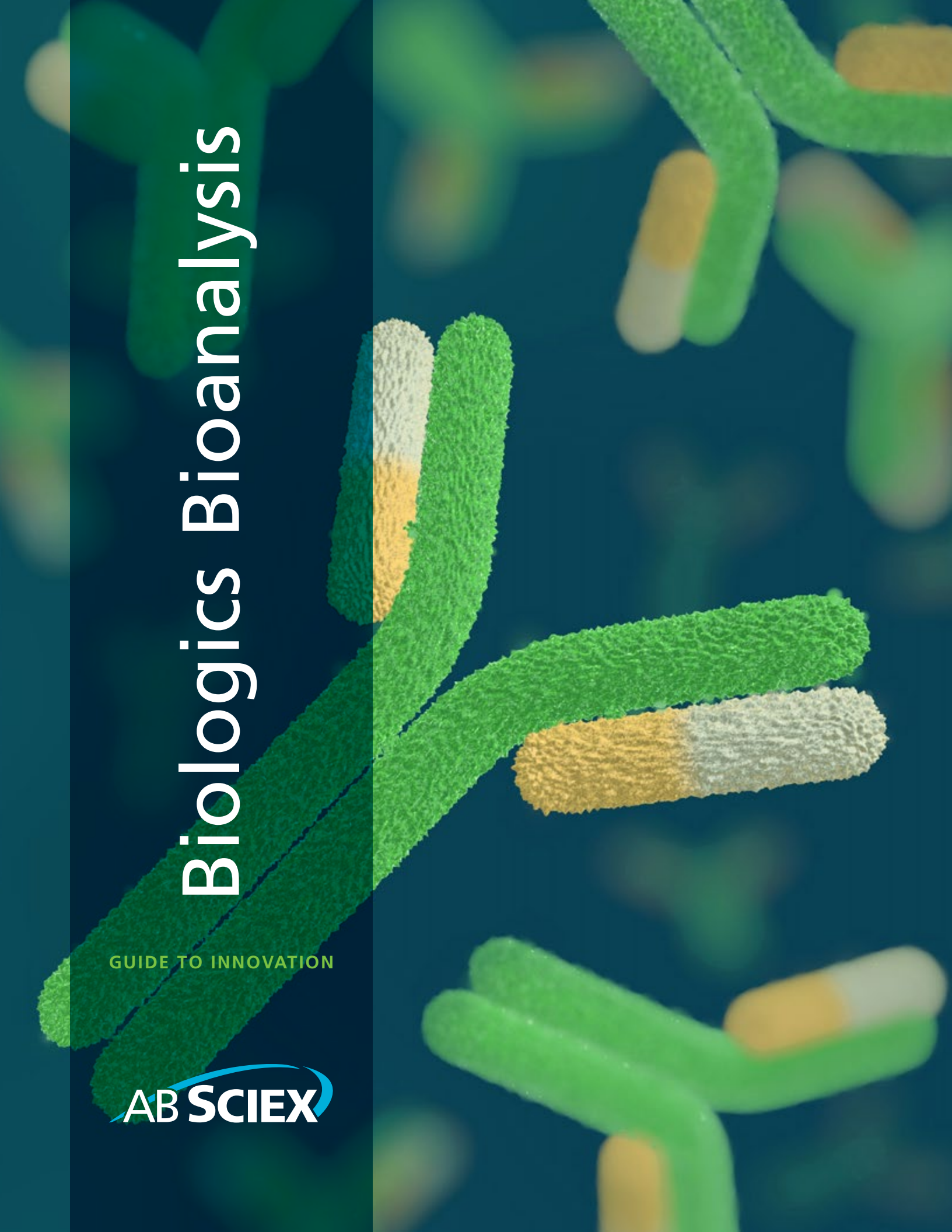
Biologics Bioanalysis

GUIDE TO INNOVATION



AB SCIEX

Biologics Bioanalysis – Guide to Innovation

Pharmaceutical companies have leveraged advancements in basic science perhaps more than any other industry. With the advent of whole genome sequencing, sophisticated analysis of metabolic pathways, and exponential improvements in computer processing, R&D organizations have expanded their drug portfolio focus on small molecules to encompass a new class of drugs — biotherapeutic compounds and biomarkers.

Helping customers by listening to their ideas, participating in discussions, and creating cutting-edge solutions to research challenges is top priority at AB SCIEX. The following compendium includes key solutions for peptide and protein bioanalysis — and, more importantly, describes in detail work done by, and in collaboration with, our customers. Your success is our success, and the AB SCIEX team will partner with you to overcome the emerging challenges of bioanalysis, now and into the future.

