INTRODUCTION

The complete analysis and characterization of pharmaceuticals is most efficient when orthogonal analytical techniques are applied to the samples. Each technique should be based on different physical properties of the protein, and can include chromatographic methods such as size-exclusion (SEC), ion-exchange (IEX) and reversed-phase chromatography. In this presentation, we will demonstrate how the ACQUITY UPLC® H-Class Bio System in combination with AutoBlend™ Plus Technology can now be used in conjunction with new ion-exchange and size-exclusion methods for improved chromatographic separations. Size exclusion method development will include the effects of salt concentration and column length. Ion-exchange method development will be outlined, including the use of pH and buffer composition. These factors will be utilized to demonstrate the improved impurity detection and faster analysis achievable with the combination of UPLC technology and new size-exclusion and size-exclusion packing materials.

METHODS

Chromatographic Conditions

LC System: ACQUITY UPLC® H-Class Bio System with

Flow Rate: 0.4 mL/min

Sample Volume: 5.0 µL

Column: ACQUITY BEH200 SEC, 1.7µm,

Injection Volume: 4.0 µL

Flow Rate: 0.4 mL/min

Mobile Phase: 25mM Sodium  Phosphate, pH 6.8, 0.15M NaCl

Temperature: 30 °C

Sample Wash: 80/20 H2O/MeOH

Column: ACQUITY  BEH200 SEC, 1.7 µm, 4.6 x 150mm

Injections: 10 - 200 µL

PDA detector

Waters Corporation, 34 Maple Street, Milford, MA 01757

pH 6.8

REFERENCES

1. Waters Corporation, 34 Maple Street, Milford, MA 01757

pH 6.8

INTRODUCTION

In a series of experiments, protein standards and monoclonal antibody biotechnologies were analyzed by UPLC SEC. The reproducibility of the calibration was tested by analysis of protein standards over the molecular weight range of 10,000-450,000 Da at regular intervals over a two day period. The elution volume for each protein standard was found to be within 0.2 % RSD (Figure 1). The consistency of the calibration curve is indicative of both the column life and instrument control of flow rate and injection volume. To test the reliability of quantitation, a humanized IgG was analyzed and found to have an average aggregate quantitation of 8.82% ± 0.3 % of the monomer species over the time period (Figure 2).

Figure 1 Protein calibration curve, ACQUITY BEH200 SEC, 1.7 µm, 4.6 x 150mm. Recommended mobile phase weight range is 10,000-450,000. Overlay of 5 calibration curves over 48 hours.

Figure 2 SEC separation of humanized IgG. Injection of unfiltered humanized IgG over 48 hours showed aggregate quantitation relative to the monomer of 6.09-6.38% with a RSD of 0.3%.

Figure 3 Effect of buffer concentration on humanized IgG aggregate measurement. At low buffer concentration, secondary interactions affect peak shape, i.e. tailing. Higher buffer concentration improves peak shape and allows for quantitation of aggregates. Buffer: 20mM Sodium Phosphate, pH 6.8.

Figure 4 Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 5 Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 6 Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 7 Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 8. Conformational C-terminal lysine variants by cation-exchange chromatography. Analysis of chroma mAbs before and after treatment with carboxyethyllysine B. Conditions: 20mM MES pH 6.0, 0.0-0.1 M NaCl in 60 min, 0.5 µm/mL.

Figure 9. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 10. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 11. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 12. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 13. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 14. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 15. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 16. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 17. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 18. Conformational C-terminal lysine variants by cation-exchange chromatography. Analysis of chroma mAbs before and after treatment with carboxyethyllysine B. Conditions: 20mM MES pH 6.0, 0.0-0.1 M NaCl in 60 min, 0.5 µm/mL.

Figure 19. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 20. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 21. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 22. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 23. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 24. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 25. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 26. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 27. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 28. Conformational C-terminal lysine variants by cation-exchange chromatography. Analysis of chroma mAbs before and after treatment with carboxyethyllysine B. Conditions: 20mM MES pH 6.0, 0.0-0.1 M NaCl in 60 min, 0.5 µm/mL.

Figure 29. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 30. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 31. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 32. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 33. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 34. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 35. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 36. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 37. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 38. Conformational C-terminal lysine variants by cation-exchange chromatography. Analysis of chroma mAbs before and after treatment with carboxyethyllysine B. Conditions: 20mM MES pH 6.0, 0.0-0.1 M NaCl in 60 min, 0.5 µm/mL.

Figure 39. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 40. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 41. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 42. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 43. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 44. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 45. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 46. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 47. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.

Figure 48. Conformational C-terminal lysine variants by cation-exchange chromatography. Analysis of chroma mAbs before and after treatment with carboxyethyllysine B. Conditions: 20mM MES pH 6.0, 0.0-0.1 M NaCl in 60 min, 0.5 µm/mL.

Figure 49. Effect of pH on IEX separation of protein mixture. Indicated by the peak height and peak width.