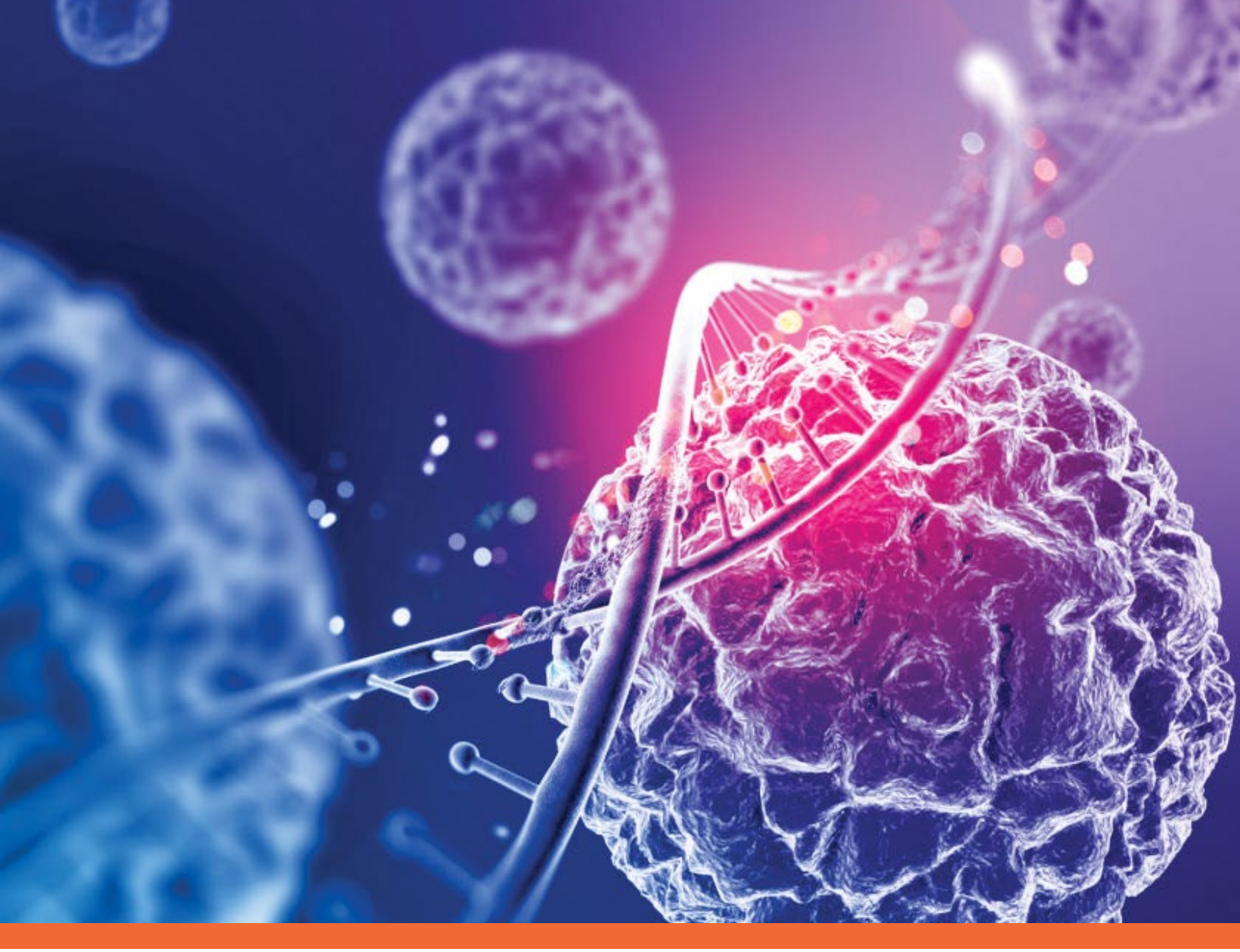

Mastering High-Resolution Mass Spectrometry (HRMS) for Preclinical Toxicology in Diverse Matrices

Sannova
Analytical

Navigating Preclinical Toxicology

This whitepaper addresses the key challenge in preclinical toxicology, namely distinguishing effects of drug exposure on endogenous molecules in various biological matrices. High-resolution mass spectrometry (HRMS) enables precise evaluation of drug effects on endogenous molecules, offering heightened sensitivity and specificity in diverse biological matrices compared to conventional methods.

The precision of HRMS allows for accurate measurement, discerning minute differences in mass-to-charge ratios (m/z) between ions that might appear identical on low-resolution instruments. Here we explore the role of HRMS in preclinical toxicology, a methodology that is increasingly gaining recognition as the gold standard for diverse applications such as biomarker research, drug metabolism and pharmacokinetics (DMPK), and bioanalytical quantification.