Joe Fox
Senior Director – Pharmaceutical Business

Application of Differential Ion Mobility Mass Spectrometry to Peptide Quantitation

Using SelexION™ Differential Mobility Separation Technology for better selectivity for peptides in complex mixtures on the AB SCIEX Triple Quad™ 5500 LC/MS/MS System

Improved Selectivity for the Low-level Quantification of the Therapeutic Peptide Exenatide in Human Plasma

MRM³ quantitation for highest selectivity in complex mixtures on the AB SCIEX QTRAP® 5500 System

Achieving Low-Flow Sensitivities for Peptide Quantitation using Microflow Rates on the QTRAP® 6500 System

High-throughput, sensitive, microflow analysis of bradykinin and other peptide standards with a hybrid triple quadrupole linear ion trap coupled with the Eksigent® ekspert™ nanoLC 425 System

A Sub-picogram Quantification Method for Desmopressin in Plasma Using the AB SCIEX TripleQuad™ 6500 System

A high-throughput method for detecting ultra-low levels (0.5 pg/mL) of a therapeutic peptide in human plasma using an AB SCIEX Triple Quad™ 6500 LC/MS/MS System and UHPLC Chromatography

Analysis of Biopharmaceutical Proteins in Biologial Matrices by LC-MS/MS II. LC/MS/MS Analysis

And many more! Coming soon...

Advances in the Bioanalysis of Protein and Peptide Therapeutics through Innovations in Mass Spectrometry

Overview of peptide and protein quantitation applications on the AB SCIEX QTRAP® System and the TripleTOF® System

Introduction

The importance of biotherapeutics as a class of drugs has increased significantly over recent years due to their enormous potential to treat a wide array of human diseases ranging from autoimmune and inflammatory diseases to cancer, cardiovascular diseases, and rare genetic disorders. These highly promising therapeutic agents, including very small peptide chains, such as insulin, up to much larger proteins, such as antibodies and novel Fc-like fusion proteins, are extremely attractive as drug candidates because of their low toxicity and high specificity, and these compounds continue to fill the pre-clinical and clinical pipelines of many pharmaceutical companies.

The rapid growth of biotherapeutics is a good indicator of its success, with the global market valued at around US$199.7 billion in 2013 and projected to grow by 13.5% through 2020. The number of clinically approved protein and peptide therapies has jumped to over 170 products with 350 antibody-based therapies currently awaiting clinical trials, making biotherapeutics the fastest growing class of drugs in the last decade. With increasing industry interest and investment and rising demand from the medical community for these unique, targeted therapies, there is a growing requirement to develop high-throughput analytical techniques to expand biotherapeutic product lines.

To overcome regulatory hurdles and advance to clinical trials, biopharmaceutical drug development and discovery requires metabolic monitoring of a candidate drug, a process which necessitates accurate quantitation during pharmacokinetic (PK), toxicokinetic (TK), bioequivalence, and clinical drug monitoring studies—all of which are conducted in a complex biological matrices (blood, plasma, or urine). With this rapid growth in biotherapies comes increased demand for an analytical platform that is flexible, robust, and is easily integrated into pre-existing drug development workflows. Widely used for small molecule drug development, liquid chromatography-tandem mass spectrometry (LC/MS/MS) has recently made a larger impact on bioanalysis applications due to recent technological developments in analyte detection. Presented here, we demonstrate how key mass spectrometry technologies from AB SCIEX can coalesce into straightforward, accurate, extremely sensitive, and, most importantly, high throughput quantitative solutions. Already considered as the preferred choice for quantitation in other areas of bioanalytical quantitation such as proteomics, anti-doping, forensics, and clinical chemistry, LC/MS/MS is poised to replace and outperform other techniques for biotherapeutic analysis.

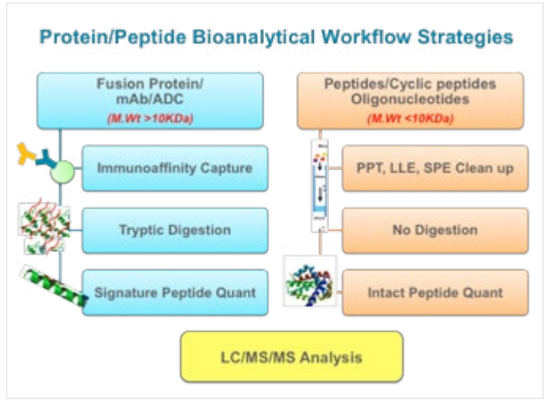


Figure 1. Peptide and protein bioanalytical workflow strategies. Protein quantitation typically involves a tryptic digestion step, which is omitted during intact peptide bioanalysis, thereby simplifying the process.

The current standard conventions for protein and peptide quantitation are based on ligand-binding assays (LBAs), such as the enzyme-linked immunosorbent assay (ELISA), or on UV detection of individual peptides using high pressure liquid chromatography (HPLC) separations. LBAs rely on immunoaffinity detection of a unique epitope on the protein or peptide of interest, and the high specificity of the antibody-based interactions can track an analyte at high sensitivity, although the dynamic range is narrowed to just one or two orders of magnitude. Because production of unique antibodies is lengthy, assay development can often be time-consuming and expensive; in addition, LBA results are often plagued by interferences and high background from antibody cross-reactivity. UV detection and quantitation of peptides is commonly used for peptide mapping, and this analytical method can be useful after extensive sample preparation and cleanup. UV detection with HPLC also does not require the expense and time commitment of antibody production, but the applicability of this method narrows as the complexity of the sample matrix increases.

