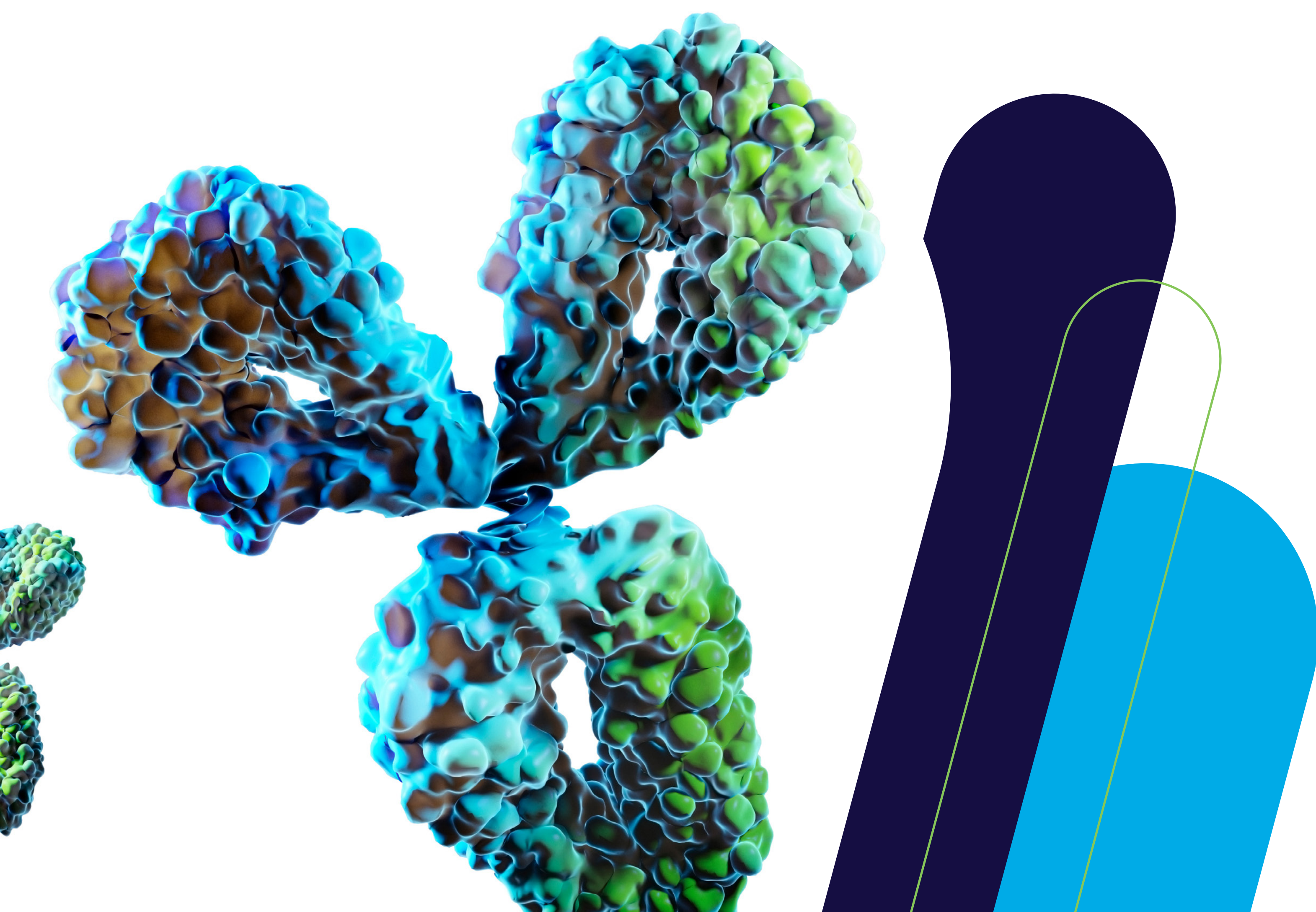
IQVIA LABORATORIES

Insight Brief

Bridging The Ligand Binding Assays And LC-MS Disciplines For Hybrid Assays



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Assessing the pharmacokinetics of biologics presents several analytical challenges, from the use of critical reagents and assay sensitivity requirements to regulatory and throughput considerations. At IQVIA Laboratories, we lead with science and in this insight brief, we'll look at all of those and more in context of combining immunoassays and LC-MS assays into a hybrid platform. The future possibilities that new frontiers in quantitative analysis of biologics may bring are also discussed.

