

Case Study

Biosimilar Insulin Aspart Development



Rapid Biosimilar Development: Inotiv Helps Xentria and Its Partners Achieve Submission of Insulin Aspart Biosimilar One Year Ahead of Schedule

BACKGROUND

At the beginning, pharmacokinetic (PK) and pharmacodynamic (PD) method development testing was conducted at Inotiv's Biotherapeutics Center of Excellence in Rockville, MD. This GLP- and GCLP-compliant facility is a recognized leader in non-mass spectrometry, non-chromatographic methods.

The lab's work spanned PK testing, immunogenicity assessments, biomarker analysis, with techniques such as immunoassays (MSD, ELISA, Luminex), flow cytometry and genomic analysis. For this project, their specialized expertise was essential as it played a pivotal role in ensuring rigorous analytical support for the project.

PROJECT OVERVIEW

Inotiv engaged with the biotherapeutics company Xentria, Inc. (Xentria) on a major undertaking to develop and validate analytical methods for a biosimilar insulin aspart, a fast-acting insulin analog designed to help people with type 1 and type 2 diabetes. The work scope ranged from developing multiple methods to analyzing clinical samples and running detailed statistical comparisons against the original reference drug.

In just 12 months, Xentria and Inotiv executed all clinical, analytical, and statistical phases of the project -- an exceptional pace that enabled the regulatory submission for the biosimilar to be submitted to the FDA a full year ahead of schedule.

Speed mattered, with global demand for insulin analogs on the rise and access to diabetes treatments more crucial than ever. Xentria in collaboration with its partners took on this initiative with a clear mission: to make insulin more affordable and expand the availability of biosimilars for people living with diabetes worldwide.



CHALLENGE

STUDY OBJECTIVE

The goal of this work was to develop and validate methods for measuring PK and C-peptide – the key pharmacodynamic (PD) marker – for the biosimilar aspart. While the immediate focus was on bioanalytical support, the work was designed with future applications in mind, including potential support for the insulin products Glargine and Lispro.

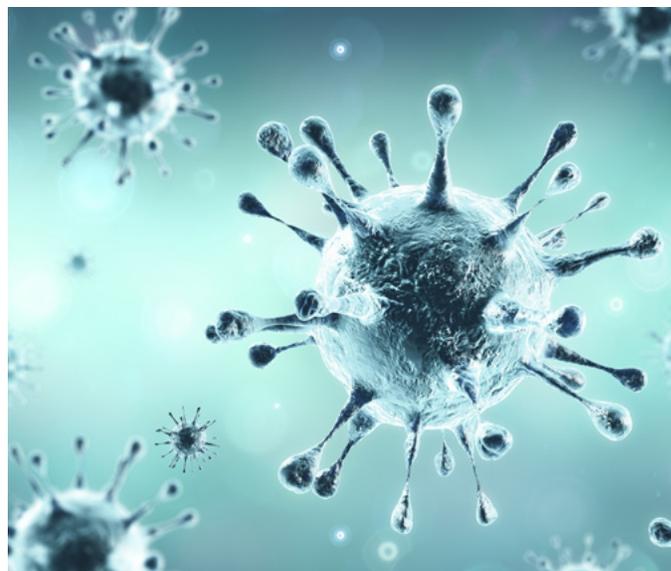
A clinical trial was conducted in a specialized euglycemic clamp unit in Bloemfontein, South Africa. Biological samples from the trial were then exported to Inotiv labs in the US for in-depth analysis, where they underwent rigorous analytical and clinical validation. The study provided a thorough evaluation of biosimilar aspart, with detailed side-by-side comparisons against the reference products – both the US and EU Prolia versions.

COURSE CORRECTION

Initially, Inotiv targeted the use of antibody-based ligand binding assays, to measure both insulin aspart and a C-peptide. However, the capturing antibody for aspart turned out to be fully cross-reactive with human insulin. This made it impossible to distinguish between the two. Attempts to find or create aspart-specific antibodies on the market were also unsuccessful.

Those limitations forced the team to consider alternative analytical methods. One option was to measure total insulin (human plus aspart) using the cross-reactive antibody, then run a human-specific insulin test and subtract the results. While this was an FDA-acceptable approach, it was not the most specific.

Ultimately, the team chose LC-MS/MS for its high specificity and strong regulatory track record. That meant transferring the insulin aspart PK analysis to Inotiv's West Lafayette, IN facility, a LCMS Center of Excellence. This GLP/GCLP-compliant facility has extensive experience in both mass spectrometry and chromatography spanning 50 years.



Inotiv's methodology first addressed peptide interference by using an immuno-capture extraction with bead-bound anti-human insulin antibodies, followed by acid dissociation to release the insulin peptide. From there, chromatography and mass spectrometry were performed, using stable isotope labeling for internal standards.

Adding to the complexity, the analysis had to support tripartite comparisons by evaluating the biosimilar alongside both US and EU reference products.

A NOVEL ASSAY DEVELOPMENT

In developing the LC-MS/MS assay, Inotiv selected a mass spectrometry-based hybrid methodology. This was done in response to the lack of antibody specificity typically required for traditional approaches.

The integrated workflow consisted of several key steps with a statistical validation approach incorporated throughout the process to ensure rigor and reliability:

- Initial ligand binding assay used for pre-capture
- Immuno-capture isolation to enrich the analyte of interest
- Chromatographic separation and MS-based quantification

While the methodology was direct, the validation stage was not. The lab had to run equivalence tests on calibration curves and quality controls. And because Inotiv was dealing with a biosimilar against two different reference products, it had to go through re-validation and triple testing to make sure the assay performed reliably and allowed fair comparisons across products.