Herein, we present an extensive resource on the quantitation of peptides using AB SCIEX mass spectrometry instruments, revealing how sensitive and selective detection can be achieved even in the presence of high background noise. To meet bioanalytical quantitative standards and assay validation parameters, peptide bioanalysis must be sensitive enough to meet the standard benchmarks for excellency in accuracy and precision. In Section 1, we explore the highest level of sensitivity by evaluating experiments conducted on the AB SCIEX QTRAP® 6500 System and the AB SCIEX Triple Quad™ 6500 System. Due to the inherent sample complexities, bioanalysis is often negatively impacted by high background noise and interfering peaks. Section 2 illustrates how realizing superb analyte selectivity—even in biological samples with numerous, highly abundant, endogenous proteins—is driven by innovative tools such as multiple reaction monitoring cubed (MRM^3) workflow and SelexION™ Differential Ion Separation Technology. Advances in high resolution mass spectrometry are detailed in Section 3, which highlights targeted workflows on the TripleTOF® 5600+ System that extend the sensitivity and selectivity of quantitative assays due to the narrow extraction widths and high resolution TOF data. Lastly, Section 4 investigates the software tools available for robust peptide quantitation workflows that give researchers intuitive tools to automate the complex, multi-step calculations for peak area quantitation.

Each section and experiment featured in this resource includes an overview of the key challenges, benefits, and features of the bioanalytical technique presented. In this way, the technique of mass spectrometry can be put into context with other bioanalytical tools and help provide insight into its many advantages. LC/MS/MS analysis offers many attractive features for supporting biopharmaceutical drug development; however, establishing LC/MS/MS in the biopharmaceutical workflow has been slow in spite of its dominance in the small-molecule laboratory. Widely accepted and easily validated, the LBA technique remains a popular method for protein and peptide bioanalysis due to its relatively lower investment in infrastructure and ease of implementation into the high-throughput environment. Yet, even LBA methods have their drawbacks, and straightforward LC/MS/MS alternatives are sought that can support the operational challenges of accelerating the further development of biotherapies.

Key challenges of peptide bioanalysis

To understand why the pharmaceutical industry has been hesitant to fully embrace LC/MS/MS strategies for peptide quantitation, the complexities and challenges of the workflow must be fully appreciated. (For a summary of excellent reviews on LC/MS/MS protein and peptide quantitation, please see Table 1.) For both proteins and peptide quantitation, standard calibration curves are used to calculate concentration values for unknowns in biological samples; in addition the amassed data must be stringent enough to meet the rigorous benchmarks prescribed by the USFDA. For therapeutic peptides, proteolysis is omitted, and the intact peptide can be directly quantitated by MS/MS after relatively limited sample preparation (Figure 1). There is appreciably much more complexity when evaluating larger molecular weight biotherapeutics (>10 kDa), which are not always suitable in their entirety for direct MS/MS analysis. Therefore, bioanalysis of larger proteins and antibodies is based on quantitation of a small portion of the protein, typically a tryptically digested signature peptide with a m/z ratio that is unique from all other peptides in the digest mixture. When coupled with a stable

Title	Article Highlights	Citation
"Analysis of biopharmaceutical proteins in biological matrices by LC/MS/MS I. Sample preparation"	• Sample-preparation aspects for quantifying biopharmaceutical proteins in body-derived fluids by LC/MS/MS • Enrichment at the peptide level after proteolytic digestion • Chemical derivatization of peptides for enhancing ionization efficiency • Automation of the entire analytical procedure for routine applications in pharmacokinetic and clinical studies	Bischoff R, Bronsema KJ, van de Merbel, NC. Trends in Analytical Chemistry. 2013; 48: 41-51.
"Analysis of biopharmaceutical proteins in biological matrices by LC/MS/MS II. LC/MS/MS analysis"	• Overview of selected reaction monitoring (SRM) strategies for quantifying peptides in biological matrices • Selection of signature peptides and internal standards • Selectivity improvements using MS^3 and differential mobility spectrometry (DMS) • Quantitative LC/MS analysis with low-resolution and high-resolution MS • Data-independent acquisition (DIA) for collection of all data in a single analysis	Hopfgartner G, Lesur A, Varesio E. Trends in Analytical Chemistry. 2013; 48: 52-60.
"Bioanalytical LC/MS/MS of protein-based biopharmaceuticals"	• Overview of topics relating to the bioanalysis of biopharmaceutical proteins in biological matrices • Compares alternative quantitative methodology, such as ligand binding assays (LBAs), to mass-spectrometry-based platforms • Review of practical aspects of the seven "critical factors" for protein sample preparation • Special focus on the quantitation of monoclonal antibodies in serum and plasma • Advances in selectivity, including high-resolution mass spectrometry	van den Broek I, Niessen WMA, van Dongen WD, Journal of Chromatography B. 2013; 929: 161-179.
"Liquid chromatography coupled with tandem mass spectrometry for the bioanalysis of proteins in drug development: Practical considerations in assay development and validation"	• Approaches for overcoming operational challenges due to complex sample preparation • Development and validation of a fast, simple, and reliable LC/MS/MS peptide quantitation method that fits into current pharmaceutical workflows • Recommendations for validating quantitative methods based on surrogate peptides	Liu G, Ji QC, Dodge R, Sun H, Shuster D, Zhao Q, Arnold M. Journal of Chromatography A. 2013; 1284:155-162.

