SCIEX
The Power of Precision

VISION

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WELCOME TO THE FOURTH SCIEX VISION

SCIEX has developed a strong reputation as a pioneer of novel technologies over the years, pushing the boundaries of mass spectrometry in terms of fundamentals like speed, sensitivity and specificity. These advances have been crucial to expanding the use of MS across various markets but, in today's fast-paced environment, simply doing the same things better isn't always enough. To use a cliché, we need to think outside the box. In this case, the 'box' is the MS system, and we need to look more closely at how people want to use our instruments, and develop solutions to better serve their needs. This is the driving force behind our research and development efforts, and is directly responsible for our latest innovations – Echo® MS and Scanning SWATH® Acquisition.

Echo MS eliminates the need for liquid chromatography for certain workflows by using acoustic energy to deliver an accurate volume of a sample directly into a novel inlet probe attached to the MS. This innovation combines a number of technologies pioneered by SCIEX and external partners. Echo MS dramatically accelerates throughput, allowing the analysis of up to three samples per second with no sample clean-up – more than 30 times faster than the incumbent technology – while delivering unparalleled standards of quantification.

At the other end of the MS workflow is Scanning SWATH Acquisition. Following its initial release in 2010, SWATH Acquisition has been continually developed in collaboration with customers. Scanning SWATH is the next significant milestone in the development of this powerful technology, allowing every single analyte in a sample to be acquired, and the fragments correlated to the correct precursor in a quantitative manner, with a high degree of confidence. This simple acquisition method provides the type of comprehensive data that would normally require multiple experiments using different approaches – a game-changer for data-independent analysis. It moves us another step closer to our ultimate goal of the push button discovery platform.

I hope you enjoy reading more about these developments in this issue, as well as numerous customer applications of our technologies.

Chris Lock
Vice President LCMS Research & Development

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REDESIGNING
FORENSIC INVESTIGATIONS

Timothy Fassette, Senior Forensic Toxicologist, Henderson Police Department Crime Laboratory

Forensic scientists in the USA are harnessing the use of mass spectrometry to analyze suspected 'driving under the influence' samples, significantly expanding the number of drugs the laboratory can rapidly identify and quantify alongside blood alcohol levels. The data collected not only enhances police investigations, but is also being used to study trends of drug and alcohol abuse, which will ultimately help state authorities to set new regulations and laws.

Henderson Police Department Crime Laboratory in Southern Nevada, USA, has completely redesigned the way its toxicology laboratory investigates cases of suspected 'driving under the influence' (DUI), leading the way forward for other testing facilities. The laboratory provides DUI services for a population of around 600,000 across various cities, handling around 800 samples a year. Senior Forensic Toxicologist Timothy Fassette explained how the change came about: "Under the old DUI testing policy – which many labs still work to – only samples with a blood alcohol concentration (BAC) below the 0.084 g/dl cut-off level were analyzed for drugs of abuse. For samples above this level, just the BAC results were reported, with no further investigation for illicit drugs."

Timothy continued: "My background is in post-mortem examinations – where everything that could have contributed to the patient's death is investigated – and so I found this approach perplexing. Around two years ago, we retrospectively studied 100 previously adjudicated cases with a BAC above the threshold that, consequently, were not tested for other drugs at the time. We found that about two thirds of these individuals did, in fact, have drugs in their system that had gone undetected. Armed with this information, we set about implementing a new testing policy to analyze all DUI samples for drugs regardless of the BAC."

"Under the old policy, only around 25 % of samples met the criteria for further drug investigation

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following BAC analysis. Now, we wanted to test 100 % of the samples, but without introducing huge turnaround times or consuming our entire budget. The challenge was figuring out how to cost-effectively scale up testing, as our existing screening method – a 12-panel ELISA followed by full scan GC-MS analysis – was going to be wholly inefficient for the more comprehensive policy. It would be too costly to implement, may not detect very low levels of some drugs and, as ELISA is non-specific, may result in false-positive results requiring further investigation, increasing turnaround times. The method was also slow, taking four or five working days to complete, and so we started to look for alternatives."

"The Society of Forensic Toxicologists (SOFT) guidelines require screening and confirmatory/quantitative analyses to be carried out using different technologies. We already had a SCIEX QTRAP 5500 System that was used for LC-MS/MS quantitative and confirmatory analysis, and began to look around for an alternative technology to complement this. However, we realized during discussions with the SCIEX team that, as this is a hybrid instrument, we already had everything we needed in this system. We could use its linear ion trap capabilities for drug screening while continuing to operate the instrument in LC-MS/MS mode for quantitative and confirmatory analysis, giving us the two different technologies required by SOFT. There was no need for significant capital investment in new instrumentation and the further staff training that might entail."

"The SCIEX team was incredibly helpful, flying an application scientist out to our lab for a week to help us set up the ion trap method, and we were soon up and running. The efficiency of this screening protocol is far greater than the old one. It's more accurate and precise, and generates far fewer false-positive and -negative results. Whereas screening by ELISA can only determine the presence of a general class of drug in a sample, mass spec allows us to identify the exact compound. In combination with the new rapid extraction method we developed, this protocol enabled us to reduce our drug screening time from five days to one. We also increased the number of drugs screened in each sample from 42 to over 100, while decreasing costs by around 70 %. The time and money we save using this method means that we are now able to screen the 75 % of samples that previously went untested, giving us a complete picture for every DUI case we receive."

"Transferring the screening method to the SCIEX system has been the driving force for a lot of the success we've had with the project over the last two years," said Timothy. "As a small toxicology lab, it was crucial to create the most efficient, robust and cost-effective method possible for DUI testing, which the QTRAP 5500 delivers perfectly. We were so impressed with its performance that we decided to move all our GC-MS confirmation testing to the SCIEX system too, combining three separate methods into a single analysis, a transition that went very smoothly, thanks to the sensitivity and selectivity of the QTRAP."

"The new DUI testing policy has not only enhanced our work, but has also benefitted other police departments. Supplying investigators with a single report containing all the analytical results from a sample saves them time as they no longer have to search around for a number of different reports, or go back and forth requesting extra tests further down the line – potentially compromising sample integrity by reopening the evidence – since everything now arrives at once. The state authorities are also using our data to help identify and monitor trends in drug use, which will ultimately be used in setting ordinances. As the sole lab performing full testing on a routine basis, Henderson is the only one that can provide the true statistics; despite being the smallest publicly-funded forensic lab in Nevada by far, we're leading the way in DUI cases," Timothy concluded.

To find out more about SCIEX QTRAP® systems, visit **www.sciex.com/products/mass-spectrometers/qtrap-systems**

> "…THIS PROTOCOL ENABLED US TO REDUCE OUR DRUG SCREENING TIME FROM FIVE DAYS TO ONE. WE ALSO INCREASED THE NUMBER OF DRUGS SCREENED IN EACH SAMPLE FROM 42 TO OVER 100, WHILE DECREASING COSTS BY AROUND 70 %."

PERFECTING **PROTEIN ANALYSIS** WITH MASS SPEC

Rachel Rowlinson, Specialist in Protein Mass Spectrometry at Peak Proteins

Proteins are valuable molecules for a broad range of applications, but their expression and purification can often require extensive engineering, analysis and troubleshooting in order to obtain a correctly folded protein with the desired therapeutic activity. Peak Proteins provides a full service for protein manufacture and structure determination, and uses mass spectrometry as an essential part of its toolkit to deliver high quality products to its customers.

Peak Proteins, based in Cheshire, UK, is a company with decades of experience in protein science, using its extensive knowledge to support a wide range of customer projects. Rachel Rowlinson, Specialist in Protein Mass Spectrometry at Peak Proteins, discussed how mass spectrometry assists the company's work and enables it to offer a precise and reliable means of investigating proteomics and protein characteristics. She explained: "Peak Proteins was founded five years ago, and our main focus is on the manufacture of proteins as tools to support small molecule and biologics drug discovery. We perform anything from large-scale protein expression to protein crystallography and structure determination. Anyone can contact us at any point during their project, from construct design through to expression and purification, and we can engineer the protein sequence according to each customer's individual requirements."

The team performs mass spec analysis to look for intact protein mass, as well as more in-depth peptide mapping. "I joined the Peak Proteins team around a year ago to form the protein mass spec analysis side of the business, where we routinely use this technique to assist our team workflow. Protein expression can be a difficult art to master, as not all proteins fold themselves as beautifully as others, and this affects their activity – a lot of our work is troubleshooting, and this is where mass spec really helps us make key decisions about the proteins we work with. I've been working in protein analysis for over 25 years now, so I've seen many of the evolutions in this field, including the advancement of mass spectrometers, which are now essential tools for this work."

The scientists at Peak Proteins use mass spectrometry as a regular part of their day-to-day work, and have chosen SCIEX systems due to their reliability and ease of use. Rachel explained: "We have a SCIEX X500B QTOF System that we regularly use to confirm protein identity, as well as for sequence and mass determination, and to look for post-translational modifications. It's fantastic for this kind of routine analysis and it's great for QC; it switches between different analyses – such as intact mass analysis or peptide mapping – seamlessly. This thorough testing gives additional information to the customer, an extra degree of confirmation that we are supplying the correct protein, and that it's 'good to go' for their needs. We also provide a stand-alone service to external customers, using the X500B. Anybody can contact us and ask for mass spec analysis – there are a number of ways we can help with customers' projects, including looking at binding partner interactions and antibody characterizations. We're always happy to discuss customer requirements and see if the X500B might suit their needs."

"We have a SCIEX ExionLC on the front end of the X500B that uses a very simple formic acid-based buffer system. The instrument has two different columns, one C4 column for intact mass, and a C18 column for peptide mapping. I only use one set of buffers for reverse phase, so it's a simple valve switch depending on what kind of analysis I want. Having a

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QTOF system is great too, as you really get the resolution, which is fantastic for what we do. It's such a simple process, when I want to use the system, it's just a case of walking up to the instrument and deciding which analysis I want to do; at the click of a button, everything else is done for you, including the calibration."

Rachel continued: "Our throughput varies depending on what projects we have on at any time; some weeks we can perform 10 to 20 intact masses, other weeks it can be more. We probably run peptide mapping analysis around 20 to 30 times, but there is the capability to run more. I regularly queue up runs overnight for both of these workflows, which means that the next day I can come in and start the analysis of all the samples – it's an ideal tool for protein work. I've previously set up a 36-hour run for intact masses and peptide mapping, and our data was ready and waiting for us afterwards with great results. The sample turnaround is very efficient; I can run intact mass analysis in five minutes, and peptide mapping is routinely only a 10-minute run."

