Comparative Performance Study Of Charge-based Separation Techniques For Biopharmaceutical Analysis

J. Kahle, H. Zagst*, R. Wiesner, H. Wätzig

Technische Universität Braunschweig, Institute of Medicinal and Pharmaceutical Chemistry, Beethovenstr. 55, 38106 Braunschweig, Germany

h.zagst@tu-bs.de

For the analysis and characterization of biopharmaceuticals, particularly with regard to monoclonal antibodies, a broad range of analytical techniques is utilized. One of the key aspects is the analysis of charge heterogeneity. Here, ion-exchange chromatography (IEX), capillary zone electrophoresis (CZE) and (imaged) capillary isoelectric focusing ((i)cIEF) are the predominantly used separation techniques.

The three techniques were compared with main focus on the performance parameters precision and resolution/peak count. An additional investigated aspect was the required analysis time. Similar experimental designs were applied to enable representative comparisons. NISTmAb and Infliximab were selected as test molecules. In most cases, their analysis was conducted with established methods obtained from current literature. In some cases, methods had to be developed according to recommended procedures.

With icIEF, a relative standard deviation (RSD) of the percentage area for the main peak (%area) of 1.3% (NISTmAb, n = 60) and 0.91% (Infliximab, n = 120) was observed. Here, 9 (NIST mAb) and 8 (Infliximab) peaks could be identified.
The obtained RSD of the %area using CZE was 1.5% (NISTmAb, n = 60) and 2.2% (Infliximab, n = 120, thereof 40 runs excluded). With CZE 7 (NISTmAb) and up to 10 (Infliximab) peaks could be distinguished.
Finally, with IEX a RSD of the %area of 1.5% (NIST mAb, n = 60) and 2.0% (Infliximab, n = 40) was achieved. A peak count of 5 was obtained for NIST mAb, while for Infliximab up to 8 peaks could be revealed.