**These review articles were reprinted with permission in the first 30 copies of this resource.*

Table 1. Selected citations for further reading on protein and peptide LC/MS/MS methodologies

isotope-labeled (SIL) internal standard, the response ratio of the signature peptide to the SIL internal standard reveals a concentration representative of the intact protein. To build this multifaceted process into the framework of regulated bioanalysis is extremely challenging in practice, which makes it easy to comprehend why LC/MS/MS quantitation of biopharmaceuticals has been slow to gain acceptance in the GLP laboratory.

Evaluation of LC/MS/MS bioanalysis reveals that the major challenges for accurate and precise quantitation lie primarily in the realm of sample preparation, which includes: 1) the lengthy and extensive workflows for producing signature peptides and 2) the diminishing accuracy of quantitative measurements in highly complex biological samples due to background interferences. Because the multi-step reduction/alkylation/digestion process generates a more complex mixture than the starting sample, bioanalysis of low-level therapeutic peptides can be extremely challenging. Achieving LLOQs in the low ng/mL range is highly dependent at this time on the optimization of sample preparation steps.[9] The numerous competing background peptides are a major consideration in sample preparation, which typically requires enrichment and semi-purification of the analyte that introduce additional complexity to the workflow. Of concern from a regulatory perspective is the potential for variable peptide release during digestion of the target protein, and if digestion conditions are not well-controlled or compensated for, then irregular signature peptide release can have a lasting impact on the overall data quality.[7] To overcome these drawbacks, strategies such as condensing sample prep steps and digestion optimization can lead to more straightforward method development with wider regulatory appeal. And to that end, as advances in technology deliver exceedingly more sensitive and selective mass spectrometry workflows for direct quantitation in the sub-picomolar range, sample prep protocols can be further streamlined and simplified, relying less on intricate sample enrichment and baseline reduction protocols, which will help propel this versatile and reliable MS methodology firmly into the domain of regulated biotherapeutic quantitation.

Summarized below are some of the current challenges of LC/MS/MS peptide quantitation:

- Limited quantitation range – Poor MS/MS sensitivity combined with often poor selectivity can compromise the desired lower limits of quantitation (LLOQ).
- Impaired sensitivity in complex matrices – Very low-level peptide detection (sub-pg/mL) can be suppressed by high background and competing ions in biological samples. The best, previously reported LOQ is 100 pg/mL, which is insufficient for extended-release pharmacokinetic studies.
- Low specificity – Complex biological matrices hamper data resolution and require sophisticated sample preparation and/or advanced instrumentation.
- Co-eluting, multiply charged interferences limit accurate quantification and also peak integration at LOQ levels.
- Isobaric interferences will limit selectivity and specificity of the assay and cause issues for accurate identification during bioanalytical method development process.
- Reduced recovery, low sensitivity – The adsorptive properties and/or polarity of peptides can compromise recovery, and interferences from biological matrices can negatively impact sensitivity and selectivity.
- Challenging physicochemical proprieties of peptides such as non-specific binding, poor solubility, and complex charge state envelope result can be problematic for the design of quantitation protocols.
- Limited MRM selectivity – MRM approaches and efficient UHPLC separations may not provide adequate signal-to-noise ratios at LLOQ due to isobaric interferences or high baseline noise.
- Systematic measurement errors – Especially for ultra-low-level quantitation, errors in measurement have a significant effect on data accuracy and precision.
- Poorly fragmenting peptides – Cyclic fragments often fragment poorly resulting in few product ions for MRM analysis.

TripleTOF vs QTRAP for Protein/Peptide Bioanalysis

TripleTOF® Workflows

- Versatile workflow for simultaneous qual and quant in drug discovery and development
- High resolution accurate mass workflows – SWATH™ Acquisition
- Characterization and comparability of biosimilar in research and development
- Bioanalysis and biotransformation workflows for PK/PD studies
- Retrospective data analysis and robust performance

QTRAP® Workflows

- When sensitivity is utmost importance – low bioavailability and high clearance
- IonDrive™ Technology for low LOQs and wider linear dynamic range
- Absolute quantitation of protein/peptide therapeutics
- GLP/non GLP bioanalysis in phase I and above
- High throughput and robust performance

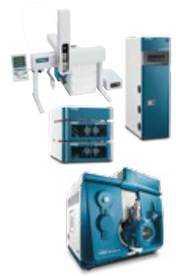


Figure 2. Comparison of mass spectrometric platforms for peptide quantitation.