"The X500B is the perfect fit for our lab space; it's great having such a compact instrument as it means that you don't need to have a specific mass spec lab. The X500B sits at one end of our protein purification lab, and the beauty of this is that the instrument is open access for a lot of our scientists. It only took me about 20 minutes to show everyone how to use it, and now they run their own intact mass samples without needing supervision. It's so simple, quicker than the alternative of running a gel, and gives us a lot more information. I've worked on many mass specs in the past, and this is by far the most reliable and intuitive instrument I've used; it's an extremely accurate machine giving us great confidence in the results we can provide to our customers."

"We use the SCIEX Bio Tool Kit routinely for intact mass analysis, as we can quickly deconvolute the data to get results of the mass of our proteins. It's a really informative tool that we wouldn't be without. I also routinely use BioPharmaView Software, as we are manufacturing bespoke proteins with unique sequences or tags that are quite different to what's in the public domain. BioPharmaView is key to our data analysis, allowing us to search all of the peptide mapping data against that bespoke sequence. We can instantly tell the customer if there's an issue, for example, if we can't see the mutation or if it looks like some of the protein has degraded. There's no end to what you can do with both BioPharmaView and the Bio Tool Kit, which dovetail perfectly with the reliability of the X500B instrument," Rachel concluded.

To find out more about Peak Proteins, visit
www.peakproteins.com

To find out more about the SCIEX X500B QTOF System, visit
www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems/x500b-qtof-system

> "THERE'S NO END TO WHAT YOU CAN DO WITH BOTH BIOPHARMAVIEW AND THE BIO TOOL KIT, WHICH DOVETAIL PERFECTLY WITH THE RELIABILITY OF THE X500B INSTRUMENT."

EFFICIENT DETECTION OF ENVIRONMENTAL POLLUTANTS

Professor Kiwao Kadokami, Faculty of Environmental Engineering at the University of Kitakyushu

Environmental pollution is an ongoing global concern, with an ever-growing list of synthetic chemicals understood to adversely affect human, plant and animal health, as well as causing climate change. In the Faculty of Environmental Engineering at the University of Kitakyushu, Japan, an innovative mass spectrometry-based method has been developed, using a SCIEX X500R QTOF System to detect chemical pollutants in environmental samples.

The Faculty of Environmental Engineering at the University of Kitakyushu in Fukuoka, Japan, has the outward looking vision 'to walk the regions, nurture the environment, connect with the world', and encourages students to develop a global outlook on environmental concerns. After retiring from the university in March 2016, Professor Kiwao Kadokami continues to teach and conduct research there as a Specially Appointed Professor. He explained his background: "I have studied the issues of chemical substances for about 40 years, and I fear that environmental pollution is adversely affecting human health and ecosystems. In order to address this situation, a highly efficient detection method is necessary to assess the current state of pollution. My research topics involve the development of such a method, and using it to detect and measure environmental pollutants."

Currently, GC-MS and LC-MS are the methods of choice for the detection of trace chemicals, due to their superior sensitivity and selectivity. However, the need for a calibration standard for every target limits the number of substances that can be measured. Professor Kadokami's research focused on streamlining this bottleneck, by developing the Automated Identification and Quantification System with a Database (AIQS-DB). By using a database instead of calibration standards, AIQS can measure hundreds of substances simultaneously. The retention time, mass information and calibration curve of each substance – obtained

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under standardized device settings – are registered in the database. Samples can then be measured using the same settings to provide reliable identification and quantification by reference to the database. New substances can easily be added, and there is no theoretical limit to the number of substances that can be included. With no need for calibration standards, running costs are low, and even difficult to obtain substances can be analyzed.

Professor Kadokami explained: "About 15 years ago, I created AIQS-GC, based on GC-MS. It has become widely recognized, adopted by the Japanese Industrial Standards (JIS), and is being used in many institutions. Since then, LC-MS has become extensively used and, in September 2016, I chose the SCIEX X500R LC-QTOF-MS to develop an LC version of AIQS. The most important criterion when choosing the instrument to replace my previous LC-MS/MS and LC-TOF-MS was the ability to perform exhaustive analysis of a large number of substances. I considered all LC-MS manufacturers, but my final decision was made based on conversations with other users."

Common environmental pollutants – such as pesticides, medicines and personal care products – that could not be measured using GC-MS were selected for the AIQS-LC database, and many candidate substances were also obtained from Professor Kadokami's collaborators. "Thanks to the performance of the X500R, it only took six months to register 500 substances in the AIQS-LC," he said. "At present, AIQS is the only method that allows this many environmental contaminants to be measured simultaneously."

Although Professor Kadokami has been responsible for the development of AIQS-LC as a detection and measurement method, he has many collaborators from China, Vietnam, Serbia and Australia – as well as from within Japan – who are using AIQS-LC to survey environmental pollution. Together, they have analyzed around 1,000 samples, including environmental water, drinking water, drainage water, soils and sediments, particulate matter in the ambient air, and indoor dust.

Professor Kadokami elaborated on his choice of instrument: "The SCIEX X500R is ideal for analyzing a large number of chemical substances because, with SWATH Acquisition, the MS and MS/MS spectra are obtained simultaneously, and the process is less susceptible to interference by contaminants. The SWATH data also allows the retrospective analysis of substances not in the database at the time of initial analysis, by using suspect screening and non-target screening methods. The ion source is not easily contaminated, the measurement data file size is small, and the data processing speed is markedly faster than that of previous instruments. My research is therefore progressing very efficiently; I believe the X500R is the most suitable LC-MS instrument for AIQS currently available."

"In the future, I would like to work on measuring other chemical substances using different instrument conditions, columns and ionization methods. We currently use ESI in positive mode, but I am considering establishing another database using negative mode ESI. This will make it possible to measure over 1,000 substances with the X500R. That's what I want to achieve, though I am fairly elderly."

To learn more about the Faculty of Environmental Engineering at the University of Kitakyushu, go to **www.kitakyu-u.ac.jp/env/lang_en/index.html**

To find out more about the SCIEX X-Series QTOF Systems, visit **www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems**

> "THE ION SOURCE IS NOT EASILY CONTAMINATED, THE MEASUREMENT DATA FILE SIZE IS SMALL, AND THE DATA PROCESSING SPEED IS MARKEDLY FASTER THAN THAT OF PREVIOUS INSTRUMENTS."

WHEN ONLY THE **HIGHEST SENSITIVITY AND ACCURACY** WILL DO

PK/PD studies are an important step in the drug discovery and development workflow. Scientists at Boehringer Ingelheim rely on mass spectrometry for these and other investigations to evaluate potential new therapies, employing both quadrupole time-of-flight and triple quadrupole technologies.

Boehringer Ingelheim is a global pharmaceutical company committed to research and development, manufacturing and marketing of novel human and veterinary medicines. A crucial step in the development of new therapies is evaluating the properties of potential drugs, which is the role of the Bioanalytical Group's Drug Metabolism and Pharmacokinetics (DMPK) department in Ridgefield, USA. Dr. Lin-Zhi Chen, Senior Research Fellow and Bioanalytical Group Leader, explained: "Drug discovery and development begins with target identification, followed by compound screening to determine potential candidates to take forward for further studies. Once the best candidate has been identified, the DMPK department provides bioanalytical services to evaluate its properties, including toxicity and PK/PD studies."

Dr. Lin-Zhi Chen, Senior Research Fellow and Bioanalytical Group Leader, Boehringer Ingelheim

Lin-Zhi continued: "I've been using mass spec for about 30 years now, and have spent much of this time working on drug metabolism and PK/PD evaluation of biologics and oligonucleotides, including quantification and biotransformation studies. Our department has a dozen SCIEX mass specs, including Triple Quads, which we have used for many years, and the more recently acquired high resolution QTOF instruments – three TripleTOF 6600s. These instruments are used extensively for preclinical analysis of small and large molecules and oligonucleotides for a wide variety of applications, including immuno- and cancer therapies, cardiovascular disease and metabolic disorders. More than 95 % of our studies are now mass spec based, complemented by LC-fluorescence and LC-UV analysis as necessary."

"We analyze plasma, urine and tissue samples from human or animal studies, looking for *in vivo* biotransformation of proteins, and quantifying both proteins and oligonucleotides. The biggest challenge for these analyses is sample clean-up and enrichment, to maximize sensitivity. For example, we routinely use immunocapture to purify and enrich protein biologics from plasma samples prior to MS/MS quantification on a Triple Quad, achieving sensitivity in the region of low nanograms per ml. The drug is captured on magnetic beads using antidrug antibodies, which are then isolated. After washing the beads, the drug is eluted and, depending on the type of analysis we want to do, may be digested. Digestion gives us better selectivity for quantitative assays, but intact protein or subunit analysis is often performed without trypsin digestion, as this gives us an overall picture of the structure of the molecule."

Until recently, most of the published literature focused on fluorescence methods for oligonucleotide analysis, but the changing face of the industry has brought high resolution MS to the fore. "Previously, it was

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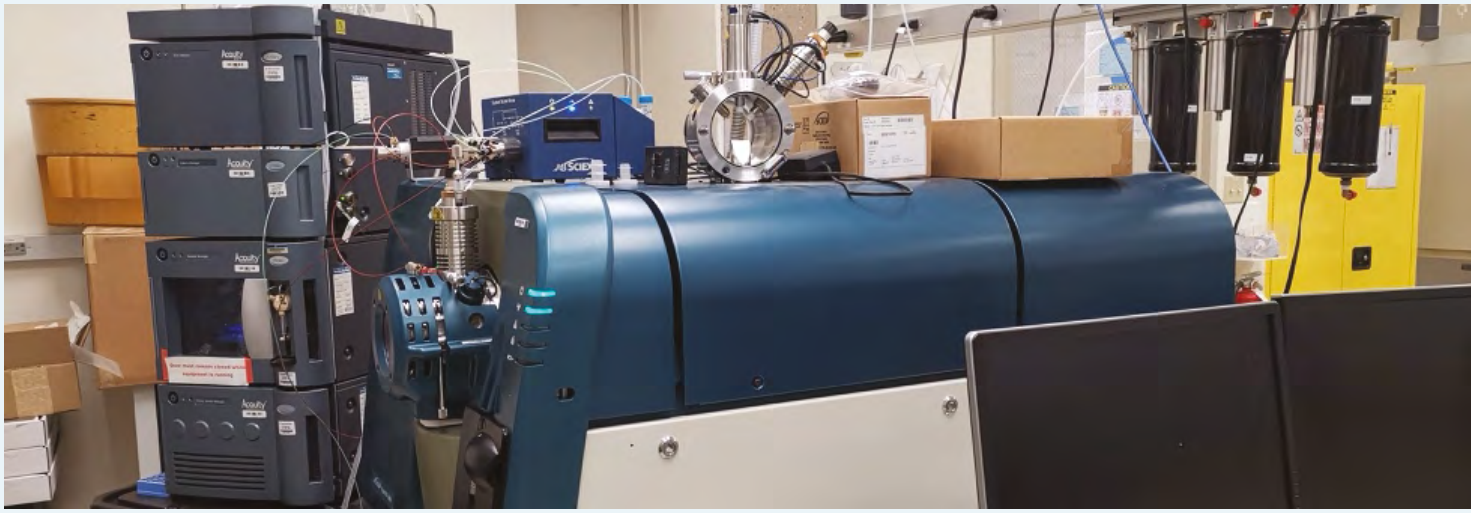

largely small biotechnology companies that engaged in early stage oligonucleotide drug development, and they tended to use LC-UV or fluorescence methods, or ELISAs, as these did not require significant investment in high tech instrumentation or staff with specialist expertise. That's history now. Today, large pharma companies are focused on oligonucleotides, and interest is turning to high resolution mass spec as the technique of choice. In our department, we rely on the QTOFs for simultaneous quantification and metabolic profiling of oligonucleotides; the high resolution and mass accuracy of these systems allow us to reduce, or even eliminate, the effect of any interfering peaks, improving sensitivity."

