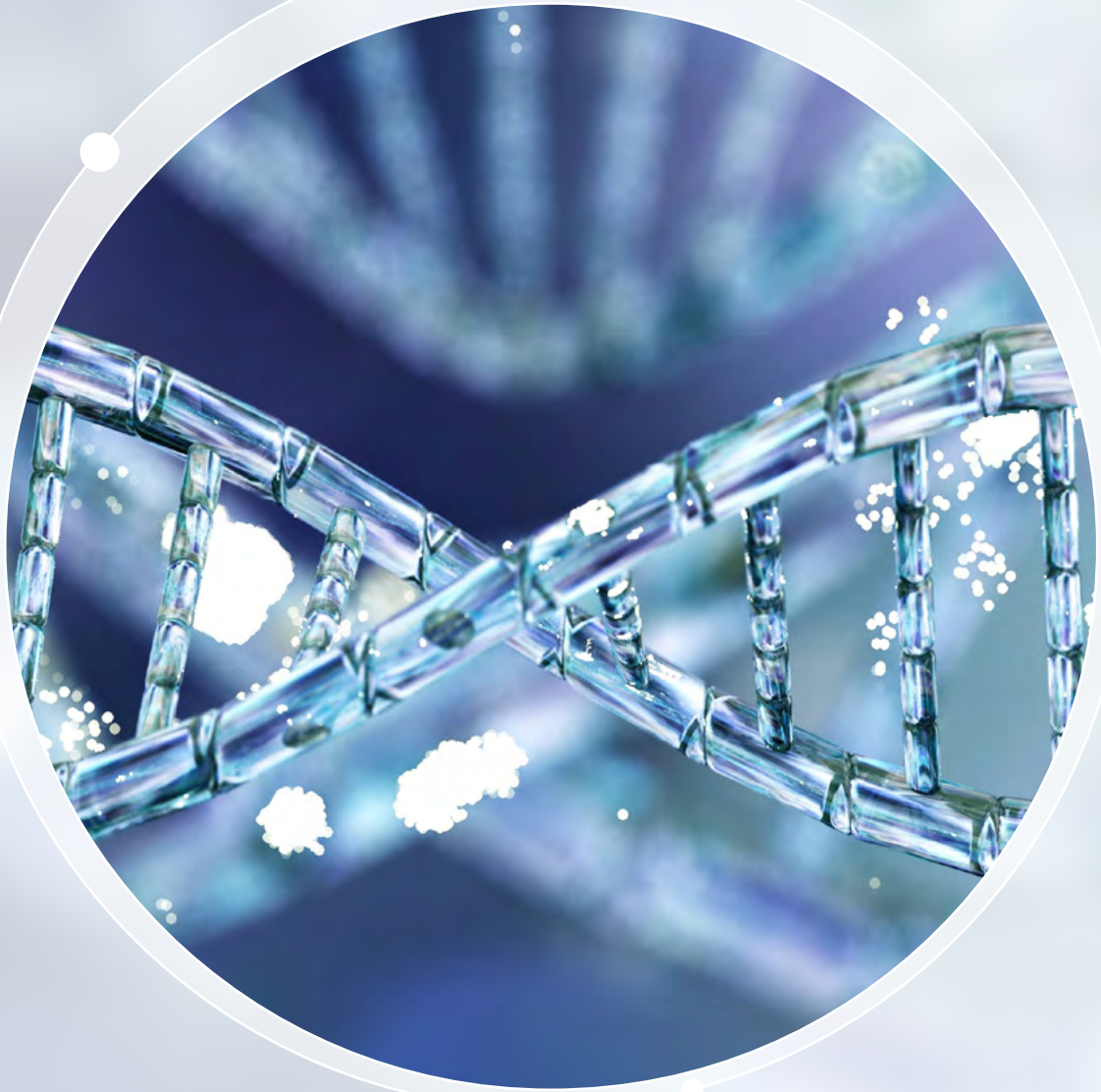
Biotherapeutic Quantification eBook

Robust methods for accurate quantification in biological matrices at every stage of the process



SCIEX
The Power of Precision

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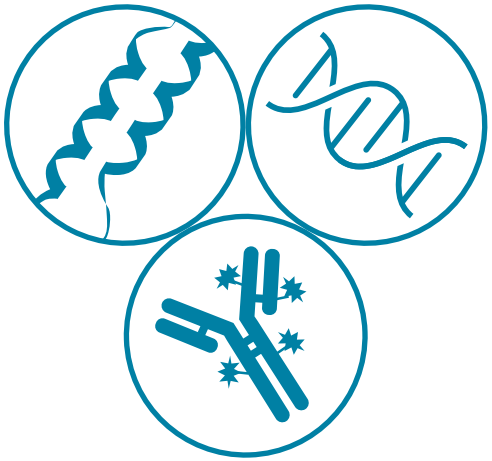
Introduction

Biotherapeutics offer novel and often revolutionary new treatments for diseases with unmet clinical needs. However, the development of this relatively new class of drugs is far from easy.

Successful biotherapeutic development requires robust methods for accurate quantification in biological matrices, at every stage of the process.



The biotherapeutic discovery pipeline has expanded to encompass a wide range of modalities. Now, researchers are not only required to contend with challenges presented by monoclonal antibodies, but also by fusion proteins, antibody-drug conjugates, and oligonucleotides.

Different molecules raise different challenges, such as peptides with poor fragmentation efficiencies, oligonucleotide samples with high background interference, and large biomolecules that must be quantified intact.

This eBook highlights:

- Techniques to overcome challenges in the development of biotherapeutic compounds
- How using LC-MS/MS offers you the sensitivity, linear dynamic range and selectivity you need to overcome biotherapeutic quantification challenges
- Market trends from around the world
- Demonstrations of robust SCIEX instruments handling complex and “dirty” biological samples

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A Global Perspective

Babburaj Kunnumal

Senior Global Market Manager, Biopharma

"Collaborating with our customers to bring life saving therapeutics to patients faster."

