Intact protein LC–MS for pharmacokinetics

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“Like small-molecule LC–MS assays, intact protein LC–MS assays bring the intact molecule to the mass spectrometer, and absolute quantitation can be performed based on the intact drug.”

Biography: John Kellie is currently a GlaxoSmithKline (GSK) fellow in the Bioanalysis, Immunogenicity, and Biomarkers group at GSK. John received his B.Sc. in Biochemistry from Indiana University (USA) and his PhD in Chemistry from Northwestern University (USA) studying under Dr Neil Kelleher. He was a post-doctoral scientist at Eli Lilly and Company, where he developed methods for intact protein quantitation of a Parkinson's Disease biomarker from human brain tissue. At GSK, John utilizes mass spectrometry for development of novel bioanalytical methods for biotherapeutic and protein quantitation from pre-clinical and clinical samples, with a focus on intact protein and large mass quantitation for pharmacokinetics, catabolism, biotransformation and product quality attribute support. John Kellie speaks to the *International Journal of Pharmacokinetics* about intact protein LC–MS for pharmacokinetic application.

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A number of recent papers have demonstrated absolute quantitation based on intact antibody therapeutic [1–3]. Examples can also be found looking at antibody-drug conjugates and biotransformation at both the intact and subunit levels [4–7]. Intact protein LC–MS has distinct differences and advantages compared with other assay formats, but is not without its challenges and obstacles. John touches on those points and more in the following interview.

Please introduce yourself & your experience working on pharmacokinetic studies

At GSK, I first worked on LC–MS methods for quantitation of therapeutic peptides and monoclonal antibodies and investigated high-resolution mass spectrometry (HRMS) for peptides and proteins in DMPK. I soon began to think about a ‘top-down’ approach for pharmacokinetics, similar to my work in graduate school on proteomics approaches for intact proteins. Proteins are inherently dynamic, complex molecules and protein digestion makes a mixture even more complex. In digestion, information about the intact molecule is lost. A peptide is easier to detect by mass than an intact protein – but that is really the only advantage of peptide-based MS methods. That's why the current bioanalysis landscape for MS favors digestion – peptide analysis was more feasible than protein analysis in the early days of large-molecule LC–MS for pharmacokinetics.

Recent progress in immunocapture assays and HRMS systems has enabled intact protein MS for biotherapeutics in plasma/serum. A small field of research has emerged, investigating what can be learned by detecting a circulating biotherapeutic via the ‘top-down’ philosophy: intact mass pharmacokinetics, mass variant quantitation, antibody subunit biotransformation and drug-to-antibody ratio determination, just to name a few. Perhaps the most interesting aspect is that intact protein MS does not have to replace existing assays, but rather can serve as another analytical tool that can complement established methods.

Can you briefly summarize the conventional bioanalytical methods used for pharmacokinetic studies?

I typically consider bioanalytical methods by molecule class, where small-molecule pharmacokinetics is achieved by LC–MS and large-molecule pharmacokinetics can be achieved by ligand-binding assay (LBA) or LC–MS. For

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small molecules, LC–MS analysis is usually achieved on a triple quadrupole (QQQ) MS. For large molecules, LBAs are based on capture and detection antibodies and LC–MS is usually achieved by use of a digestion step to generate a surrogate peptide for quantitative monitoring. LBAs have traditionally been the most sensitive, most widely used, and highest-throughput assays for large molecules. In the last decade, hybrid LC–MS methods that employ immunocapture followed by digestion-based LC–MS analysis have progressed impressively to rival sensitivity of LBAs. Hybrid methods for monoclonal antibodies typically employ the digestion step; however, intact protein LC–MS assays have emerged that utilize the immunocapture step to purify the protein prior to LC–MS.

Similarities can be found between intact protein LC–MS and traditional assay formats. Like small-molecule LC–MS assays, intact protein LC–MS assays bring the intact molecule to the mass spectrometer, and absolute quantitation can be performed based on the intact drug. Like LBAs, intact protein LC–MS assays feature immunocapture and thus functional binding to the capture reagent/antibody.

