Ion exchange chromatography (IEC) is a method of choice for the analysis of charge heterogeneity encountered with biopharmaceutical drug candidates. Traditionally, IEX separations require high salt gradients to achieve significant separation and mass spectrometry (MS) analysis, which has left a gap in the characterization of charge variants. It has been shown that direct MS-based characterization of these charge variants is possible if volatile salts are employed. In this study, MS-compatible IEX separations are combined with a new small footprint benchtop flow-through (TOF) MS instrument and applied to a case study on identifying the charge variants formed upon forced degradation.

**METHODS**

Forced Degradation of Trastuzumab

A sample of Trastuzumab (NS) was buffer exchanged into 100mM sodium phosphate, pH 8.0 using BioSprint Micro Bio-Spin-6 chromatography columns (W32-0221), according to manufacturer instructions. The buffer exchanged Trastuzumab sample was further diluted to 2 mg/mL in 100mM sodium phosphate, pH 8.0 and was rapidly split. Two lots were kept at 4°C until analysis and the other was incubated at 25°C for 1 week.

**Histidine Digestion of Trastuzumab and Other mAb samples**

For each Trastuzumab (T0 & 1 week stressed) sample was digested with 1 μL of 1 mg/mL sample of enzyme (Genovis, A050) was incubated at 25°C for 1 hour. The digested samples were then analyzed by IEX separation and MS for detection and deconvolution of each peak.

**EX-MS**

Analyses were performed on the new BioAccord compact TOF MS instrument, which consists of an ACQUITY UPLC with TOF detector and RDa detector.

**RESULTS**

**Figure 6.** Left panel shows combined raw spectra for each peak. The right panel shows combined raw spectra for Trastuzumab T0 & 1 week stressed Trastuzumab (T0 & 1 week stressed) samples digested with 1 μg/mL IdeS enzyme (Genovis, A050) was incubated at 25°C for 1 hour. The digested samples were then analyzed by IEX separation and MS for detection and deconvolution of each peak.

**Table 1.** 1D histidine digestion IEX-MS results and possible assignments for each peak.

- **Deamidation**
- **Terminal lysine addition**
- **Possible disulfide or conformational variants**
- **Terminal lysine addition**

**CONCLUSION**

- **Successful establishment of IEX-MS method using a di-uf and volatile salt gradient with optimization at the column level.**
- **Streamlined data acquisition with a compact high-performance TOF with increased user accessibility and performance standardization.**

**IEX-MS is ideal for high throughput monitoring of charge heterogeneity, with the added benefit of direct investigation of variants by native mass spectrometry.**

**IEX-MS successfully employed for monitoring of charge variants in pH-stressed Trastuzumab sample**

**Further characterization (e.g. modification site confirmation, potency assay) is made possible by a fraction collection.**

**REFERENCES**

1. Yan, Y et al. (2016) "A quick method for the detection and quantification of charge heterogeneity with minimal sample preparation." J. Proteome Res. 15: 13013-13020.

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**Figure 7.** Left panel shows combined raw spectra for peaks as in Figure 6. The raw data for Trastuzumab sample digested with 1 μg/mL IdeS enzyme (Genovis, A050) was incubated at 25°C for 1 hour. The digested samples were then analyzed by IEX separation and MS for detection and deconvolution of each peak.