Development and Application of an LC-UV/MS^E Peptide Mapping System for Detailed Characterization of Protein Therapeutics

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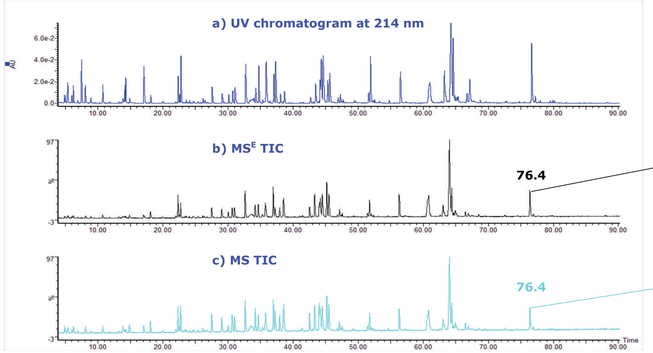
OUTLINE

- Monoclonal antibodies are large, heterogeneous glycoproteins that are susceptible to a number of posttranslational modifications and chemical degradations common to other classes of therapeutic proteins. As such they represent an appropriate model system for illustrating a new peptide mapping system for recombinant protein characterization.
- An UPLC-UV/MS^E system is developed for mapping an IgG1 antibody tryptic digest, which collects UV, MS (precursor) and MS^E (fragments) data in 3 parallel chromatograms (Figure 1) for peptide characterization.
- MS/MS^E data are able to identify extremely large/small, and co-eluting peptides, ensuring high (≥97%) sequence coverage (Figures 1, 2 and 3).
- LC-eluted peaks are assigned by BiopharmaLynx and ready to be validated (Figures 2, 4, 5), which automates and speeds up the data processing procedure.
- MS/MS^E enables monitoring the site and level of modifications such as N-deamidation (Figure 4) and N-linked glycosylation (Figure 5).
- Optimized method can be easily transferred to UPLC-UV only method for quality control purpose.

SYSTEM SUTUP AND METOHDS

1. Antibody Digest: RapiGest SF-assisted 4-hours tryptic digestion of IgG1 antibody Trastuzumab (MW ~148 kDa).
2. LC System: Waters ACQUITY UPLC® configured with a TUV detector and a 2.1 x 150 mm BEH300 C18 1.7 µm PST column. Column temperature 65 °C; Mobile phase A—0.02% TFA in water, B—0.018% TFA in acetonotrile; Gradient 1-40% B in 90 min; Flow rate 200 µl/min; Injection amount & volume—9 µg in 10 µl.
3. MS System: Waters SYNAPT™ HDMS, operated in positive ESI V-mode. An alternating MS acquisition mode was applied for both peptide precursor (MS, acquired at collision energy 4 V) and fragments (MS^E, acquired at collision energy ramping from 25 to 45 V). Total duty cycle time of 1 second was applied.
4. Data Processing: BiopharmaLynx 1.2

Figure 1. UPLC-UV/MS^E data of the IgG1 antibody tryptic digest. An example MS and MS^E spectrum for the peak eluted at 76.4 min was plotted, which was identified to be the antibody largest tryptic peptide HT15 (MW 6712.31, with 63 amino acids) by BiopharmaLynx processed (charge-reduced & isotope-deconvoluted) MS/MS^E spectrum. a) UV chromatogram at 214 nm; b) TIC of MS collected at elevated collision energy (MS of peptide fragments, called MS^E) ; c) TIC of MS collected at low collision energy (MS of peptide precursors, regular MS); d) MS spectrum of the peak at 76.4 min; e) MS^E spectrum of the peak at 76.4 min; f) MS^E spectrum of the peak at 76.4 min after BiopharmaLynx processing.



