
Case study

EVOSEP

# Alphalyse accelerates GMP-compliant HCP characterization by LC-MS using Evosep Eno

**Highlights**

- LC-MS based HCP analysis de-risks biologics development across the whole process pipeline.
- 2–3× higher sample throughput for routine HCP workflows.
- Evosep Eno provides the reproducibility, robustness, and uptime required for GMP laboratory operations.

HCPs are process-related impurities that can reduce the efficacy of a biologic drug, decrease its stability, or trigger unwanted immune responses in patients. Regulatory agencies classify HCPs as critical quality attributes (CQAs) and require rigorous monitoring and control of their levels in biopharmaceutical products.

Thomas Kofoed is CEO and co-founder of Alphalyse, an analytical CRO specialized in protein characterization using mass spectrometry. Developers of biologics — from monoclonal antibodies to recombinant proteins, vaccines, and cell and gene therapies — rely on the expertise of Alphalyse to gain insights into their products at preclinical and clinical stages.

"*We've developed a method that uses mass spectrometry to identify and quantify individual host cell proteins (HCPs) in client samples,*" explains Thomas, "*something highly valuable for biologics development.*"

> *"The Evosep Eno has increased our capacity and uptime while providing highly reproducible data. Using a single standardized LC method simplifies operations significantly, and after several months of running the system we have had no issues. We are very satisfied."*

**Thomas Kofoed**
CEO & Co-Founder,
Alphalyse

## The power of LC-MS in HCP analysis

While ELISA remains the gold standard for HCP quantification, its limitations have shifted the methodological spotlight to liquid chromatography–mass spectrometry (LC-MS).

LC-MS provides protein-level visibility, enabling more precise risk assessment and data-driven purification optimization. On the other hand, ELISA typically delivers a single "total HCP" value without distinguishing individual proteins or detecting protein modifications.

ELISA also relies on batch-dependent reagents that require bridging studies for long-term and high-throughput analyses. It often fails to detect low-abundance or weakly immunogenic proteins, as well as proteins structurally similar to the therapeutic molecule. As a sensitive physical measurement technique, LC-MS avoids these issues and maintains methodological continuity.

"*While LC-MS is not yet a formal requirement, regulatory interest has increased significantly,*" says Thomas. "*The trend is clear: regulators are moving in this direction.*"

This trend is reflected in the publication of USP Chapter 1132.1, "*Residual Host Cell Protein Measurement in Biopharmaceuticals by Mass Spectrometry.*"

Analytical workflows at Alphalyse identify and quantify hundreds of individual HCPs — often with >95% coverage— detecting problematic proteins that ELISA may miss and reporting properties such as molecular weight and the isoelectric point (pI) that support data-driven process development.

## Added value of LC-MS-based HCP analysis compared to ELISA

**Granular quantification**
Identifies and discriminates individual HCPs comprehensively with >95% coverage.

**Greater sensitivity**
Detects low-level, drug-similar, and weakly immunogenic proteins that ELISA misses.

**Richer information**
Provides protein properties that inform process optimization.

## Operating where GMP is not negotiable

Offering customers high-quality analytics that span the complete development lifecycle — from preclinical studies to commercial manufacturing — requires GMP-level operations.

Thomas makes no compromise: "*We must run fully qualified, validated instruments under GMP.*"

Three aspects are essential to this mandate:

1. Instruments must be robust and generate reproducible output.
2. Instruments must be qualified according to USP Chapter <1058> Analytical Instrument Qualification.
3. Maintaining high-capacity operational continuity must be straightforward and non-disruptive.


## Evosep standardization drives reproducibility at scale

Alphalyse adopted the Evosep Eno to scale HCP analyses while preserving robustness and reproducibility in a GMP-qualified environment.

Evosep's standardized LC-MS methods facilitate method transfer and consistent results across instruments and projects, a prerequisite for high-volume analytical services.