The biopharma industry has changed significantly over the past 25 years. Initially, many of the therapeutics coming through development were monoclonal antibodies (mAbs). Indeed, five of the top 10 best-selling drugs in 2017 were mAbs. Issues with efficacy, however, led to a new generation of biotherapeutics in the last decade, such as antibody-drug conjugates (ADCs), bispecific antibodies and fusion proteins. Very recently, there has been renewed interest in oligonucleotide-, mRNA- and cell-based therapies. While these types of molecules have been studied for their therapeutic potential, difficulties with stability and delivery reduced their ability to reach the market. Many of these problems have been resolved in the last few years, paving the way for increased new drug development in this area.

Conversations with customers reveal that current drug development pipelines contain many of these different types of biological molecules. This presents significant challenges for routine biotherapeutic quantitative analysis. Different types of molecules mean different demands for selectivity, specificity and dynamic range. Researchers need an array of different workflows that can be used for quantification, often in extremely challenging biological matrices, throughout different stages of clinical development. When working with customers, our focus is on delivering reliable solutions that can adequately address their needs for various types of biotherapeutics.

One area we are currently watching with interest is vaccine development. The field has shown a resurgence recently within the industry as pharma companies seek single vaccines that can be used to prevent multiple diseases. Significant investment is being made in this space by some of the largest global funding bodies. After many years on the backburner, the discovery side of vaccines is becoming profitable again. As the field develops further, researchers will need new, reliable methods for quantification, which SCIEX is poised to deliver.

Success in these areas are due to the reputable robustness, sensitivity and wide dynamic range offered by SCIEX CE and LC-MS instruments. As the interest in oligonucleotides increases for both therapeutic use and reagent manufacture, SCIEX will continue to work with customers to understand their challenges and deliver analytical solutions that help bring their important lead molecules through the drug development pipeline.

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Global Biotherapeutic Trends
Americas

Yi Zhang

Market Development Manager, Americas

One trend we see in the Americas market is the greater demand for accurate quantification of more challenging assays, such as the analysis of cyclic peptides and oligonucleotides. For these types of workflows, there is increasing interest in our high-resolution accurate mass spectrometry (HRAMS) systems such as the SCIEX TripleTOF® 6600+. This instrument is extremely versatile, with exceptional selectivity and the capability to handle simplified sample preparation. It enables a range of workflows to meet the evolving needs of the biopharma industry. However, even with the flexibility and selectivity of the TripleTOF® 6600+, challenges remain for intact protein quantification, which requires an extremely wide dynamic range, high sensitivity, and novel software for data processing.

Peptide analysis using the SCIEX QTRAP® 6500+ is the most popular biotherapeutic quantification workflow for our customers in the Americas. This instrument offers renowned sensitivity for routine quantification throughout drug development, even when working with biological matrices such as tissues and cell cultures. For cases where dosing levels are extremely low and extra sensitivity is required, our microflow solution with the QTRAP® 6500+ is also gaining traction.

"We pride ourselves on finding solutions for our customers that are easy to implement and reliable. We will continue working on technology developments to deliver routine and accurate quantification of intact proteins."

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Global Biotherapeutic Trends
EMEAI

Ferran Sanchez

Market Development Manager, EMEAI

"SCIEX aims to stay at the forefront of high-performance technology."

The major trend we see in our region is that the market is moving more towards high-resolution mass spectrometry solutions for biopharmaceutical quantification. Although the instruments that are most in-demand for our customers are currently the SCIEX Triple Quad™ systems, requests for high-resolution instruments are beginning to accelerate. These instruments can provide high sensitivity, mass accuracy and robustness for applications such as the TOF MS-based quantification of large peptides. The high-quality quantification data that is achievable can give greater confidence in compound identification and will be of increasing importance to companies involved in biopharmaceutical development.

One area where further development is requested is laboratory automation, particularly when it comes to sample preparation. Simplified and automated sample preparation shortens workflow timelines and reduces the chance of human errors. As an additional area for further development, improvement of linear dynamic range for quantification would broaden the scope of drug development for both small and large molecules. A growing interest in analyzing large intact proteins, and post-translational modifications, is another trend we recognize. Taking advantage of CE to handle large protein quantification is under consideration for future applications. By turning customer feedback into innovation, SCIEX aims to stay at the forefront of high-performance technology.

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Global Biotherapeutic Trends
Korea

Jason Neo

Director, Marketing & Field Applications Support, Rest of Asia (ROA)

Korea is rapidly becoming a major player in the pharmaceutical industry. The top global biopharma manufacturers are leading this trend by building a local presence. These prominent companies are innovators who, with the support of the Korean government, are creating a boom in Korea's biopharma industry. During the last decade, these companies began to heavily invest in better analytical tools. More recently, the importance of LC-MS/MS based analysis has increased. The method has become essential in many parts of biologics development, both in characterization as well as in quality control. Biotherapeutics quantification is an essential part of this workflow and SCIEX Triple Quad™ systems are the preferred choice for Korean researchers.

Performing biopharmaceutical analysis with LC-MS/MS presents several challenges that can be difficult to overcome. The molecules are large and heterogeneous, with different variants impacting function, stability, efficacy, and safety. All of these characteristics need to be accurately and reliably determined during development. We help support the Korean biopharma industry by developing robust workflows that can provide accurate identification of the mass of a molecule, including any modification. This is a key factor for the Korean industry, allowing companies to better quantify their products so that they can lower their risk of failure in development.

"We help support the Korean biopharma industry by developing robust workflows that can provide accurate identification of the mass of a molecule, including any modification."

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Global Biotherapeutic Trends
Japan

Kazunari Kessoku

Market Development Manager, Japan

"Continuous follow-up is the hallmark of a relationship with customers in Japan. Constantly ensuring that their experience with our technology is pushing the boundaries of what is achievable."

In Japan, the general challenges in the field of biotherapeutic quantification are sensitivity and selectivity in the development of antibody-drug conjugates, monoclonal antibodies, glycan peptides and oligonucleotides. There is also an increasing need to look at endogenous biomarkers such as TCA cycle peptides and neurotransmitters. In the average lab, the more everyday question is how to rationalize the sample preparation and collection to shorten the timeline of the entire workflow.