"We have shown that mass spec can now match or even exceed the sensitivity of fluorescence techniques, achieving up to 10 times more sensitivity using MS/MS analysis. Typically, we reach a sensitivity of less than 10 nanograms per ml using high resolution MS quantification – as low as picograms per ml using MS/MS – which is probably better than most LC-fluorescence methods or ELISAs. At the same time, we have been able to halve the run time from roughly 10 minutes a sample by LC-fluorescence to approximately five minutes by MS, increasing throughput significantly; LC-fluorescence requires a long gradient run to separate the parent drug and metabolites for individual quantification, whereas MS distinguishes between compounds based on their mass, so the gradient run time can be shorter. Even more of an advantage is that the QTOFs allow us to quantify the parent oligonucleotide and, simultaneously, perform metabolite profiling and quantification, which is hard to achieve by LC-fluorescence, and not possible with ELISA or qPCR."

"As we support GLP and GCP studies, once we identify good instruments, we tend to stay with them, because it makes validation, training and regulatory compliance easier. Generally, we regularly upgrade our older systems, so we always have the most recent generation of instruments. As well as that, we often invite SCIEX application scientists to come to our site to deliver training, demonstrations and seminars on new applications, ensuring that we keep abreast of the latest technology," Lin-Zhi concluded.

To find out more about Boehringer Ingelheim, visit **www.boehringer-ingelheim.com**

To find out more about the SCIEX TripleTOF® 6600 System, visit **www.sciex.com/products/mass-spectrometers/qtof-systems/tripletof-systems/tripletof-6600-system**

> "TYPICALLY, WE REACH A SENSITIVITY OF LESS THAN 10 NANOGRAMS PER ML USING HIGH RESOLUTION MS QUANTIFICATION – AS LOW AS PICOGRAMS PER ML USING MS/MS."

HIGH THROUGHPUT PROTEOMICS WITH SCANNING SWATH® ACQUISITION

Prof. Dr. Markus Ralser, Charité – Universitätsmedizin and Francis Crick Institute

Christoph Messner, Analytical Chemist, Francis Crick Institute

Vadim Demichev, Computational Scientist, Francis Crick Institute

Proteomics has huge potential for the elucidation of disease mechanisms, as well as rapid diagnostics and precision medicine. However, current proteomics methodologies are tailored to small-scale research applications. Prof. Dr. Markus Ralser and his colleagues are aiming to address this issue through the development of high throughput proteomics based on SCIEX's recently launched Scanning SWATH Acquisition.

Prof. Dr. Markus Ralser is a pioneer of metabolism research, dividing his time between Charité – Universitätsmedizin Berlin in Germany, where he is Director of the Institute of Biochemistry, and the Molecular Biology of Metabolism Laboratory at the Francis Crick Institute in London, UK. His multidisciplinary team is interested in cellular homeostasis and response to external stimuli, with the aim of better understanding metabolic processes and identifying potential targets for drug development. They use mass spectrometry to monitor small changes in protein production across large sample cohorts, and have a long history with SCIEX. Markus explained:

"Detecting these, often very small, changes in protein expression or post-translational modification requires us to perform measurements on a much larger scale than most biological laboratories – something traditional proteomics approaches simply aren't designed to cope with. Over the years, this has led to ever-closer collaboration with SCIEX, to find innovative solutions and develop new methods to address this need for high throughput. Through this partnership, we have been lucky enough to have early access to a number of exciting technologies to assist with application development. The most recent of these is Scanning SWATH Acquisition, which has proven ideal for high throughput proteomics."

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Scanning SWATH Acquisition, available exclusively on the TripleTOF® 6600+ System, is the next chapter in the SWATH story. It allows every single MS/MS iteration to be acquired and directly correlated to the correct precursor, providing a single, all-encompassing dataset. Christoph Messner, an Analytical Chemist in the Francis Crick lab, explained: "With standard SWATH Acquisition, you cycle over the entire mass/charge range in a step-wise manner, with some overlap between the isolation windows. This works very well, and produces high quality data, but the speed of the step-wise acquisition is naturally limited. In contrast, Scanning SWATH relies on continuous scanning of the quadrupole, allowing much faster cycle times without losing selectivity. Using this approach, we can go down to a window size of three or five daltons and cycle times of half a second."

"Scanning SWATH basically decouples isolation window size from cycle time," added Computational Scientist Vadim Demichev. "This allows us to have very short cycle times, which is ideal for high throughput approaches using short chromatography gradients of around five minutes, while maintaining a reasonable window size. We can therefore choose the most appropriate window size for our experiment and sample type, whether we're working with primary cells, cultured cells or plasma. This would be impossible with normal SWATH Acquisition, due to inefficiencies inherent in any step-wise process."

Vadim continued: "Our high throughput proteomics workflow essentially consists of three elements. The first part is our high flow chromatography set-up, which combines a flow rate of 800 µl/min with a fast, five-minute gradient to achieve separation. Next comes the Scanning SWATH Acquisition, which has been developed for the TripleTOF 6600+ system by the SCIEX team with continuous interactions and input from our laboratory. Then the third part of the workflow is data analysis, which is performed by our DIA-NN (data-independent acquisition by neural networks) software tool. Using this approach, we can achieve the same performance with a five-minute gradient as our previous generation SWATH-based method could with a 50-minute gradient – a 10-fold increase in throughput – allowing up to 200 samples a day to be analyzed with a single instrument."

Markus continued: "This increased throughput enables us to run much larger scale experiments than could previously have been undertaken, due to the sheer scale of the studies. A good example of this is a collaboration with the UK Medical Research Council's Epidemiology Unit. As part of an ambitious epidemiology project called the 'Fenland Study', the MRC unit has been following the health of about 12,000 individuals for over a decade. This is a classical epidemiology study looking at who develops certain metabolic diseases – fatty liver disease, Type 2 diabetes, metabolic syndrome, etc. – and our role in this project is to systematically perform proteomic analysis of plasma samples for every individual, with the aim of identifying the protein expression signatures that correspond to the occurrence of these diseases. MS-based high throughput proteomics approaches, such as the one we've developed, are well suited to this type of investigation, providing a rapid, cost-effective and more dynamic alternative to genome sequencing."

"We now have three TripleTOF systems in our laboratory at the Francis Crick Institute, and are using this high throughput capacity to refine our workflow and further increase the robustness of the technique. These will soon be complemented by additional systems in our Berlin lab, which is situated within the medical faculty of Charité – Universitätsmedizin Berlin. This lab is ideally placed to translate the technology to medical applications, which is where we believe this approach has real potential," Markus concluded.

To find out more about the Francis Crick Institute, visit **www.crick.ac.uk**

To find out more about the Charité – Universitätsmedizin Berlin, visit **www.charite.de/en**

To find out more about Scanning SWATH Acquisition, visit **www.sciex.com/scanningswath**

> "SCANNING SWATH BASICALLY DECOUPLES ISOLATION WINDOW SIZE FROM CYCLE TIME… THIS ALLOWS US TO HAVE VERY SHORT CYCLE TIMES, WHICH IS IDEAL FOR HIGH THROUGHPUT APPROACHES."

OPEN YOUR EYES
TO THE POTENTIAL OF MASS SPEC

Dr. Thomas Lam, Associate Professor in the School of Optometry at Hong Kong Polytechnic University

Myopia – or short-sightedness – is an increasingly common eye disorder that causes distant objects to appear blurry. Scientists in Hong Kong are turning to proteomics to investigate factors that affect normal development of the eye and can potentially result in this condition.

Proteomics can provide a wealth of information on the proteins involved in particular disease states, offering insights into the external environmental or genetic factors that affect normal development in both humans and animals. Dr. Thomas Lam, Associate Professor in the School of Optometry at Hong Kong Polytechnic University, is using proteomics to investigate various eye disorders, with a particular focus on myopia. Thomas discussed how the lab uses SCIEX instruments to support its work: "I graduated from Hong Kong Polytechnic University around 15 years ago, before going on to achieve a PhD at the same institute. I have always had an interest in myopia, which was the main focus of my PhD, but this was long before we were fully aware of proteomics and the in-depth information that this approach could provide. There are many clinical situations where proteomics could be helpful, such as myopia and glaucoma, but also diseases such as macular degeneration and corneal infections. Discovery of new protein interactions could be groundbreaking, and potentially allow us to provide better treatments to our patients, and I was determined to explore this."

"Eyes are the perfect model for this type of research, as you can directly compare one diseased eye and one

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healthy eye, as the genetic model is the same. During my studies, I was introduced to 2D gels to try and profile the proteins in animal eyes. These are time consuming and labor intensive but, at that time, there was little other choice, and almost nothing was known about proteomics in myopia. In addition, it was not possible to run gels for some tissue types – ocular fluid, for example – which limited the work we could perform. It wasn't until some years later that people started to move from 2D gels to LC-MS, which led me to purchase both ion trap and MALDI-TOF mass spec instruments around seven years ago. Unfortunately, I didn't have time to understand the complexities of the systems, and I struggled with both the hardware and the software, which put my proteomics work on hold for a couple of years; but this was before I was aware of SCIEX."

"In around 2014, I took up a teaching position in Singapore, and met many different groups working on various projects. It was here that I encountered other brands of mass spectrometry instruments, saw how others used SCIEX instruments to support their work, and the kind of results they were able to produce. When I returned to Hong Kong, I was determined to give the technique another go, and continue my proteomics work."