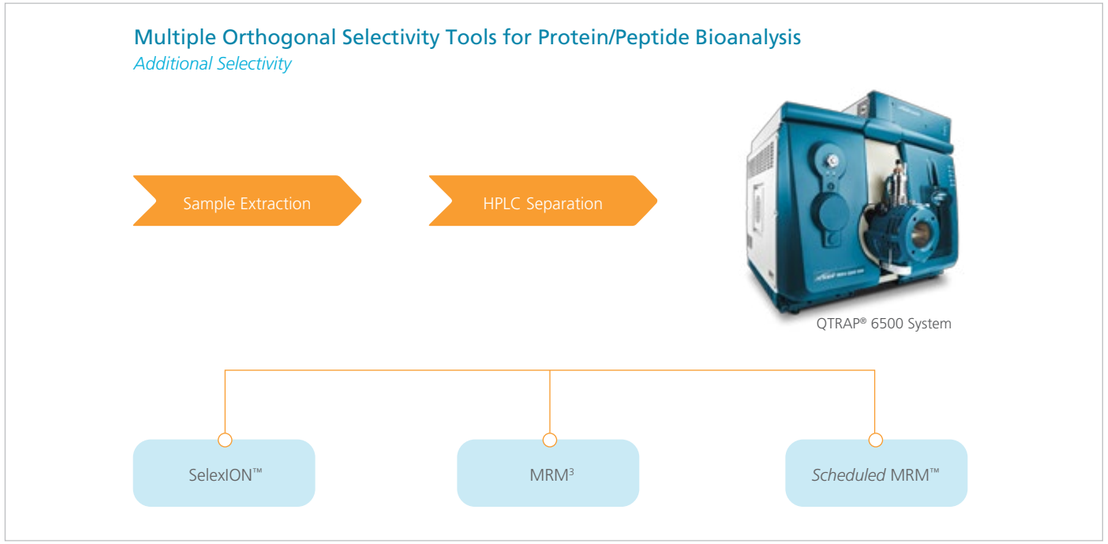


Figure 3. Mass Spectrometry based additional orthogonal sample clean up tools such as SelexION (differential mobility spectrometry), MRM^3 scan function on QTRAP LC/MS/MS System and *Scheduled* MRM for increase in duty cycle

Key benefits of the mass spectrometry based peptide quantitation workflow

While LBAs may be primarily used in industry at this time, LC/MS/MS techniques provide many potential benefits that are grounded in the direct evaluation of the analyte's chemical nature, rather than indirect signals stemming from an immunological interaction. Quantitative data obtained by LC/MS/MS methodology correlates well with LBA-derived concentrations.[7] Unlike LBA assays that require specific antibodies for each analyte, mass spectrometry platforms have universal applicability, providing one technique for a large diversity of analytes. All types of proteins and peptides can be evaluated by LC/MS/MS without exception, and a wide diversity of other biomolecules such as lipids and carbohydrates can also be identified, providing researchers with a flexible platform for identifying non-protein impurities. LBAs are generally more limited in their applicability because of auto-antibody cross-reactivity and the lack of commercial kits for every protein of interest.[11] Non-specific binding and molecular class limitations are surpassed with LC/MS/MS, which can even quantify highly homologous isoforms that are impossible to distinguish using immunoaffinity techniques. Low-level biomolecule quantitation is analogous to finding a needle in a haystack; yet LC/MS/MS is able to deliver quantitative data with excellent accuracy and precision over a wide linear dynamic range, often over 3 to 4 orders of magnitude.[7] Additionally, in contrast to the repeated expense and time-consuming nature of antibody production, LC/MS/MS methods can be developed and validated within a relatively shorter amount of time for multiple targets all at once. All of these characteristics taken together, including its flexibility, good data quality, and excellent selectivity, make LC/MS/MS an attractive method for biopharmaceutical quantitation in the regulated laboratory.

Key features of AB SCIEX instruments for MS/MS peptide quantitation

Ongoing optimization of sample preparation steps will continue to enhance the LC/MS/MS quantitation process, but the most significant gains in protein and peptide quantitation will be realized through technological innovations in mass spectrometry instrumentation. Focused on improving sensitivity and selectivity for the detection of very low levels of proteins and peptides in very complex backgrounds, AB SCIEX delivers high performance instruments that can rapidly and simultaneously measure multiple analytes—powering pharmaceutical discovery and development into the future (Figure 3).

1. **Sensitivity.** Biopharmaceuticals are very potent, highly targeted therapies that are administered in low concentration doses and exhibit a narrow therapeutic range. Often found at circulating levels in the sub-ng/mL range, detection of biotherapies requires very highly sensitive methods, and the enhancement of ionization efficiency and ion transmission have made it possible to detect drugs and metabolites in the sub-femtogram levels. New technologies such as the IonDrive™ QJet® Ion Guide underpin the sensitivity enhancements in the QTRAP 6500 System and the AB SCIEX TripleQuad™ 6500 System, bringing more ions to the detector through improved collisional focusing of ions. Heating and desolvation improvements in the IonDrive™ Turbo V™ Source and increased size and improved design of the aperture release more ions into the instrument. To fully detect the augmented signal, improvements to the dynamic range of the detector allow for accurate ion counting; the high energy conversion dynode (HED) detection system measures high ion signals without saturation to produce a linear dynamic range of over 6 orders of magnitude. These technologies are pivotal for providing continued improvements to sensitive bioanalysis.

2. **Selectivity.** Even if the pinnacle of sensitivity is reached, researchers will still be faced with the challenges of separating low levels of pharmaceutically active biomolecules from the highly complex biological matrix, where every endogenous compound can potentially interfere with the target signal. On the sample prep side, several strategies exist for the selective removal of competing background ions as well as enrichment of the analyte fraction. However, the required time and the potential for sample loss with additional cleanup steps makes this approach much less appealing. Currently, advances in MS selectivity are focused on methods that provide an additional degree of separation subsequent to the entrance to the MS or post MS/MS selection to help improve separation capacity in highly complex biological matrices. To maximize instrument performance when detecting low-level analytes masked by high background, AB SCIEX offers MRM³ scans and the SelexION™ Differential Mobility Separation Device for improved peak shapes and signal-to-noise ratios during protein and peptide quantitation.