SCIEX is addressing these demands with thorough research to gain a deeper understanding of the samples, the candidates and the analytic conditions. This enables us to develop novel applications and deliver the best performance from SCIEX instruments. The most important technological offerings for our customers are the TripleTOF® 6600 LC-MS/MS System for accurate mass analyses, and the SCIEX Triple Quad™ 5500+ and 6500+ series for nominal mass analyses. By incorporating microflow, our customers are greatly improving their oligonucleotide analysis in terms of sensitivity and overall quantitative performance. An area we are currently working on is to further develop technology for characterization and analysis of anti-drug antibodies (ADA). Better analytical quantification of ADAs will allow for the close monitoring needed to establish any cross-reactivity, which can pose problems for both patient safety and product efficacy. Continuous follow-up is the hallmark of a relationship with customers in Japan. Constantly ensuring that their experience with our technology is pushing the boundaries of what is achievable.

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Global Biotherapeutic Trends
China

Zhe Zhou

Market Development Manager, China

"An exciting challenge for SCIEX in this market is developing novel applications that push current lower-end technologies in terms of sensitivity and reliability."

China is pursuing a comprehensive, long-term strategy to become a driving force in biotechnology, especially medical biotechnologies. This includes biologic-based therapeutics, technologies to support individualized drug treatments and breakthrough technologies. As a result of these policies, the biotechnology industry is supported by the government and has initiated regulatory reforms in recent years to address challenges in this area.

For the biopharmaceutical labs, the major encounters in the field of mass spectrometry are large molecule quantification with HRMS that has a resolving power over a hundred thousand. The most prevalent equipment used from SCIEX for peptide quantification is the QTRAP® 6500+ LC-MS/MS System. Also, the TripleTOF® 6600+ LC-MS/MS System with SWATH® acquisition is a highly advanced and appealing selling point. However, any smaller companies in the Chinese biopharma space are operating on tight budgets that put some of the more advanced biotherapeutic quantification solutions out of reach. An exciting challenge for SCIEX in this market is developing novel applications that push current lower-end technologies in terms of sensitivity and reliability.

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Over 800 products on the new **SCIEX Online Store**

Visit **www.us-store.sciex.com**

SCIEX
The Power of Precision

Technology Overview

SCIEX recognizes that biotherapeutic quantification covers a broad range of radically different molecules, from small therapeutic peptides and oligonucleotides to multi-subunit fusion proteins. That is why, through in-house research using a wide variety of different molecule classes, SCIEX has repeatedly shown that our technologies can deliver the speed, sensitivity and selectivity required to support the growing development of the next generation of biotherapeutics.

The TripleTOF 6600 system

Built with versatility in mind, the TripleTOF technology unleashes sensitivity, speed, and productivity in a single multifaceted platform to help you dig deeper into complex samples. The system was designed to be as flexible as possible, allowing biopharma labs to take on new challenges and adapt their workflows accordingly. The TripleTOF® 6600 accurate mass system provides exceptionally fast data acquisition without compromising speed, resolution, or sensitivity—making it an excellent fit for biotherapeutic development and analysis.

Learn More

QTRAP® 6500+ series

The QTRAP 6500+ system for the most difficult, challenging analyses involving complex matrices, you need the most sensitive instrument available. The QTRAP 6500+ System is the fastest and most sensitive QTRAP system available, delivering enhanced selectivity and improved levels of quantification. This single instrument pushes the boundaries of LC-MS/MS further than ever before. It provides a simple route to achieving comprehensive quantification throughout biotherapeutic development.

Learn More

X500 QTOF Series

These revolutionary benchtop high resolution accurate mass spec instruments have been designed exclusively to boost productivity in high-throughput sample analysis laboratories. X500R workflows are simple to implement and maintain, and offer high-throughput and sensitive detection capabilities, allowing researchers to perform quick, comprehensive analyses and obtain data they can trust. To overcome the challenges encountered when working with complex matrices such as cell cultures and serum, the X500R series is highly robust. In addition, the system has an independent calibrant delivery path, which maintains highly reliable mass calibration through long runs, maximizing machine uptime—an important feature in busy biopharma labs.

Learn More

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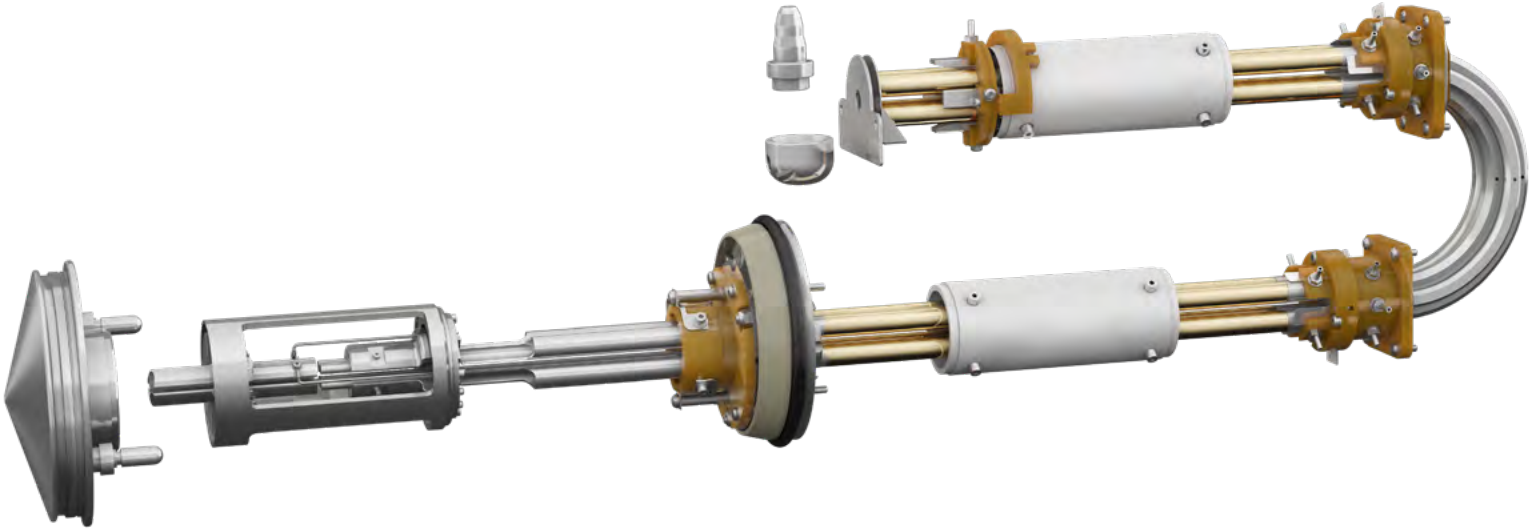
The Tools to Meet the Challenge

A quantitative biotherapeutic LC-MS assay is only as good as its power to discern your target compound from everything else. Real-world biotherapeutic samples, as we know, can exhibit a wide range of behavior. They often behave quite unpredictably, with coeluting compounds, background interferences, and other matrix effects that can severely degrade assay quality. SCIEX has invested time and research into developing mass spectrometry tools with exceptional selectivity and specificity to bring our customers their required data quality.

