The mass spectrometry innovations simplifying drug discovery workflows

Mass spectrometry (MS) has long been a valuable tool for drug discovery, and steady advances in its capabilities and performance have generated powerful insights for the pharmaceutical industry. The latest MS innovations are now helping biotherapeutics developers overcome challenges around sample preparation and large molecule analysis.

The use of MS in pharmaceutical discovery and development is rich and varied, spanning a wealth of applications from target discovery, medicinal chemistry, pharmacokinetics and bioanalysis workflows, to name just a few. Offering the selectivity, specificity and sensitivity required to answer a broad range of analytical questions, MS has become one of the most powerful tools in the drug discovery toolbox. While the traditional coupling of MS with liquid chromatography (LC) and gas chromatography (GC) continues to be a robust method, technological advances have seen the rise of other techniques, such as matrix-assisted laser desorption (MALDI) being directly coupled with MS, making it adept for even more applications. For example, the latest MS technologies can simultaneously analyse many features in one cell, which could help researchers discover novel targets with the potential for translation into new therapies.

MS is a particularly powerful tool for the discovery and development of biotherapeutics. Today, biotherapeutic products account for a growing proportion of new drug approvals. Increased focus on the development of much larger, more complex therapeutics such as monoclonal antibodies, fusion proteins and antibody-drug conjugates has placed new demands on MS. While small molecule drugs can easily be analysed on a system with a mass range of 1,250 Daltons (Da), the analysis of higher order structures such as peptides, proteins and oligonucleotides can require the visualisation of ions from the 2,000 Da to the tens of thousands of Da range.

This increased molecular complexity means that much greater instrument resolution is required to confidently elucidate atomic-level structure and function. Where a single or triple quadrupole mass spectrometer offering unit-mass resolution may be sufficient for early metabolite, impurity and degradant screening, high resolution accurate mass (HRAM) has become increasingly important for the analysis of large biotherapeutics and protein complexes.

“The evolution of mass analysers to acquire high resolution data at high mass ranges, in time frames amenable to coupling with ultra-high performance LC (UHPLC) has been incredibly important for drug discovery efforts,” says Rowan Moore, Senior Manager of the European Pharma and BioPharma Customer Solutions Center at Thermo Fisher Scientific. “The commercialisation of mass spectrometers with high mass, high resolving capabilities has undoubtedly opened new avenues in the study of protein drugs and their targets.”
The exceptional power offered by Orbitrap™-based instruments is leading the way when it comes to high mass, high resolution analysis. The latest Orbitrap™ instruments cover an ‘ultra-high’ mass range of up to 80,000 Da, with quadrupole mass selection up to 25,000 Da and pseudo-MS3 fragmentation.

Such advances in MS technology have also liberated drug discovery scientists to explore the coupling of traditionally non-MS friendly separation techniques such as ion exchange chromatography (IEX) and size exclusion chromatography (SEC) with MS. Historically the coupling of these techniques was only possible via offline LC separations from which fractions were collected for subsequent static nanospray HRAM MS analysis. Through the direct combination of native protein chromatographic separations and HRAM detection, researchers now have at their disposal the possibility of automated, high-throughput native intact mass analysis of complex therapeutics and drug targets/complexes.

**Removing the analytical bottleneck**

While advances in mass range and resolution are generating ever more powerful insights, sample...
preparation is still a major challenge, especially for bioanalysis applications. Currently, many MS workflows rely on expert users to prepare samples, which can be the rate-limiting step in many drug discovery workflows. Although there are kits available that can reduce preparation time for some samples from days to hours, introducing new ways of working that simplify or bypass these complex processes is still a key priority for many developers of MS products.

“Analytical chemistry can often feel like a never-ending game of moving the bottleneck,” says Dr Robert Plumb, Director of Omics Sciences, Scientific Operations at Waters. “Conventional MS still relies, in the most part, on the sample being prepared in or transferred to a liquid state for analysis. Too often, this results in time-consuming and expensive sample preparation.”

New MS innovations are helping to overcome this issue. Acoustic sampling, for example, is enabling thousands of measurements to be made every hour with minimal to no sample preparation. As a result, this is allowing high-throughput screens or activity assays to be developed. Moreover, the implementation of direct MS analysis based on ambient ionisation techniques such as direct analysis in real time (DART) and desorption electrospray ionisation (DESI) are further simplifying bioanalysis workflows. Because these techniques can operate in the open laboratory environment, they do not require sample pre-treatment steps – streamlining workflows and enabling researchers to collect more data, faster.

