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VISION

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WELCOME TO THE FIFTH SCIEX VISION

SCIEX enjoys a strong reputation for pushing the envelope of innovation and performance – continually offering groundbreaking MS technologies that provide new levels of sensitivity and productivity – but there is no secret to how we achieve this. By engaging our customers early in the development cycle, we understand their 'unsolvable' problems, and develop the instruments to help solve them. This was the blueprint for our latest instruments – the SCIEX Triple Quad 7500 and ZenoTOF 7600 systems – which continue to expand laboratory capabilities worldwide.

The SCIEX Triple Quad 7500 system enters a new era of precision, sensitivity and robustness for nominal mass instrumentation. This innovation minimizes sample preparation, accelerates workflows and, where low-level detection and quantification are critical, offers heightened levels of sensitivity. This is the first of our nominal mass systems to use our next generation SCIEX OS software, which is easier to use and learn, and is more intuitive than our legacy software. It also offers advanced features designed to enhance 'real world' applications – like the ability to rapidly re-analyze samples – for real-time decision-making and more streamlined workflows.

This year we also launched a high-end accurate mass instrument, the ZenoTOF 7600 system, which combines two unique capabilities. Firstly, our Zeno trap technology advances the sensitivity of accurate mass in an unparalleled way, and enables users to quantify and characterize on the same platform – effectively in the same analysis – breaking the chains of traditional accurate mass spectrometers. Our EAD technology comprises an alternative fragmentation technique, offering an orthogonal, complementary view of a molecule to CID approaches, and providing significantly more structural information compared to other techniques.

Our technologies enable previously unachievable applications across sectors, including biopharmaceutical and RNA vaccine development, metabolite studies, and protein expression, characterization and post-translational modification, as well as for the screening of food and environmental samples. This issue of SCIEX VISION showcases these game-changing technologies, and the unmatched investigative power and research capabilities they offer to our customers. I hope you enjoy reading it.

Dominic Gostick
CTO and VP/GM of the LC-MS business

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GENDER MATTERS

Wouter Bruins, co-founder and Managing Director of In Ovo

The poultry industry needs to maintain high ethical standards of animal welfare while operating at maximum efficiency. Dutch company In Ovo has taken advantage of the Echo MS system to develop a high throughput gender typing method that can distinguish between male and female chicks prior to hatching, to ensure optimal and irreproachable production.

The poultry industry has long needed a reliable means of *in ovo* gender typing to ensure that only female chicks are hatched. Female chicks are commercially valuable as they grow up to produce eggs, but males are usually euthanized at birth. This practice is not only inefficient – on average, one male chick is discarded for every hen produced – but also an ethical problem, and it will shortly be banned in some European countries. Finding a way to address this issue was the challenge facing Wouter Bruins and Wil Stutterheim, the founders and managing directors of In Ovo, a spin-out from Leiden University in the Netherlands. Wouter explained: "I was keen to start a biotech company, but one that was focused on solving a problem. That led me to the poultry industry and the issue of how to perform diagnostic tests on chicken eggs, and resulted in Wil and I setting up the business in 2011."

Wouter continued: "Wil and I met as students at Leiden University – I was doing a master's degree in biology, he was studying biomedical sciences. We also did some entrepreneurship courses together, and we were still studying when we set up In Ovo. Our aim was to find a way to gender type an egg. It was already known that estrogen levels are different in males and females, but this is relatively hard to measure, and so we decided to look for potential biomarkers that could be more quickly and easily evaluated. We took samples from lots of different eggs – brown and

white, from various breeds of chicken – and used PCR to gender type them, eventually finding a novel biomarker that we named sabinamin in the allantois, which is easily accessible for testing."

Once sabinamin had been identified, the challenge was to find a rapid, cost-effective screening technique suitable for use in a hatchery, to determine the concentration in each egg. "Our application probably demands more speed than most workflows and, when Thomas Hankemeier, Professor of Analytical Chemistry at Leiden University, first suggested mass spec, we felt that it would probably be too slow and expensive for our purposes. However, we then heard that SCIEX was launching the Echo MS system, an ultra-high sample throughput system for Acoustic Ejection Mass Spectrometry (AEMS). The Echo MS system uses acoustic dispensing to introduce samples to the SCIEX Triple Quad 6500+ mass spectrometer, removing the need for LC and significantly increasing the speed of analysis. We had a prototype instrument installed in September 2019 and, since then, have collaborated with SCIEX to push the system to its limits and maximize our throughput."

In Ovo has now installed an Echo MS system in a Dutch hatchery as part of an automated gender typing workflow, with more to follow in the coming year. "The Netherlands has four hatcheries, which supply all the chicks for the whole country. To give you an idea of numbers, the hatchery where we have an Echo MS system in use produces 22 million chicks a year; without gender typing, half of these would be euthanized. We've now created an innovative sampling platform and coupled this with the Echo MS system for high throughput analysis. Currently, we can process 1,800 eggs an hour, but we are developing a new sampling platform that will increase this to 10,000, making this a very demanding application where speed is concerned. After nine days of incubation, the eggs are introduced into the sampling machine in trays, where they are turned and visualized using a camera system to identify the best place to insert a needle and remove a sample of the allantois. These samples are then analyzed in 384-well plates, using the Echo MS system to rapidly determine the concentration of sabinamin; the AEMS technology gives us far greater speed than chromatography-based techniques. Depending on the concentration of sabinamin, we stamp each egg as male or female, and then sort them into groups. The whole process takes around 10 minutes, after which our 'girls only' chicks are returned to the incubator and hatched, while the other eggs are taken out of production, spray dried and used as a source of high quality protein for pet foods."

"AEMS TECHNOLOGY GIVES US FAR GREATER SPEED THAN CHROMATOGRAPHY-BASED TECHNIQUES."

In Ovo's novel gender typing method is a big step forward for animal welfare, as every chick that hatches is raised. "At the moment, our test is achieving over 95 % accuracy, which is a real benefit for our customers, increasing their efficiency as almost all the chicks produced will be female. Importantly, any male chicks are reared for meat rather than being destroyed at birth, which is a better use of resources than can be achieved without gender typing, and more ethical. There are still things we can optimize – for instance, there may be additional biomarkers we can monitor – and I think a goal of 99 % accuracy is perfectly possible in the near future," said Wouter.

"When we first talked about putting a mass spec in a hatchery, people understood our reasoning but thought that it was a crazy idea. However, hatcheries are actually relatively clean environments, and the sampling platform and mass spec are installed in a dedicated room. The SCIEX team put a lot of effort into helping us achieve our goal, and we've shown how using the Echo MS system can benefit the poultry industry and help address animal welfare concerns. But the challenge isn't over yet – we want to work towards performing 100,000 tests a day at a single location. It's a demanding target, and we look forward to working with SCIEX to see how much further we can push the boundaries of AEMS technology," concluded Wouter.

To find out more about In Ovo, visit **www.inovo.nl**

To find out more about the SCIEX Echo MS system, visit **https://sciex.com/products/integrated-solutions/Echo-ms**

CUTTING-EDGE MASS SPEC TO ACCELERATE HEALTH SCIENCE RESEARCH

Dr. Moriah Sandy, Metabolomics Specialist and Director of QMAC

There is an increasing need in many research institutions for robust mass spectrometers that go beyond the current limits of sensitivity, while offering high throughput and reliable workflows. Dr. Moriah Sandy at the University of California San Francisco (UCSF) discusses the benefits of using SCIEX systems for different projects on campus – such as building a metabolite library – and for collaborations with external organizations.