As the step preceding regulatory submission, preclinical toxicology serves as a critical bridge between laboratory findings and clinical trials. Regulatory bodies like the United States Federal Drug Agency (FDA) and the European Medicines Agency (EMA) expect to see a high degree of detail in submission data. Meeting these regulatory requirements in preclinical toxicology entails overcoming challenges related to conducting sensitive analyses for low-concentration endogenous molecules across various biological matrices.

In this context, HRMS emerges as a valuable tool, offering exceptional sensitivity and adaptability across a range of animal matrices. Its distinguished reputation among regulators further underscores its capability to consistently deliver results with a high level of confidence. Maximizing the benefits of HRMS at lower quantification limits is a complex task, demanding the expertise of experienced individuals.

Due to budgetary constraints, a dearth of qualified personnel, and limited resources, small and emerging biotech and pharmaceutical companies may find it advantageous to streamline operations by outsourcing rather than undertaking the in-house development of bioanalytical methods. This approach allows for efficient resource allocation while avoiding the substantial time and financial investments associated with internal development.

Ensuring a high level of confidence in preclinical toxicology necessitates the development of robust assays that adhere to good laboratory practices (GLP) specifically in the creation of accurate calibration curves. This meticulous approach is crucial for attaining precision in characterizing potential toxicities.

Regulatory advances aimed at lowering costs of drug development, such as the FDA's recent Omnibus Appropriations Act, permits manufacturers to reference information from previous applications related to manufacturing and toxicity when submitting subsequent applications.

Consequently, the utilization of GLP in preclinical stages becomes even more advantageous. By incorporating the most informed data delivered through HRMS and leveraging the confidence derived from GLP, a substantial impact can be observed in terms of optimizing costs and enhancing efficiency throughout the drug development process.

EXPERTISE IN PRECLINICAL BIOANALYTICAL STUDIES

In the course of drug development, sponsors often engage with various partners. Opting for a single source of analytical capability ensures a technical team intimately familiar with your molecule, capable of progressing methods study after study. Sannova serves as a central lab supporting toxicology testing from preclinical phases to late-stage clinical trials.

As a leading contract research organization (CRO) dedicated to facilitating drug development, we strive to assist developers in implementing high-resolution techniques for efficient drug development. Equipped with a state-of-the-art lab and seasoned scientists well-versed in analyzing a range of molecules in diverse animal matrices, high-resolution ligand binding, and immunogenicity studies, our track record encompasses over 300 validated methods across 21 different species and matrices. This attests to our proficiency in method development and validation for DMPK studies, contributing to the streamlined progression of drug development efforts.

Good Laboratory Practices

In preclinical toxicology, challenges arise when differentiating endogenous from exogenous compounds in biological matrices such as blood, plasma, serum, urine and tissues. In conducting these studies, we encounter three primary challenges.

The first set involves the complexity of the matrix, along with considerations of specificity and sensitivity. The second challenge revolves around establishing confidence and robustness in methodology, crucial for ensuring the reliability of generated data in future trials across different matrices.

The last category of challenges delves into the technical aspects, focusing on the selection of internal standards (IS) to facilitate the creation of biologically relevant calibration curves, effectively overcoming matrix effects. Addressing these challenges systematically enhances the precision and applicability of study outcomes.

The implementation of GLP to ensure the quality, reproducibility and confidence of experimental methods is instrumental in surmounting these hurdles. This includes addressing matrix interference, cross-reactivity in ligand binding assays (LBAs), and ensuring that calibration curves accurately reflect biological contexts where even low changes in concentrations can significantly impact DMPK parameters.

By adopting a standardized approach to method development, tailored to the unique characteristics of both the compound and matrix under consideration, confidence is heightened, ensuring that insights gained from one study are optimally informative for subsequent studies.

Z FACTOR & GLP SYNERGY OVERCOMES MATRIX EFFECTS

Matrix effects can significantly impact identification and quantification of a target analyte. The presence of coeluting substances in a sample can furthermore impact analysis by altering ionization efficiency during HRMS analysis. This can potentially cause ion suppression and lead to deviations in mass accuracy, thereby reducing the accuracy and confidence of results.

To overcome matrix effects, it is necessary to both reduce the presence of components that may introduce errors and/or to calibrate to compensate for their influence. Fulfilling our high internal GLP standards, Sannova Analytical's Z factor approach successfully fulfills this initial criterion, minimizing extraneous variables to enhance confidence. The second criterion is effectively achieved through our focus on robust methods that deliver high sensitivity, specificity, and consistency.