Immunoassay vs. IA/LC-MS: Role of critical reagents

Immunoassay performance is entirely dependent on critical reagents because it requires a capture and a detection reagent. Those critical reagents can also affect IA/LC-MS methods, but typically not to the same degree. Whereas both assay formats involve a capture step, IA/LC-MS methods aren't dependent on the downstream binding of the analyte to the detection antibody. The IA/LC-MS method is specific for that signature peptide derived from the digested analyte, and less reliant on a capture antibody binding to a specific protein epitope. There are also other robustness aspects for IA/LC-MS methods that can improve quantitation, such as the use of a stable isotopically labeled protein level internal standard to normalize variability in the whole analysis process.

Validity of a bottom-up approach

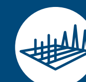
There has been much effort to measure large proteins by LC-MS in an intact state or with minimal fragmentation approaches that retain whole molecule information. Those methods haven't made their way into routine regulated bioanalysis at this point. The concern over using a peptide fragment as a representation of the intact protein with bottom-up methods is understandable. It's known that the measured concentration of a protein with a bottom-up strategy may depend on the selected signature peptide. The challenge is how to control for that. Careful selection of the peptide with respect to uniqueness and the lack of post-translational modification liabilities is important. We can also improve sequence coverage of the targeted measurement by following additional peptides for qualitative information where possible. That can increase confidence in our quantitative measurements, especially when those additional peptides can give information about the intactness of the protein. While an immunoassay may measure the target protein in its intact state, such a measurement doesn't give whole molecule information either as it's based on binding events. Ultimate confidence will come from intact protein measurements by LC-MS, when those methodologies become more mainstream.

Aggressive LLOQs: Immunoassay versus LC-MS

The sensitivity of immunoassays is limited by the quality of critical reagents. If you need an aggressive LLOQ and you are using the best critical reagents, you can try changing the Minimum Required Dilutions (MRD), different assay buffers and blocking reagents, and different immunoassay platforms. If those don't yield the required sensitivity, there are limited techniques left to achieve that LLOQ while ensuring that the assay is robust in accuracy and precision evaluations. One also needs to ensure that there are no matrix effects in each of the individual lots, both normal and disease.

One can either raise the LLOQ on the immunoassay platform or move to LC-MS detection. With LC-MS, there are more tools available to achieve those aggressive LLOQs. Sensitivity can be increased through low-flow chromatography and downstream use of an anti-peptide column as an antibody capture of the signature peptide we are following. Coupling this to high-resolution mass spectrometers that give better sensitivity through greater selectivity, one can achieve that ultimate sensitivity when it's needed.

In terms of the analysis itself, when a low LLOQ is required or there is a question on the quality of the critical reagents, it can be worthwhile to move directly to a hybrid LC-MS analysis at an early stage. When immunoassays fail to achieve the required LLOQ, that leads to shortened timelines for the customer who still needs an assay ready to run their samples. That takes up valuable time that can delay data delivery.



At IQVIA Laboratories, we have **SOPs specific for the validation and sample analysis for hybrid methods** based on the diverse experience of our **team comprising individuals from both the LC-MS and the immunoassay fields**.

Regulatory procedures for IA/LC-MS assays

From a regulatory standpoint, IA/LC-MS methods can be considered immunoassays that use a mass spectrometer as a detector. That makes them hybrid methods that span both immunoassay and LC-MS disciplines. Because of that, it's important to consider regulatory guidance for both when validating and using these methods for sample analysis. At IQVIA Laboratories, we have SOPs specific for the validation and sample analysis for hybrid methods based on the diverse experience of our team comprising individuals from both the LC-MS and the immunoassay

fields. Each SOP section should be reviewed and aligned with appropriate guidance. The experimental design and the acceptance criteria need to be applied based on the understanding of the regulatory guidance, the intent of the experiment, the familiarity of the method being validated, and the knowledge of both LC-MS and immunoassays.

Speed of analysis: Development, validation and run time

The development of an LC-MS assay can be done quickly, depending on the specific assay configuration. Often, when presented with a protein therapeutic assay challenge, it's because the immunoassay development efforts didn't yield the performance needed to support that program. That's usually related to the quality of the reagents. However, those same reagents may work perfectly well with a hybrid IA/LC-MS assay where more degrees of selectivity are available. Therefore, the long timelines that are associated with reagent generation or optimization can be eliminated. The exception to this is when using anti-signature peptide antibody enrichment when maximum sensitivity with an aggressive LLOQ is needed. Those reagents aren't likely to be commercially available nor are they likely to have been generated previously under an LBA assay format.

When reagents don't need to be generated, an assay can be developed from scratch quickly. However, the analysis time with LC-MS is longer than a ligand binding assay. Additional steps like denaturation, reduction, alkylation, and digestion all add time — sometimes days — to the sample preparation aspect. Chromatographic separation adds to the run time compared to LBA. Bringing in low-flow and multi-dimensionality in the liquid chromatography adds additional throughput challenges. However, it's possible to run a sample using three-column chromatography and 600 nL/per minute flow rates in 10 to 12 minutes, injection to injection, by optimizing routines.