The University of California San Francisco focuses exclusively on health sciences and, in July 2020, it established a new core facility for metabolomics research: the Quantitative Metabolite Analysis Center (QMAC) at the Benioff Center for Microbiome Medicine. Dr. Moriah Sandy, Metabolomics Specialist and Director of QMAC, was tasked with hitting the ground running. She explained: "There is a huge interest in using mass spec – over 173 researchers across our biomedical institution and medical school use it for their work – but around 93 % of these faculty members are outsourcing their samples. Partnering with SCIEX to accommodate two high performance mass spectrometers – a TripleTOF 6600+ system and a SCIEX 7500 system – within QMAC has allowed us to provide these services to scientists on site, saving them precious time and money."

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"WE ARE HAPPY WITH THE INSTRUMENTS AND OUR ONGOING RELATIONSHIP WITH SCIEX, AND EVEN MORE IMPRESSED BY THE EASE OF GETTING OUR INSTRUMENTS RUNNING AT UCSF."

Moriah continued: "I have previous experience using a SCIEX instrument – the QTRAP 6500 system – to perform method development and untargeted metabolomics workflows, so I was familiar with both the company and its products. This meant that I found it straightforward when the SCIEX 7500 system arrived. To strengthen my understanding of the updated interface, I watched over the installation process, and made calibration curves with some of the standards while the engineers were still on site. This was very beneficial, and helped with the first phase of our implementation, which involved validating basic methods for untargeted and targeted workflows. With the TripleTOF 6600+ system, I was trained by someone from SCIEX, who went through the SWATH acquisition methods, some untargeted workflows and some flux analysis protocols."

"Once I was familiar with the instruments, the priority was to train a few postdoctoral researchers and graduate students to prepare samples, run MS experiments from start to finish, and analyze chromatograms," Moriah added. "These 'super users' could then train others, maintain the instruments, and solve technical issues. Alongside this training initiative, we started on a project that ties into my broader interest, which is building a metabolite library of all identified compounds over the past decade at UCSF. This is complementary to ImmunoX, a proven concept for collaborative sciences, and is at the heart of our CoProjects and CoLabs initiatives here at UCSF. The aim of this, with respect to metabolomics, is to collect large amounts of data and store it in a centralized library accessible to the greater UCSF community. The advantage of using the SCIEX MS systems is that, if these compounds show up again in the future, or are initially unknown, we can go back and retrospectively look at them based on parameters such as mass retention times and fragmentation patterns."

"Over the last year, we have been able to establish the role of each instrument. The SCIEX 7500 system is very sensitive, and is therefore well suited to performing targeted quantitative work when we know what to observe. For example, we were analyzing a lipid-type molecule for a project, and I was amazed to see detection in the low picomolar range. An additional benefit of this is that we can get data from a single mouse, instead of having to pool samples from 15 mice. In contrast, we use the TripleTOF 6600+ system for tracking specific molecules and quantifying them across different conditions, or for stable isotope-labeled experiments to track compounds through metabolism. The instrument's SWATH acquisition methods make it very easy for projects where we don't know exactly where to look, and are really great for untargeted analyses."

"We are happy with the instruments and our ongoing relationship with SCIEX, and even more impressed by the ease of getting our instruments running at UCSF. Now that we have a variety of credible and sustainable workflows on our MS platforms, the goal is to showcase what we can do in publications or presentations. Our vision is to expand our capacity and services to other UCSF departments – such as the Helen Diller Family Comprehensive Cancer Center and the Bakar Computational Health Sciences Institute – as well as to external collaborators and industry partners. This will accelerate the transition of exploratory and more targeted research into various therapeutic areas, from discovery to lead, and ensure the full potential is achieved," concluded Moriah.

To find out more about QMAC, visit **https://microbiome.ucsf.edu/quantitative-metabolite-analysis-center-colab-incubator**

To find out more about SCIEX MS systems, visit **https://sciex.com/products/mass-spectrometers**

TRACKING ENVIRONMENTAL CUES WITH LC-MS TO IMPROVE HEALTH

Professor Jochen Mueller, Theme Leader for Emerging Environmental Health Risks at QAEHS

Professor Kevin Thomas, QAEHS Director

Examining the life cycle of chemicals around the world is an important means of understanding how they impact the environment and human health. Mass spectrometry is a powerful tool in this field of research and is regularly used for the detection of trace amounts of unknown chemical compounds from a variety of complex matrices. As a result, this technology is at the core of the workflow of the Queensland Alliance for Environmental Health Sciences (QAEHS), a renowned Australian research center dedicated to addressing emerging global environmental and health challenges.

Mankind currently faces a number of threats, including climate change, water pollution and loss of biodiversity. While the current global pandemic may have overshadowed these in the public awareness, it is more important than ever to understand the factors driving these challenges in order to tackle them. This is the goal of the University of Queensland's QAEHS research center, which brings together a diverse group of experts covering a wide range of subjects – from toxicology to microbiology and epidemiology – to improve human health.

The team at QAEHS has been using a range of LC-MS/MS systems in their research for a number of years, allowing them to monitor both known and unknown contaminants of emerging concern (CECs) for programs covering everything from wastewater-based epidemiology and human biomonitoring to PFAS and plastics. Professor Kevin Thomas, QAEHS Director, explained: "There is a lot of overlap between all the programs we run at the center. Each research theme is championed by a senior researcher working with a team of postdoctoral scientists and PhD students, but there is a lot of crossover between the programs, leading to a high degree of internal collaboration."

One of the challenges of this multidisciplinary, collaborative approach is that the team is working with a large variety of different matrices, from fresh water and wastewater to human matrices, such as urine, blood and breastmilk. This sample diversity is one of the reasons QAEHS has several SCIEX mass spectrometry systems, allowing it to optimize sample preparation, protocols and instruments for each matrix, and rapidly and efficiently test more samples without instrument bottlenecks. Professor Jochen Mueller, Theme Leader for Emerging Environmental Health

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"THEY ARE JUST INCREDIBLY RELIABLE INSTRUMENTS, AND THIS IS ONE OF THE REASONS WE HAVE STUCK WITH SCIEX ALL THESE YEARS."

Risks at QAEHS, commented: "We started out with an old API 3200 system back in the early 1990s, and from there we acquired several newer SCIEX instruments. We now have a QTRAP 5500 system, two SCIEX Triple Quad 6500+ systems and we have just installed two SCIEX Triple Quad 7500 systems. We also have a couple of high resolution mass spec machines – a TripleTOF 5600+ system and an X500R QTOF system – that have mainly been used for plasticizer work so far, although we have ambitious plans for these in the future. They are just incredibly reliable instruments, and this is one of the reasons we have stuck with SCIEX all these years."

This huge mass spec capability allows the team to, for example, monitor illicit drug traces – as well as alcohol, tobacco and various biomarkers for prescription medication use – in wastewater as part of a national surveillance program. The data generated by this program is then used to help guide campaigns to tackle specific societal problems, like localized drug issues. Kevin added: "Through this type of program, we do a lot of routine screening for compounds of interest – whether it's identifying illicit drug use or tracking and reducing particular pollutant levels – but we are also constantly developing new methods and protocols. The SCIEX technical team in Australia has been really valuable in this, and has provided us with a lot of technical support and input that has helped with moving forward and resolving complex problems that sometimes arise. It's been a real partnership."

The center's close university ties and cutting-edge research facilities mean that QAEHS attracts a large number of skilled researchers and students every year. New users are therefore constantly being trained on how to operate the mass spec instruments. Fortunately, this has not been an issue for the lab, nor in any way slowed down its research. Jochen explained: "The SCIEX platforms are very easy to operate, so new staff that come into the lab can quickly get the hang of the instruments and hit the ground running. Even inexperienced students rapidly gain operator-level aptitude with the instruments, without the need for continuous supervision, which says a lot. And then they later take this knowledge and apply it to their own labs, across the world. A lot of our collaborators also come to us for expert knowledge in this area, and we get to hear their thoughts and experiences with various mass spec systems, which confirms that we really are using the right instruments for our purposes."