"From what I'd seen in Singapore, I knew that the SCIEX systems would be suitable to investigate different proteins for eye research. I spoke to other universities and labs that use mass spec, and many commended the local SCIEX team here in Hong Kong, which gave me further confidence that the company doesn't just supply systems, but also provides ongoing support. This was incredibly important to me, given the previous experience I'd had with other mass spec instruments, and was ultimately the reason I chose SCIEX, investing in a TripleTOF 6600 and QTRAP 6500+."

"A lot of labs do not have access to their own mass spec instruments, and send their results to a core facility. This isn't always the best option, as these facilities can be busy, and often follow a standard protocol that cannot cater for specific sample types, putting a limitation on their work. We have found that our results are a lot more in depth and revolutionary than other publications looking at proteomics and myopia, due to access to our own mass spec equipment. With the support we receive from SCIEX – which is second to none – we can get the best results out of the systems. We have developed many collaborations worldwide, and we are often sent different samples to test. I am expanding my team to further our proteomics research, and have found that the SCIEX systems are fantastic to train on, as they are incredibly intuitive and easy to use; everyone in the lab group enjoys working with them, which is very important to me. We also benefit from the OneOmics Project, integrated into SCIEX Cloud, which makes analysis and sharing of data easier."

"Now that we have many years of research, we are looking more into translation. Our primary goal is to try and develop drugs that can be used to treat myopia or glaucoma, which would be amazing. Proteomics is also incredibly useful in detecting biomarkers. For example, some work has already been performed looking at the proteins found in human tears to detect systemic diseases – such as diabetes or glaucoma – so we can apply our knowledge of proteomics to target these markers for clinical applications. These are the two main areas for proteomics for us now; drug development and biomarker detection and, thanks to SCIEX's mass spec instruments, these goals are hopefully within reach in the very near future," Thomas concluded.

To find out more about the School of Optometry at Hong Kong Polytechnic University, visit **www.polyu.edu.hk/so**

To find out more about the SCIEX TripleTOF® 6600 System, visit **www.sciex.com/products/mass-spectrometers/qtof-systems/tripletof-systems/tripletof-6600-system**

> "SCIEX SYSTEMS ARE FANTASTIC TO TRAIN ON, AS THEY ARE INCREDIBLY INTUITIVE AND EASY TO USE; EVERYONE IN THE LAB GROUP ENJOYS WORKING WITH THEM, WHICH IS VERY IMPORTANT TO ME."

MASS SPECTROMETRY AT THE SPEED OF SOUND

Tom Covey,
Principal Research Scientist, SCIEX

In 1989, SCIEX launched the first mass spectrometer designed to serve solely as a detector for high performance liquid chromatography, based on atmospheric pressure ionization. The API 3 shocked the field of mass spectrometry and the many scientific and industrial disciplines that adapted it, and laid the foundations for the instruments that followed. So, it seems ironic that we are now describing a new innovation – Echo® MS – that emancipates the mass spectrometer from its role as a detector for the liquid chromatograph; MS is no longer a hostage to the chromatographic time scale.

In the late '90s, pharmaceutical companies were improving the efficiency of the drug discovery process through innovations like combinatorial chemistry and high throughput screening. They have continued to build and evolve these approaches ever since, with ever-increasing throughput requirements. This created a need for a high throughput technology – expanding capacity from a few hundred samples a day to over 100,000 a day – which could only be achieved with optical plate reader technologies at that time.

Many mass spectrometry systems were developed over the next 20 years with the aim of meeting the demand for higher throughput by increasing the speed of sample injection. Each fell short of fully achieving this goal because they failed to address all the key bottlenecks in high throughput workflows. Sample injection speed was only part of the problem, and solving it only moved the bottleneck somewhere else. By 2008, after the launch of SCIEX's FlashQuant® technology, it became clear that all bottlenecks in the high throughput workflow (Figure 1) must be addressed in a single system. SCIEX researched ways to address these bottlenecks one by one.

Figure 1: The six bottlenecks to high throughput mass spectrometry.

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The first bottleneck to be resolved was isobar separations in the millisecond timeframe, resulting in the development of SelexION® Differential Ion Mobility technology in 2009. A joint effort between SCIEX and the technology's Russian inventors, this was launched on its own merits, rather than as part of a sans LC system. For SelexION to reach its full commercial potential, the other five bottlenecks needed to be tackled.

Three additional bottlenecks were addressed with a breakthrough in the transfer of samples into the MS. The Open Port Probe (OPP) eliminated valves, autosamplers, high pressure pumps and complex plumbing, simplifying the fluidic pathway. It also addressed the sample preparation and carry-over bottlenecks, by providing online sample dilution and continuous self-washing. This was the result of a cooperative research and development agreement between SCIEX and Oak Ridge National Labs that began in 2008, leading to the emergence of a promising advanced prototype.

The OPP also enabled a potential solution to the injection speed and high density sample format bottlenecks. With a few design modifications, the OPP could capture, process and transfer samples dispensed with acoustic energy. Labcyte and EDC had developed microplate replicators based on acoustic dispensing at this time, and SCIEX worked with instruments from both companies to explore this application. A trial using the OPP to capture acoustically-dispensed droplets was conducted in collaboration with Labcyte at SCIEX's Redwood City facility. This study indicated that all bottlenecks were effectively addressed, and throughputs in the order of 100,000 samples per day might be possible. The results were replicated on a second prototype in Framingham, MA, which was then transferred to Pfizer in Groton, CT, to run real-world samples. The success at Pfizer – measuring *in vitro* drug discovery assays, plasma-based pharmacokinetics, enzyme kinetics and nanoscale synthesis products – accelerated efforts to commercialize this technology.

Echo MS – the future of contactless sampling

Echo MS is set to redefine high throughput workflows, eliminating the need for LC (Figure 2). Combining SelexION Differential Ion Mobility technology, an OPP optimized for nanoscale droplet capture, and acoustic dispensing, it brings together all the elements necessary to overcome the bottlenecks identified in SCIEX's research efforts. Mass spectrometers with technologies that maximize ion sampling efficiency – such as the QJet® Ion Guide – are also important to the success of this approach, as online dilution requires high sensitivity mass spectrometers to achieve low limits of detection and ensure meaningful results.

With Echo MS you can:

- **Accelerate analysis** – analyzing up to 260,000 samples per day
- **Deliver rich datasets up to 50 times faster** – reducing the risk of missing lead compounds
- **Enjoy unparalleled standards of quantification** – ensuring high reproducibility, regardless of the matrix
- **Analyze samples without sample preparation** – eliminate LC-associated carry-over and errors

Echo MS has been successfully tested in the field, and is expected to launch commercially in 2020.

To find out more about the SCIEX Echo MS, visit **info.sciex.com/echo-ms**

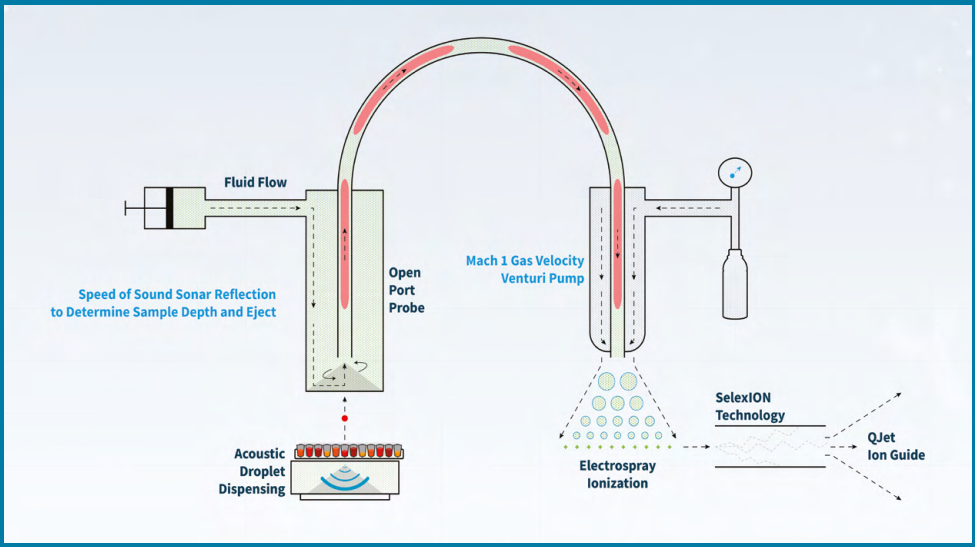

Figure 2: Schematic representation of Echo MS. Gas expanding at the speed of sound pumps a transport fluid into the electrospray ion source. A sonar pulse measures the sample depth by timing its reflection from the surface.

SEARCHING FOR IMMUNOTHERAPEUTIC DRUG TARGETS

Researchers at Pure MHC are using mass spectrometry to compare thousands of peptides recovered from HLA complexes extracted from cancerous, infected and normal cells, in a drive to identify novel disease targets for drug discovery.

Dr. Curtis McMurtrey, Director of Immuno-Proteomics, Pure MHC

Pure MHC, based in Austin, Texas, is a platform technology company focused on immunotherapeutic drug development for cancers, infectious and autoimmune diseases, and allergies. Harnessing the immune system's innate ability to distinguish foreign or aberrant protein targets in diseased or infected cells, the company aims to identify and validate disease-specific peptide sequences that offer potential targets for drug discovery and diagnostics. Dr. Curtis McMurtrey, Director of Immuno-Proteomics at Pure MHC, explained: "We work with the human leukocyte antigen (HLA) system – also known as the Class I major histocompatibility complex (MHC) – which forms transmembrane protein complexes that play a major role in the adaptive immune system. HLA acts as a scavenger, binding to short peptides – around nine amino acids in length – that originate from intracellular protein degradation. Once captured by HLA, these peptides are brought to the cell surface, where they are presented for T lymphocyte recognition. This provides clues as to what is happening inside the cell, as the presence of aberrant or foreign peptide sequences – such as those from cancerous cells or viruses that have infected the cell – will be recognized by the T cells, which stimulates the immune system to destroy the affected cell."

Curtis continued: "We are interested in those target peptides that are recognized as 'foreign' by the immune system, identifying the sequences that best differentiate between a diseased cell and a normal cell, or diseased and normal tissue. The aim is to discover peptides that are unique to – or only expressed in – diseased cells, or are so highly upregulated that they are druggable targets, giving us a disease-specific target for the development of novel immunotherapies and diagnostics. What makes our approach unique is our proprietary soluble HLA (sHLA) technology. Normally, HLA is anchored to the cell membrane by its transmembrane domain. We have patented the truncation of that transmembrane domain, so that HLA is secreted into the culture media instead of being tied to the surface. This allows us to make large amounts of HLA for our research."

"One method we use begins with isolating HLA, either biochemically from diseased or normal tissue samples, or by purifying the sHLA complexes present in the media of transfected cell lines. We then denature the HLA complexes by acidification to release the captured peptides, creating a pool containing hundreds of thousands of different amino acid sequences. We use a number of mass spectrometry techniques to analyze

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these peptide pools, including *de novo* identification, semi- and absolute quantification."