Additionally, as many new, powerful MS technologies can directly analyse substrates, without reagents, fewer artefacts are generated, which in turn increases confidence in results. Not only does better data quality aid researchers with their drug discovery efforts, but the costs saved on reagents can be reinvested to help expedite other parts of the pipeline. For instance, using the funds to perform more large-scale compound screens could help to identify more chemical starting points and so potentially increase the potential of drug discovery success.

“These technologies are beginning to remove
barriers that can increase timelines, not only for high-value activities such as tissue imaging, but also for purity assessment and confirmation of identity in medicinal chemistry synthesis too,” says Plumb. With the average time to market for most therapies being around a decade (and costing upwards of $1 billion), innovations such as these are playing an important role in accelerating the delivery of new therapies to the patients who need them.

In addition to the challenge of sample preparation in bioanalysis workflows is the increased chemical background of biological samples. “Matrix interference adds a complexity to the analysis which means we then have to look at additional innovations,” says Neil Walsh, Global Marketing Manager, Pharma/CRO at SCIEX. Here, the use of technologies such as differential mobility spectrometry and capillary electrophoresis MS (CE-MS) is allowing researchers to push the boundaries when it comes to generating meaningful analyses from complex samples.

Differential mobility spectrometry (DMS) has proven to be a valuable addition to ion mobility spectrometry methods, providing separations that are orthogonal to traditional LC-MS workflows. The technique makes use of a fast gas stream at right angles to an electric field, which causes ions of different mobilities to pass through the instrument with different trajectories. The use of DMS can sometimes circumvent the need to use LC techniques, due to its ability to provide reduced isobaric and isomeric chemical noise, without the need for extensive sample preparation steps. As a result, it is considered to be a powerful approach for bioanalysis workflows.

CE-MS is a powerful analytical technique that combines the high separation efficiency of capillary electrophoresis with the detailed characterisation that can be achieved using MS or HRAM analysis. As the method allows the analysis of intact proteins and various small molecules, including peptides, CE-MS is helping researchers obtain answers to questions that would not otherwise be possible using conventional LC techniques.

“These technologies are real milestones for orthogonal techniques,” says Walsh. “Their application is truly helping researchers overcome complex challenges, especially those relating to difficult to analyse sample matrices.”

**Commerically-available MS technologies**

A wide range of products are available on the market that are reducing the complexities associated with drug discovery workflows. SCIEX offers instruments for the confident characterisation of both small and large molecule therapeutics. Its latest TripleTOF® 6600 Quadrupole Time-Of-Flight (QTOF) mass analyser, for example, enables additional insights for more complex samples through its single multifaceted platform. The ability to perform data-independent SWATH® (sequential window acquisition of all theoretical fragment ion spectra) allows the collection of comprehensive high-resolution MS data for every detectable analyte. The system also allows for large numbers of Q1 isolation windows at user-controlled
widt hs, at rates of up to 200 per cycle, to achieve better specificity even in complex samples.

Combining capillary electrophoresis with electro-spray ionisation in a single process, the SCIEX CESI 8000 Plus ESI-MS capillary electrophoresis system is designed for rapid analysis of biopharmaceutical, proteomics and metabolomics samples. The system enables researchers to achieve reliable and sensitive separation of intact mAbs and proteoforms with online MS detection, eliminating the need for pre-fractionation and reinjection. The system also allows the study of molecular interactions and structure of proteins and peptides under native conditions.

Waters also supplies a broad range of technologies for high resolution mass analysis. Its Xevo G2-XS QTOF instrument is specifically designed to help researchers achieve confident analyses in even the most challenging of samples. With class-leading quantitative sensitivity, as well as exceptional mass accuracy, dynamic range and speed, thanks to the use of QuanToF™ technology, the system offers superior quantitative performance, even in the presence of interferences from complex matrix components.

The Waters Xevo TQ-S micro triple quadrupole mass spectrometer is a compact instrument that is designed for reproducible low-level analyte detection, but with a minimal laboratory footprint. This small-but-mighty system is capable of delivering consistent quantitative performance with a wide dynamic range. The instrument’s next generation T-Wave™ collision cell permits acquisition of 500 multiple reaction monitoring channels per second, while minimising cross-talk and maintaining intensity. The increased MS full scan speed capability of up to 20,000 Da per second reduces the impact on the duty cycle when switching between full scan and MS/MS acquisition.

