Monoclonal Antibody Analysis with Compact Capillary LC Instrumentation

Benjamin Libert[1,2], Samuel Foster[1], Taylor Harmon[2], and James P. Grinias[1]

1) Department of Chemistry and Biochemistry, Rowan University, Glassboro, NJ 08028

2) Advanced Materials Technology, Wilmington, DE 19810

Abstract

The analysis of monoclonal antibodies (mAb) and their various critical quality attributes (CQAs) by LC-MS is a critical aspect of biopharmaceutical characterization. Optimally, these techniques could be coupled directly to bioreactors for real-time process monitoring of biopharmaceutical manufacturing by LC-MS. Recently, a method for on-line monitoring of small molecule reactions was demonstrated using compact capillary LC-MS instrumentation. Here, the same compact capillary LC instrument is utilized for a variety of ultra high performance liquid chromatography (UHPLC) methods adapted for the characterization of mAbs. Capillary columns (0.2/0.3mm i.d.) were packed with 2.7 µm superficially porous particles and were operated in reverse phase (RP). Particles with 1000 Å pores were used for the analysis of intact mAbs and mAb fragments. Particles with 160 Å pores were used for the analysis of a mAb tryptic digest.

Introduction & Theory

Commercial biotherapeutic product development and process control by UHPLC often involves the selection of a column containing a specific column packing material, with an optimal particle pore size for the analyte(s) being investigated, with surface chemistry that can obtain useful peak selectivity and high efficiency separations while maximizing for the chromatographic resolution of all components in the separation. While previous studies have highlighted the Axcend Focus LC for its ability to perform many routine separations on small molecules, here we demonstrate the instruments' ability to work on peptides, antibody fragments, and intact IgG1 (trastuzumab).

Chromatographic Conditions: A custom column oven was implemented to maintain column experiments at 60-80°C for RP-HPLC peptide and protein separations performed on the Axcend capillary scale LC. Column temperatures were monitored and recorded. For mAb separations, a HALO® 1000Å Diphenyl 0.2x150 mm 2.7 µm column was used. For analysis of peptides a HALO® 160Å ES-C18 0.2x150 mm 2.7 µm column was used.

mAb Digests and Modifications: Separations of intact trastuzumab (mAb), IdeS (FabRICATOR, Genovis) digest, mAb digest (Trypsin Gold, Promega), and a reduced heavy and light chain mAb were performed. The mAb (1mg/mL) was digested by FabRICATOR at 37°C for 30min, then acidified; the reduced mAb denatured [in 6 M Urea (Thermo), 50 mM Trizma (Aldrich), 1 mg/ml mAb, and 5 mM Dithiothreitol (Thermo)] by heating to 60° C for one hour. The mAb was digested by trypsin at 37°C overnight.

Axcend Focus LC: The system utilized a new cartridge allowing for coupling to these commercially available columns. Additionally, this cartridge utilizes a new commercially available Z cell to increase detector sensitivity by lengthening the flow path from 0.15 mm to 1.2 mm, resulting in an ~ 8 fold increase in sensitivity. The cartridge also included an oven component; however, this was bypassed since the present LC model had a 50°C maximum oven temperature, which did not meet the ≥60°C analytical method requirements,.

Intact mAb and mAb Fragment Separations

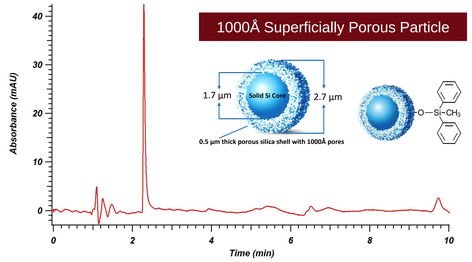

Figure 1: Traztuzumab 1 mg/ml in water. Run at 3.1 µL/min with a gradient of 25-80% ACN over 10 minutes.

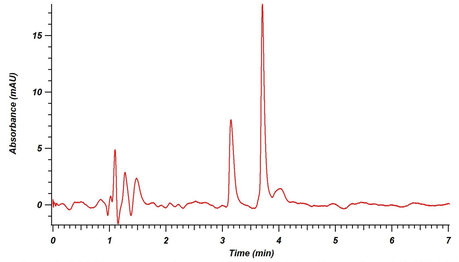

Figure 2: IDES Digest 1 mg/mL in water. Run at 3.1 µL/min with a gradient of 20-50% ACN over 7 minutes.

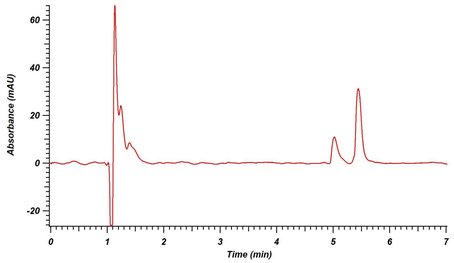

Figure 3: Reduced Traztuzumab at 1 mg/mL in Tris Buffer. Sample was run at 3.1 µL/min with a gradient of 10-50% ACN over 7 minutes.

