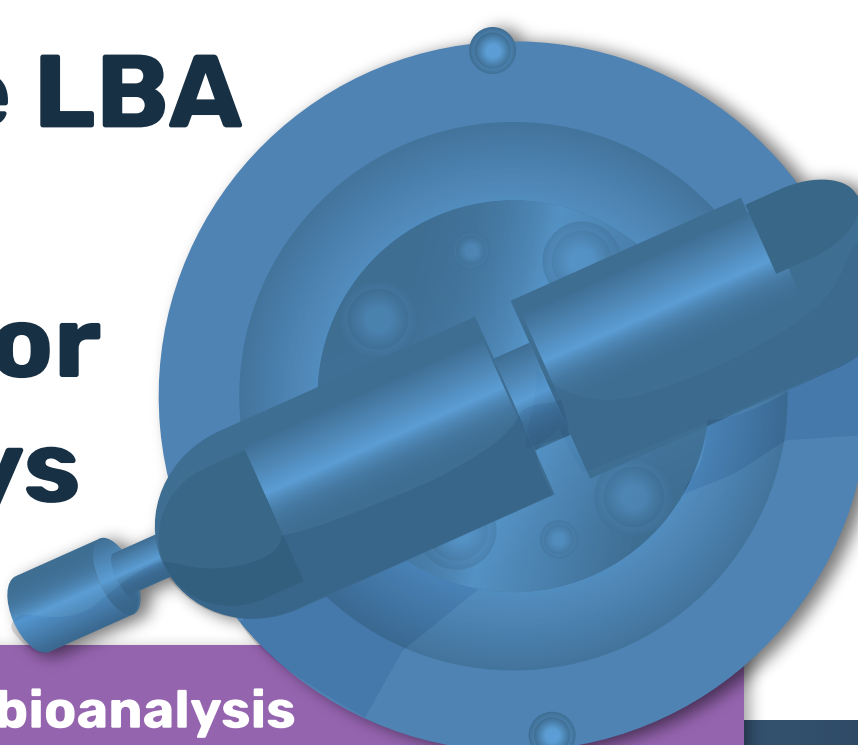


# Bridging the LBA and LC-MS disciplines for hybrid assays



## Becoming bilingual in bioanalysis

The increasing complexity of large molecule therapeutics has driven the need for additional approaches to quantitation in regulated bioanalysis where challenges with traditional **ligand binding assays (LBA)** are encountered for PK assay establishment. Hybrid immunoaffinity/**mass spectrometry (IA/MS)** techniques have been able to provide solutions such as improved selectivity, sensitivity, and the ability to multiplex measurements. This combination of techniques offers the advantages of specific antibody-antigen interactions together with the selectivity provided by the multiple dimensions of separation with **liquid chromatography and mass spectrometry (LC-MS)**.

**Ligand binding assays**

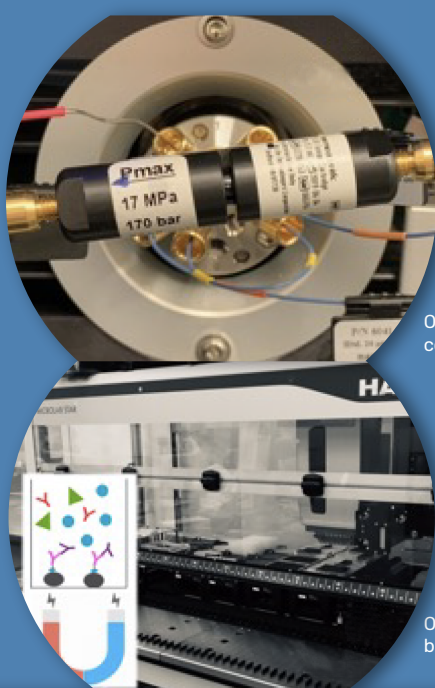
**Industry-leading LBA/LC-MS assay expertise**

**LC-MS assays**

## Hybrid approaches

Due to the emergence of hybrid IA/**MS** approaches, the boundaries between immunoanalytical and **LC-MS** assays continue to blur in the application to **biologics** and large molecule biomarkers. However, this is not without scientific challenge. At Q² Solutions, we've been addressing these challenges for 13+ years by embracing novel technologies and techniques with a diverse team, accumulated experience, and a focused effort. The convergence of **ligand binding** sample purification with **MS** detection for hybrid IA/**MS** large molecule quantitation begs research teams with a wide breadth of experience.

**Modes of immunoaffinity for hybrid IA/MS at Q² Solutions**



Online column-based

Offline bead-based

The first selectivity dimension of a hybrid assay is the immunoaffinity capture of the target analyte. At Q² Solutions, we leverage two approaches to immunoaffinity purifications: bead-based and column-based. Our offline magnetic bead-based approaches utilize automated liquid handling robotics (Hamilton STAR) for protein and peptide analyte purification. Our column-based, inline approaches are used for ultimate sensitivity/selectivity applications and are incorporated into multidimensional nano-LC assays.



**Adriane Spytko**  
 14 years of LBA industry experience



**Barry Jones**  
 17 years of LC-MS industry experience

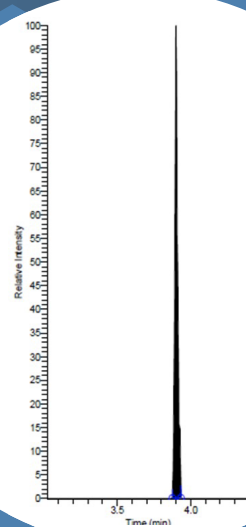
**16 years of combined hybrid IA/MS experience**

## Regulations in the hybrid space

The lack of regulatory guidance furthers the call for breadth of bioanalytical experience applied to these assays. Thoughtful creation of standard operating procedures that are considerate of existing guidance for both chromatographic and **LBAs**, as well as relevant industry White Papers on the hybrid assay science, is critical to guide scientifically sound practices in the absence of prescriptive regulations. This extends to method development approaches as we learn from the experience within our teams, sponsors, and collaborators. An understanding of the underlying biology of the disease and the therapeutic mechanism-of-action directly translates to the details of the assay, much more so than historical small molecule method development.

## Case study

One of the early challenges we encountered when applying LC separation and **MS** detection for large molecule quantitation was the need for sensitivity not easily achieved with standard approaches. Low-flow chromatography can drive the needed sensitivity for challenging applications, and **HRMS** can provide added selectivity and often further sensitivity. Both bead-based and column-based immunoaffinity techniques also add options to tailor the technology to the challenge. The tools are available to solve these problems, however the resulting assay complexity requires discipline in execution. Here again, the breadth and depth of experience in an interactive team is critical for success of production mode assays.



**Human plasma sample spiked with 100 pg/mL of a bispecific antibody therapeutic and assayed using immunoaffinity-based extraction, trypsin digestion, nano-LC, and HRMS.**

At Q² Solutions, we continue to encounter applications where hybrid IA/**MS** assays can solve bioanalytical challenges to advance our clients' programs where either platform alone cannot. There are many opportunities in the future to leverage the technological advances being made in both **LBA** and **LC-MS** technologies that will make the hybrid platform more approachable, powerful, and elegant.

This infographic has been created as part of a Bioanalysis Zone feature in association with Q² Solutions.