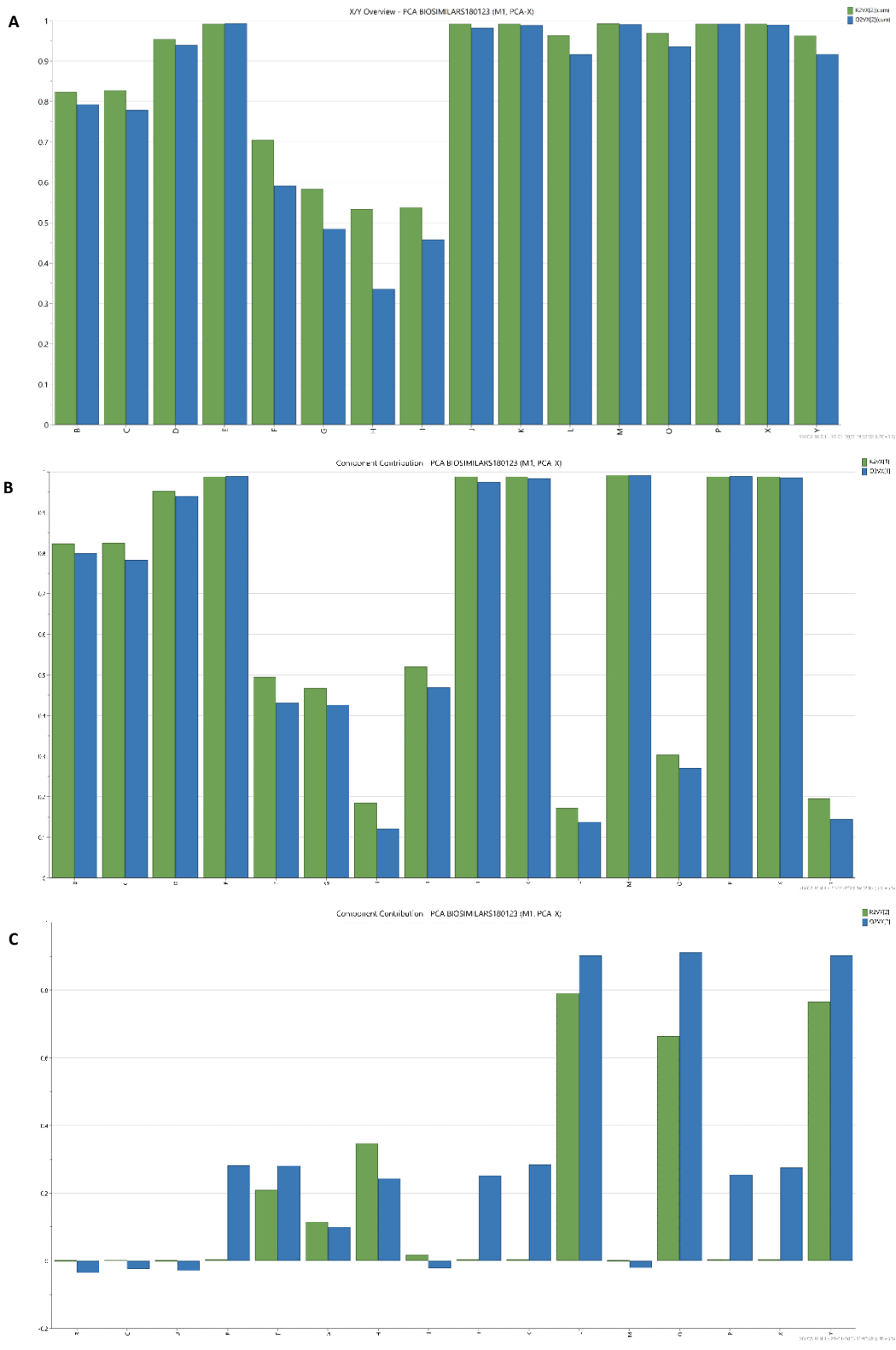
K: No. of Peptides detected in the formulation from peptide mapping data;

L: PTM based ratio of oxidized peptide vs un-oxidized peptide for M122 from data of peptide mapping analysis;

M: PTM based ratio of oxidized peptide vs un-oxidized peptide for M138 from data of peptide mapping analysis;

N-Y: Presence/Absence of peptides from peptide mapping data.

S-9:X/Y Contribution Plots Displaying individual parameters contribution in PCA analysis. Cumulative Contribution Plot A); Contribution in PCA 1 component analysis B); and Contribution in PCA 2 component analysis C).



Note: X axis represents different input Parameters used in the study where Y axis represents its contribution in PCA. R2 tells us how much of variability of a variable that is fitted by the model (as fraction) shown as Green colour bar; and Q2 tells us how much variability of a variable that is predicted by model (as fraction).