Being successful in the hybrid space

Hybrid LC-MS methods incorporate elements of immunoassay and LC-MS platforms, of course, but also of underlying biology, chemistry, physics, proteomics, bioinformatics, etc. All of this is done under an umbrella of compliance to regulatory rigor. Success in LC-MS analysis of biologics requires a diverse team. One person or even a few can't cover all aspects of this. IQVIA Laboratories hires teams with a breadth of expertise. They are critical to tackle complex biological and analytical problems with a comprehensive approach, and ultimately to help advance new therapies with confident, careful measurements.

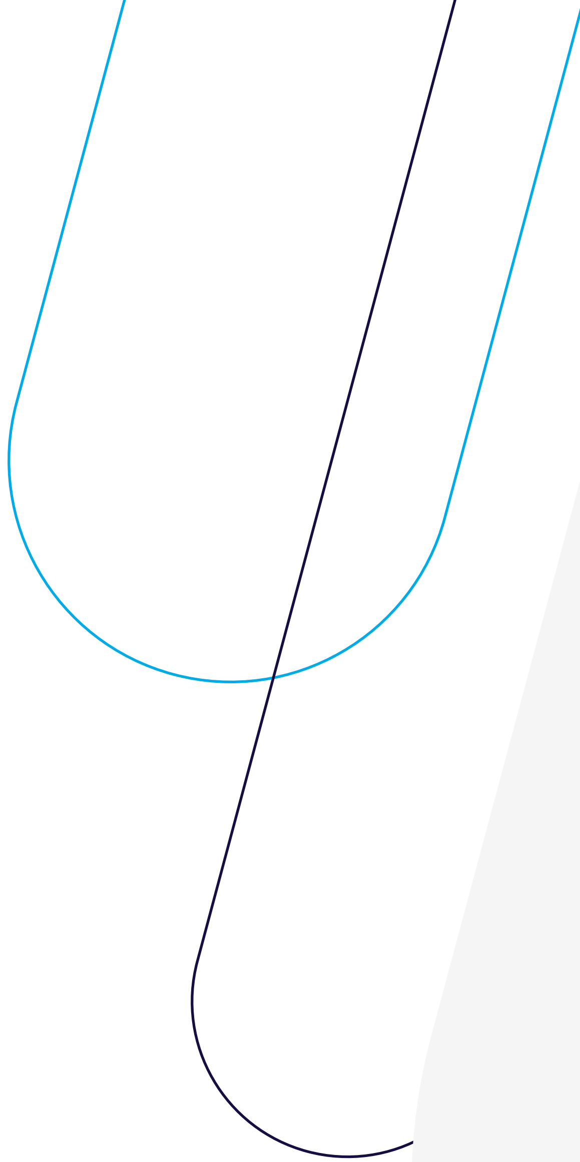
Future directions for biologics

IQVIA Laboratories is currently working on an Anti-Drug Antibody (ADA) by LC-MS method. We see value in one method that can screen, confirm, and measure the level of ADA and even isotype if needed. Developing and deploying one method that can give all that information with a single analysis of the sample, as opposed to three or more analyses using the immunoassay platform, comes with significant challenges. Based on published industry research, the main challenge among many for LC-MS immunogenicity methods is nonspecific binding of Human Ig to the beads. Using our experience of running an ADA by LC-MS assay in

the past and our overall expertise with hybrid IA/LC-MS methods and immunoassays, we are able to mitigate nonspecific binding of Human Ig to the beads. We are currently proceeding with further evaluation of the LC-MS immunogenicity method, incorporating the positive control to assess specificity, drug tolerance, and mitigating target interference.

In terms of the chromatography, there are promising options at both ends of the spectrum. The dimensionality of low-flow LC configurations provides options for those applications where ultimate sensitivity is required. IQVIA Laboratories continues to investigate new approaches to improve robustness and throughput. We have used affinity chromatography and size exclusion chromatography separately in conjunction with reverse phase chromatography, and there are more combinations to explore.

On the other end of that spectrum, where bioanalytical applications don't require high sensitivity, is it possible to improve existing LBA assay formats using mass spectrometry without liquid chromatography? Some will question the acceptance of a regulated PK assay without LC, but this is done with immunoassays all the time. Could replacing the detection antibody in a typical ligand binding assay with a mass spectrometer — using a quick analysis approach like acoustic ejection Mass Spectrometry (AEMS), or Matrix-Assisted Laser Desorption Ionization (MALDI-TOF) — add value to an immunoassay by providing additional degrees of selectivity without chromatographic separation? That's an avenue we believe is worth exploring.



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