To learn more about QAEHS, visit
www.qaehs.centre.uq.edu.au

To find out more about LC-MS systems from SCIEX, visit
https://sciex.com/products/mass-spectrometers

SENSITIVITY GAINS IN MASS SPECTROMETRY

The evolution of mass spectrometry systems over the last 30 years has addressed key bottlenecks in efficiency to enable high throughput workflows. The new SCIEX Triple Quad 7500 system takes this approach to the next level, going beyond the current limits of sensitivity while providing pharmaceutical, food and environmental labs with the ruggedness and robustness they need to meet their productivity targets.

Ian Moore, Senior Technical Product Manager for Nominal Mass Platforms at SCIEX

Hassan Javaheri, Research Scientist at SCIEX

Recent rapid advances in the biopharmaceutical drug development pipeline have driven an ever-increasing demand for mass spectrometry systems capable of achieving even lower detection and quantification limits. This issue is further compounded by the analytical complexities of working across a diverse range of molecule types and therapeutic classes.

In July 2020, SCIEX launched the next generation of its flagship nominal mass spectrometer, the SCIEX 7500 system. Ian Moore, Senior Technical Product Manager for Nominal Mass Platforms at SCIEX, explained: "The SCIEX Triple Quad story began with the API 3000 system in 1989, which was extremely popular with pharmaceutical customers, becoming a revolutionary technology in that sector. Since those early days, we have introduced a new flagship Triple Quad instrument every five years or so and, while the key selling point has always been sensitivity, there have been countless other improvements along the way, particularly in terms of the control software.

This ongoing success has been driven by continuous innovation and, even though our QTRAP 6500+ system was the most sensitive mass spec on the market when it was launched, our R&D team was already working on the next generation of instrument."

The market for mass spectrometry systems has expanded enormously since the first Triple Quad systems were launched for the pharmaceutical sector, and it now includes a wide range of other life sciences disciplines, such as food and environmental sciences. This has changed the nature of the mass spectrometry business, with each sector having its own requirements. Despite these changes, speed and sensitivity remain the central tenets of Triple Quad system design. Hassan Javaheri, a Research Scientist at SCIEX, has been involved in the design and development of the SCIEX 7500 system from the project's early days, and explained the aims of the new instrument: "We had two main goals at the start of the project – improving sensitivity to achieve lower limits of detection, and further increasing throughput. To achieve the first of these, our aim was to improve the transmission of target ions from atmospheric pressure to the deep vacuum required for mass analysis. Increasing the atmosphere-to-vacuum aperture diameter is an effective way to improve the ion-to-gas ratio, and therefore the limit of detection, but the resulting intense gas expansion can scatter ions in the vacuum region. We therefore needed to design a completely new ion guide – the D Jet ion guide – with a unique geometry to counter the ion losses from scattering collisions with the gas and maintain a high ion transmission efficiency."

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> **"... THE SCIEX 7500 SYSTEM [OFFERS] A REMARKABLE INCREASE IN SENSITIVITY OF UP TO TENFOLD OVER PREVIOUS INSTRUMENTS."**

Hassan continued: "Another challenge created by increasing the orifice size more than fourfold was that we could not achieve the required vacuum in one stage. The SCIEX 7500 system is therefore our first system to feature a two-stage ion guide. The D Jet ion guide provides an optimized flow field to the second stage vacuum, where a QJet ion guide is used to further improve the ion-to-gas ratio, resulting in a five to six times improvement in ion transfer efficiency."

"The D Jet ion guide has been an enabling technology," Ian added. "It has revolutionized the efficiency of one of the fundamental issues in mass spectrometry – trying to get your sample from the atmosphere into an ultra-low vacuum without losing any of the analyte ions. These ions are in a very fast-moving stream, and retaining your analyte while getting rid of everything else that is coming through is the real challenge, requiring the optimal combination of the ion guide, orifice size and pressure to improve sensitivity. Together with our latest E Lens probe within the ion source, the D Jet ion guide and larger orifice size have resulted in the SCIEX 7500 system offering a remarkable increase in sensitivity of up to tenfold over previous instruments."

Ian continued: "The final component that makes the SCIEX 7500 system so unique is the software, as this is our first Triple Quad platform to be controlled by SCIEX OS software for data acquisition. This solution was originally developed for our QTOF instruments, and first appeared on the X500 series systems intended for routine testing applications. As a result, this software is very intuitive, even for users with limited mass spec experience, allowing them to get up to speed quicker. As well as being very easy to learn, it is designed to simplify routine tasks and do everything with fewer clicks, which essentially improves productivity."

As a class-leading instrument, the SCIEX 7500 system has been developed to offer unrivaled sensitivity while addressing recent trends in mass spectrometry, particularly in the pharmaceutical industry. "The emergence of biological therapeutics, which are more potent than their small molecule predecessors, means that much lower drug concentrations are now required to have the desired therapeutic effect," Ian said. "This means that you need a much more sensitive mass spec to effectively study and characterize the very small amounts of biotherapeutics or metabolites – especially in complex matrices like human plasma, blood or urine – and the SCIEX 7500 system is the ideal tool for this."

"The other major pharma trend addressed by this system is the move towards getting far more data at a much earlier stage in the drug development workflow. The aim of this approach is clear – to avoid costly late-stage failures for new drug candidates – but it requires the use of much more sensitive techniques to extract the relevant information from the low sample volumes available at this early stage of the pipeline. By enabling much lower limits of detection, the SCIEX 7500 system reduces or completely eliminates the need for complicated sample prep and concentration protocols, saving time and money and decreasing the chance for sample loss. This improves the accuracy and reproducibility of results, while simultaneously increasing productivity. It's a win-win situation," Ian concluded.

To find out more about the SCIEX 7500 system, visit **https://sciex.com/products/mass-spectrometers/triple-quad-systems/triple-quad-7500-system**

SUPPORTING PRECLINICAL DMPK STUDIES

Dr. Vishwottam Kandikere, Head of DMPK at Syngene

Mass spectrometry is a key technique used by contract research organizations to support drug discovery. Syngene International in India is one such company, and it relies on a range of SCIEX instruments – including SCIEX Triple Quad, QTRAP and TripleTOF systems – to deliver preclinical drug metabolism and pharmacokinetic (DMPK) services to its global customer base.

Syngene International, based in Bangalore, is a subsidiary of India's largest biotechnology company, Biocon. It is a contract research organization that provides customized solutions in synthetic chemistry and molecular biology for early-stage drug discovery and development to a worldwide customer base, including many of the top 10 pharma companies, as well as numerous Boston-based and European biotechs. Dr. Vishwottam Kandikere, Syngene's head of DMPK, explained: "Our department provides preclinical DMPK services – both integrated drug discovery and high throughput DMPK – to companies in sectors including pharma, nutraceuticals and animal health, as well as taking part in collaborative ventures."

Vishwottam continued: "Typically, large companies come to us either because they don't have the necessary capacity, or because they need a more efficient and less expensive way of doing things. Usually, they have validated protocols that they want to run in a high throughput mode that is faster, fully traceable and readily auditable. For smaller companies, which may not have the infrastructure or sufficient staff to carry out a particular study, we offer a 'one-stop shop', where they can get everything done under the same roof. Often, they have a specific problem that they need help to solve, so it's about understanding the science and trying multiple experiments to come up with a solution, rather than working in high throughput."

"We work with a wide variety of species in preclinical research – mouse, rat, rabbit, guinea pig, ferret, monkey and dog – and diverse matrices, such as blood, plasma, various tissues, microsomes, hepatocytes, bile, urine and feces," Vishwottam added. "These samples are typically only available in very small volumes – five to 10 microliters per analysis – and can be challenging to work with. Generally, we are working with LLOQs in the region of less than 20 nanomolar; around five to 20 nanomolar

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"DATA-INDEPENDENT ACQUISITION USING THE SWATH ACQUISITION CAPABILITY IS A REAL ASSET DURING THE DRUG DISCOVERY PHASE."

for *in vitro* assays, one to two nanomolar for *in vivo* assays, and five to 10 ng/ml for PK studies. Some form of sample preparation is usually necessary, ensuring that the samples are as clean as possible to achieve the sensitivity required and minimize the need for instrument maintenance. Around 90 % of the time, this will be an acetonitrile 'crash', but we also use simple 'dilute-and-shoot' analysis, as well as both solid phase and liquid-liquid extraction techniques."

