

## mAb charge variant analysis

How are you optimizing your method for maximum reproducibility?



The biopharmaceutical industry has seen increasing numbers of new monoclonal antibodies (mAb) drug products emerging in recent years, increasing the demand for robust characterization methods to support drug development and meet regulatory requirements. The challenging nature of the analysis and a choice of separation strategies has led to a need to understand the optimal approach for developing an ion exchange method.

### What are the most important considerations for reproducible salt gradients?

Salt gradients represent the most filed method type for mAb charge variant analysis. With a salt gradient, proteins are pushed down the column as they compete with the salt ions in the mobile phase. Familiarity and comfort mean we often default to this technique, but method optimization can be tricky.

- Do your samples have a high salt concentration?**  
 These samples can be troublesome, the salt from the sample acts as secondary eluent, preventing the protein from binding consistently to the top of the column leading to issues with retention times.
- Which buffers are you using and at what concentration?**  
 Charge variant profiles typically display partially resolved peaks on the shoulders of larger peaks which can be difficult to sufficiently and reliably separate, requiring significant investment in method development. Correct buffering ensures

the pH is kept constant during separation and maintains the method robustness. Ensure you are selecting the best buffer for your separation.

- How stable is the pH of your mobile phase during separation?** Maintaining control of the pH throughout a salt gradient is difficult and hard to troubleshoot without an in-line pH meter. Salt is known to affect pH, and the introduction of salt to non-salt containing buffers will change the pH value during the separation. As the pH changes, the interaction of the protein with the column is impacted and can result in poor chromatographic consistency

Thermo Fisher Scientific has years of experience troubleshooting and researching charge variant analysis. The lessons learnt have been written up in an open access journal article “New Insights into the Chromatography Mechanisms of Ion Exchange Charge Variant Analysis: Dispelling Myths and Providing Guidance for Robust Method Optimization” ([Anal.Chem. 2020, 92, 13411-13419](#)). Our objective was to support analysts to understand and troubleshoot their methods as efficiently as possible.



Thermo Fisher Scientific can support you with every aspect of your charge variant analysis – from columns to mobile phase buffers and liquid chromatography systems

Find out more at [thermofisher.com/chargevariants](https://thermofisher.com/chargevariants)