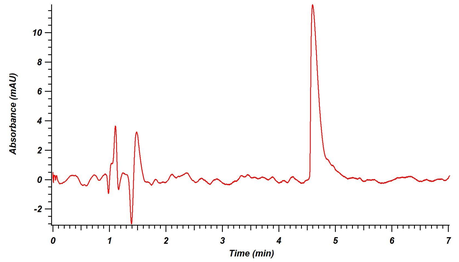

Figure 4: Insulin in water. Sample was run at 3.1 µL/min with a gradient of 10-50% ACN over 7 minutes.

mAb Tryptic Peptide Separations

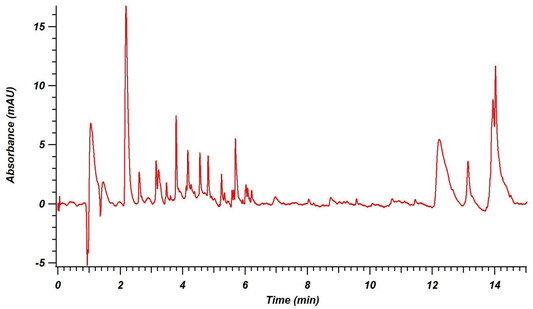

Figure 5: A tryptic digest of Traztuzumab at 1 mg/ml. Sample was run at 3.6 µL/min with a gradient of 3-95% ACN over 15 minutes. Column was washed before injection and given 3 minutes to equilibrate at the given flow rate before sample was injected. Of interest is the compounds from 12-14 minutes which are seemingly larger undigested fragments.

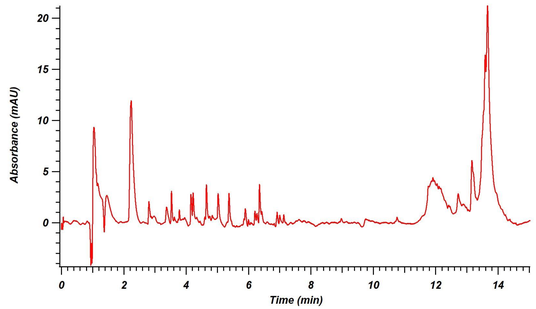

Figure 6: A tryptic digest of Traztuzumab at 1 mg/ml. The sample was again run at 3.6 µL/min however a multi step gradient consisting of 3-55% ACN over 10 minutes, followed by a gradient of 55-80% ACN from 10-11 minutes, where it was held until the run ended at 15 minutes. This was in an attempt to further separate the compounds between 12 and 14 minutes.

Axcend Cartridge

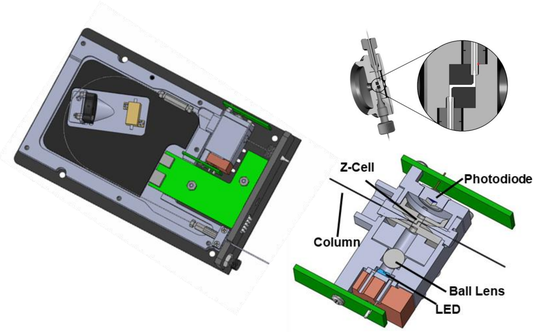

Figure 7: The new cartridge and flow cell used for this analysis. The cartridge allows for the coupling of commercially available capillary scale columns, as well as heating up to 50° C. Also pictured is the commercially available Z flow cell as well as its custom housing. This housing allows for single wavelength detection through the 1.2 mm flow path, increasing the sensitivity ~8 fold from on capillary detection.

Conclusions

Here we have demonstrated the Axcend Focus LC's ability to perform separations of recombinant therapeutic antibody (trastuzumab), mAb fragments, and peptides using HALO® 1000 Å Diphenyl and 160 Å ES-C18 columns. By using a newly designed cartridge for the Axcend, it was possible to couple commercially available capillary scale columns to the system. Additionally, a commercially available Z flow cell was introduced, resulting in an ~8 fold increase in detector sensitivity when compared to on-capillary detection. Using this setup, the system was able to separate and detect mAb, as well as mAb IdeS digest and reduced mAb fragments. Insulin separation on the 1000Å pore size material was also demonstrated. Finally, a tryptic digest was analyzed using 2.7µm (160Å pore-size) particle column packing bonded with sterically protected C18 (ES-C18). The LC's performance demonstrates utility for the analysis of small and large biomolecules. Future projects will explore aspects of coupling the LC system to a high-resolution mass spectrometer to obtain mass and charge information from these biomolecule separations.

Acknowledgements

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References

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