An Integrated Workflow for Automatic Mapping of Disulfide Linkages of Therapeutic Proteins Using High-Resolution Mass Spectrometry and a Targeted Software

Hongwei Xie and Weibin Chen

Biopharmaceutical Sciences, Waters Corporation, 34 Maple Street, Milford, MA 01757

OVERVIEW

- featuring high-resolution UPLC • An integrated workflow separation, multiplexed MS data acquisition (MS^E), and automatic batch processing of data by BiopharmaLynx (ver 1.3), is developed for fast mapping of protein disulfide bonds
- Highly reproducible, fully annotated peptide maps for therapeutic proteins can be routinely acquired using the workflow
- The workflow enables automatic assignment of disulfide bonded peptides (including scrambled ones) in the same run as peptide profiling

EXPERIMENTAL METHODS

Samples:

- RapiGest SF[™] aided Lys-C digested IgG1

Instrumentation:

- Liquid Chromatography, ACQUITY UPLC®
- Mass spectrometers Xevo[™] G2 QTof or Synapt[™] G2 HDMS

Columns:

– Peptide Separation Technology, ACQUITY UPLC[®] C4 BEH300 1.7µm, 2.1×150 mm

Informatics:

– BiopharmaLynx[™] ver 1.3 application manager









Figure 1. Waters biopharmaceutical LC/MS system: ACQUITY UPLC[®] LC, *Xevo™ G2 QTof/Synapt G2 HDMS, and BiopharmaLynx Informatics*



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	▼ ¹ Protein	Fragment Number	Modifiers	Calculated Peptide Mass (Control RT (Min)	Control Intensity (Counts)	C
1	Trastuzumab	2:K14-4:K14		5004.4878	75.93	1309166.0	
2	Trastuzumab	1:K6		2101.1208	51.58	1030857.0	
3	Trastuzumab	2:K16-2:K21		3144.5142	46.36	994145.0	
4	Trastuzumab	1:K7-1:K13		3883.9236	48.71	925899.0	
5	Trastuzumab	2:K7-2:K8		7389.6489	76.73	918845.0	
6	Trastuzumab	2:K29		1872.9146	53.18	898695.0	
7	Trastuzumab	2:K3		2558.2917	50.23	729825.0	
8	Trastuzumab	2:K7-2:K8-9		7916.9194	74.18	638662.0	
9	Trastuzumab	2:K28		2543.1240	50.70	606959.0	
10	Trastuzumab	2:K26		2342.1689	30.29	570005.0	
11	Trastuzumab	2:K27-2:K31		4087.9568	40.81	547834.0	
12	Trastuzumab	1:K10		1501.7512	37.72	531996.0	
13	Trastuzumab	2:K17		1676.7947	39.59	462691.0	
14	Trastuzumab	2:K18-19*	Glycosylation G0F N(1)	4904.3237	53.42	407729.0	
15	Trastuzumab	2:K18-19*	Glycosylation G1F N(1)	5066.3774	53.12	393583.0	
16	Trastuzumab	2:K6		1185.6394	34.68	341814.0	
17	Trastuzumab	1:K1-1:K4		10657.1172	70.59	309054.0	
18	Trastuzumab	1:K9		2134.9614	17.66	291272.0	
19	Trastuzumab	2:K2		1569.8052	27.40	109031.0	
20	Trastuzumab	2:K32		659.3490	17.97	99315.0	
21	Trastuzumab	2:K3-4		3721.8853	76.74	62188.0	
22	Trastuzumab	2:K4		1181.6040	14.32	61359.0	
23	Trastuzumab	2:K23		837.4960	20.94	54750.0	
24	Trastuzumab	2:K19*	Glycosylation GOF N(1)	4675.1812	56.71	39512.0	
25	Trastuzumab	2:K25-26		2541.3010	51.75	33107.0	
26	Trastuzumab	2:K1-2:K5		8303.9404	66.93	32320.0	
27	Trastuzumab	2:K19*	Glycosylation G1F N(1)	4837.2349	56.42	28100.0	
28	Trastuzumab	2:K14-4:K14-15		5229.6357	72.82	21149.0	
29	Trastuzumab	2:K28x2		5086.2480	50.69	12634.0	
30	Trastuzumab	2:K29		1872.9146	52.36	12387.0	
31	Trastuzumab	1:K8		559.3118	4.84	10113.0	
32	Trastuzumab	2:K1-2:K5		8303.9404	67.51	9723.0	
33	Trastuzumab	2:K17		1676.7947	38.51	7720.0	
34	Trastuzumab	2:K30		574.3326	14.33	6560.0	
35	Trastuzumab	2:K29x2		3745.8291	53.17	6190.0	
36	Trastuzumab	2:K14-4:K14		5004.4878	72.65	6005.0	
37	Trastuzumab	2:K19-20*	Glycosylation GOF N(1)	5095.3818	76.00	4693.0	

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CONCLUSIONS

- Fast mapping of disulfide linked peptides has been achieved using LC-MS^E methodology coupled with BiopharmaLynx informatics
- Assignment of disulfide bonded peptides is automated, based on accurate MS measurement and confirmed by elevated-energy MS^E fragmentation data
- Effective sample preparation in combination with robust analytical workflow facilitates routine analysis of disulfide bonded peptides within a non-reduced peptide map
- Automatic detection and confirmation of both expected and scrambled disulfide bonded peptides have been achieved in a same workflow.