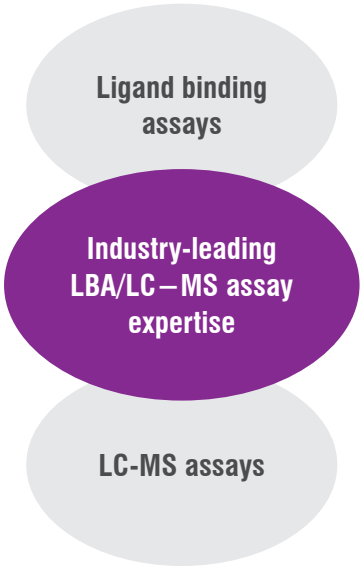
Q² Solutions®

Turning Hope Into Help™

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 in the establishment of PK assays. Hybrid immunoaffinity/mass spectrometry (IA-LC-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).



Hybrid approaches

Due to the emergence of hybrid IA-LC-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 challenges. At Q² Solutions, we've been addressing these challenges for 30+ 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-LC-MS large molecule quantitation demands research teams with a wide breadth of experience.

Modes of immunoaffinity for hybrid IA-LC-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.

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.

50+ years of combined of combined hybrid IA-LC-MS experience

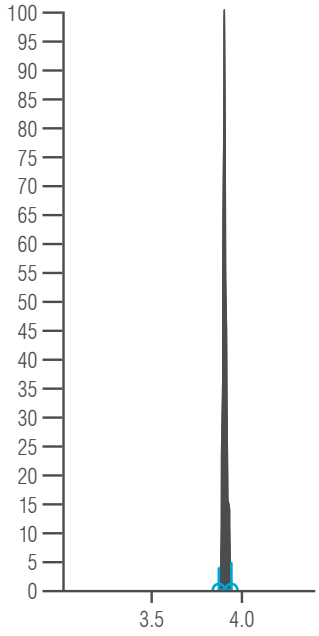
Adriane Spytko
16 years of LBA industry experience

Steve Lowes
35 years of LC-MS industry experience

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, which was 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.

At Q² Solutions, we continue to encounter applications where hybrid IA-LC-MS assays can solve bioanalytical challenges to advance our clients' programs. 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 platforms the bioanalytical strategies of choice.



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.

Contact us

US: 1-833-793-5298
UK: +44 800 002 9832
Email: Bioainfo@Q2labsolutions.com