   MRM³. Peak measurements obtained by multiple reaction monitoring (MRM) scans are occasionally challenged by interferences that cannot be removed without further, more elaborate sample clean-up. To provide additional specificity, the technique of MRM³ can be applied using the QTRAP Series of instruments—extremely sensitive, hybrid triple quadrupole instruments with a linear ion trap for further fragmentation of the primary product ions. Quantitation of the secondary product ions is usually not affected by competing or overlapping ions, which are filtered out in previous MRM selection steps. This reduction in baseline results in improved peak shape, higher signal-to-noise ratios, and superior LLOQs. The QTRAP® 5500 System and 6500 System are powered by eQ™ Electronics for scan speeds that are fast enough to be compatible with fast LC flow rates; and these instruments are equipped with single frequency excitation for highest selectivity of the product ion prior to secondary fragmentation. The Linear Accelerator™ Trap Electrodes provide 100-fold more sensitivity for the detection of low-level secondary fragments resulting from the use of MRM³ to resolve issues of high background noise.

   Differential mobility spectrometry (DMS). In some cases, if secondary product ions are not specific enough or are too low for MRM³ to be used, or method development time is too limited for prolonged MRM³ development, then additional selectivity can be gained through differential mobility spectrometry (DMS). This technique selects ions of interest based on their inherent mobility difference between a set of planar plates with high and low energy fields applied, where co-eluting interferences can be tuned out prior to analyte entrance into the mass spectrometer. AB SCIEX offers the SelexION™ Differential Mobility Separation Device for quickly resolving isobaric species and single and multiple charge state interferences on a timescale compatible with UHPLC and multiple MRM acquisitions, thus providing an additional, orthogonal level of separation for difficult-to-address overlapping peaks.

3. **High resolution accurate mass spectrometry.** Improvements to selectivity can also be gained through high resolution mass spectrometry on instruments such as the AB SCIEX TripleTOF® 5600+ System, which combines qualitative exploration and high resolution on a single platform. When using an MRM^{HR} workflow, the TOF analyzer detects all the fragments from the precursor at high resolution and high mass accuracy. Using narrower extraction widths than the unit resolution of triple quadrupole-based experiments, difficult separations between background peaks and analytes can now be achieved and improved to such an extent that minimal interferences are observed. When fragment ions are extracted at these narrow extraction widths, analytes can be detected at higher specificity and at accurate mass in complex matrices.

4. **Software.** Evaluating the results of protein and peptide quantitation can often be time-consuming and repetitive, relying on manual peak identification and data integration—a process that does not lend itself well to the high-throughput environment. AB SCIEX has developed comprehensive, powerful, and easy-to-use solutions such as MultiQuant™ Software and DiscoveryQuant™ Software that simultaneously process multiple analytes. Not only do these software packages rapidly process MS scans and data, but they also support improved data integrity and security, combining unique audit trail functionality for improved regulatory compliance and an embedded digital link to the Watson LIMS system for increased confidence in data safety.

Advantages of the diversity of mass spectrometry systems

In this resource, we primarily focus on experiments conducted on two hybrid triple quadrupole instruments, the TripleTOF 5600+ System versus the QTRAP 6500 System. Each platform has distinct advantages (Figure 2): The TripleTOF is uniquely suited to qualitative discovery (as well as quantitation) due to the underlying acquisition of a full spectrum of secondary fragments at high resolution, while the QTRAP System and its augmented ion generation, transmission and detection works best for applications requiring high sensitivity and expanded linear ranges. The AB SCIEX QTRAP 6500 System is fully accepted for regulated bioanalysis at the Phase 1 level and above, but the TripleTOF System dominates in ease of method development and non-targeted analysis during drug discovery protocols. In the event that one application demands the benefits and strengths of an alternative MS platform, transferring methods is easy and intuitive; the two MS systems have identical source and collision cell designs based on the innovative LINAC® Collision Cell, which allows for seamless coordination of quantitative data with qualitative analysis (Figure 4).

Perspectives for the future

As technological innovations surpass the limitations imposed by biological sample complexity, LC/MS/MS biopharmaceutical quantitation will become more fully established as a routine methodology in the regulated laboratory. Time-consuming and complicated sample preparation steps will evolve to become better suited to the automated requirements of the MS-based bioanalytical workflow, and sample extraction procedures are likely to become more highly selective to achieve the sensitivities required for monitoring sub-picomolar concentrations of biotherapeutic agents. Working with highly sensitive methods based on the enhanced MS ionization efficiency and transmission has yielded promising results on the QTRAP System, producing sufficient LLOQs for low-level