QTRAP®

SCIEX QTRAP® systems are triple quadrupole instruments with the added value of ion trap scan functionality. The third quadrupole operates as a linear ion trap. When challenged with coeluting analytes, an MRM3 workflow can be used. By capturing MRM data and MS/MS data in one injection, compounds can be quantified and identified simultaneously, thereby confirming the selection of the analyte versus other possible compounds. Scans done on the QTRAP® can deliver new and exciting discoveries for identification, characterization and quantitation of your samples. From high-throughput analysis to detailed analyte investigations, the QTRAP system gives you the ability to both quantify and find unknowns in your sample, without any sacrifice to sensitivity.

Learn More



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Microflow Chromatography

The development of novel therapeutics and biotherapeutics is a challenge for bioanalytical scientists that need to accurately detect and quantify these compounds. When people are looking to advance quantitative assays from a sensitivity point of view, without having to do extensive sample prep, they typically move from analytical flow LC-MS into lower flow rate regimes for those sensitivity gains.

Microflow LC-MS is the best compromise between sensitivity and robustness. It is more sensitive than traditional analytical flow LC and more flexible and more robust than nanoflow LC, giving you the potential to have this tool in your assay development.

With microflow you can inject less and get a better signal. The lower volumes reduce contamination in your mass spectrometer. The cleaner your instrument and source, the less downtime you have because of troubleshooting activities. Microflow combined with our optimized OptiFlow™ Turbo V Source, based on the trusted Turbo V™ Source technology, gives the robustness and simplicity you've come to expect from traditional analytical flow LC-MS. Now, those advantages are available in the more sensitive microflow regimes.

Engineered with enhanced gas flow dynamics and new features for maximizing ion production and robustness, the OptiFlow® source helps to ensure consistency of results. Intelligent probe sensing technology presets system source settings to an optimal range for best spray conditions. This eliminates manual adjustments of the source and decreases optimization time which, with lower flow chromatography, had previously been an art rather than science.

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High Resolution Mass Spectrometry

With the SCIEX QTOF X500 series and TripleTOF® systems, MS/MS spectra are collected at high-resolution and fast scan speeds without sacrificing sensitivity. Fragment ions are extracted post-acquisition to generate high resolution MS/MS (MRM^{HR}) peaks for integration and quantification. MRM^{HR} can provide better selectivity by reducing the effects of interferences. Because MS/MS data are also always acquired, assay development is simplified since fragment ion selection for quantification is done post-acquisition. Additionally, the full scan MS/MS data acquired across the entire data set provide confirmation for every analyte.

TripleTOF® systems

Learn More

QTOF X500 series

Learn More

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SelexION®

SelexION® DMS Technology is used to separate hard to resolve ions based on their gas-phase mobility. With a SelexION® device, you can overcome coeluting matrix interferences, separate isobaric compounds, reduce background, and improve data quality. Additionally, because the SelexION® device provides an additional layer of separation and selectivity, sample preparation can be greatly simplified and tedious LC optimization strategies can be minimized.

Learn More

CESI 8000 Plus

An often overlooked approach is to use a capillary electrophoresis separation strategy rather than an LC-based strategy. Capillary electrophoresis electrospray ionization MS (CESI-MS) can enable high-resolution separation and enhanced sensitivity while reducing ion suppression bias. The SCIEX CESI 8000 Plus, OptiMS cartridge, and adapter kit enable seamless CESI-MS analysis with SCIEX mass spectrometers for challenging samples, while consuming minimal sample quantities. SCIEX solutions and workflows address both the separation process and the MS methodology.

Learn More

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Software Integration to **Power Your Workflows**

Powerful, workflow-driven software ties everything together to deliver a new benchmark in efficiency, throughput, and productivity. Take full advantage of all the power that the SCIEX 5500+ System puts at your fingertips with fully integrated software designed to enhance fast adoption and easy operation.

Analyst® Software – Your Mass Spec Operating System

Provides state of the art functionality for instrument control, data analysis and reporting with enhanced performance and ease of use.

Try It For Free

SCIEX OS-Q Software – Your Workflow Software Optimized for Qualitative and Quantitative Data Analysis

Advance your data processing capabilities with ease and confidence. SCIEX OS-Q software is the integrated platform for multiple workflows. From performing high-throughput screening MRM data processing, to non-targeted investigations – all the tools you need are at your fingertips, without sacrificing time, valuable samples, or results. The software simplifies compound identification, quantitation, and data review, so your lab can master the speed, power, and accuracy offered by LC-MS/MS technology.

Try It For Free

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Biotherapeutic Quant Application Development

Lei Xiong

Senior Manager, Biopharma Qualification Technical Marketing

"At SCIEX, our global field teams are continuously in discussions with our customers in the biopharmaceutical industry, striving to understand their needs, as well as their pain points. This relationship with our customers is the driving force behind the development of new applications for our mass spectrometry technologies."

Le Xiong is a the forefront of innovative application development at SCIEX. Here, she discusses the challenges customers in the biopharmaceutical industry are facing, and the various applications of mass spectrometry that can overcome these challenges:

The application notes presented in this compendium were generated to directly address the many and varied challenges our customers face, with several of the application notes created in close collaboration with SCIEX customers.