"I've used SCIEX systems for around 20 years now, from the API 3000 up to the SCIEX Triple Quad 6500+ system. We currently have 12 mass spectrometers in DMPK, and will shortly add a thirteenth, a SCIEX Triple Quad 7500 system, to complement our existing QTRAP and TripleTOF systems. These instruments are used for a variety of applications – both quantitative and qualitative – such as biomarker research and small molecule analysis, including newer technologies such as proteolysis targeting chimeras (PROTACs). We also work with peptides and oligonucleotides and, further down the line, hope to expand this to include proteins and vaccines."

"The choice of instrument depends on the sensitivity and complexity of the method," Vishwottam continued. "For Cacos, and some Log D and MetStab assays – where we do a three-in-one or five-in-one cassette dosing (N-in-one) analysis – we use the high-end SCIEX Triple Quad 5500 and SCIEX Triple Quad 6500+ systems. Where the sensitivity requirements are less challenging – for instance, where N is equal to one or two – and for quick quantitation and discovery phase decision-making, rather than a regulatory submission, we use the SCIEX Triple Quad 4500 system. The important thing for these studies is to have an instrument that acts as a workhorse for the efficient generation of reliable data. For drug discovery metabolite identification, where we look at fragmentation, we use our QTRAP systems – a 4500 and a 5500 – and the TripleTOF 5600+ system. The TripleTOF system, along with our SCIEX Triple Quad 6500+ system, is also used for the analysis of peptides and other large molecules. Data-independent acquisition using the SWATH acquisition capability is a real asset during the drug discovery phase, as it allows us to look at everything in the sample without specifically targeting any particular analyte. Typically, we provide high volume services to large pharma companies that require processing of around 150 to 300 compounds a week, and this technology allows us to perform MetStab assays much faster than we otherwise could."

"The impact of preclinical research should not be underestimated," Vishwottam emphasized. "Incorrect results could cause a potentially successful drug to be abandoned unnecessarily, or lead to a company spending a great deal of money developing the wrong compound. Traceability is essential, and we have to make sure that all our systems are set up to record every action taken, and the reason for it, so that the data is audit ready. Many of our clients have specific record keeping and regulatory requirements, which are usually met using a LIMS. We find the Analyst Software is extremely good and equally robust. It's customizable and offers all the scripts we need to track, monitor, review and export data, and prepare reports that meet the client's needs."

"SCIEX is an innovative company that never stands still, with a strong R&D team that constantly looks to do things even better by designing and launching new instruments and, similarly, software. As we move from small molecule peptides towards the larger oligos and proteins, we're looking to procure ever-more sensitive equipment to support our continued growth in the biologic and biomarker arena. We're constantly in touch with the SCIEX team in India and its applications specialists, as well as the global team, to make sure that new methods are well established and transferred in a way that will allow us to provide some good solutions for drug or vaccine development," Vishwottam concluded.

To find out more about Syngene International, visit **www.syngeneintl.com**

To find out more about the SCIEX range of mass spectrometers, visit **https://sciex.com/products/mass-spectrometers**

RESOLVING CRIMES USING THE POWER OF MASS SPECTROMETRY

Dr. Adolfo Caballero Quintero, Head of the Forensic Chemistry Laboratory at the FGJ NL

The high discrimination power of mass spectrometry has proved invaluable in the field of forensic toxicology, where it is commonly used to resolve cases involving drugs of abuse and confiscated drugs, often within a 48-hour turnaround time. The speed and sensitivity of the latest MS systems have made them a nationwide forensic laboratory staple in Mexico, for both routine testing and specialized analyses.

Criminal investigations and prosecutions in the state of Nuevo Leon, Mexico, are overseen by the State Attorney General's Office – known locally as the Fiscalia General de Justicia de Nuevo Leon (FGJ NL) – which is the primary law enforcement body for the region. Under the FGJ NL's supervision, trained scientists in the forensic chemistry laboratory work to resolve crimes at the state level, while occasionally providing support for confiscated drugs cases under the national jurisdiction.

Dr. Adolfo Caballero Quintero, Head of the Forensic Chemistry Laboratory at the FGJ NL, explained how forensic chemistry has evolved over the years: "When I joined the Fiscalia 24 years ago, we had just started exploring the different applications of chemistry to forensics. Today, we have an established knowledge base and analytical tools, and expertise in forensic toxicology and serology, as well as the analysis of trace evidence and confiscated drugs. I supervise the lab's technical operations and safeguard the operational integrity by ensuring compliance with the relevant policies and procedures. As well as my forensic work, I am also involved in some collaborations with the Tec de Monterrey university, using mass spectrometry to help identify contaminants in pharmaceutical drugs."

"In forensic sciences, we rely on confirmatory techniques that can conclusively identify compounds of interest," continued Adolfo. "Mass spectrometry rose to prominence in this field due to the in-depth quantitative

"A RELIABLE SYSTEM SUPPORTS THE CONTINUOUS DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL PROTOCOLS... WE WERE ALREADY LEANING TOWARDS SCIEX WHEN WE STARTED LOOKING FOR MS INSTRUMENTS FOR OUR LAB, DUE TO THE COMPANY'S REPUTATION."

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and qualitative information it can deliver. We have been using liquid chromatography-mass spectrometry since 2004, and we were the first forensic laboratory in the country to use LC-MS/MS, years before it became a mandatory requirement in ANAB-accredited laboratories. Primarily, we use the technique to confirm the presence of drugs of abuse and their metabolites in forensic cases, and for the identification of confiscated drugs. Having these systems has helped us to shorten analysis times, enabling us to deliver services to our customers in a timely manner – often within 48 hours."

Adolfo added: "On an average day, our caseload is around 12 toxicology cases – 10 post- and two ante-mortem, for example – which are carried out according to standard operating procedures to ensure reliable results. In toxicology, it is recommended that highly discriminatory techniques such as mass spectrometry are used. We typically run around 20 samples a day, plus standards, quality controls and blanks. The workflow begins with either small-scale liquid-liquid sample extraction or solid phase extraction, followed by LC-MS/MS analysis. Prior to the LC-MS/MS sample analysis, we conduct routine quality tests, running verified standard solutions to make sure that the instrument is performing according to specifications. After this, we analyze the samples interspersed with quality controls. The end results go through a thorough evaluation process comprising a technical review by another analyst, and an administrative review by a supervisor. Once this meets our stringent quality control standards, the results are released to the client."

"From our years of experience, we know that a quality LC-MS/MS system determines the output of a forensic laboratory. A reliable system supports the continuous development and validation of new analytical protocols and helps in expanding our services repertoire. We were already leaning towards SCIEX when we started looking for MS instruments for our lab, due to the company's reputation. We evaluated instruments from various manufacturers and decided that SCIEX was the best fit for our lab and budget. Over the years, we have acquired several SCIEX systems, which support us well in our daily caseload. The software is highly intuitive to use, while also being consistent across the different systems that we work with. We have an API 2000 system and a 4000 QTRAP

system that we use for routine toxicology analysis, and a QTRAP 6500+ system when we need greater sensitivity. We are also considering acquiring another 4000 QTRAP system, so we can run sample analyses in parallel and increase the speed of our work while still using our optimized protocols."

"One of the main advantages of SCIEX is the excellent maintenance service that the company offers," noted Adolfo. "We renew the service contract annually without fail, and this helps to ensure that our systems are in top working condition. The SCIEX applications specialists have also been extremely helpful in providing us with access to methods, application notes and scientific articles on our request."