"The main challenge with this particular approach is that all the peptides are biochemically similar, and so tend to elute around the same time. One way we have overcome this is by using a two dimensional analytical technique: offline reversed-phase HPLC, fraction collection and concentration, followed by nano LC-MS. Many of our methods are built around nano LC-MS, because this gives us the sensitivity we need when working with tissue samples, which are only available in very limited quantities."

Pure MHC performs nano LC-MS analysis on SCIEX TripleTOF® 6600+ and Triple Quad™ 6500+ systems equipped with OptiFlow® ion sources. "We chose our first system – a TripleTOF 5600 – in 2013, based on past experience and personal recommendations," said Curtis. "We liked the instrument and found it ideal for HLA peptides, and have since upgraded to the TripleTOF 6600+. When we wanted to introduce absolute quantification, it was a straightforward decision to select the Triple Quad 6500+, as SCIEX Triple Quad systems have an excellent reputation. The sensitivity of the instrument is good, it has a large dynamic range of $1x10^{14}$, and is very reliable."

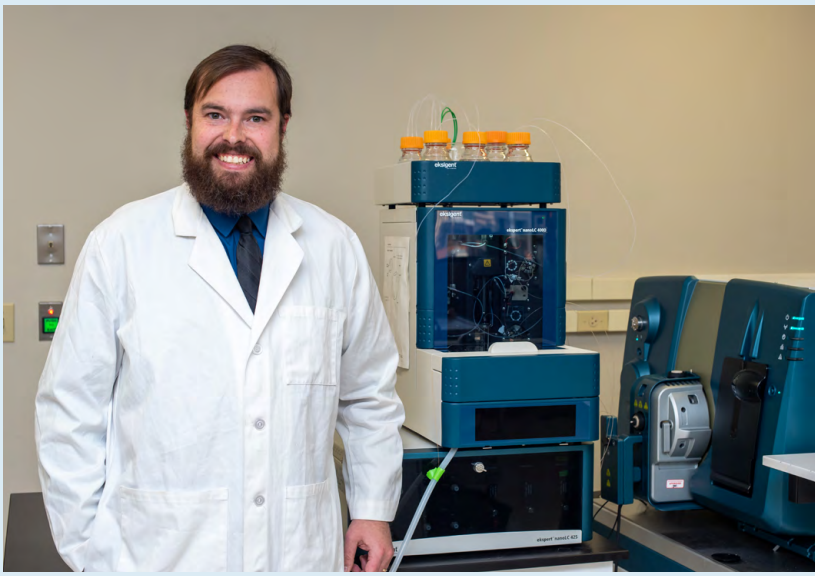
"Typically, we have to create *de novo* libraries, as there are few publicly-available resources. We generally use our sHLA platform to generate spectral libraries by information dependent acquisition on the TripleTOF 6600+, as this allows us to easily produce milligrams of HLA proteins to work with. Once we have the library, we apply a number of different methods to identify suitable targets, such as using variable window SWATH Acquisition to perform semi-quantitation of the HLA peptides, and drawing up a shortlist for absolute quantification by *Scheduled* MRM on the Triple Quad 6500+. *Scheduled* MRM is ideal for this, giving us the sensitivity and cycle times we need to perform absolute quantification on a large number of transitions."

"A particular challenge with HLA peptides is reproducibility; CVs have tended to be high compared to those from tryptic peptides, which ionize more reproducibly than HLA peptides and are less sensitive to ion suppression. We upgraded to the OptiFlow Ion Source a few months ago, and saw a great improvement. While there was little difference for the tryptic peptide results, it was like night and day with the HLA peptides. Sensitivity tripled, and we don't really see any ion suppression now. There will always be considerable variation stemming from the biochemical extraction process, but our CVs are now routinely below 30 %, which was not previously the case. We are looking forward to our next MRM project, as I expect the lower limit of quantification to decrease significantly using this source," Curtis concluded.

To find out more about Pure MHC, visit **www.puremhc.com**

To find out more about the SCIEX TripleTOF 6600+ System, visit **www.sciex.com/tripletof6600plus**

> "WE UPGRADED TO THE OPTIFLOW ION SOURCE A FEW MONTHS AGO… … IT WAS LIKE NIGHT AND DAY WITH THE HLA PEPTIDES. SENSITIVITY TRIPLED AND WE DON'T REALLY SEE ANY ION SUPPRESSION NOW."



FOOD, GLORIOUS FOOD

Analytical laboratories have an important role to play in ensuring the safety, provenance and regulatory compliance of food for human consumption. A crucial part of this process is the analysis of foodstuffs for veterinary drug residues, an application that Swiss scientists are performing with high resolution mass spectrometry.

Anton Kaufmann, Department of Veterinary Drug Analysis at KLZH

Kantonales Labor Zürich (KLZH) in Switzerland is responsible for ensuring food safety in the Canton of Zurich. This requires the analysis of samples from various stages of the food chain, from raw materials to the final product, to make sure that they comply with the current legislation. Any foodstuff that is found to be non-compliant can then be removed from the market.

Veterinary drugs have a known history of abuse in meat production, either through the administration of high doses of a legal therapeutic or, in some cases, the use of drugs that (although effective) are not licensed for treating animals. Anton Kaufmann, leader of the Department of Veterinary Drug Analysis at KLZH, described the work of his laboratory, and how it has come to depend on high resolution mass spectrometry for the analysis of these compounds: "Our laboratory is responsible for routine screening and quantification of a wide variety of foods to check for the presence of veterinary drugs. This includes antibiotics, hormones and tranquilizers, and anything else used to treat animals destined for the food chain. We are also the reference laboratory for veterinary drugs in Switzerland."

"We analyze in the region of 1,500 samples a year," Anton continued. "As every sample is screened for a range of different compounds, each of them quantitatively, the actual number of analyses is many times greater than this. As well as targeted analysis, we have an interest in non-targeted

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work, to find unlicensed drugs that could potentially be abused. We also do a lot of method development, focusing on analysis by high resolution MS rather than the more conventional triple quadrupole approach."

Over time, scientific advances have led to significant changes in the way food analysis is performed, as Anton explained: "When I joined the laboratory about 20 years ago, my department relied on UV fluorescence methods of analysis, while another group was using ELISAs. A couple of years later, we began to use MS, starting with a triple quadrupole instrument. More recently, we have moved over to high resolution MS for routine sample analysis."

"My interest in high resolution MS began quite early on, as I realized its potential for non-target analysis. It allows us to look at anything we want to. In contrast, triple quadrupole instruments are used for targeted analyses, where you know what you are screening for. In the early days, the technique had limitations in terms of dynamic range, sensitivity, software, etc., but things have come a long way since then. The dynamic range and ability to handle complex matrices are far better now, and a lot of effort has gone into improving the sensitivity, closing the gap between this technology and triple quadrupole systems. As more and more people have adopted high resolution MS, there has been significant investment in the software too; today's instruments offer good, user-friendly software, which wasn't always the case in the past. Together, these advances have helped to progress the development of high resolution systems considerably, and we are now in a position to really harness this potential. There is no reason not to use it."

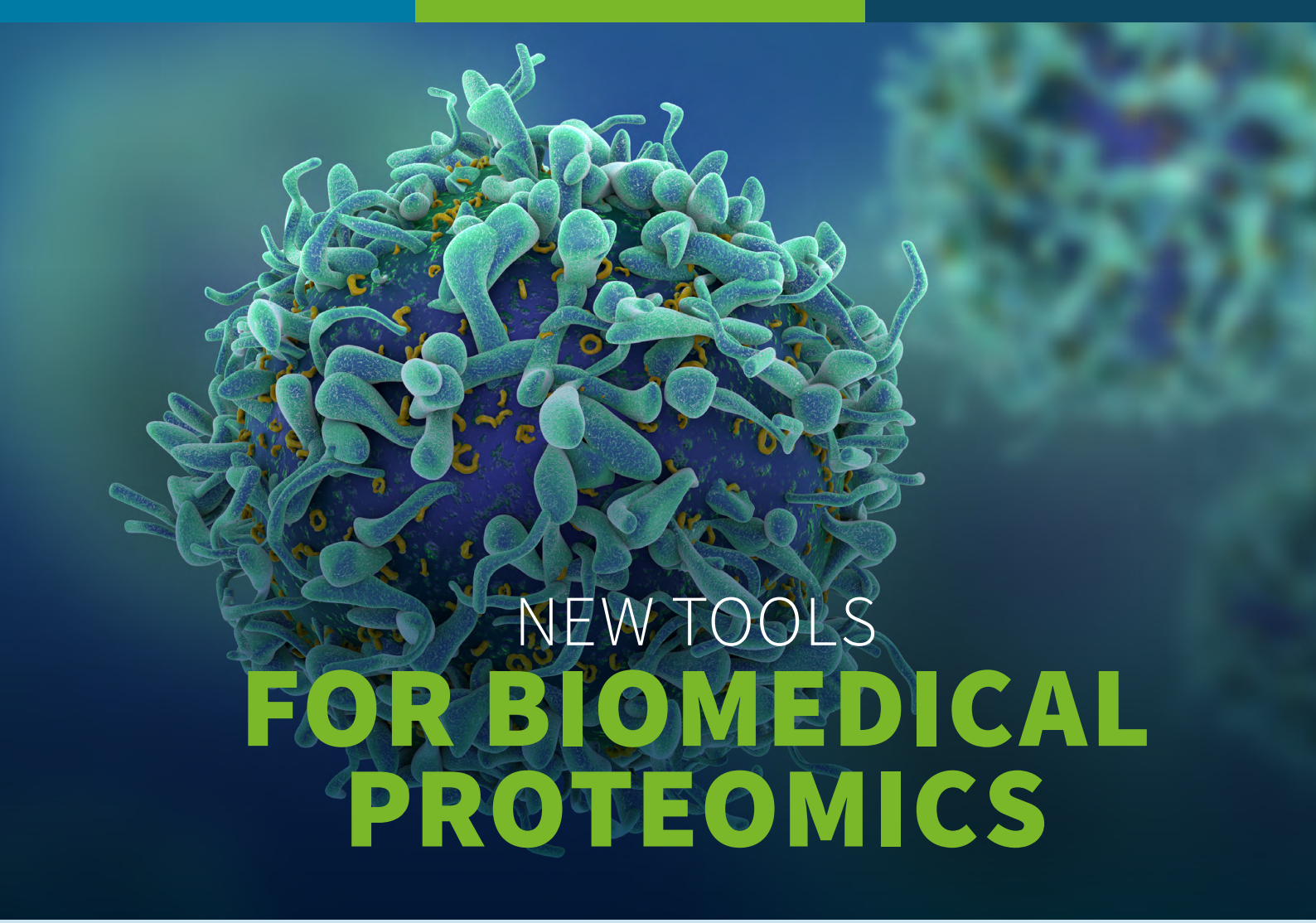
"Around two years ago, we invested in a SCIEX X500R QTOF System. We had always liked the company's hardware, but it was the software that really convinced us that this was the right instrument for our lab. Being able to download and test the software beforehand was a big advantage. We were able to process samples by ourselves, without assistance from an application chemist, and that convinced us that our laboratory staff would be able to learn to use the software very quickly, without external help, in order to become rapidly productive. Very often, software is developed by engineers and technicians who don't really know how it is used in the laboratory. SCIEX OS Software, on the other hand, was the outcome of a collaboration between developers and end users, which makes a big difference. The software is particularly important when we are using SWATH Acquisition, which has great potential for our work. SWATH Acquisition gives us the sensitivity we need for low level analyses, and the selectivity to handle challenging complex matrices, but that capability can only be harnessed because we have good software support."