Mass spectrometry workflows for quantifying biotherapeutics involve dealing with extremely challenging matrices from cell, tissue or patient samples. These matrices often require considerable cleanup steps to make them suitable for analysis, and part of our work is to streamline this process and develop easy-to-implement solutions. Even after cleanup, biological matrices still require careful analysis, which can be negatively affected by high background interference and low analyte concentrations. Specific types of biomolecules also come with their specific demands on instrumentation, be it exceptional sensitivity, high selectivity or rugged robustness.

The SCIEX QTRAP® 6500+ LC-MS/MS System provides an elegant solution to many of these challenges. Its applications take advantage of its best-in-class sensitivity, a linear dynamic range spanning five orders of magnitude, and industry-recognized robustness. In conjunction with innovative SCIEX trap-elute microflow solutions, this technology enables high-throughput analysis of some of the most challenging biotherapeutic targets. The application note detailing a microflow workflow for the analysis of trastuzumab emantasine is a highlight of this compendium, as is the technical note describing improved oligonucleotide quantification. For slightly more niche biotherapeutic applications, the SCIEX TripleTOF® 6600 LC-MS/MS System is a high-resolution accurate mass platform, ideal for applications dealing with high interference from biological matrices and quantification of intact molecules.

As the biopharma industry evolves, so do the challenges facing analytical laboratories. Novel oligonucleotide and cell therapies are entering the drug development pipeline, and researchers need suitable mass spectrometry workflows to overcome the challenges that these new biotherapeutics present. We are continually developing applications to meet industry needs, with some of our current work focusing on application notes for targeted cell culture media analysis, oligonucleotide quantification, and host cell protein quantification and identification. Whichever direction the biopharma industry moves into over the coming years, SCIEX is well-positioned to continue delivering a complete line-up of quantitative solutions.

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The Power of Precision
Meet the Technology Behind the Numbers

• Evolving Sensitivity

Option 1 **3500**
Option 2 **4500 System**
Option 3 **5500+ System**
Option 4 **OptiFlow**

6500+ System

- More Versatile with QTRAP®
- More Selective with MRM³
- More Robust with Turbo V™ Ionization Source

• Extra Dimensions

Option 1 **SelexION®**
Option 2 **CESI 8000 Plus**

M5 MicroLC

- More Sensitive with Microflow
- More Selective with SelexION®
- More Integrative with Sheathless-flow CE

• High Resolution

Option 1 **5600+**
Option 2 **X500R**
Option 3 **X500B**
Option 4 **SWATH® Acquisition**

6600+ System

- More Comprehensive with SWATH® Acquisition
- More Flexible with OptiFlow™ Turbo V
- More Information with 100 Hz MS/MS Scanning

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SCIEX Biotherapeutic Quantification
Technical Notes

This section gives an overview of technical notes that detail comprehensive biotherapeutic quantification from traditional peptide quantification to hybridized immuno-enrichment strategies. The technical notes in later sections showcase the application of SCIEX tools to meet various challenges in biotherapeutic quantification.

Overview of LC-MS Quantitative Solutions for Biotherapeutic Analysis

Accelerating the growth of the biopharmaceutical industry has resulted in drug development pipelines containing a variety of different classes of biotherapeutics, from monoclonal antibodies to peptides and oligonucleotides. This has increased the need for analytical solutions for quantification in different biological matrices across multiple stages of development. SCIEX provides a comprehensive array of biotherapeutic quantification solutions that address the many challenges associated with analyzing complex biological moieties to deliver superior quantification results. This technical note provides a summary of the tools provided by SCIEX across a wide variety of applications.

Read Now

Hybrid Immunoaffinity-LC-MS/MS Method for Quantifying Insulin Aspart in Human Plasma

Insulin aspart, used in the treatment of diabetes mellitus, is one of the top-selling pharmaceutical products, creating a tremendous interest in analyzing and studying insulin aspart in clinical samples. However, challenges remain for routine pharmacokinetic studies: long sample preparation times and wide sample concentrations frequently reduce linearity. A hybrid immunoaffinity-LC-MS/MS analysis method for insulin aspart has therefore been developed. The exceptional sensitivity of the QTRAP® 6500+ LC MS/MS system coupled with an ExionLC™ system provides a broad dynamic range for peptide MRM studies, achieving an LLOQ of 50 pg/mL. The immunoaffinity sample preparation reduces sample complexity and eliminates interference from the matrix.

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Quantification of Trastuzumab in Rat Plasma using an Improved Immunoaffinity-LC-MS/MS Method

Trastuzumab, a monoclonal antibody, is a TNF inhibitor approved for the treatment of early-stage breast cancer that is Human Epidermal growth factor Receptor 2-positive (HER2+) and has spread into the lymph nodes, or is HER2-positive and has not spread into the lymph nodes. Marketed as Herceptin®, trastuzumab has been one of the top-selling pharmaceutical products in the past five years. As such, there is tremendous interest within the clinical research community in analysing and studying trastuzumab in preclinical samples. However, the procedures are lengthy and complicated for measuring active or free circulating biotherapeutic drug in complex matrices. Active debate on technique selection between LBA and LC-MS has been going on for many years. Key is the ability to achieve the required linearity across the expected pharmacokinetic (PK) sample range. Here, a universal hybrid LBA/LC-MS workflow is introduced combining the advantages of both technologies for protein biotherapeutic PK analysis.

Read Now

Quantification of Adalimumab in Rat Plasma using an Improved Immunoaffinity-LC-MS/MS Method

Adalimumab, a monoclonal antibody, is a TNF inhibitor approved for the treatment of rheumatoid arthritis, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, chronic psoriasis, hidradenitis suppurativa, noninfectious uveitis, and juvenile idiopathic arthritis. Marketed as Humira®, adalimumab has been one of the top-selling pharmaceutical products in the past five years. As such, there is tremendous interest within the clinical research community in analyzing and studying adalimumab in preclinical samples. While active debate on technique selection between LBA and LC-MS has been going on for many years, herein we introduce a universal hybrid LBA/LC-MS workflow combining the advantages of both technologies for protein biotherapeutic PK analysis.