Choosing Internal Standards

Small biotech and pharma face multiple hurdles in securing the ideal internal standard (IS) – juggling availability, accessibility, and cost. Balancing analogs, meeting timelines, and thinking ahead about an internal standard that aligns well with future studies across matrices are crucial considerations. Navigating these challenges strategically is vital for success in small-scale biotech and pharma ventures.

Whether analysis is targeted or untargeted, the selection of IS is a critical aspect of preclinical toxicology. Analysts must consider the type of internal standard and its potential for biotransformation, requiring a comprehensive understanding of both the analyte, matrix and any inactive ingredients. When sourcing IS, attention should be paid to purity and radioactive enrichment to make sure they match with product specifications.

Additional scrutiny should be given to antibody-based therapeutic classes due to their high susceptibility to biotransformation. In this context, selection of IS for antibody-related studies requires a holistic approach to DMPK and immunogenicity.

STREAMLINING TOXICOLOGY EXCELLENCE

Toxicology distinguishes itself in the scientific field by often requiring a multidisciplinary approach. Coordination among diverse laboratory personnel is crucial in developing robust methodologies to streamline regulatory review processes, including biomarker qualification. For a comprehensive understanding of the criteria involved in selecting methods, we invite you to explore our previous *whitepaper* on high-resolution techniques for biomarker analysis, offering insights into the regulatory criteria of most interest to the FDA and EMA.

Predicting Toxicity Earlier in Development

Recent research suggests that predicting potential toxicity earlier in drug development using biomarkers is becoming a key area of focus. [1] By utilizing endogenous biomarkers to qualify drug-drug interaction (DDI) and DMPK toxicity indications, we can confidently make toxicity predictions at an early stage of development in a range of matrices, including plasma, serum and tissue.

Understanding the role of transporters is crucial in these analyses. These proteins have a significant impact on the absorption, distribution, and elimination of drugs, influencing their efficacy and potential adverse effects. The identification of several endogenous drug transporters, including OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K, has already contributed significantly to toxicity testing. [2-3]

However, inconsistencies between in vitro and in vivo assessments have been observed.[3]

These inconsistencies often arise from the low concentrations of endogenous molecules, where even small changes can result in significant biological effects. These limitations highlight the need to move beyond traditional methods and embrace high-resolution approaches to analyze endogenous transporter reactions during preclinical development, allowing for more accurate DMPK analyses.

To meet these challenges, MS techniques coupled with chromatography provide powerful systems for constructing detailed pharmacokinetic profiles for test compounds. This enables laboratories to assess precisely how these compounds affect endogenous biomarkers in diverse matrices and fulfill regulatory requirements with ample detail.

TARGETED VS. UNTARGETED MS

Targeted MS studies focus on specific compounds and involve chromatographic separation, using isotopically labeled internal standards for absolute quantification. On the other hand, untargeted MS methods play a vital role in the comprehensive analysis of complex mixtures. This approach often employs relative quantification utilizing IS.

In both contexts, HRMS effectively addresses the challenges posed by matrix effects and the fluctuating ionization and fragmentation efficiency of various compound classes in complex mixtures, which can lead to signal suppression during ionization.

Calibration Accuracy

In the realm of quantitative analysis, adhering to international guidelines for analytical method validation is crucial. We have refined our methodology during the development and validation of more than 300 methods to deliver high quality insights that adhere to international guidelines and regulatory acceptance criteria.

The multipoint calibration curve, widely employed in GLP toxicology, represents a significant evolution in estimating the calibration function for quantitative analysis. At Sannova Analytical, our calibration curve preparation involves two control blanks, two zero standards, and 10 non-zero calibration standards to achieve highly accurate quantification.

The analyte to IS peak area ratio values are then compared with quality control standards at four concentration levels, enabling precise quantification. The weighting factor that gives the smallest value of the sum of the percent relative error is taken to be the simplest model that gives the best results and adequately describes the concentration-response relationship.

The calibration curve is then applied to analyze each batch for precision and accuracy, ensuring confident and consistent results.

ABSOLUTE QUANTIFICATION

Absolute quantification in MS analysis of complex mixtures is a formidable challenge, requiring the resolution of matrix effects arising from the intricate nature and variable ionization efficiency of diverse compound classes, commonly leading to signal suppression; the pivotal step involves the development or definition of suitable standards, with recovery criteria dictating a coefficient of variation (%CV) within 15% and an IS recovery not surpassing 115%.