BIOLOGICS BIOANALYSIS

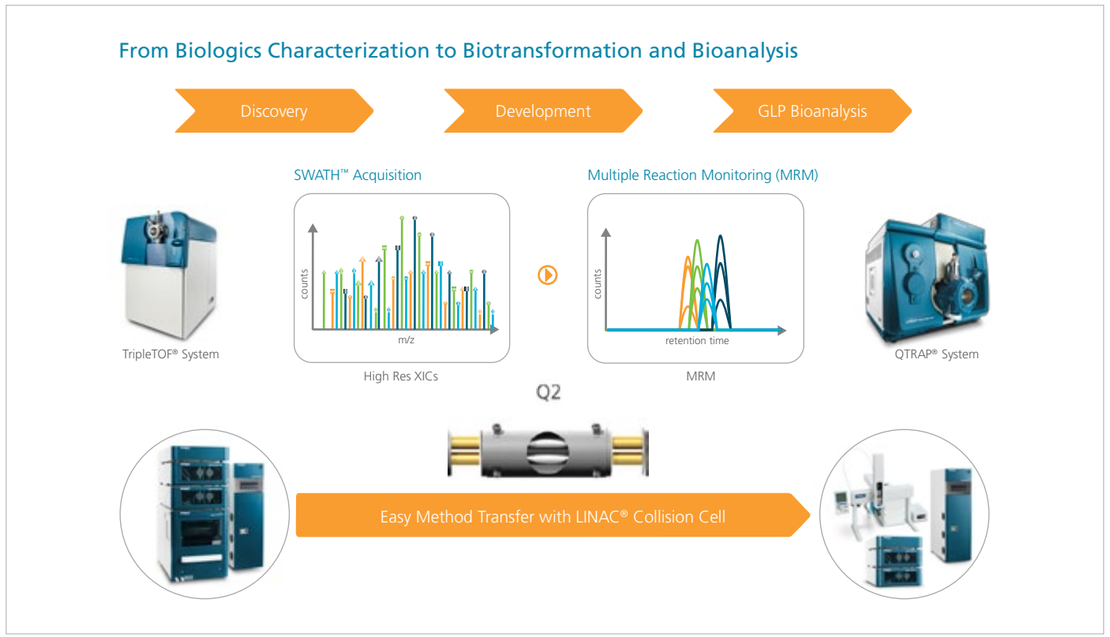


Figure 4. Continuity of workflows between TripleTOF to QTRAP. From product characterization during research and development process to biotransformation and bioanalysis during PK/PD analysis in preclinical and clinical studies

biomolecule quantitation needed for PK and TK studies. Additionally, distinct gains using DMS and MRM[3] are adding an additional layer of selectivity, removing hard-to-separate background and leading to better signal-to-noise parameters. The potential of high resolution mass spectrometry to measure intact, high molecular weight biomolecules will gain increasing interest as technological advances push TOF sensitivities towards those of the hybrid linear ion trap instruments. By reducing the need for additional sample preparation steps with enhanced MS detection and selectivity capacities, LC/MS/MS techniques are becoming more closely aligned with the high-throughput workflows necessary for regulated bioanalysis.

References

[1] "Biopharmaceuticals – A Global Market Overview." November 2013. The abstract from a market research report from Reportbuyer.com. Accessed online at: http://www.prweb.com/releases/2013/11/prweb11314067.htm

[2] Zhong X, Neumann P, Corbo M, Loh E. "Recent Advances in Biotherapeutics Drug Discovery and Development." Drug Discovery and Development – Present and Future, Dr. Izet Kapetanovic (Ed.) ISBN: 978-953-307-615-7, InTech. Accessed online at: http://www.intechopen.com/books/drug-discovery-and-development-present-and-future/recent-advances-in-biotherapeutics-drug-discovery-and-development

[3] Reichert J. "Which are the antibodies to watch in 2013?" mAbs. Jan/Feb 2013; 5(1): 1-4.

[4] Shi T, Su D, Liu T, Tang K, Camp DG 2nd, Qian WJ, Smith RD. "Advancing the sensitivity of selected reaction monitoring-based targeted quantitative proteomics." Proteomics. Apr. 2012; 12(8): 1074-92.

[5] Hopfgartner G and Gougongne E. "Quantitative high-throughput analysis of drugs in biological matrices by mass spectrometry." Mass Spectrom Rev. May/Jun 2003; 22(3): 195-214.

[6] Ezan E and Bisch F. "Critical comparison of MS and immunoassays for the bioanalysis of therapeutic antibodies." Bioanalysis. Nov. 2009; 1(8): 1375-1388.

[7] van den Broek I, Niessen WMA, van Dongen WD. "Bioanalytical LC/MS/MS of protein-based biopharmaceuticals." Journal of Chromatography B. 2013; 929: 161-179.