**With the Evosep Eno integrated into their HCP analysis, Alphalyse now:**

**Processes 2–3× more samples per mass spectrometer**

**Achieves 200% longer analytical column lifetime**

**Operates with 30% higher instrument uptime**

The streamlined workflow also optimizes instrument utilization.

"*We operate as a production site with significant sample throughput, so downtime is costly,*" says Thomas. "*The Evosep Eno has increased our capacity and uptime while providing highly reproducible data. Using a single standardized LC method simplifies operations significantly, and after three months of running the system we have had no issues. We are very satisfied.*"

Thomas' team reports a two- to three-fold increase in throughput per mass spectrometer, with stable retention times that underpin consistency across projects.

Marcella Nunes de Melo-Braga, Project Manager in the Product Characterization team at Alphalyse R&D, highlights the day-to-day robustness of the system: "*We've seen improvements in sensitivity and in column lifetime thanks to the tip-based separation of Evosep. The system also provides stable retention times.*"

## Evosep reliability is based on meticulous IQ/OQ

All LC-MS systems at Alphalyse undergo Installation Qualification (IQ) and regular Operational Qualification (OQ) to support GMP-compliant processes.

The IQ/OQ Service for Evosep Eno uses calibrated, traceable tools for an MS-free full-system evaluation, documented in a tamper-proof report. This qualification ensures data traceability and reproducibility, facilitating auditing and troubleshooting — essential requirements for production-style proteomics analyses.

Marcella emphasizes the importance of a reliable analytical environment: "*Reliable results require reliable instruments. A qualified environment ensures that the data we produce meets the necessary standards, and when issues arise we can quickly determine whether they stem from the instrument or the sample itself.*"

> *"Evosep Eno delivers reproducible results, is easy to operate, and supports a smooth workflow. It has made our daily work more efficient and reliable."*

**Marcella Nunes de Melo-Braga**
Project Manager, Product Characterization, Alphalyse

Thomas adds: "*When clients know our instruments are qualified and routinely audited, they trust the data they receive from us. That confidence helps our customers move from one development phase to the next more efficiently.*"

As regulatory agencies increasingly request LC-MS data to complement ELISA results, developers working with Alphalyse rely on audit-ready datasets and documentation to reduce risk and streamline approvals.

## Evosep design and support simplify the workload

Just as important as qualified performance and workflow standardization, the Evosep Eno is simple to install and operate.

"*Our experience has been very positive,*" says Marcella. "*The system is straightforward to use, and the qualification process performed by the technicians was smooth. Having documented proof that the instrument meets the required criteria gives us confidence in all subsequent analyses.*"

She also highlights the system's practical usability: "*The diagnostics features help us resolve issues independently when needed.*"

Through online troubleshooting tools, remote diagnostics, and guided service workflows, around 90% of support cases are resolved without an on-site visit, reducing downtime and making operating costs highly manageable.

## From lab bench to industry: Driving adoption of LC-MS

With standardized methods, full IQ/OQ qualification, and consistent sample separation, Evosep Eno eliminates bottlenecks in daily laboratory workflows.

*"The system delivers reproducible results, is easy to operate, and supports a smooth workflow. It has made our daily work more efficient and reliable,*" says Marcella.

Beyond laboratory efficiency, LC-MS-based HCP analysis is becoming an increasingly important tool for biotherapeutic quality assessment and patient safety.

As regulatory interest grows and developers increasingly rely on LC-MS for protein-level characterization of HCPs, Alphalyse is helping drive a shift toward production-scale LC-MS analytics in a verified GMP environment.

Their mission to deliver high-coverage, audit-ready proteomic data aligns with Evosep's commitment to providing reliable instrumentation and service solutions that simplify workflows and enable robust analytical results.

The result is dependable, comprehensive data that helps advance biologics development quickly, confidently, and safely.

**About Alphalyse**

Founded in 2002 and headquartered in Odense, Denmark, Alphalyse provides LC-MS–based HCP impurity analysis and drug product characterization to an international client base ranging from small biotech companies to large pharmaceutical organizations, CROs, and CDMOs across preclinical, clinical, and manufacturing phases.


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