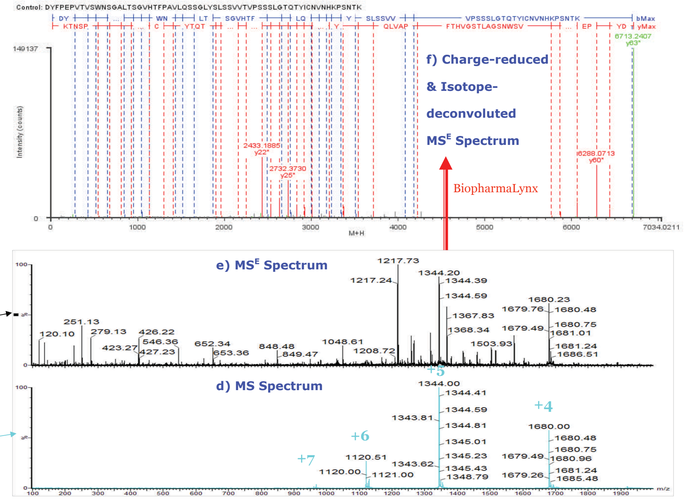


Figure 2. Automated peak assignment by BiopharmaLynx processed MS/MS^E data and sequence coverage from the identified peptides. Two continuous injections were assigned and plotted in mirror (A), demonstrating the reproducibility of the method and its ability for batch-to-batch comparison. The identified peptides covered ~98% sequences of both light (B) and heavy (C) chains. In the sequence coverage map (B and C), amino acids marked in green—identified by both injections, yellow— only by one injection, others—not identified.

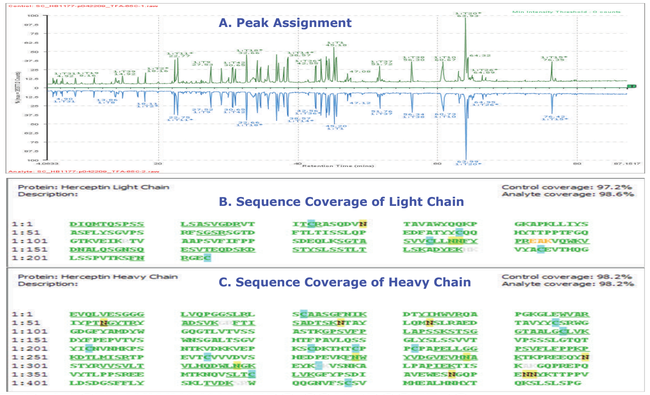


Figure 3. MS/MS^E identification of co-eluted peptides. Peptides HT6 (IYPTNGYTR, MW 1083.53) and HT11* (AEDTAVYYC*SR, MW 1333.56, *-carbamidomethyl C) co-eluted at 22.73 min were confidently assigned by BiopharmaLynx processed MS^E spectrum at 22.73 min. a) UV chromatogram at 214 nm; b) MS TIC; c) XIC of HT6 and HT11* double charged ions; d) MS spectrum at 22.73 min; e) fragments of MS^E spectrum at 22.73 min assigned to HT6 and HT11*.

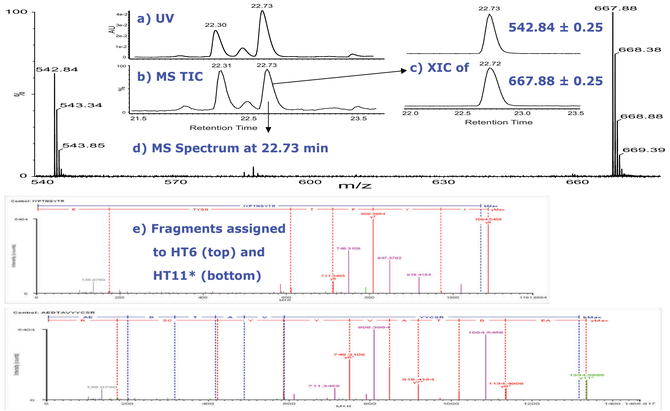
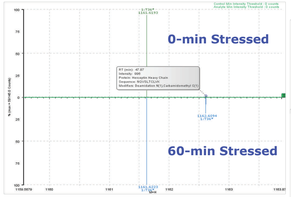
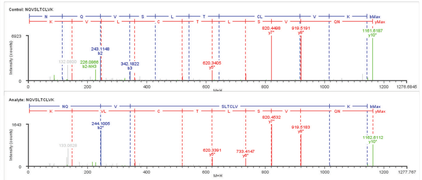


Figure 4. MS/MS^E identification of N-deamidation sites and monitoring deamidation level in an *in vitro* stress experiment with digest exposed at pH 10 and 60 °C for 0, 5, 10, 30 and 60 min. a) BiopharmaLynx processed MS detection of deamidation in peptide HT36 (NQVSLTCLVK); b) MS^E identification of the deamidated N-site; c) Deamidation level monitored by BiopharmaLynx with MS intensities; d) Deamidation level showed by XICs. The quantification data obtained by BiopharmaLynx and by the integrated area of XICs were comparable. No UV quantification data was available due to co-elution issue.

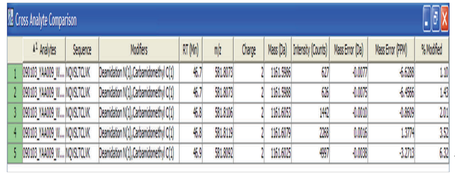
a) MS Detection of Deamidation



b) MS^E Identification of the deamidated N-site
top— before deamidation, bottom— after deamidation



c) Deamidation level monitored by BiopharmaLynx



	Analyte	Sequence	Modifiers	RT (Min)	m/z	Charge	Mass (Da)	Intensity (Counts)	Mass Error (Da)	Mass Error (PPM)	% Modified
1	090103_HA0806_III...	NQVSLTCLVK	Deamidation N(1),Carbamidomethy C(2)	45.7	591.8073	2	1181.5985	6327	-0.0077	-6.5268	1.10
2	090103_HA0806_III...	NQVSLTCLVK	Deamidation N(1),Carbamidomethy C(2)	45.7	591.8073	2	1181.5985	6326	-0.0076	-6.4966	1.49
3	090103_HA0806_III...	NQVSLTCLVK	Deamidation N(1),Carbamidomethy C(2)	45.8	591.8195	2	1181.6325	1442	-0.0010	-0.8459	2.01
4	090103_HA0806_III...	NQVSLTCLVK	Deamidation N(1),Carbamidomethy C(2)	45.8	591.8135	2	1181.6079	2268	0.0016	1.3774	3.52
5	090103_HA0806_III...	NQVSLTCLVK	Deamidation N(1),Carbamidomethy C(2)	45.9	591.8092	2	1181.6525	4997	-0.0038	-3.2212	6.70

d) Deamidation level showed by XICs

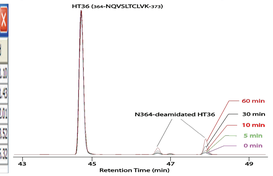
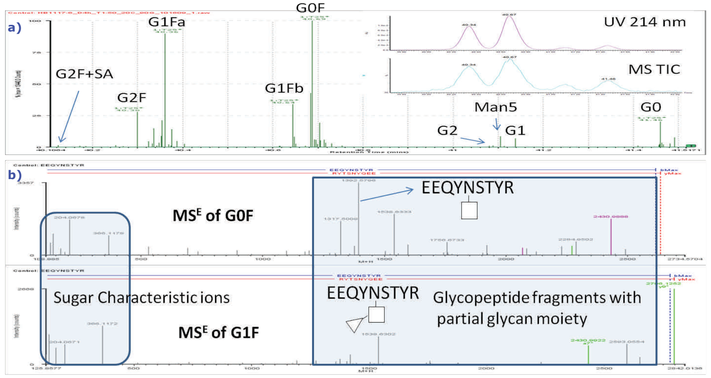


Figure 5. MS/MS^E identification of N-linked glycopeptide and glycoforms. a) Glycoforms assigned by BiopharmaLynx processed MS. The UV and MS TIC chromatograms were inserted to show the UPLC separation of glycoforms (at 20°C column temperature); b) MS^E spectra of G0F and G1F glycoforms.



References: Waters Application Notes (2009), 720003227en, 720003168en, 720003173en

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