Read Now

A Sub-picogram Quantification Method for Desmopressin in Plasma using the SCIEX Triple Quad™ 6500 System

A reliable, fast, and sensitive method for the detection of desmopressin was developed. Therapeutically, desmopressin reduces urine production, restricting water elimination from the kidneys. The longer half-life of desmopressin over vasopressin offers some therapeutic advantages, and typical doses of desmopressin to treat diabetes insipidus and bedwetting range between 0.200 to 1.20 mg per day, resulting in very low plasma concentrations. This method established a sensitive and selective LC-MS/MS method for the quantification of desmopressin in human plasma, detecting peptide levels as low as 0.500 pg/mL with excellent accuracy and precision.

Read Now

High Sensitivity Quantification of Triptorelin Deca Peptide using the QTRAP® 6500 System

Triptorelin, a ten amino acid synthetic peptide, is a gonadotropin releasing hormone agonist (GnRH agonist) used in the treatment of hormone-responsive cancers such as prostate cancer and breast cancer. Used in men, triptorelin works to reduce the amount of testosterone in the blood, which can act to limit the growth of prostate cancer. When given to women, triptorelin reduces the amount of estrogen the body produces. A high sensitivity, robust LC-MS/MS detection strategy is required for monitoring triptorelin in rat and human plasma at the low pg/mL levels during drug development.

Read Now

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QTRAP®
Technical Notes

Quantification of the Therapeutic Peptide Exenatide in Human Plasma

Exenatide is a large therapeutic peptide, approved for the treatment of Diabetes mellitus type 1 and 2. Due to other compounds in biological matrices having similar physiochemical properties, exenatide analysis requires methods exhibiting high specificity and selectivity. Traditional exenatide analysis methods, such as immune-enzymatic assays for pharmacokinetic studies, do not meet these criteria. An MRM3 LC-MS strategy has therefore been successfully developed using a SCIEX QTRAP® 5500 system to deliver enhanced selectivity. The method significantly reduced baseline noise, and the accuracy, reproducibility and high linearity were compatible with the requirements for biopharmaceutical development assays.

Read Now

Quantification of Prostate Specific Antigen (PSA) in NonDepleted Human Serum Using MRM3 Analysis

The use of Multiple Reaction Monitoring (MRM) combined with stable isotope labeled proteins / peptides for the quantification of proteins has been actively explored over the last few years and shows great promise for clinical research. We present here a novel approach that combines the use of analytical chromatography with a new, highly selective mass spectrometry technique called MRM^3 that is able to significantly reduce sample complexity. The approach enables robust detection of protein biomarkers from human serum at concentrations down to the low ng/ml level.

Read Now

Powerful Scan Modes of QTRAP® System Technology

The unique combination of a QqQ and LIT analyzer within the QTRAP instrument enables both quantitative and qualitative experiments on the same platform with the same performance one would expect from two separate high quality, high performance instruments. High sensitivity quantitation experiments can be performed in MRM mode with no compromise in performance. When required, the unique configuration can be leveraged to obtain qualitative information on samples. In fact, the unique configuration of the two analyzers together actually enables higher sensitivity and higher quality MS/MS scanning beyond what is possible with traditional triple quadrupole and linear ion trap instruments alone. Additionally, the QTRAP system configuration enables valuable new workflows that can link QqQ scans with LIT scans within automated acquisition strategies to provide the ultimate flexibility for a wealth of different analytes and applications.

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Microflow Chromatography
Technical Notes

Ultra-Sensitive Quantification of Trastuzumab Emtansine in Mouse Plasma using Trap-Elute MicroLC MS Method

When developing small animal pharmacokinetic (PK) assays, sensitivity and throughput are key factors used to evaluate performance. A highly sensitive assay has been successfully developed for quantifying trastuzumab emtansine – a large antibody-drug conjugate (ADC) used in the treatment of breast cancer – in mouse tissue. The assay utilizes optimized immunoaffinity sample preparation that decreases matrix interference, and also takes advantage of the trap-elute function of the M5 microLC system. This enables the capability for high flow sample loading before microflow elution for increased sensitivity. The QTRAP® 6500+ LC-MS/MS system achieves ADC quantification at an LLOQ of 0.5 ng/mL with high throughput and wide linear dynamic range.

Read Now

Improving Sensitivity for Trastuzumab Emtansine Quantification using Trap-Elute MicroLC-MS with Large Volume Sample Loading

LC-MS based quantification of monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) has been routinely adopted in multiple stages of the biotherapeutic development, serving as an orthogonal technology to the traditional ligand binding assays (LBAs). For small animal PK analysis, the assay sensitivity and throughput are the key factors to evaluate the assay performance. Moreover, scientists have started to work on improving the quantification confidence by generating replicate data from multiple sets of sample preparation. Therefore, each individually prepared sample no longer needs to be fractionated and injected multiple times into the LC-MS system. Instead, it can be fully injected at higher injection volumes, to improve the assay sensitivity. Herein, a trap-elute microLC-MS/MS workflow with large volume sample loading is demonstrated for ultrasensitive quantification of Trastuzumab Emtansine in mouse plasma. Without affecting the throughput, a two-fold improvement on assay sensitivity with the LLOQ as low as 0.5 ng/mL is observed compared with previously published data.

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Sub-Picogram Level Quantitation of Desmopressin in Small Volumes of Human Plasma Using a Trap-Elute Microflow

Desmopressin is a synthetic peptide analog, used to treat disorders affecting urine production. Typical doses of desmopressin are <1 mg per day, resulting in very low plasma concentrations. This, combined with high matrix interference, makes quantification of desmopressin in patient samples extremely challenging. A Trap-Elute microLC-MS workflow was therefore developed to analyze desmopressin in human plasma, taking advantage of the sensitivity gains from microflow LC. This method achieved an LLOQ of 0.5 pg/mL and required 70 % less sample than previously reported methods. Additionally, the method demonstrated good selectivity, with no matrix interference detected.

Read Now

Extending the Lower Limits of Quantification of a Therapeutic Oligonucleotide Through Microflow LC-MS/MS

Oligonucleotide therapeutics offer the exciting potential to target and modulate gene expression. Traditionally, oligonucleotides have been analyzed using ion-pairing reversed phase liquid chromatography mass spectrometry (IP-RP LC-MS). However, ion pairing reagents can reduce MS sensitivity and build up in MS instruments over time, requiring frequent downtime in order to clean.