The Thermo Fisher Scientific Orbitrap™ instruments are delivering exceptional performance when it comes to high resolution biopharmaceutical analysis. The Thermo Scientific™ Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap mass spectrometer incorporates a high capacity transfer tube for maximum ion loading, and an electrodynamic ion funnel capable of accommodating and transmitting ions over a broad mass range. The system also incorporates a high-field Orbitrap™ mass analyser for the rapid identification and analysis of peptides, label-free and tandem mass tag-based (TMT) quantitation, top-down proteomics analyses, sophisticated data-dependent acquisition (DDA) and data-independent acquisition (DIA), and dynamic retention time parallel reaction monitoring (PRM). Combined with the powerful Thermo Scientific™ Q Exactive™ BioPharma option, the system is capable of supporting highly sensitive biotherapeutic characterisation – from peptide mapping to the analysis of intact proteins under native conditions.

Some of the most recent Orbitrap-based instruments, such as the Thermo Scientific™ Q Exactive™ UHMR Hybrid Quadrupole-Orbitrap™ mass spectrometer, also offer enriched sensitivity in the high mass range through their novel in-source trapping. This innovation is enabling improved intact protein transmission in the gas phase and allows researchers to fine-tune protein desolvation (such as in the removal of detergent micelles) and perform consequent fragmentation of protein drugs, drug targets and protein-ligand complexes in their native state.

The future of the field
Improvements in sensitivity, precision and accuracy of instruments for research purposes have largely driven the remarkable advances in instrument design that have taken place over the last decade. However, with biotherapeutics set to play an even

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greater role in the future of medicine, developers of MS technologies are increasingly looking to better meet the needs of biopharma discovery workflows. Here, there is a clear focus on automation, miniaturisation and making both instruments and analysis software more user-friendly.

“In the future, we should expect to see greater use of miniaturised systems that can be implemented directly into automated drug synthesis and testing processes,” says Plumb. “Direct ionisation techniques are also likely to mature from the early research-focused products to more mainstream solutions geared towards non-expert users.”

With MS becoming more widely used in drug discovery, the requirement for user-friendly technologies that meet the needs of non-MS specialists does not simply relate to instruments themselves. The analysis workflows and software used to control them is also set to become increasingly accessible and here, computer algorithms are likely to take up the slack.

“The biggest change that I expect over the next three to five years is the incorporation of artificial intelligence into MS data analysis, enabling the searching of not only well-organised databases but also external data such as journal text,” says Plumb. “This will greatly increase the knowledge generation from MS data sources.”

MS has long been a valuable tool in the drug discovery toolbox, however recent advances are set to open up an array of new opportunities for biopharmaceutical developers and patients alike. The ability to combine increasingly powerful MS analysis with orthogonal techniques, such as capillary electrophoresis, SEC and IEX is allowing researchers to obtain answers to questions that would otherwise not be possible using traditional separation methods. Moreover, the latest MS tools are helping to minimise or even eliminate the complex sample preparation steps that are a current bottleneck in many MS workflows. By providing researchers with effective solutions, these technologies are helping to accelerate the delivery of safe and effective biotherapeutics to patients.

How much should a drug discovery company spend on marketing?

**By Paul Avery, Managing Director, and Craig Townsend, Director of Sales and Marketing Services, BioStrata**

Creating a great product or service is only half the battle when it comes to developing and growing a successful drug discovery business: often the company that markets itself most effectively will outcompete its rivals (even if these competitors have superior offerings on paper). So how much should a company invest in these activities?

The US Small Business Administration suggests that small businesses (defined as firms with less than $5 million in revenue) should spend 7-8% of their revenue on marketing. However, in reality, these companies are often competing with larger operations that spend more than this benchmark. For example, according to a 2016 survey of US and UK marketers, respondents from businesses with revenues of $250-500 million spent approximately 10% of that amount on marketing. Those from businesses with more than $5 billion in revenue spent even more – around 13%.

The BioStrata team has decades of combined experience working across the breadth of the life science industry, including the drug discovery sector. During this time, we have estimated that some companies are spending as little as 1% of their annual revenue on marketing, while at the opposite end of the spectrum, others are routinely investing as much as 10% (i.e. in line with suggested benchmarks).

Regardless of budget, all companies working in drug discovery are facing growing pressure to make their marketing plans smarter and their investment work harder. It is therefore essential to identify and execute marketing strategies and tactics that have a proven track record of delivering an effective return on investment and that will help you achieve your marketing and sales goals.


Helen Stewart-Miller is Director of PR Services and Dr Richard Massey is a science writer at BioStrata, a life science specialist marketing agency. The company’s growing team in Cambridge (UK) and Boston (US) includes a significant number of people with deep scientific experience and knowledge. The agency offers everything from strategy, branding and message development through to content creation, creative services, digital marketing and public relations.