"Historically, triple quadrupole instruments have been used for quantitative bioanalysis, while high resolution systems have been more popular in research environments. I believe our laboratory is unique in almost exclusively using high resolution MS for routine screening and quantification in the veterinary drugs field, and I am keen to convince others to take the same approach. We participate in various proficiency tests, and have seen no difference between our results with the X500R QTOF and those of other labs using triple quadrupole systems. Laboratories tend to be cautious about the instruments they use for proficiency testing, but we have the data to show that these two technologies deliver the same accuracy and precision in a routine environment. Our work has shown that high resolution MS compares favorably with triple quadrupole instruments, and I believe that this will convince other labs that the technology is ready to be adopted for routine screening and quantification in complex matrices," Anton concluded.

To find out more about KLZH, visit **www.kl.zh.ch/internet/gesundheitsdirektion/klz/de/service/international.html**

To find out more about the SCIEX X500R QTOF System, visit **www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems/x500r-qtof-system**

> "WE ARE NOW IN A POSITION TO REALLY HARNESS [HIGH RESOLUTION MASS SPECTROMETRY'S] POTENTIAL. THERE IS NO REASON NOT TO USE IT."



NEW TOOLS FOR BIOMEDICAL PROTEOMICS

Professor Pengyuan Yang, Executive Vice Director of the IBS and Principal Researcher of the Biological Mass Spectrometry Group, Fudan University

Mass spectrometry has become the 'go to' tool for proteomics over the last decade, with potential applications across the entire spectrum, from basic cell biology research to clinical diagnostics. Researchers at Fudan University in China are using the technology to better understand the fundamental biology of disease, helping to guide future drug discovery and diagnostics.

The Institutes of Biomedical Sciences (IBS), part of Shanghai Medical College at Fudan University, is an interdisciplinary center focused on understanding disease mechanisms and developing novel therapeutic tools for human health. IBS's Biological Mass Spectrometry Group combines novel sample preparation techniques with the latest MS technologies to explore the fundamental biology of numerous diseases, identifying and validating new biomarkers and therapeutic targets. Professor Pengyuan Yang, Executive Vice Director of the IBS and Principal Researcher of the Biological Mass Spectrometry Group, explained: "As we have moved into the 'omics' era, mass spectrometry has emerged as the predominant tool for proteomics and metabolomics studies. My group focuses on using mass spec-based proteomics methods to investigate the biological mechanisms of disease, including a number of cancers."

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"We use a combination of advanced sample preparation protocols with a variety of MS acquisition technologies to identify disease-related changes to the proteome. We begin any project by developing fast and efficient methods for protein enrichment and digestion, then establish a 'normal' proteome profile in healthy tissue. We can then use this data to identify changes that occur as a result of disease, highlighting potential biomarkers or therapeutic targets. The Shanghai Medical College includes 16 hospitals across the city, so we are in the fortunate position of having very good access to both diseased and healthy tissue samples for our research."

"We have a large number of MS instruments within the department, around 20 in total, and these systems are usually running at almost full capacity, due to the large number of in-house and collaborative projects underway at any time. Among these are many SCIEX systems – including three TripleTOF 5600+s, a TripleTOF 4600, three QTRAP 6500+s, a 4000 QTRAP and a TOF/TOF 5800 – and, although the workflow will vary from project to project, each of these instruments has been chosen for a specific role. For example, we developed a novel method for glycoprotein analysis, initially using the TOF/TOF 5800 for intact protein analysis, to help us optimize sample preparation conditions and screen for potential key proteins. We then switched to the TripleTOF systems for 'bottom up', untargeted proteomic analysis to identify differential expression patterns between healthy and diseased samples. Finally, these differentially expressed proteins were validated in large-scale studies using a targeted approach on the QTRAP 6500+ systems, identifying a number of potential glycoprotein biomarkers."

"The SWATH Acquisition capabilities of our TripleTOF 5600+ systems are particularly useful for our research, allowing us to rapidly analyze all the peptide species in a proteomic data set. This unique technology enables us to conduct quantitative studies without having to rely on conventional labeling techniques, which are both costly and tedious, as well as being poorly suited to quantification of low abundance proteins. SWATH Acquisition allows us to qualify and quantify all the proteins present in a single run, without any loss of sensitivity, making large-scale studies easier and more cost effective."

"We first began working with SCIEX in 2010, due to the company's reputation for high performance in the proteomics field. We evaluated a number of instruments from potential vendors at the time and, in my opinion, the SCIEX systems offered best-in-class performance for proteomics, with high sensitivity and extremely good robustness. They are able to generate vast quantities of excellent proteomics data in a very high throughput manner, helping us to investigate novel disease biomarkers and publish quality results," Professor Yang concluded.

To find out more about the Institutes of Biomedical Sciences, visit **ibs.fudan.edu.cn/#**

To find out more about SCIEX mass spectrometers, visit **www.sciex.com/products/mass-spectrometers**

> "SWATH ACQUISITION ALLOWS US TO QUALIFY AND QUANTIFY ALL THE PROTEINS PRESENT IN A SINGLE RUN, WITHOUT ANY LOSS OF SENSITIVITY, MAKING LARGE-SCALE STUDIES EASIER AND MORE COST EFFECTIVE."

A CLEANER AND SAFER FUTURE

Charles Neslund, Scientific Officer, Eurofins Lancaster Laboratories Environmental

Man-made compounds can take thousands of years to degrade in the environment, earning themselves the nickname 'forever chemicals'. There is a growing concern about the impact that the rising levels of these contaminants could have on nature, as well as on human health. Scientists in the US are employing mass spectrometry to investigate the levels of these compounds, paving the way for regulatory authorities to control their production and legislate to protect the environment for future generations.

Eurofins, a world leader in environmental testing, has a central ethos of a cleaner and safer environment, and examines and analyzes a range of substances to ensure the highest quality is met. Eurofins Lancaster Laboratories Environmental in Pennsylvania – founded in 1961 and now with over 50 years of testing experience – serves a large variety of businesses, industries and government authorities across the globe. Scientific Officer Charles Neslund talks about the escalating concerns of one particular group of man-made chemicals; per- and polyfluorinated alkyl substances (PFAS). "PFAS are used in a number of industries, and are found in a range of commercial products, such as non-stick coatings, stain- and water-resistant materials, and cleaning products. The problem with these chemicals is that they don't decompose when they end up in the environment. Consequently, they settle and contaminate soil and water supplies."

Charles continued: "PFAS also accumulate in the human body, and there is a growing amount of evidence to indicate that they can have adverse effects on human health, including raising cholesterol, increasing the risk of developing certain cancers, and harming the immune system. The initial interest in PFAS began over a decade ago, but it has really grown over the last few years and is now attracting a lot of attention. Now that these chemicals are being identified as contaminants

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in the environment, alongside ongoing research that suggests they have detrimental effects on health, there is increasing concern for these compounds to be regulated by authorities such as the US Environmental Protection Agency (EPA)."

"At Eurofins, we test a wide range of substances and materials for traces of these compounds. Around 75 % of our PFAS work is centered on analyzing water from various sources, whether that is groundwater, treated drinking water, wastewater or landfill leachates. In the past, we've worked with the EPA to validate a method for analyzing PFAS in drinking water and, in the last few months, it has put into place an action plan for establishing acceptable levels in drinking water supplies. This was the first ever initiative of its sort to be implemented by the US EPA. Individual states are also moving forward, setting maximum contaminant levels for drinking water along with other legislation for some of these compounds. The remainder of our work is split between solids, soil and food samples, including fish, vegetables and dairy products, like milk. The final area we work in is product testing. Over the last three years or so, we've been receiving more regular requests from companies to analyze their products for PFAS, to ensure that they are safe and free from these chemicals before they are sold to customers."

"PFAS exist naturally in the environment as anions, which means that LC-MS/MS in negative ion electrospray mode is the perfect tool for analyzing them. When the demand for PFAS analysis took off in 2015, we had one MS system that was used full time for this application. As the complexity of the analysis grew – the number of compounds and transitions that we needed to monitor, and working with and regressing the data – we found it was no longer suitable for the application. That's when we moved over to the SCIEX systems. We now have several SCIEX mass spectrometers, as well as other SCIEX instrumentation, that we plan to use for forensics applications."

"When we acquired our first system, there was concern that the solvents might leach out low levels of PFAS from the Teflon tubing and Teflon-coated parts of the LC system, causing background interference in the MS analysis. Since then, we've had discussions with SCIEX that have resulted in what I call a 'PFAS kit' that the company fits to the systems, and this resolved the potential for leaching to occur. The support we received from the SCIEX applications team was important too. Although we had already set up the application, the team came out to our laboratory and made suggestions for further refinements, which was a great help."

"In the space of a few years we've experienced huge growth, and are now the largest single site environmental laboratory within the US. We've gone from using a single MS system for PFAS analysis to having several SCIEX instruments that are now permanently dedicated to this application, and find that their sensitivity out-competes others in the market, setting them apart from their rivals; where we inject two to three microliters of a sample extract, others in the field using different systems are having to inject between 15 and 20 microliters. The SCIEX instruments are so sensitive and consistent that we have been able to duplicate the application with relative ease, which is essential when we're running all these systems side by side, as we don't want different results from each instrument. That's an important asset to have in a production lab. It gives us the flexibility to move between different systems, which is a real plus," concluded Charles.

To find out more about Eurofins Lancaster Laboratories Environmental, visit **www.eurofinsus.com/LancLabsEnv**

To find out more about SCIEX mass spectrometers, visit **www.sciex.com/products/mass-spectrometers**

> "THEIR SENSITIVITY OUT-COMPETES OTHERS IN THE MARKET, SETTING THEM APART FROM THEIR RIVALS."