A novel microflow LC-MS strategy for oligonucleotides has therefore been developed to deliver enhanced sensitivity and reduce instrument contamination. A two-fold reduction in flow rate from conventional LC led to significant sensitivity improvement. The new microflow strategy also cut instrument contamination by 60 times, greatly reducing the need for system maintenance.

Read Now

Hybrid Immunoaffinity-LC-MS/MS Method for Quantifying Insulin Aspart in Human Plasma

Insulin aspart is often used by adults and children with type 1 diabetes and adults with type 2 diabetes. Marketed as NovoLog, insulin aspart has been one of the top-selling pharmaceutical products in the past five years. As such, there is tremendous interest within the clinical research community in analyzing and studying insulin aspart in clinical samples.

Presented here a hybrid immunoaffinity-LC-MS/MS method for quantifying insulin aspart in human plasma is reported. The QTRAP® 6500+ LC MS/MS system coupled with ExionLC™ system provides high sensitivity, robustness and broad dynamic range for MRM quantification of peptides. The immunoaffinity sample preparation significantly eliminates interference from matrix. Combined together, this method enables scientists to confidently quantify insulin aspart at 50 pg/mL in rat plasma.

Read Now

Universal Solution for Monoclonal Antibody Quantification in Biological Fluids Using Trap-Elute MicroLC-MS Method

The implementation of microflow chromatographic technique and immunoaffinity based sample preparation method provides significant improvement on assay sensitivity. Microflow LC provides multiple fold boost on signal intensity, while immunoaffinity based sample preparation dramatically improves the sample cleanness, thereby reducing baseline interference. Herein a hybrid LBA/microflow LC-MS/MS workflow for ultrasensitive quantification of SILuLite SigmaMAb universal antibody (SILuLite) in mouse plasma is presented. This method can be simply transferred to quantification assays for any other human mAbs in an animal matrix with minimum modification.

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High Resolution Mass Spectrometry
Technical Notes

An Immunoaffinity-High Resolution Accurate Mass Assay for the Pre-Clinical Quantification of Trastuzumab in Rat Plasma

The generic approach can be utilized in the pre-clinical setting for fast method deployment. For example, using anti-human IgG (Fc specific) capture antibodies allows for a simple and generic methodology for monoclonal antibodies such as Trastuzumab. In addition, multiple candidates can be rapidly screened using the same capture process. Routinely, immunoaffinity prepared samples are analyzed by signature peptide quantification on triple quadrupoles. In this study, we present an immunoaffinity high resolution accurate mass (HRAMS) assay as a complimentary analytical technique for signature peptide quantification.

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Quantification of Large Oligonucleotides using High Resolution MS/MS on the TripleTOF®5600 System

Current LC-MS approaches to oligonucleotide quantification predominantly use multiple reaction monitoring (MRM), however the complex fragmentation pathways of oligonucleotide species coupled with the variability of matrix effects mean that it can be difficult to predict the sensitivity and selectivity of a given MRM transition without significant optimization. These effects limit the utility of low resolution quantification methods both in terms of the achievable limits of quantification and in sample throughput, particularly when quantifying large numbers of potential drug candidates of different sequences, and their metabolites.

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An Immunoaffinity Coupled High Resolution-MS Workflow for Quantifying Biotherapeutics in Rat Plasma

Quantification of monoclonal antibodies (mAbs) and antibody drug conjugates (ADCs) in biological matrix is required during multiple stages of the biotherapeutic development process. LCMS is serving as an orthogonal methodology to ligand binding assays (LBA) for biotherapeutics quantification. Increased interest has been observed in simplifying LC-MS method development and exploring new quantification workflows including high resolution accurate mass spectrometry (HRAMS). The HRAMS technology provides quantification based on the precursor ion of the target analyte, which requires minimum MS optimization compared to MRM based quantification. Herein, we report a HRAMS method to quantify SILuLite SigmaMAb and trastuzumab emtansine in rat plasma, using SCIEX X500B QTOF system with ExionLC™ UHPLC system.

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High Resolution Mass Spectrometry
Technical Notes

A Highly Robust and Sensitive SPE-LC-HRMS Workflow for Quantifying KR Rich Peptide RTP004 in Rat Serum

Sample preparation using solid phase extraction (SPE) coupled with high resolution mass spectrometry (HRMS) has become more applicable and important for bioanalytical quantification of large peptides in biological matrices. The high resolving power and mass accuracy allows quantification based on the precursor ion of the target analyte, thereby significantly improving the assay sensitivity for the large peptides with low fragmentation efficiency. Herein a highly robust and sensitive SPE-LC-HRMS workflow is reported for quantifying the 35-mer proprietary peptide RTP004 in rat serum.

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Quantitation of Intact Therapeutic Protein in Plasma Matrix by LC/MS

For intact quantification in matrices there are several challenges including distribution of signal over many charge states, interference from matrix species and limited guidance on data analysis. A common practice for quantification is the use of extracted ion chromatograms (XICs) from the non-deconvoluted full-scan mass spectra. This approach may not be feasible in the case of intact therapeutic monoclonal antibodies in biological matrix, due to interference from matrix proteins and low intensity of target ions at low concentrations. Another approach is to quantify using the reconstructed spectrum of the target peak in the chromatogram. This may lead to the loss of original information during peak selection, however, and impact the robustness of the method, especially for complex samples.

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With the advancement of data analysis software, we developed a novel data processing strategy for quantification purpose, utilizing the available protein deconvolution algorithm to reconstruct the entire data file by deconvoluting every full-scan mass spectrum recorded in the data file. XICs of major glycoforms of the target intact therapeutic monoclonal antibody in the reconstructed data are used for quantification.

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SelexION® Differential Mobility Separation
Technical Notes

Differential Mobility Separation Mass Spectrometry for Quantitation of Large Peptides in Biological Matrices

The ability to quantify large peptides in biological matrices with adequate selectivity and sensitivity depends on several factors: 1) peptides are multiply charged, 2) peptides have varied fragmentation efficiency due to size and structure, and 3) different matrices can cause varied background signal and interferences. Tryptic digestion can reduce the size of the peptide and improve fragmentation but introduces an additional step, which is undesirable in a high-throughput environment.

