Defining the Peaks:

cIEF-MS FOR METHOD ROBUSTNESS AND VALIDATION

Coupling Charge Separation with Mass Identity for Confident Characterization

A biopharma team preparing for method validation needed to confirm the identity of peaks observed in their capillary isoelectric focusing (cIEF) assay. While the UV-based cIEF method delivered clean charge separation, the team lacked direct evidence of what each peak represented — raising concerns about the method's specificity and interpretability. To eliminate ambiguity and strengthen their analytical package, the customer turned to Solvias for cIEF-MS analysis.

THE CHALLENGES

- **Ambiguous Peak Assignments:** UV detection provided separation but no molecular identity — leaving open questions about charge variants, degradation products, or fragments.
- **Risk to Method Confidence:** Without knowing what each peak represented, the analytical team faced challenges in defending the method's specificity and robustness — potentially raising concerns during validation or regulatory review, especially given the role of charge variants as a critical quality attribute.
- **Complex Variant Profile:** Observed peaks could include deamidated species, low-level fragments, or other charge-related post-translational modifications (PTMs).

OUR COLLABORATIVE APPROACH

- **Partial Filling cIEF Coupled to MS:** Solvias applied a partial filling technique — where the capillary is only partially loaded with focusing reagents — to maintain charge separation while preventing non-MS-compatible buffers from entering the mass spectrometer. This enabled clean hyphenation of cIEF with high-resolution MS without compromising peak resolution.
- **Direct Peak Characterization:** Using high-resolution mass spectrometry, each charge-separated species was identified based on its intact mass, allowing confident assignment of variants such as deamidated forms, clipped products, and glycoform-related shifts. This approach was applied to an IgG1 molecule but is fully transferable to other monoclonal antibodies, offering broad utility across mAb programs.
- **Comparison of UV vs. MS Profiles:** UV and MS data were reviewed in parallel. While UV alone showed a consistent peak pattern, MS revealed the actual molecular identities underlying each signal — including distinctions between intact 2K, 1K, and 0K species, deamidated variants, and low-abundance fragments — adding critical interpretability to the charge profile.

THE RESULTS

- Each cIEF peak confidently assigned to a specific variant.
- Method robustness and validation supported with structural evidence.
- Customer advanced toward GMP implementation with fewer unknowns.

CONCLUSION

By integrating cIEF with mass spectrometry, Solvias helped the customer elevate their method from separation-only to structurally defined—removing ambiguity and unlocking confidence in product characterization. This approach not only strengthened their validation package but also provided a durable analytical tool for future comparability, stability, and release testing efforts. Whether applied to IgG1 or other mAb formats, cIEF-MS delivers the resolution of electrophoretic separation and the molecular clarity of mass spectrometry—making it a powerful asset for biologics developers seeking both precision and depth.

WHY PARTNER WITH US?

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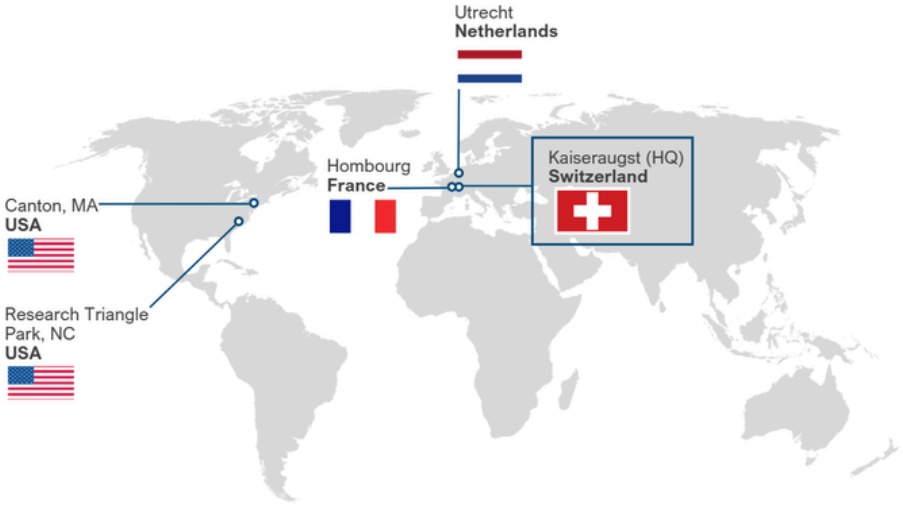
- Founded in 1999
- 800+ team members
- 175+ PhD-level scientists
- GMP, GLP, ISO9001 certified
- 23K m^2 of lab capacity
- 700+ customers worldwide
- 5 centers of excellence



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