Quantifying Endogenous Biomolecules

High-resolution techniques also unlock the potential to obtain quantitative information, enriching our understanding of the time profiles of drug transporter inhibition following drug administration. However, accurately quantifying endogenous biomarkers presents challenges similar to qualification, requiring highly sensitive methods and precise calibration curves to deal with low concentrations and small, yet significant effects.

Quantification of endogenous molecules offers a window into time profiles of important biological reactions. MS methods, especially tandem MS (MS/MS) coupled with chromatography techniques such as liquid chromatography (LC) offer many advantages for both targeted quantification of biomolecules and for novel discovery. These benefits derive from the fragmentation of analytes, making it possible to separate and identify ions with very similar mass to charge ratios (m/z).

For example, the endogenous cyclic nucleotide, cyclic guanosine monophosphate (cGMP), is a commonly used biomarker used to assess cellular toxicity in preclinical toxicology. Unfortunately, the coexistence of naturally occurring cGMP and low cellular concentrations can often make it difficult to interpret the significance of toxicity testing.

To enhance the detection of low-abundance biomolecules, various methods involve fractionating serum or plasma to concentrate the target analytes and then employing antibody to enrich the target molecule from a proteolytic serum digest. To ensure the consistency of the immunoaffinity enrichment step, stability is introduced by including stable-isotope labeled peptides during the process. These enriched peptides are subsequently quantified using MS/MS.

Combining fractionation, isotopic labeling and MS/MS effectively overcomes the difficulties of coexistence and low concentrations, achieving high-quality, robust results. As interest in precision medicine using oligonucleotides continues to rise, regulatory bodies are progressively endorsing the utilization of MS methods, particularly in the field of biological drug development that encompasses interference Ribonucleic Acid (RNA) (RNAi), Antisense Oligonucleotides (ASOs), Small-Interfering RNAs (siRNAs), and Messenger RNAs (mRNAs) based therapeutics.

CHALLENGES IN BIOTHERAPEUTIC BIOANALYSIS

The rising prominence of biotherapeutics, which often utilize sophisticated modalities and novel delivery routes, introduces new challenges in bioanalysis. One sophisticated technique that has gained prominence is bioconjugation, which offers enhanced therapeutic efficiency and improved diagnostic accuracy.

Quantifying the inherent complexity of conjugated molecular structures is crucial for preclinical toxicology studies. MS methods address challenges in measuring excipients, potential drug metabolites and drug-drug interactions, along with matrix effects.

Strategic Partnerships in Preclinical Toxicology

This whitepaper introduces the key challenges of preclinical toxicology for modern drug development. As the need for achieving high-quality clinical predictions earlier in development grows, HRMS techniques have emerged as solutions to modern challenges in preclinical drug development.

These techniques offer sensitivity, specificity and are adaptable across a range of biological matrices. Notably, MS/MS coupled with LC offers a powerful approach to the targeted analysis of endogenous biomolecules.

Qualification and quantification of endogenous molecules both encounter similar challenges when measuring the biological impacts of drugs. Selecting appropriate IS and creating precise calibration curves using GLP is an integral part of addressing these challenges. Within this whitepaper, we have outlined Sannova Analytical's approach to constructing robust calibration curves, optimized through extensive refinement across numerous projects.

This methodology, employing our Z factor approach, serves to minimize errors, enhancing confidence in later stages of development and mitigating risks tied to inadequate preclinical data.

In summary, modern drug development demands both sensitivity and accuracy. However, many laboratories encounter challenges in accessing high-resolution methods and the required expertise for effective utilization. Outsourcing emerges as a practical solution, generating confident and efficient results that streamline the regulatory review process for preclinical submissions.

Choosing a partner with a proven track record in developing validated methods and a commitment to quality control, as demonstrated by Sannova Analytical's GLP and Z factor approach, plays a crucial role.

This decision supports the development of strong methodologies and provides a foundation for future drug development initiatives.

MANY HANDS MAKE LIGHT WORK

We would like to emphasize another key strength – Sannova Analytical's commitment to interdisciplinary communication within our laboratories.

As discussed earlier *(See Streamline Toxicology Excellence)*, toxicology frequently demands multidisciplinary expertise.

Expertise across various areas is essential to overcome matrix effects in diverse animal and tissue matrices and to effectively predict the potential for biotransformation.

Sannova Analytical's collaborative environment ensures a holistic approach, delivering comprehensive insights and optimal solutions for the challenges presented in preclinical toxicology studies.

To learn more about Sannova Analytical's experience and capabilities, visit our WEBSITE or contact one of our knowledgeable representatives.

5 Reasons to Choose Sannova Analytical

17 Years in Business
21 Different Species & Matrices Experience
300+ Validated Methods
11 Successful Regulatory Agency Audits
168 Active Customers Worldwide

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