Differential mobility separation (DMS) mass spectrometry adds an additional level of selectivity to LC-MS/MS providing gas phase separation of isobaric species and co-eluting interferences to reduce background noise. Here, the utility of a SIM workflow combined with DMS for specificity was investigated for quantifying large therapeutic peptides in a protein precipitated plasma matrix.

Read Now

Quantification of Peptides with Poor MS/MS Fragmentation using Novel Jet Injector SelexION® + MIM Workflow

MRM is widely used for the quantification of peptides, but frequently a peptide is not suitable for MRM analysis. Poor fragmentation or fragmentation that produces common low m/z ions can result in high background and a poor LOD. Peptides that do not fragment well include cyclic peptides. Low fragmentation efficiency typically translates to poor sensitivity. An alternative to MRM that has the potential for higher signal is SIM (Selected Ion Monitoring). However, the increase in signal response is accompanied by an increase in the noise, resulting in poor selectivity. Here we use MIM (Multiple Ion Monitoring) coupled with SelexION®+ Differential Mobility Separation Technology to achieve low level selective quantification of Somatostatin.

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SelexION® Differential Mobility Separation
Technical Notes

Sensitive Quantitation of Cyclic Peptide by Differential Mobility Separation Analysis

Differential Mobility Separation (DMS) using SelexION® Technology adds an additional level of selectivity, providing gas phase separation of isobaric species based on their chemical properties and ion mobility. In addition to adding an additional level of ion separation before entering the instrument orifice, SelexION® Technology is also compatible with the fast cycle times required for quantification workflows, including MRM or selective ion monitoring (SIM). Herein, a workflow combining DMS and SIM on SCIEX Triple Quad™/QTRAP® 6500+ LC-MS/MS System is demonstrated for cyclic peptide quantification in plasma.

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Rapid Quantitation of Substance P in Plasma Using Differential Mobility Spectrometry and Micro flow Liquid Chromatography

Substance P is an 11 amino acid neuropeptide that is known to modulate neural responses primarily associated with pain perception. Recent studies have shown that this peptide also plays a significant role in regulating the immune system, and that its increased production is part of the pathology of several autoimmune/inflammatory disorders including Inflammatory Bowel Disease and Rheumatoid Arthritis. Consequently, there is significant interest in analytical strategies that enable detection of Substance P at physiologically relevant concentrations. Here we describe a fast and robust method to detect and quantify Substance P in protein precipitated plasma. We demonstrate that sub-femtomole limits of quantification (LOQs) are obtained by combining traditional Multiple-Reaction Monitoring with micro-flow liquid chromatography and Differential Mobility Separation (DMS).

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Benefits of Differential Ion Mobility Spectrometry for High-Sensitivity Quantification of Peptides

Some modern biopharmaceuticals (exenatide, a glucagonlike peptide-1 agonist, for example) are active at very low concentrations (down to several pg/mL). To detect these drugs in complex biological backgrounds at ultra-low levels, highly sensitive and selective assays are required for robust and accurate quantitative analysis. Quantitative analysis of proteins and larger peptides can lead to dilution of the signal due to multiple charge states. Signature peptide quantification can improve sensitivity but increases the chance for overlapping transitions due to competing peaks and matrix impurities that can affect the signal-to-noise. This work examines the use of SelexION (Differential Mobility Separation) as a means of orthogonal separation to remove overlapping interferences and reduce the background noise to obtain accurate and selective quantification.

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Capillary Electrophoresis
Technical Notes

Improving the Detection Limits for Highly Basic Neuropeptides Using CESI-MS

Quantifying neuropeptide hormones can be immensely demanding. Researchers must contend with low concentrations in scarce samples and the fact that these very basic peptides bind to LC auto-sampler components, leading to very poor separation results. SCIEX has developed a new workflow using the powerful selectivity of the CESI 8000 Plus coupled to a QTRAP 6500+. Utilizing the CESI 8000 Plus reduced carry-over by two orders of magnitude compared to previous work on the same mass spectrometer. The assay successfully detected neuropeptides with 20-fold greater sensitivity than traditional LC-MS approaches.

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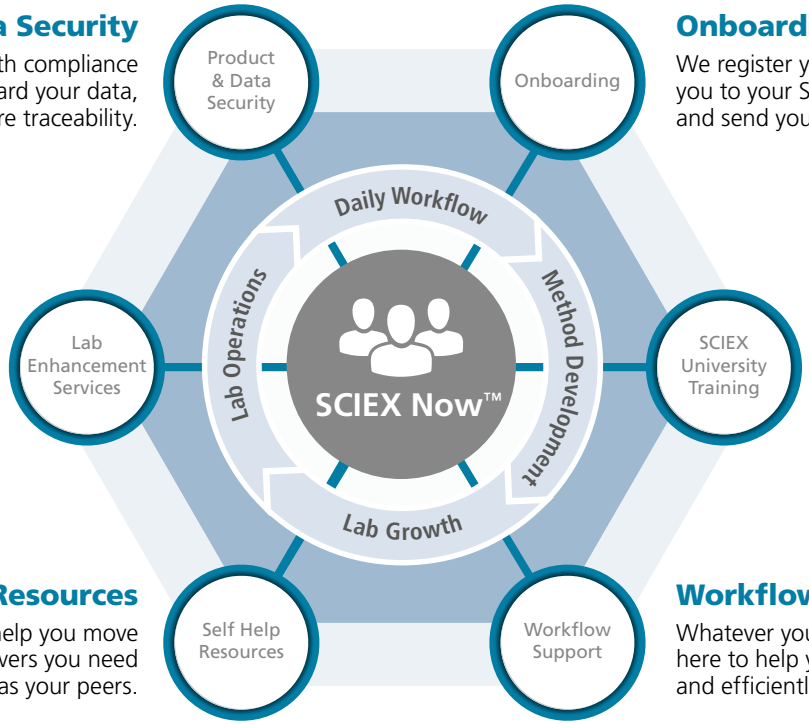

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Our Knowledge Base and Community help you move your science forward and find the answers you need from SCIEX experts as well as your peers.

Workflow Support

Whatever your challenge, the SCIEX Support team is here to help you achieve your scientific goals quickly and efficiently.

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