TAKING A METABOLOMICS APPROACH TO DRUG DETECTION

Alberto Salmone, Forensic Toxicologist and Associate Professor of Analytical Chemistry, University of Turin

Mass spectrometry is widely used for drug testing in competitive sports and the detection of illicit drug use in the wider community. Researchers at the "Alessandro Bertinaria" Regional Anti-Doping Center (CAD) are conducting studies to gain further insight into doping in sports and recreational drug use, using SCIEX instruments to develop MS-based screening methods.

Italy's "Alessandro Bertinaria" Regional Anti-Doping Center was established in 2004 as the official World Anti-Doping Agency (WADA) laboratory for the 2006 Winter Olympics in Turin. After the Olympics, the laboratory's focus changed, with CAD turning its attention to applied analysis and research into the use of drugs of abuse, doping substances, alcohol and, more recently, the detection of new psychoactive substances. Forensic Toxicologist Alberto Salomone, Associate Professor of Analytical Chemistry at the University of Turin and a consultant at CAD, discussed how the laboratory is using mass spectrometry to detect a wide range of prohibited substances that impact on the integrity of sports and public health: "After the Olympics, CAD diversified to provide much more than drugs testing in sports. Today, we offer alcohol and drugs of abuse testing, including the determination of the pharmaceutical composition of drug seizures, as well as analyzing nutritional supplements used by athletes, to certify that they do not contain concentrations of prohibited substances above specified levels. We are also involved in research projects to determine indirect markers of steroid use, along with epidemiological studies monitoring trends in drug abuse and, more recently, the application of metabolomics to drug screening."

Alberto continued: "We receive samples for routine screening from the local Piedmont area, and also from further afield, including the UK and the USA. The majority of this workload is the analysis of hair for markers of drug or alcohol abuse, although we also test blood and urine. Typically, we analyze around 20,000 samples a year for substances such as cannabis, cocaine, heroin and other drugs prohibited by WADA, as well as ethyl glucuronide – a breakdown product of ethanol – using SCIEX Triple Quad and QTRAP instruments. The systems are perfect for these applications, giving us the sensitivity and specificity we need for our ISO 17025-accredited, qualitative and quantitative screening methods."

"Hair is particularly interesting to work with, offering a long detection window. We can analyze, for example, a four centimeter length to determine the average abuse over a number of months, or perform segmental analysis of smaller sections of hair to get a more detailed, chronological story month by month. Epidemiological studies allow the identification of trends and patterns of drug abuse – or even co-use of different substances – over time. Black market drugs sold as one substance may be something completely

different, for instance, synthetic cathinones rather than amphetamine, or fentanyl instead of heroin, which is incredibly dangerous. However, this can be identified by segmental hair analysis, and users warned of the risk it poses. It's fascinating."

"We have always used SCIEX systems and are incredibly happy with the technical capabilities and high sensitivity of these instruments. Quite recently, we acquired an X500R QTOF System – which is integral to our ongoing research – and are currently collaborating with SCIEX to use its high resolution capabilities to develop new metabolomics approaches for drug screening in urine. At the moment, our focus is on identifying markers for steroids and fentanyl. Currently, we are at the proof-of-concept stage, identifying the presence of baseline metabolites and potential markers of abuse, and the early results look very promising."

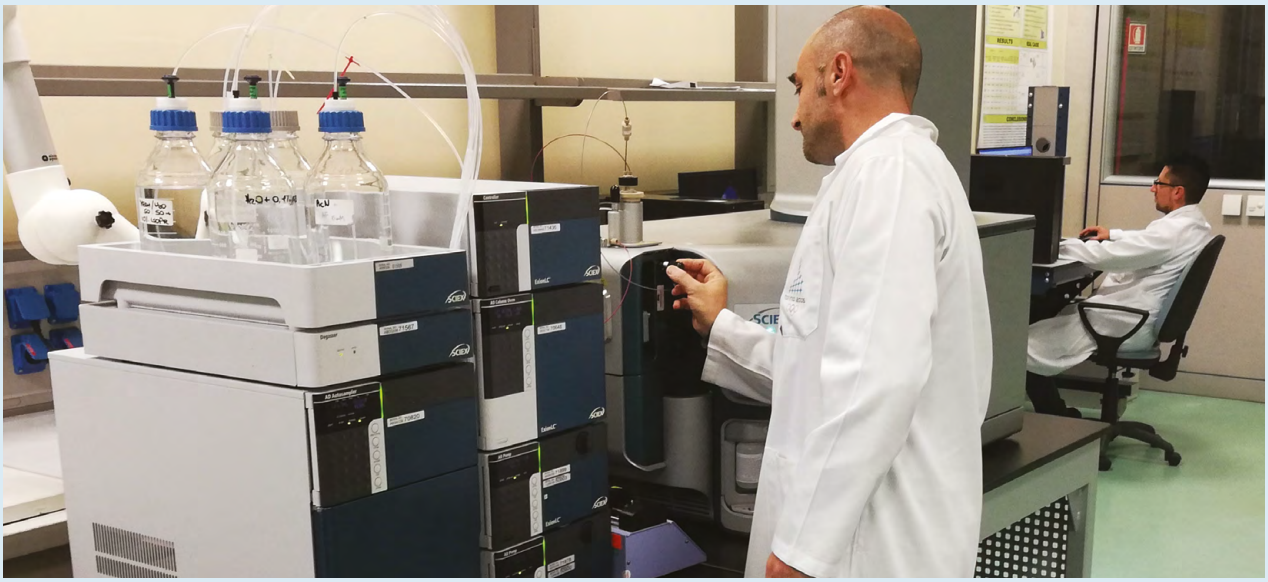
"Establishing markers for specific drug classes will also give us a head start when new compounds come onto the market, as we will be able to detect previously unknown substances belonging to any class of drug that we have identified a marker for. It's likely that each drug class will have different markers, and our aim is to use chemometrics to interpret the data and identify metabolites for confirmation of the presence of drugs that are undetectable using current techniques. SWATH Acquisition is a major benefit for this type of work, allowing us to collect and store data for every detectable analyte in a sample. This means that we can retrospectively analyze the data at a later date. For example, if we subsequently learn of a new psychoactive substance arriving on the market, we can reanalyze past sample data to see when the drug started to come into use."

"The X500R has really boosted our facility, and we are extremely pleased with the support and technical assistance that we receive from SCIEX generally and the local team in Italy. We always feel encouraged and supported with our research, and the collaboration with SCIEX is fantastic. At the beginning of the project, specialists from the company visited our laboratory to help us to set up the instruments and refine our methods, adjusting different software parameters for post-processing of the results, as high resolution, untargeted analysis is very different to the target analysis methods that we are used to. With the X500R, the sky is the limit for our metabolomics studies. It has opened up a world of possibilities, which is very exciting," Alberto concluded.

To find out more about the "Alessandro Bertinaria" Regional Anti-Doping Center, visit **www.antidoping.piemonte.it/cms**

To find out more about the SCIEX X500R QTOF System, visit **www.sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems/x500r-qtof-system**

> "WITH THE X500R, THE SKY IS THE LIMIT FOR OUR METABOLOMICS STUDIES."



GEL-FREE BIOPHARMACEUTICAL ANALYSIS

Gokben Yildirir, Associate Scientist, Paragon Gene Therapy

SDS-PAGE is commonly used in the pharmaceutical industry for purity and stability testing of biopharmaceutical products, but it is time consuming, labor intensive and subjective. Capillary electrophoresis (CE) offers a genuine alternative to this technique, enhancing the throughput, precision and reproducibility of results.

The number of clinically-approved gene therapies is growing rapidly, with ever-more under development. Scaling up production of these biological therapeutics to meet the demands of clinical trials and commercial manufacture requires specialist knowledge and GMP-compliant manufacturing laboratories. For this reason, many companies – both small biotechs and large pharmaceutical manufacturers alike – choose to outsource this work to a dedicated contract development and manufacturing organization (CDMO).

Paragon Gene Therapy in Baltimore, Maryland – an affiliate of Catalent Pharma Solutions, Inc. – is a CDMO specializing in the production of gene therapies, next generation vaccines and immunotherapies based on scalable adeno-associated virus vector platforms. Gokben Yildirir, an Associate Scientist in the Analytical Development Department at Paragon, explained: “Our focus is on scale-up production of virus-like particles and recombinant viral vectors. We work for a broad range of clients – predominantly in the United States, but also in Europe and around the world – to develop transformative technologies for oncology and rare diseases. We have

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extensive experience with recombinant adeno-associated virus (rAAV) vectors – as well as insect and transient mammalian production models – developing custom scale-up processes tailored towards our clients' individual requirements."

"In most cases, a client comes to us with a product that is ready to make the step from a research environment into preclinical or clinical testing. Our job is then to develop a regulatory-compliant process that allows manufacturing of this product to be scaled-up in a GMP environment. In-process analysis and QC testing is integral to this, so our Analytical Development Department is involved at almost every stage – from initial process development through to lot testing of final therapeutic products – to ensure quality and safety."

"Our main analytical considerations are the purity, heterogeneity and stability of the rAAV products," Gokben continued. "The most commonly used technology in the industry for this type of work is SDS-PAGE – and we still use this ourselves in some studies – but a growing number of clients are requesting the use of more advanced technologies that are both less subjective and less prone to operator-to-operator variability. We already had a SCIEX PA 800 Plus Pharmaceutical Analysis System in the department, which had previously been used for isoelectric focusing, and so looked at developing a CE-SDS method for analyzing rAAV vectors – which we believe is a first in the sector. SCIEX had already developed a number of methods for biopharmaceutical analysis, and we were able to easily adapt these to our needs. The results from CE-SDS with the PA 800 Plus can be compared directly with SDS-PAGE, simplifying interpretation, and both methods use the same preanalytical sample preparation, making it easy to fit into our existing workflows. The ease of use of the PA 800 Plus also makes it very straightforward to optimize the method of individual clients' projects, depending on the sample types and concentration."

"Automated CE-SDS offers a number of advantages over manual SDS-PAGE in terms of throughput and reproducibility, both important considerations in a GMP manufacturing environment. The automated workflow is obviously less labor intensive; it's completely walkaway once you load the samples onto the system, which can process 15 samples at a time. We can set it up to run overnight, and simply interpret the results the next morning. The reduced inter-operator variability this offers, combined with automated data analysis, has also increased the consistency of our results. This has the added benefit of giving us higher resolution, making it easier to detect any impurities."

"Gene therapy is still a fairly new area for the industry, but it's picking up very quickly, and we're working with both pharmaceutical manufacturers and government agencies to develop robust scale-up and production processes for novel therapeutics. SCIEX is continuously developing and optimizing methods to support these efforts, working closely with us and other companies in the sector to provide the tools we need," Gokben concluded.