In PCA analysis, XY contribution displays how much a model was able to fit a particular variable. As shown in Fig 4, the two component PCA analysis shows different clusters for three formulations, wherein the differentiation observed is a result of the parameter whose contribution is least in

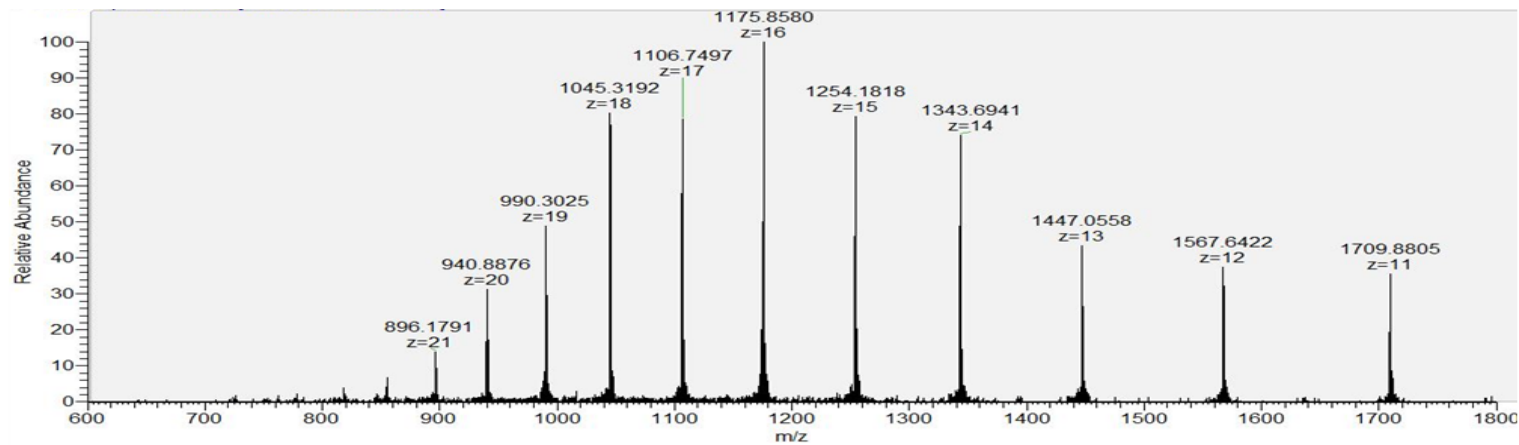
XY contribution plot. It is clear from the plot that HDX-MS variables F-I have the least contribution, therefore this technique can serve as most discriminative one.

Description for input:

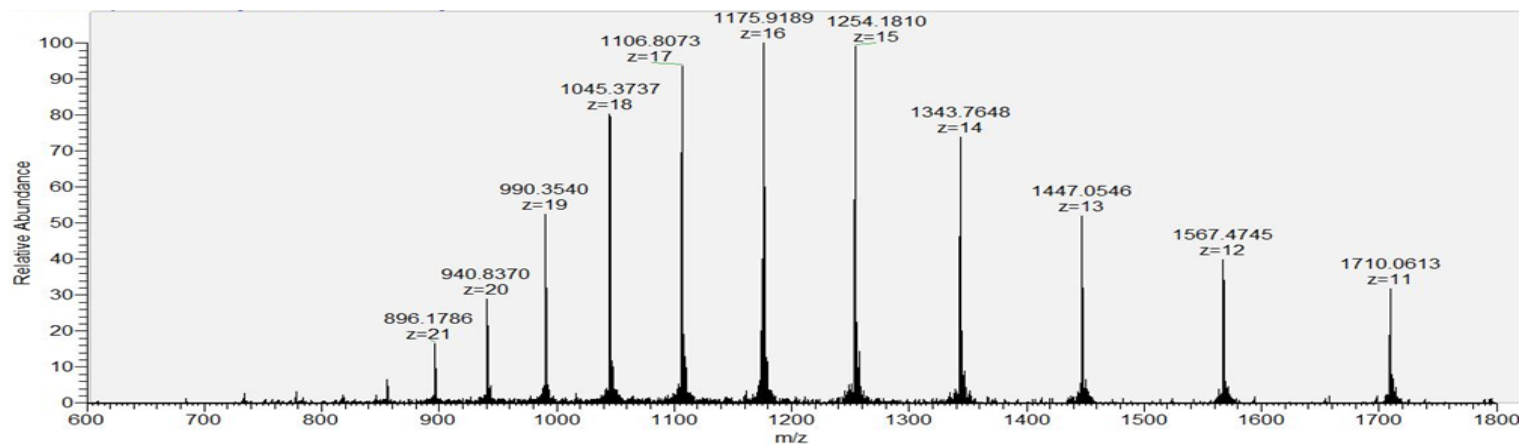
- B: Relative intensity of major isoform-I based on Intact Mass Analysis;
- C: Relative intensity of major isoform-II based on Intact Mass Analysis;
- D: Relative ratio of oxidized protein in formulation
- E: Number of charged species from Iso-Electric Focusing;
- F: Log of relative H-D exchange contribution for major isoform-I from data of HDX-MS;
- G: Log of relative H-D exchange contribution for major isoform-II from data of HDX-MS;
- H: Difference in deconvoluted molecular mass of major isoform-I post H-D Exchange;
- I: Difference in deconvoluted molecular mass of major isoform-II post H-D Exchange;
- J: Sequence Coverage Score from peptide mapping;
- K: No. of Peptides detected in the formulation from peptide mapping data;
- L: PTM based ratio of oxidized peptide vs un-oxidized peptide for M122 from data of peptide mapping analysis;
- M: PTM based ratio of oxidized peptide vs un-oxidized peptide for M138 from data of peptide mapping analysis;
- O: Presence/Absence of peptide no.2 from peptide mapping data.
- P: Presence/Absence of peptide no.3 from peptide mapping data.
- X: Presence/Absence of peptide no.11 from peptide mapping data.
- Y: Presence/Absence of peptide no.12 from peptide mapping data.

S-10: Line Spectra for IMA of Formulations

Formulation-I



Formulation-II



Formulation-III