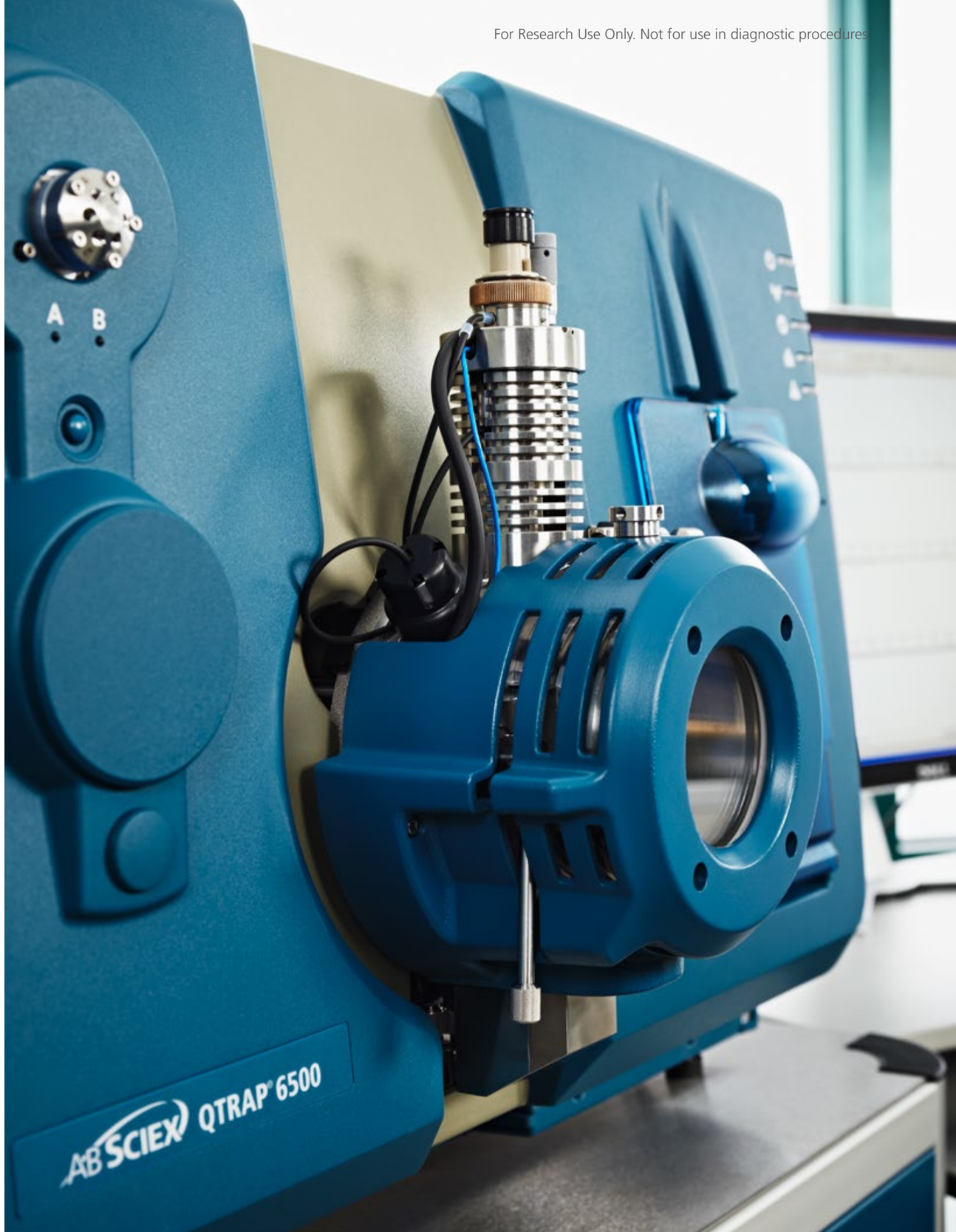
[8] Food and Drug Administration. "Guidance for Industry; Bioanalytical Method Validation." US Department of Health and Human Services. FDA. Center for Drug Evaluation and Research, Rockville, MD, 2001.

[9] Bischoff R, Bronsema K, van de Merbel NC. "Analysis of biopharmaceutical proteins in biological matrices by LC/MS/MS 1. Sample preparation." Trends in Analytical Chemistry. 2013; 48:41-51.

[10] Liu G, Ji QC, Sun H, Shuster D, Zhao Q, Arnold M. "Liquid chromatography coupled with tandem mass spectrometry for the bioanalysis of proteins in drug development: Practical considerations in assay development and validation." Journal of Chromatography A. 2013;1284: 155-162.

[11] Hopfgartner G, Lesur A, Varesio E. "Analysis of biopharmaceutical proteins in biological matrices by LC/MS/MS II. LC/MS/MS analysis." Trends in Analytical Chemistry. 2013; 48:52-61.

[12] Thompson B. "Driving high sensitivity in biomolecular MS." Genetic Engineering and Biotechnology News. Nov 2012; 32(20). Accessed at: http://www.genengnews.com/gen-articles/driving-high-sensitivity-in-biomolecular-ms/4603/?kwrd=high%20sensitivity%20in%20biomolecular%20MS.



Your success is our success

We take it personally

As an AB SCIEX customer you have access to an excellent customer support organization. Wherever you are, we're there with you as a trusted partner to answer questions, provide solutions, and maximize lab productivity.

Our customer support organization has access to the latest product updates, software revisions, methods and repair procedures to make sure that you stay on top of your game.

When you have questions, we have answers.

Learn more at www.absciex.com/customersupport, or locate your local account representative at www.absciex.com/contactus

9680314-01 03/2014

Headquarters
500 Old Connecticut Path | Framingham, MA 01701 USA
Phone 508-383-7700
www.absciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices