Comprehensive Characterization of Trastuzumab using a Single-Shot Peptide Mapping Approach with CESI-MS

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INTRODUCTION

In the biopharmaceutical industry, comprehensive characterization of therapeutics-based monoclonal antibodies (mAbs) is crucial, as impurities and heterogeneity can impact therapeutic safety and/or efficacy. mAbs are large glycosylated proteins (≈ 150 kDa) containing several post-translational modifications (PTMs). Peptide mapping of mAbs can provide critical qualitative and quantitative information about impurities and heterogeneity.

Capillary electrophoresis (CE) has exceptional separation efficiency and capabilities for peptide mapping of mAbs by mass spectrometry. We have previously reported on the development of an electrospray ionization (ESI) emitter integrated with capillary electrophoresis (CE), combining CE separation and ESI into a single dynamic process (CESI).[1] We present data for the bottom-up proteomic peptide mapping of a leading, representative monoclonal antibody, Trastuzumab (Herceptin) using a single protease, trypsin. A Beckman Coulter CESI 8000 system sold through SCIEX Separations, a part of AB SCIEX, coupled to a Thermo Q-Exactive was used for the analysis.

The results illustrate the benefits of the integration of CE and ESI in a single dynamic process with high resolution mass spectrometry. That is, the CESI separation provides both the separation efficiency of CE and low-nanoflow electrospray conditions to provide the sensitivity to achieve 100% sequence coverage of the heavy and light chains of this mAb. At flow rates below 20 nL/min, ionization efficiency is maximized and ion suppression is dramatically reduced, allowing strong ionization of modified peptides and glycopeptides. As all analytes migrate through the capillary, all peptides, both large and small (3 – 65 amino acids) were identified, separated, and quantified. With the comprehensive (100%) sequence coverage, identification and quantification of mAb purity, stability, and glycoform heterogeneity is possible from a single CESI-MS analysis. In particular, we can identify degradative hotspots like aspargine deamidation, methionine oxidation, and glutamic acid cyclization from a mere 100 fmol. Exploiting the reduced ion suppression effects from the low-nanoflow ESI regime, low abundance PTMs were identified and quantified in the presence of peptides with higher responses and abundances. A glycosylation hotspot is highlighted by comprehensive characterization at N300. Collectively these results indicate the capabilities for CESI-MS coupled with high resolution mass spectrometry to quickly and comprehensively characterize a therapeutic mAb using peptide mapping from a small amount of sample with a single protease.

Representative mAb (Trastazumab)

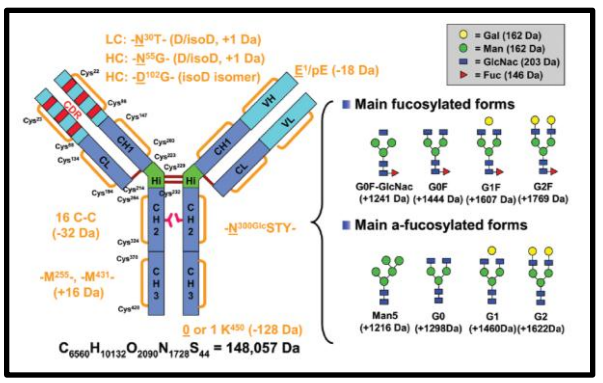

Figure 1. Trastuzumab schematic representing the major PTMs identifiable by mass spectrometry (disulfide bridge pairings, main glycoforms, heavy chain N-terminal pyroglutamic acid, C-terminal lysine clipping, deamidation hot spot in CDRs) and their associated mass differences.[2]

METHODS

Samples were prepared following a classical but short (2-hour digestion) protocol using DTT, iodoacetamide, RapiGest, and trypsin. CESI experiments were carried out with a CESI 8000 prototype system equipped with a temperature controlled autosampler and a power supply with the ability to deliver up to 30 kV. Prototype fused-silica capillaries with porous tip were used. Solutions of 10% acetic acid and 200 mM ammonium acetate (pH 4) were employed as background electrolyte (BGE) and leading electrolyte, respectively. CESI separations were performed at 20 kV, and about 100 fmol (45 nL of a 2 mM solution) of each tryptic digest were injected per analysis. Eluted peptides were analyzed using a bench-top quadrupole Orbitrap mass spectrometer with a data-dependent top ten HCD method. High resolution HCD spectra were analyzed using Protein Pilot™ (AB SCIEX software) and Byonic (ProteinMetrics).

CESI 8000 Configuration

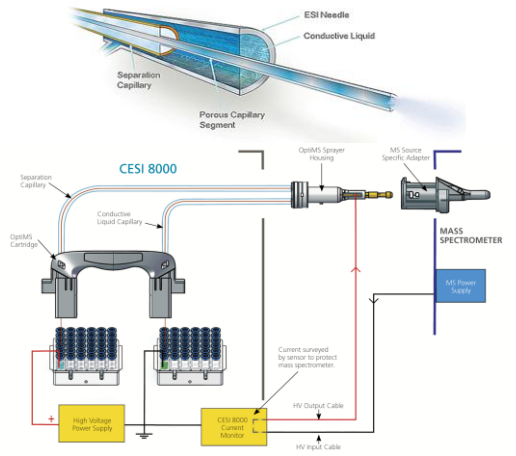

Figure 2. Illustrations of CESI 8000 sprayer and cartridge.

RESULTS

100% Sequence Coverage of Trastuzumab

Figure 3. Triplicate sequence coverages of heavy (left) and light (right) chains

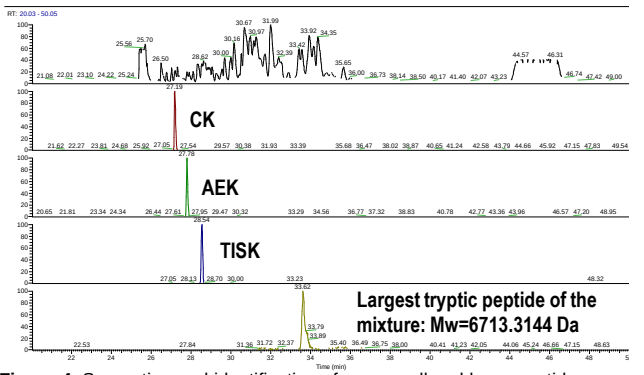

Figure 4. Separation and identification of very small and large peptides

Degradative PTM hotspot analysis

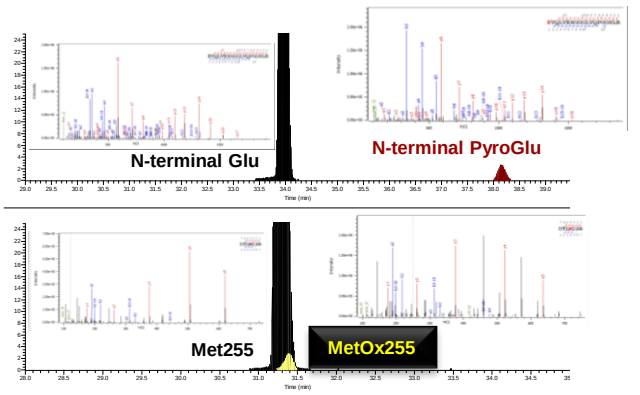

Figure 5. Separation, identification, and relative quantification of N-terminal pyroglutamate and methionine oxidation on the heavy chain.

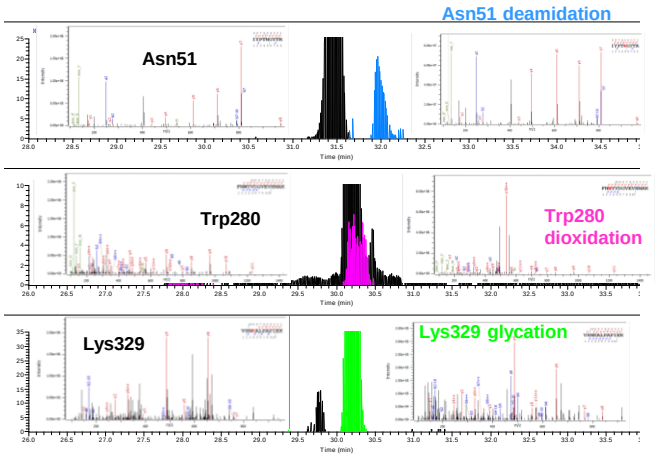

Figure 6. Separation, identification, and relative quantification of asparagine deamidation, tryptophan dioxidation, and lysine glycation on the heavy chain.

Modification	Modified peptide sequence and associated mass shift	Monoisotopic mass [M+H]+	Mass accuracy (ppm)	Average relative abundance (%)
Methionine Oxidation	*K.DTLM(+15.9949)ISR.T*	*851.4291*	*0.33*	*2.39 ± 0.98*
Pyroglutamate formation	*-.E(-18.0106)VQLVESGGGLVQPGGSLR.L*	*1863.9923*	*0.95*	*1.60 ± 0.19*
Asparagine deamidation	*R.IYPTN(+0.9840)GYTR.Y*	*1085.5262*	*0.06*	*15.57 ± 2.92*
Lysine glycation	*K.VSNK(+162.0528)ALPAPIEK.T*	*1428.7944*	*-0.85*	*91.99 ± 1.45*
Tryptophan dioxidation	*K.FNW(+31.9898)YVDGVEVHNAK.T*	*1709.7818*	*-1.62*	*0.26 ± 0.03*

Table 1. Summary of quantified degradative PTMs from triplicate analyses.

Asn300 glycosylation hotspot analysis

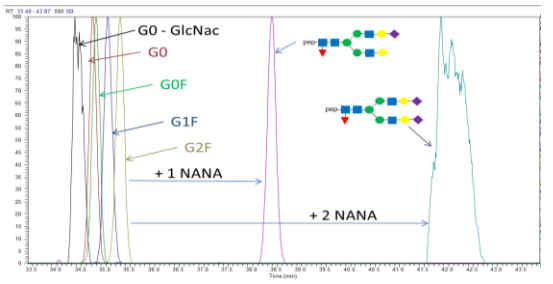

Figure 7. Separation by glycan structure and relative quantification of glycopeptide forms (EEQY**N**STYR) from the heavy chain.

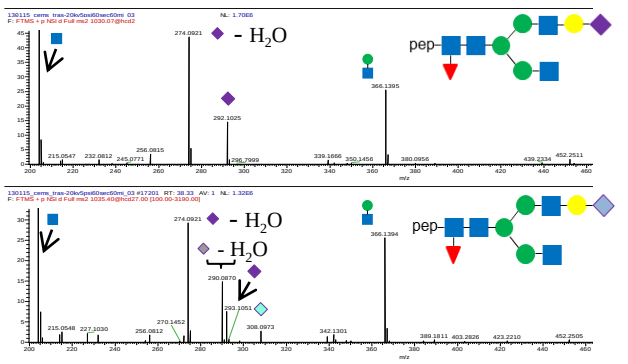

Figure 8. MS/MS spectra of NANA- and NGNA-containing glycopeptides (EEQY**N**STYR) from the heavy chain.

Glycopeptides identified as R.EEQYN(Glycan)STYR.V	mAb glycan abbreviation	Glycan mass (Da)	Monoisotopic mass [M+H]+ (Da)	Mass accuracy (ppm)	Average relative abundance (%)
	G1F	*1606.5867*	*2796.0987*	*-0.41*	*54.49 ± 1.45*
	G0F	*1444.5339*	*2634.0459*	*0.25*	*17.28 ± 0.97*
	G2F	*1768.6395*	*2958.1515*	*-0.01*	*8.59 ± 0.40*
	G0	*1298.476*	*2487.9880*	*0.67*	*5.58 ± 0.31*
	G1	*1460.5288*	*2650.0408*	*0.93*	*4.07 ± 0.09*
	-	*1403.5073*	*2591.0037**	*3.04*	*1.52 ± 0.06*
	A1F	*2059.7349*	*3249.2469*	*0.78*	*1.41 ± 0.11*
	-	*1241.4545*	*2430.9665*	*0.40*	*1.25 ± 0.06*
	Man5	*1216.4228*	*2405.9349*	*0.09*	*1.13 ± 0.66*
	-	*1897.6821*	*3085.1785**	*3.10*	*1.02 ± 0.48*
	-	*1257.4494*	*2446.9614*	*0.44*	*0.78 ± 0.40*
	-	*1694.6027*	*2884.1147*	*-3.15*	*0.57 ± 0.03*
	G2	*1622.5816*	*2812.0936*	*-2.77*	*0.55 ± 0.03*
	-	*1095.3966*	*2284.9086*	*0.57*	*0.51 ± 0.32*
	A2F	*2350.8303*	*3540.3423*	*1.77*	*0.44 ± 0.05*
	-	*1548.5448*	*2738.0568*	*-2.81*	*0.32 ± 0.15*
	-	*1809.6661*	*2999.1781*	*-3.00*	*0.17 ± 0.01*
	-	*1710.5976*	*2900.1096*	*-1.62*	*0.14 ± 0.01*
	-	*1419.5022*	*2609.0142*	*-1.92*	*0.14 ± 0.07*
	A1	*1913.677*	*3103.1890*	*-1.74*	*0.09 ± 0.03*

Table 2. Summary of quantified glycoforms from N300 glycosylation on the heavy chain from triplicate analyses.

CONCLUSIONS

- CESI-MS was capable of analyzing very small (< 300 Da) and very large peptides (>8000 Da) under the same experimental conditions
- 100% sequence coverage was reproducibly achieved for each chain with a single run and single enzymatic digestion from 100 fmol of a monoclonal antibody
- Multiple degradative PTM hotspots were reproducibly identified and quantified with relative abundances over three orders of magnitude
- Reproducible, in-depth characterization and quantification of a glycosylation hot spot with relative abundances over three orders of magnitude was achieved

REFERENCES

1 Busnel *et al., Anal. Chem*. **2010**, 82, 9476–9483.
2 Beck *et al*., *Anal. Chem.* **2012**, 84, 4637−4646.

TRADEMARKS/LICENSING

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