To find out more about Paragon Gene Therapy, visit **www.paragonbioservices.com**

To find out more about the SCIEX PA 800 Plus Pharmaceutical Analysis System, visit **www.sciex.com/products/capillary-electrophoresis**

> "AUTOMATED CE-SDS OFFERS A NUMBER OF ADVANTAGES OVER MANUAL SDS-PAGE IN TERMS OF THROUGHPUT AND REPRODUCIBILITY."

THE EVOLUTION OF CANNABIS TESTING

Seth Wong, President of TEQ Analytical Laboratories

Colorado was the first US state to legalize cannabis for both recreational and medicinal use, but there are strict regulations governing the quality of the products on sale. Laboratory testing of cannabis samples is therefore routinely performed, both to screen for contaminants and to determine their potency. TEQ Analytical Laboratories is recognized as an industry leader in cannabis potency and contaminant testing, and uses SCIEX mass spectrometry instruments to assist with its work.

TEQ Analytical Laboratories – located in Fitzsimons Innovation Community at the University of Colorado, Anschutz Medical Campus – was established to meet the nascent demand for cannabis testing as part of the state's legalization of the drug. Regulation of this newly created market required the development of advanced analytical techniques to ensure the quality and safety of products on sale. Seth Wong, President of TEQ, discussed how the company started, and how it tests cannabis products for various contaminants. "Our sister business, Industrial Laboratories, has been working in the food and drug testing arena since 1945 so, once cannabis was legalized, we realized that we already had the necessary experience and quality systems for a cannabis testing lab. We wanted to raise the bar for cannabis testing laboratories, especially in Colorado, and went on to establish TEQ in 2014. This became the first lab in this sector to be accredited to ISO 17025 – the pinnacle of laboratory accreditation."

"It's now incredibly common to find cannabis dispensaries in population centers across the state, for both medicinal and recreational use, which makes laboratory testing and regulation extremely important. Products come in a range of forms – from oils and topical creams to cannabis-infused candy – and these can vary tremendously in cannabinoid content and, therefore, potency. The state has specific rules and testing workflows based on the product type and what it needs to be tested for, but generally we are looking at the cannabinoid potency profile for THC and CBD, plus other components such as terpenes. Potency is typically analyzed using HPLC, but we do have the capability for a more in-depth analysis using mass spectrometry, if necessary."

"State regulators also require microbiological analysis on specific samples, so we can test for shiga toxin-producing *E. coli*, pathogenic *Salmonella*, and yeasts and molds. In some cases, depending on the product, we also look for residual solvents, pesticides or even mycotoxins. We routinely use LC-MS for pesticide and mycotoxin contaminants; we prefer to use mass spectrometry wherever possible, as the technique increases our scientific capabilities and gives us more concrete knowledge – an additional layer and depth of understanding – compared to HPLC. When we started TEQ, we immediately purchased a 4000 QTRAP, which has been amazing for our work due to the low-level sensitivity that it provides. SCIEX instruments are at the core of our sister lab, so we were already familiar with the equipment, and knew that the systems were robust and performed well. We were also at ease with the operating software, which was exceptionally helpful, and made it easy for our staff to switch between instruments."

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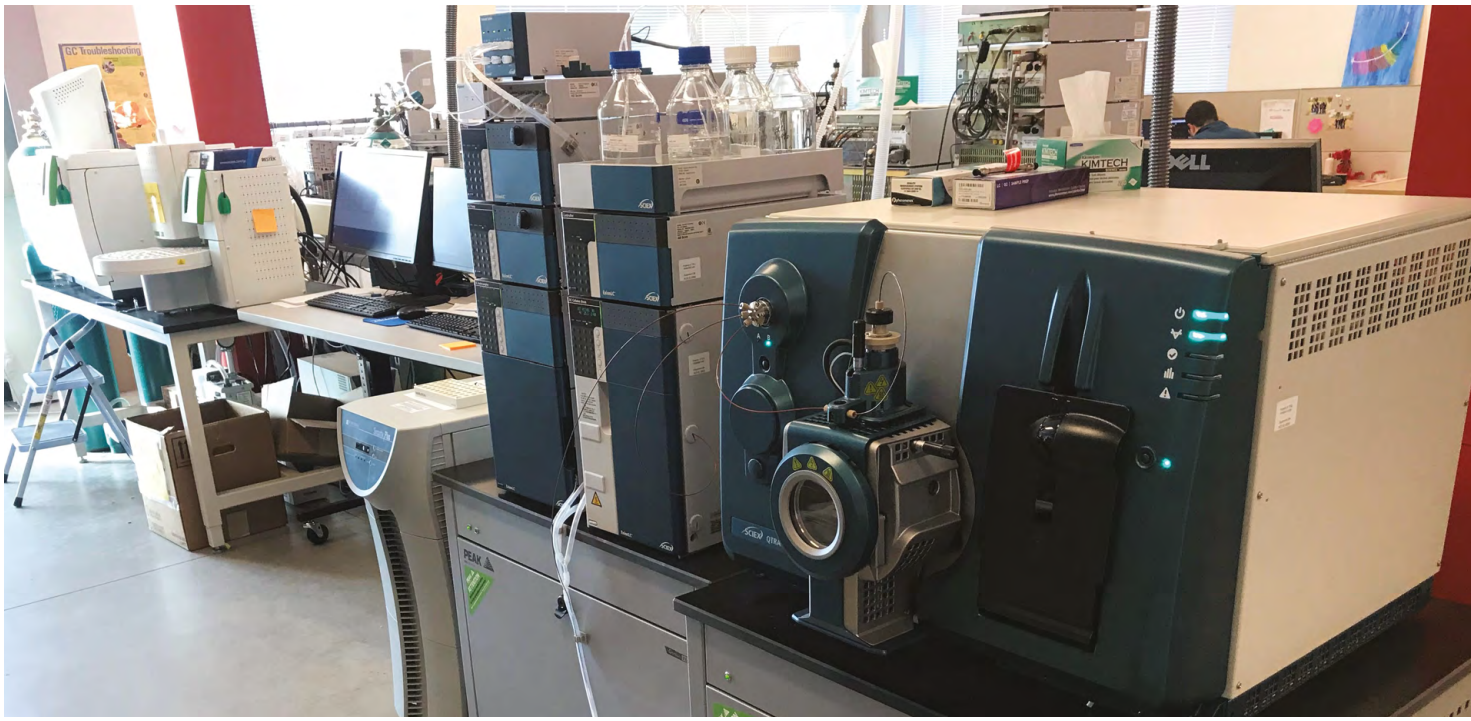

"We only accept substances from licensed producers, so our samples are either from the growers directly, or from manufacturers of marijuana-infused or concentrated products. Samples therefore arrive in different formats – such as oils or finished products – meaning that they all get processed slightly differently prior to analysis, with some requiring additional clean-up or filtration. We begin by determining the best processing method for each matrix, to ensure our analysis is accurate and efficient. For pesticide detection, for example, we use a form of solid phase extraction called QuEChERs before loading the samples. This pre-analytical work helps to ensure that our SCIEX instruments stay cleaner for longer – despite our high throughput of different sample matrices – which is very important to us."

"Our workload is increasing as the industry grows, and we are simultaneously working on method development and research, as well as taking on specific requests from clients. As a result, we recently decided to invest in a second instrument – SCIEX's high throughput QTRAP 6500 – to expand our testing capabilities; we are excited to get it up and running! The QTRAP 6500 will allow us to standardize our processes more, with fewer samples needing to be processed beforehand, saving time and making our workflows even more efficient."

"We've been using SCIEX for years now, and it's a brand we know and trust. We are extremely happy with both the powerful and easy-to-use systems, and the fantastic support offered by the SCIEX service team, and we look forward to what the future holds for TEQ with these instruments," Seth concluded.

To find out more about TEQ Analytical Laboratories, visit **www.teqanalyticallabs.com**

To find out more about the SCIEX QTRAP® 6500 System, visit **www.sciex.com/products/mass-spectrometers/qtrap-systems/qtrap-6500-system**

> "WE'VE BEEN USING SCIEX FOR YEARS NOW, AND IT'S A BRAND WE KNOW AND TRUST. WE ARE EXTREMELY HAPPY WITH BOTH THE POWERFUL AND EASY-TO-USE SYSTEMS, AND THE FANTASTIC SUPPORT OFFERED BY THE SCIEX SERVICE TEAM."

UPCOMING EVENTS

DON'T MISS SCIEX'S EVENTS IN 2020

NORTH AMERICA & CANADA

ASMS
5/31-6/4
Houston, TX

AACC
7/26-7/30
Chicago, IL

MSACL
3/29-4/3
Palm Springs, CA

SOFT
9/21-9/25
San Diego, CA

EMEA

Analytica
3/31-4/3
Munich, Germany

SLAS
6/2-6/5
Vienna, Austria

HUPO
10/18-10/22
Stockholm, Sweden

CHINA

2020 China Mass Spectrometry Conference (2020 年全国质谱大会)
9/19-9/23
Hangzhou, China

The 3rd CIIE (第三届中国国际进口博览会)
7/6-7/10
Shanghai, China

The 16th International Conference of the Metabolomics Society (第十六届国际代谢组学会议)
7/6-7/10
Shanghai, China

ASIA-OCEANIA Mass Spectrometry Conference (AOMSC 2020)
1/5-1/7
Macau, China

JAPAN

JASIS 2020
11/11-11/13
Chiba, Japan

KOREA & SINGAPORE

ASIA-OCEANIA Human Proteomics Organisation Conference (AOHUPO 2020)
3/25-3/28
Busan, Korea

Food Safety Analysis 2020
8/25-8/26
Singapore

SCIEX helps to improve the world we live in by enabling scientists and laboratory analysts to find answers to the complex analytical challenges they face. The company's global leadership and world-class service and support in the capillary electrophoresis and liquid chromatography-mass spectrometry industry have made it a trusted partner to thousands of the scientists and lab analysts worldwide who are focused on basic research, drug discovery and development, food and environmental testing, forensics and clinical research.

With over 40 years of proven innovation, SCIEX excels by listening to and understanding the ever-evolving needs of its customers to develop reliable, sensitive and intuitive solutions that continue to redefine what is achievable in routine and complex analysis. For more information, please visit sciex.com.

SCIEX Headquarters
Legal Entity: AB Sciex LLC, 500 Old Connecticut Path, Framingham, MA 01701, U.S.A.
www.sciex.com

Singapore – ROA
SCIEX Distribution

Mon–Fri: 9:00 AM–6:00 PM

Email: sea.orders@sciex.com
Phone: +65 6559 4338
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Technical Support and Service
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Toll free number: 00800 2255 2279
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USA – Americas
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