Can intact protein LC–MS assays be held to the traditional pharmacokinetic assay performance standards?

I think so, although intact protein LC–MS may need separate categorization from LBAs and small-molecule LC–MS. Owing to commonalities with other assay formats, it may be tempting to categorize assays as 'LBA' or 'LC–MS'. However, LBA versus LC–MS implicates different acceptance criteria based on previous regulatory positions. Furthermore, intact protein LC–MS data has inherent complexities arising from the many charge states generated during ESI (and to a lesser degree, multiple isotopes within a charge state).

From practical experience, intact LC–MS assays perform as well as LBAs in terms of precision and accuracy. Whatever acceptance criteria are used for any given intact protein LC–MS assay, data should accompany a justification for the applied criteria.

Are there any variations in the sample results from intact LC–MS assays compared with other established formats?

I think the best way to address this question is from an aspect of study design. Examples can be found in the literature that may indicate intact LC–MS matches or doesn't match other formats – but coherence, or lack thereof, might be due to specific issues and not general differences in assay format.

For example, in comparing a direct digest LC–MS to a hybrid LC–MS assay, the difference in the capture step may lead to a fundamentally different molecular measurement. Since the purification is different, the background ions (and thus ion suppression during ESI) may also be different, possibly impacting MS measurement.

The most useful comparisons for any two bioanalytical assay formats are when the assay ranges, dilution factors and capture antibodies (if used) are similar/identical. Then, head-to-head comparisons can be made with the least risk. Importantly, good study design and proper method testing enables smart decision making based on the data. So, if the intact LC–MS result varies from the peptide LC–MS result, the conclusive outcome can be that the intact mass may change thus impacting sample concentration and not that different assay formats produce different sample concentration results.

What are the key challenges in the development of intact LC–MS assays for pharmacokinetics?

Different challenges for intact protein LC–MS may exist depending on the expertise of the researcher, application of the method, or time/budget restrictions.

First, HRMS and an established immunocapture LC–MS platform are critical steps for implementation of intact protein LC–MS methods. If a laboratory is not equipped with HRMS or has little experience with immunocapture, success will be difficult to achieve. Also, if a researcher is not experienced with large-protein MS data, interpretation and analysis of the raw data will be challenging. Applications such as absolute quantitation, DAR determination, or biotransformation/stability screening may face obstacles such as linear dynamic range, sensitivity, or suitable reference standards.

Currently, the most common challenge for groups running the assays is centered around data processing and software for analysis. HRMS protein data contains multiple charge states corresponding to a single mass and several publications have examined the options and optimal use of intact protein LC–MS data for absolute or relative quantitation. Furthermore, no vendor-agnostic software exists, leading to possible differences or inconsistencies in data processing practices depending on the manufacturer of the mass spectrometer used for data acquisition.

What emerging technologies are you particularly excited about, which are likely to change the future of pharmacokinetic studies?

Intact protein LC–MS for pharmacokinetics is still emerging and I think great progress will be made in the next decade – particularly as immunocapture platforms and HRMS systems improve with respect to sensitivity. Eventually intact protein LC–MS will progress to GLP application, and it will be interesting to see outcomes and learning from such a milestone.

Looking forward, native protein MS for pharmacokinetic applications holds a great deal of promise, especially if methods can be developed, which preserve native protein–therapeutic complexes. Target engagement, anti-drug antibodies and off-target binding could, theoretically, be assessed in a single assay, which could also inform intact mass or biotransformation. Progress in immunocapture of complexes, front-end separation approaches (such as hydrophobic interaction chromatography or size-exclusion chromatography) and extended mass range detection will continue to push native MS for pharmacokinetics forward.

Overall for the future, I expect we will soon have a variety of intact protein MS assays with different formats and applications that can answer a variety of key questions regarding circulating biotherapeutic, which I hope will lead to improved molecule selection as well as overall performance in the clinic.

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