Throughout, innovation was a central theme to the development work. Even though at the outset Inotiv started with published methods for other peptides, it had to make substantial adaptations and custom tweaks for this application.

The result was something that borrowed from earlier work in concept but was truly novel in execution. To validate the outcome, Inotiv ran a similar process for the Insulin Glargine study as well, which helped show just how adaptable and advanced this assay design was.

CLINICAL CLAMP STUDIES

The clinical testing took place at Farmovs in Bloemfontein, South Africa at one of the world's largest and renowned euglycemic clamp study units. In operation since 1977, Farmovs has built an impressive track record with roughly 70 to 100 clamp studies to date. They also combine the technical capability with full audit clearance from major regulators, including the FDA and the EU.

The study team used the dual-arm method, with highly trained staff assigned to each patient to make sure the highest standards of care are maintained. During the blood sampling phase, samples were taken in precisely timed five minute intervals, supported by a team of five to six trained staff per patient. This meticulous setup played an immense role in maintaining testing integrity and accuracy.

ANALYTICAL EXECUTION AND TIMELINE OPTIMIZATION

The study was designed with parallel enrollment across six arms, which enabled rolling sample analysis. This prevented an analyzation crunch of approximately 2,600 samples taken by the study's end. To keep variability low, samples were processed at the individual subject level.

Resources were applied and streamlined by assigning two Principal Investigators, running two instruments in parallel while doubling up the analytical staff, and adding regular weekend shifts. On top of that, daily and weekly team meetings kept everyone aligned on scientific decisions, quality assurance, quality control, and reporting. Visual execution plan maps made it easy to track progress and adjust resources as needed.

The result was speed. Inotiv cut the usual three-to-five-month validation window down to just 2.5 months for three full validations, a 70 percent drop in method development and validation (MDV) time.

ASSAY IMPLEMENTATION

C peptide analyses were performed in Inotiv's Biotherapeutics Center of Excellence in Rockville, MD, using commercial kits validated according to FDA Guidelines. When C-peptide is measured during Clamp studies, the main goal is to accurately assess endogenous insulin secretion, compared to the insulin administered externally. Several different commercial kits were assessed to make sure the results matched the well established reference ranges seen in healthy individuals.

The data from these assays lined up closely with the aspart profiles and the findings from the Clamp study, adding another layer of confidence that all the methods used in the different laboratories were solid and that the results could be trusted for clinical evaluation.

“The result was speed! Inotiv’s ingenuity reduced the MDV time by 70%.”



OUTCOME

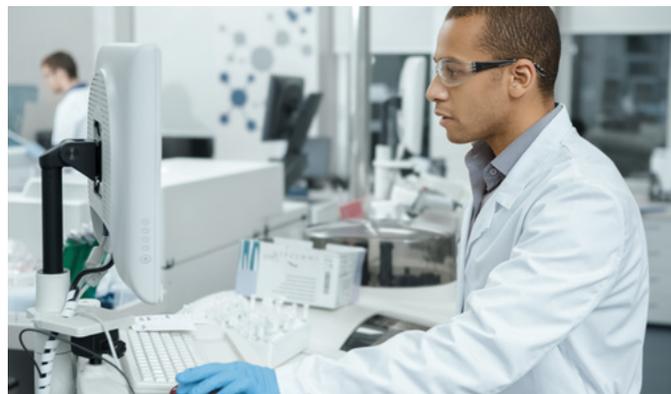
RESULTS AND OUTCOMES

Inotiv delivered standout pharmacokinetic (PK), clinical, and bioanalytical results. The data showed that insulin aspart biosimilar measurements for all three products consistently landed within the regulatory acceptance range of 80 to 125 percent.

This level of performance was made possible by developing a novel, validated method for aspart quantification—one with high specificity and selectivity, ensuring accurate and reliable measurement of the biosimilar’s properties.

For Xentria, the impact was substantial. The combination of strong scientific data and robust analytical methods meant they could submit their biosimilar application ahead of schedule, potentially accelerating their time to market. Just as important, the validated, reproducible bioanalytical method solidified both the scientific and regulatory strength of the submission.

On a larger scale, this work focused on a major global healthcare priority: making insulin more affordable. By supporting the development of accessible biosimilar options, the project aimed not only to advance public health goals for cost effective diabetes care but also to potentially expand treatment access for patients worldwide.



LESSONS LEARNED AND STRATEGIC APPROACHES

Inotiv took away important lessons from this project that will shape how it approaches similar work in the future.

Scientifically, pairing hybrid mass spectrometry (MS) with immuno capture strategies proved practical and worthwhile, especially in situations where non cross reactive antibodies were unavailable.

Team structure turned out to be just as essential. Cross department collaboration facilitated the flow of scientific ideas, while flexible staff assignments and backup instrumentation helped maintain operational efficiency.

Success came down to careful planning and agility. The front loading of planning efforts, using rolling sample analysis, and maintaining strong teamwork throughout paid off.

The result: a dramatic cycle time reduction with faster results delivered.



CONCLUSION

Inotiv’s laboratories showcased speed, innovation, and cross disciplinary teamwork to keep all aspects of the project on track and at the highest quality. The study stands as a strong example of how scientific creativity, effective operational project management, and robust clinical infrastructure can come together to address urgent healthcare needs while accelerating the development of biosimilar drugs.