"In addition to standard toxicological analysis of blood and urine, and pharmaceutical contaminant analysis, we hope to start providing analytical services for other biological sample types, such as liver, in the near future, once we have optimized and validated the different sample processing methodologies," added Adolfo. "We are also looking into buying a SCIEX quadrupole time-of-flight system, which would speed up analyses of unknown substances, and we are excited that the increased sensitivity that SCIEX systems offer will soon allow us to develop and validate new analytical applications," he concluded.

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

BUILDING A HEALTHY AND SUSTAINABLE FUTURE

Sang-Hoon Lee, PhD Researcher in the Food and Nutrition Division at the National Institute of Agricultural Sciences

Agriculture is an essential industry that sustains civilization, helping to feed the world and support the livelihood of farmers. However, global concern over the impact that rapidly expanding agriculture has on both the environment and our health has hit an all-time high. This imbalanced narrative calls for a harmonious solution for the sustainable growth of agriculture. The Rural Development Administration in Korea is an integral part of a science research institute that actively promotes the sustainable production of healthy food in vibrant, rural areas of the region. A part of its vital work is enhancing the value of Korea's agri-food, and the Food and Nutrition Division is using mass spectrometry to compile information on the nutritional and functional components of the population's diets, in order to provide dietary guidance and encourage healthy eating.

The Food and Nutrition Division of the National Institute of Agricultural Sciences – part of Korea's Rural Development Administration (RDA) – specializes in promoting healthy and sustainable improvements to Korean agriculture. The department was founded to establish a scientific basis for improving the dietary health of the population, promoting nutritious food consumption and enriching the value of the agricultural sector's produce. To do this, researchers have been using mass spectrometry to evaluate the nutritional content of various ingredients, and compile an online database for the government and industry leaders to use when analyzing the quality of foodstuffs.

Sang-Hoon Lee, a PhD Researcher in the Food and Nutrition Division, explained: "We have been examining the nutritional and functional content of traditional Korean foods, for leaders to make informed decisions about the future of agriculture and food production, including the labeling of processed foods and nutrient intake standards. Following this work, the RDA was able to release the Korean Standard Food Composition Database 9.3 in 2021, which was a revision of the original document released in 1970 and updated every five years. This edition profiles over 3,113 types of food, with information on 130 types of nutrients, including macronutrients – such as carbohydrates, proteins and lipids – and micronutrients, including vitamins and minerals. In addition, the RDA also developed a Functional Components Database specifying the composition and content of flavonoids and phenolic acids in foods – including details such as molecular weight, UV absorbance, compound structure and MS ion fragment patterns at positive and negative ionization modes – to evaluate the intake of functional ingredients and select indicator substances for

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health foods. This work is vital for the thorough and reliable quantification of functional and nutritional components of food to provide healthy, honest and sustainable dietary guidance to the people of Korea."

"To achieve this, our dedicated team of 16 researchers is performing HPLC, GC, GC-MS, and LC-QTOF-MS with the SCIEX X500R QTOF system," he continued. "We analyze 130 types of nutrients in around 350 different foods per year, and our work has demonstrated that the composition ratio of functional ingredients in some foods was previously underestimated. For example, vitamin B6 has seven derivatives, but only one of those – pyridoxine – was ever analyzed. We have now established and verified a multicomponent simultaneous analysis method for all seven derivatives – as well as other components – using the X500R system, allowing us to process 50 samples per week. This has greatly improved our workflow efficiency, reducing the time and workforce needed for analysis."

"CRUCIALLY FOR US, THE MACHINE IS VERY DURABLE, AND HAS BEEN ABLE TO PROCESS MORE THAN 10,000 SAMPLES CONTINUALLY FOR SIX MONTHS, WITH NO IMPACT ON EQUIPMENT STABILITY."

Sang-Hoon added: "The X500R system is ideal for this research, as it supports both ESI and APCI ion sources, providing an in-depth analysis of a wide range of molecular weights and polarities for the thorough investigation of various food groups and functional ingredients. The MRM^{HR} mode makes it possible to quantify components that have a low degree of separation during LC, and the high resolution quadrupole time-of-flight mass spectrometer allows the quantification of unknown compounds or foods, by confirming the mass ion fragment pattern of each compound. In addition, the convection during spraying is minimized by the patented ion source, helping to prevent source contamination and deliver highly reproducible data. Crucially for us, the machine is very durable, and has been able to process more than 10,000 samples continually for six months, with no impact on equipment stability. Even if there is a minor problem with the system, SCIEX is always on hand to offer technical support within 48 hours."

To find out more about the National Institute of Agricultural Sciences at the Rural Development Administration in Korea, visit **www.naas.go.kr/english/about/organization.do**

To find out more about the Korean Standard Food Composition Database and the Functional Components Database in Korea, visit **www.koreanfood.rda.go.kr/eng/fctFoodSrchEng/engMain**

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**

MAKING THE LEGALIZATION OF CANNABIS SAFER

Scott Churchill, Director of Scientific Operations at MCR Labs

Legalizing cannabis in several states in the US created a demand for analytical testing to help ensure that harvested plant materials and derivative products comply with regulations for potency, and chemical and microbial contamination. MCR Labs is a certified cannabis testing laboratory that uses mass spectrometry and other analytical procedures to help its clients ensure the safety and quality of their products, as well as providing assistance with regulatory compliance.

MCR Labs was the first company in Massachusetts, US, to become certified for cannabis testing, working with a range of clients, from doctors and caregivers to craftsmen creating their own cannabis products. The company uses methods developed in the pharmaceutical industry to help ensure that cannabis products comply with individual state regulations. This includes testing for potency throughout cannabis processing – from the flower through to concentrates and infused products – and testing for microbial contamination and chemical residues at various points during the cannabis production cycle. Scott Churchill, Director of Scientific Operations at MCR Labs, explained: "The company was founded in 2013, when medicinal cannabis was voted in and the need for safety screenings emerged. We decided to apply our experience in pharmaceutical sciences to public safety by analyzing these newly legalized samples. That has basically been our mission since – to protect public health – but also to legitimize the cannabis industry by using rigorous scientific methods for safety and potency screening. Clients come to us to check if their product is contaminated in any way, and whether it has the active profile that they are interested in. We usually start with a qualitative screening to check that all the regulated substances are within the allowed limit and, if anything is flagged, we follow up with a quantitative analysis to get the actual concentration of the unwanted compound. After the screening, we provide the clients with an ISO-certified test report containing all the information about the analysis."

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"We work with several types of cannabis that have a lot of variation in terms of the terpene profiles, but the final products can be quite similar. The largest differences can be found in the infused products; if the products are created for medicinal use, they are allowed to be more potent, while if the purpose is recreational, an edible cannot contain more than 5 mg of the active ingredient per serving. This creates unique challenges, since every sample needs to be processed and analyzed in a different way."

Samples must also be tested for heavy metals, microbial contamination, mycotoxins, pesticides and residual solvents. Although many pesticides used for cultivation are authorized by the US Food and Drug Administration (FDA), heating, burning and inhaling those chemicals has the potential to negatively impact consumer health. Each state government in the US has set its own limit for the amounts of these chemicals that are acceptable in cannabis products, which is something that MCR Labs needs to monitor, since it has clients that distribute their wares in several states. "We need to keep track of the pesticide levels allowed in the different states," Scott continued. "This can be difficult when the limits vary so much from place to place, and some can be extremely low; the lowest one is enforced in Massachusetts and is 10 ppb for all types of pesticides. What complicates the matter further is that everything is constantly evolving; the clients search for new ways to innovate their products and new pesticides are introduced all the time, 14 of them were added to the [Massachusetts] list just in the last six years."

"TO DO THIS EFFICIENTLY, WE REQUIRE AN INSTRUMENT THAT IS BOTH FAST AND SENSITIVE. THE QTRAP 6500+ SYSTEM FROM SCIEX HAS EVERYTHING THAT WE NEED FOR OUR WORK, ALLOWING US TO USE THE SAME INSTRUMENT FOR ALL THE DIFFERENT ANALYSES."

"On a regular day, we are handling over 100 samples, and all of them need to be screened for many different compounds with varying concentrations. To do this efficiently, we require an instrument that is both fast and sensitive. The QTRAP 6500+ system from SCIEX has everything that we need for our work, allowing us to use the same instrument for all the different analyses. The reason we turned to SCIEX is that, in my opinion, it produces the best mass spectrometers. However, before buying the instruments, we sent out some samples to SCIEX for testing, to make sure that its technology had the required sensitivity and accuracy. The extracts we supplied had different concentrations of pesticides, some as low as 5 ppb, and a 'trick sample' that could give a false positive. Needless to say, it resulted in us buying the instruments and we have enjoyed working side by side with SCIEX ever since. They are sharp people and genuinely interested in high quality science, something that the cannabis industry has been lacking for a long time, when all the purchases were done through the black market," Scott concluded.

To learn more about MCR Labs, visit **www.mcrlabs.com**

To find out more about the QTRAP 6500+ system, visit **https://sciex.com/products/mass-spectrometers/qtrap-systems/qtrap-6500plus-system**

A COMMUNITY EFFORT TO DEVELOP MS-BASED TESTING FOR COVID-19

Dr. Maarten Dhaenens, Postdoctoral Fellow at the University of Ghent

Efficient and rapid patient diagnosis is essential for limiting the spread of a virus, and the sudden demand for diagnostic testing during the COVID-19 pandemic posed one of the greatest challenges for modern-day medicine. Researchers at the University of Ghent have been leading a community-based effort to develop mass spectrometry-based assays for the clinical diagnosis of COVID-19, providing an alternative method for monitoring the spread of the SARS-CoV-2 pathogen through the population.

The Laboratory of Pharmaceutical Biotechnology at the University of Ghent, Belgium, is a research institution specializing in proteomics and genomics. The laboratory's main focus is on the development of novel assays to broaden the scope of mass spectrometry-based techniques, primarily for fundamental epigenetic research on histones. The COVID-19 pandemic changed all of that, bringing the scientific community together to help meet the demand for rapid, diagnostic tests.

Researchers in the lab have been pioneering the clinical use of MS-based diagnostic tests to complement current testing methods for COVID-19. Efficient population testing is essential to actively monitor the spread of a pathogen such as SARS-CoV-2, providing quantitative data to support policy makers in reducing transmission. At the beginning of the pandemic, COVID-19 was diagnosed almost exclusively by reverse transcription polymerase chain reaction (RT-PCR) methods, but this reliance on one technique had notable disadvantages. Dr. Maarten Dhaenens, a Postdoctoral Researcher in the lab, explained: "The unprecedented worldwide demand for RT-PCR resulted in chronic shortages of tests and other necessary reagents, limiting testing capacity. We realized the need for an entirely novel approach to testing that would be as efficient and universal as RT-PCR, in order to relieve pressure on laboratories. Mass spectrometry, with its different reagents and workflow, is entirely orthogonal to other diagnostic methods, with the potential to double the capacity of testing within a very short timeframe. MS-based viral protein detection also offers a number of other benefits, including the ability to quickly adapt to new variants as the virus mutates – such as the recent SARS-CoV-2 UK variant with the N501Y mutation – and the capacity for straightforward multiplexing assays to include testing for other diseases, such as influenza."

"We developed an alternative MS test that identifies structural protein digests from a COVID-19 infection and, with a cohort of other laboratories, assessed its validity and robustness for clinical use. Previously, we had shown how SWATH acquisition – a data-independent acquisition method from SCIEX – provides the breadth of analysis, specificity and reproducibility to find novel biomarker peptides in non-model organisms when targeting *in silico* predicted spectral libraries. This was inspired by our search for a method that could

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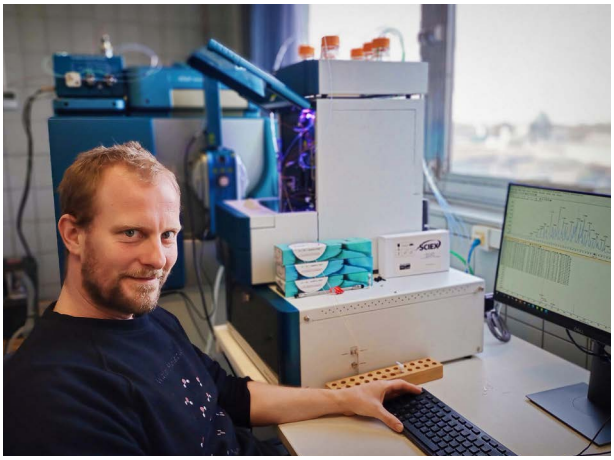
handle the complexity of histones, but we decided to apply this same approach to identifying peptide biomarkers in COVID-19 patients. SWATH acquisition, performed on the SCIEX TripleTOF 6600+ system, enabled us to identify 17 peptide biomarkers from two proteolytically-digested SARS-CoV-2 proteins: nucleocapsid and spike. The nature of SWATH acquisition data allowed easy translation of this information into multiple reaction monitoring (MRM) assays on quadrupole instruments, selectively quantifying the presence of the 17 peptides in nasal swab samples from COVID-19 patients. The strong linear correlation between our MS intensities and the Ct values generated through PCR testing encouraged us to move into the next phase, checking if the assays could be transferred to other settings."

Compared to PCR-based testing, MS assays are considerably more heterogenous, requiring a broader perspective to tackle different sample matrices and instrument technologies. Maarten continued: "In April 2020, the preliminary results were shared with a newly established consortium (Cov-MS) of 15 academic laboratories and industrial partners – including SCIEX – that were provided with a kit containing negative samples spiked with SARS-CoV-2 proteins and an SOP. The 17 peptide biomarker targets were distributed in a comprehensive, vendor-independent Skyline document for translation to any MS system, reflecting the variety of apparatus found in clinical settings. Using our kits, members of Cov-MS performed their own analyses to optimize our method, select peptides that complemented the sensitivity of their MS equipment, and develop their own targeted MRM assays. The latest generation quadrupole instruments proved able to detect below 50 amol amounts of

"THE ENTIRE SCIENTIFIC COMMUNITY – FROM ACADEMIC CIRCLES TO INDUSTRY – JOINED FORCES WITH THE SHARED GOALS OF ALLEVIATING THE PRESSURE ON TESTING AND MONITORING THE SPREAD OF COVID-19."



peptides on a column, the approximate equivalent of between 25-30 Ct in PCR patient testing, proving mass spectrometry technology offers clinically-relevant results."

"From the start, it was clear to us that this could only be tackled as a community. The entire scientific community – from academic circles to industry – joined forces with the shared goals of alleviating the pressure on testing and monitoring the spread of COVID-19. The Cov-MS consortium worked together to validate the adaptability and robustness of mass spectrometry across different vendors. For further collaboration, we created the Cov-MS Digital Incubator; a transparent and browsable platform for the sharing of expertise on assays, optimizations and data acquisition with other labs and clinics. SCIEX and all other members of Cov-MS were open, receptive and helpful throughout, and it was inspiring to see the walls come down as the scientific community worked as one – something that I hope will last into the future and long after the COVID-19 pandemic," Maarten concluded.

To find out more about the Laboratory of Pharmaceutical Biotechnology at The University of Ghent, visit **https://www.ugent.be/fw/pharmaceutics/pharmbiotech/en**

You can read more about the test on MedRXiv, and gain access to the Cov-MS Digital Incubator by sending an e-mail request to **covms@ugent.be**

To find out more about SWATH acquisition on SCIEX TripleTOF systems, visit **https://sciex.com/technology/swath-acquisition**

BREAKING THE CHAINS

OF TRADITIONAL ACCURATE MASS SPECTROMETRY

Dominic Gostick, CTO and VP/GM of the LC-MS business at SCIEX

SCIEX has a long history of developing high performance accurate mass spectrometers that provide heightened levels of precision and sensitivity. However, clinical, pharma and environmental labs continue to demand more from their instruments, leaving no time for the company to rest on its laurels. That's why the R&D team at SCIEX always keep customers at the forefront of innovation, incorporating their insights during the development cycle to ensure each new technology expands on previous capabilities. This rigorous process led to the development of the recently launched SCIEX ZenoTOF 7600 system – a high resolution accurate mass system – that combines multiple groundbreaking technologies to deliver unsurpassed sensitivity and quantification.

Advancements in mass spectrometry have redefined research opportunities and opened new doors to biopharmaceutical drug development. New technologies, however, aren't solely devised in the R&D labs of biotech companies – real-world customer input is crucial to the creation of innovations that truly make an impact. This was certainly the case in the development of the latest accurate mass spectrometer from SCIEX, which was released this year. Dominic Gostick, CTO and VP/GM of the LC-MS business, explained: "Customer engagement early in the R&D phase has contributed to the success of many of SCIEX's market-leading instruments. An early customer evaluation (ECE) stage during the development of a new system ensures that it matches current and future research requirements, while applying the feedback to improve the final product further. This was a real challenge during the COVID-19 pandemic – customers usually visit the labs in Toronto, Canada, to spend time with the instrument – but obviously, this wasn't possible. Instead, we got our customers to send us challenging samples to run on the instrument while still early in development. This way, we could actually understand the capabilities of the system and – to an extent – modify and change the implementation of the technology as a result of this feedback. This is such an important part of the innovation process, and ensures we deliver instruments that solve our customers' problems."

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"The ECE played a pivotal role in the development of the ZenoTOF 7600 system, which is our latest high-end accurate mass spectrometer. This system showcases two new technologies, the Zeno trap – which has further increased the limits of sensitivity in accurate mass spectrometry – and an alternative fragmentation technique called EAD (electron activated dissociation) that can uncover structural information like never before."

Traditionally, quantification is done in a nominal mass system, but the Zeno trap technology, in combination with MRM^{HR}, enables customers to characterize and quantify on the same platform – effectively in the same analysis – which is new to accurate mass spectrometry. Heightened sensitivity also sets the ZenoTOF 7600 system apart from other systems. Zeno trap pulsing delivers up to 20 times more sensitivity and enables the detection of low abundance molecules, resulting in more useful MS/MS information from each TOF experiment. This is especially important for proteomics experiments, where increased sensitivity leads to greater coverage for protein identification. In fact, up to 40 % more proteins can be quantified compared to previous technologies, with heightened protein identification allowing for five times lower sample loading.

Dominic continued: "The other innovation incorporated into the ZenoTOF 7600 system is our EAD technology. Typically, fragmentation on these platforms is done with collision-induced dissociation (CID), but EAD provides a different mode of fragmentation solution that is amenable to different types of molecules. EAD gives an orthogonal, complementary view of a molecule, providing significantly more structural information than traditional methods like CID. With EAD, the instrument can perform various analyses in a single experiment – from small molecule or intact protein analysis to complete lipid characterization. This complete solution has given our customers a benchtop accurate mass system that is unparalleled in terms of power and capability."

EAD maximizes the utility of the ZenoTOF 7600 system with the ability to tune electron kinetic energy within an experiment to analyze a wide range of molecule types. At lower kinetic energies, researchers can characterize intact biomolecules to rapidly identify and confirm structural features. Medium kinetic energies can rapidly fragment post-translationally modified peptides such as phosphopeptides and glycopeptides with high reproducibility, which is especially valuable to biopharmaceutical drug development in glycopeptide mapping experiments. Finally, high-energy EAD fragmentation produces precise MS/MS information on small molecules for accurate and highly specific quantification.

"WE'VE SEEN THE ACCELERATION OF VACCINE DEVELOPMENT THROUGH NEW TECHNOLOGIES, AND WE'RE EXCITED THAT THE ZENOTOF 7600 SYSTEM WILL BE PART OF NEW DISCOVERIES IN THIS RESEARCH FIELD."

The ZenoTOF 7600 system expands critical research capabilities in various sectors, including – most significantly at present – biopharmaceuticals, where drug and vaccine development is becoming increasingly complex, and requires instrumentation to match. Lipidomics, for example, is a research field that has exploded in recent years and, as a result, lipid nanoparticles have now been used as a drug delivery system for various therapies and SARS-CoV-2 vaccines. EAD fragmentation can characterize an individual lipid from a single spectrum, enabling the development of such therapeutic delivery systems, and will potentially lead to the discovery of lipid biomarkers for prostate and breast cancers.

"We've seen the acceleration of vaccine development through new technologies, and we're excited that the ZenoTOF 7600 system will be part of new discoveries in this research field. This instrument is also capable of performing metabolite work – such as ADME studies in omics workflows – and even applied markets for the general screening of food and environmental samples. The ECE really helped to maximize the potential of this system and, as a result, SCIEX has produced an accurate mass spectrometer like never before," Dominic concluded.

To find out more about the SCIEX ZenoTOF 7600 system, visit **https://sciex.com/products/mass-spectrometers/qtof-systems/zenotof-7600-system**

THE MISSING MOLECULES:
HELPING CE-MS REACH ITS FULL POTENTIAL

Dr. Takayuki Kawai, Associate Professor of Chemistry at Kyushu University

Analyzing trace levels of biological molecules from *in vivo* samples can prove challenging with equipment that does not have the sensitivity required to detect low concentration metabolites. Researchers at Kyushu University in Japan are using SCIEX mass spectrometers and capillary electrophoresis instruments to overcome this challenge by developing ultra-sensitive trace bioanalysis methods to detect single-cell metabolites for pharmaceutical applications.

Understanding *in vivo* microenvironments, such as tumor cells, at the single-cell level can provide a thorough overview of the landscape and open up possibilities for pharmaceutical applications. However, detecting low-level, trace concentrations of biological molecules requires highly sensitive methods of analysis. Researchers at Kyushu University are optimizing methods on SCIEX mass spectrometers and capillary electrophoresis (CE) instruments to create ultra-sensitive trace bioanalysis methods for the identification of molecules in single cells. Dr. Takayuki Kawai, Associate Professor of Chemistry at Kyushu University, explained: "My research group is interested in developing methods for analyzing trace levels of biological molecules, as even the rarest stem cell or metabolite can play an important role in complicated life systems. There is often a trade-off between sensitivity – the smallest concentration that you can detect – and analytical reliability when detecting such molecules. Capillary electrophoresis coupled with mass spectrometry (CE-MS) is currently the most reliable method for single-cell metabolome analysis, but even this technique cannot provide the sensitivity needed to detect trace concentrations of metabolites. This is where our work comes in. We are aiming to optimize these methods to achieve femtomolar sensitivity and beyond."

Dr. Kawai continued: "I have worked with SCIEX CE instruments – including P/ACE MDQ, PA 800, PA 800 Plus and CESI 8000 Plus systems – and QTRAP 5500 and TripleTOF 5600 mass spectrometers since 2007. I was first introduced to these systems in my previous employment at a leading Japanese research institution, where I was working on developing ultra-sensitive analytical systems. Now, my current team is building on

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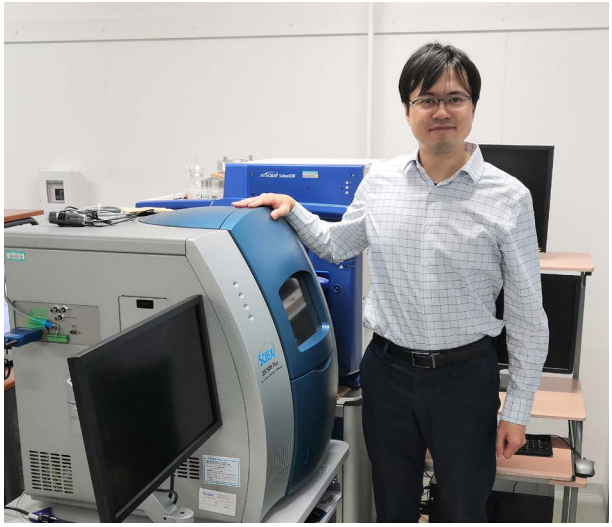
this research to further advance these methods and apply them to real-world pharmaceutical applications in oncology and medicine."

"The extensive customizability of the SCIEX CE equipment allowed the installation of additional external features that could increase the sensitivity of our analyses," said Dr. Kawai. "For instance, I was able to install a relatively high power laser (30 mW) and band-pass filter using fiber optics, improving the sensitivity approximately sixfold without causing stress to the CE instrument. In another example, I attached an innovative, sensitive, tapered sheathless emitter – known as a nanoCESI emitter – to the automated PA 800 Plus system, which avoids contact between the capillary and the vial cap and dust build-up, improving the reliability of CE-MS analysis. Modifying systems in this way involves taking large risks with electrical equipment, so it was critical that the SCIEX service team was on-hand to help with any technical complications. A further benefit of SCIEX CE equipment is that the flexible flow control system places virtually no limitations on parameters such as pressure, duration and vial combination, enabling the seamless modification of protocols. This allowed me to successfully develop an online sample preconcentration method – LDIS – that improved the sensitivity of N-linked glycan analysis by 2,000-fold."

"The MS instruments also offer a degree of flexibility that allows me to apply novel protocols, and having two systems using different technologies broadens the scope for research. CE-MS analysis using the TripleTOF 5600 system is the fastest method for the non-targeted profiling of metabolism – whether for data-independent SWATH acquisition or data-dependent methods – whereas the QTRAP 5500 system has been shown to offer us the highest sensitivity for targeted analyses. For ultra-sensitive analyses, I use the LDIS preconcentration method and a laboratory-customized MS ionization system, achieving a 500 zmol sensitivity for metabolome analysis, which has been successfully used for single-cell profiling of a human HeLa cell."



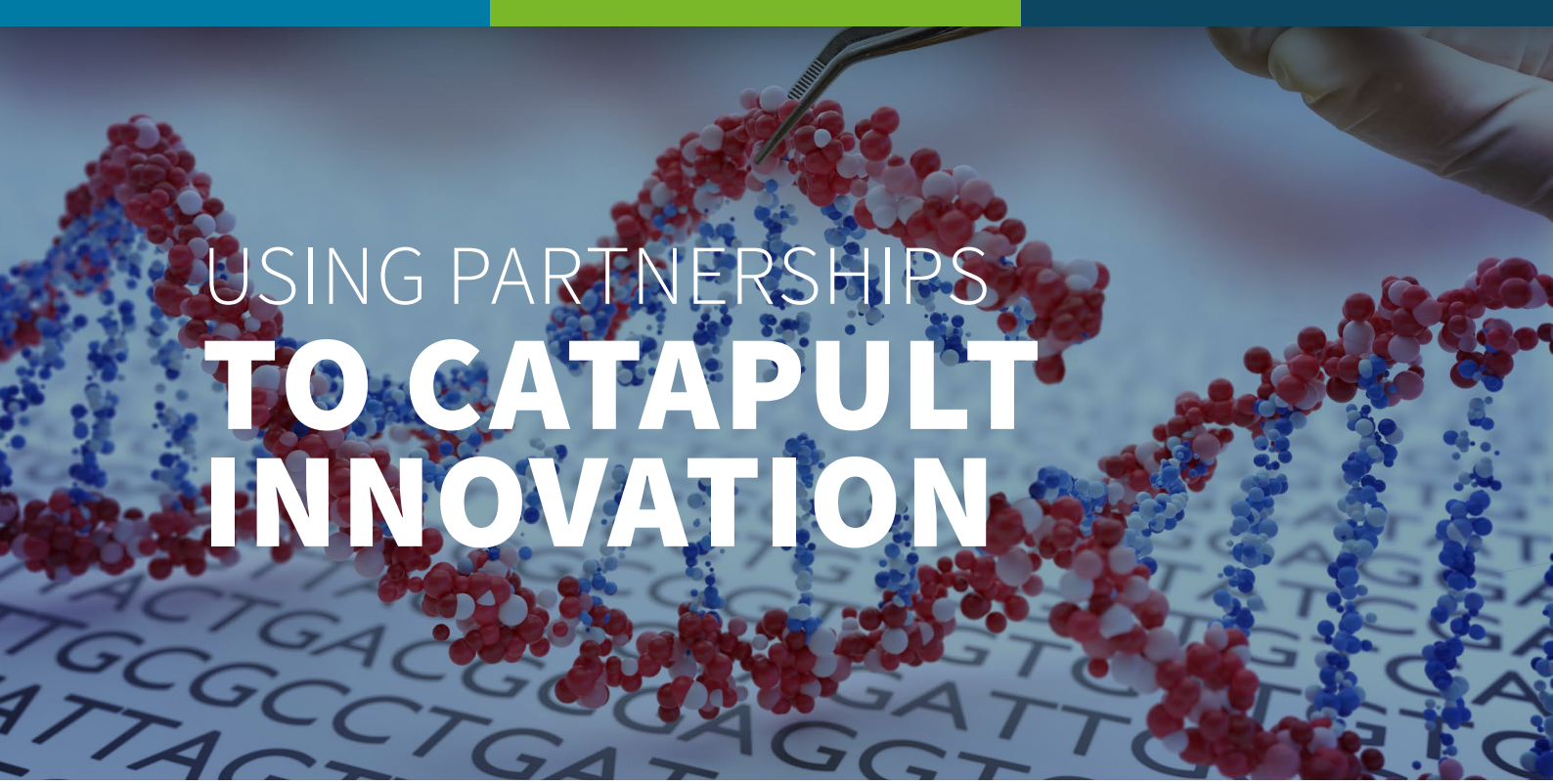
"CE-MS ANALYSIS USING THE TRIPLETOF 5600 SYSTEM IS THE FASTEST METHOD FOR THE NON-TARGETED PROFILING OF METABOLISM... WHEREAS THE QTRAP 5500 SYSTEM HAS BEEN SHOWN TO OFFER US THE HIGHEST SENSITIVITY FOR TARGETED ANALYSES."

"I am confident that we are yet to realize the full potential of CE. We are on the brink of developing cutting-edge analyses – such as single-cell metabolomics or highly sensitive proteome exploration – using CE-MS, but we have to take more risks. Currently, it is a reliable method used largely for routine analyses but, by optimizing our current research methods, we can go further and find out more about microenvironments in complicated life systems. I look forward to collaborating with SCIEX in the future, and contributing to progress in the field of CE and analytical chemistry," concluded Dr. Kawai.

To learn more about Kyushu University, visit **https://www.kyushu-u.ac.jp/en**

To understand more about CE instruments from SCIEX, visit **https://sciex.com/products/capillary-electrophoresis**

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



USING PARTNERSHIPS TO CATAPULT INNOVATION

Louise Taylor, Team Leader in Downstream Processing at CPI's National Biologics Manufacturing Centre in Darlington

Vicky Smith, Principal Scientist in the Analytical Team at Darlington

Innovation and R&D are key drivers of development that bring with them highly skilled jobs and economic growth. However, the process of getting products to commercialization can be costly. CPI is an independent technology innovation center and a founding member of the UK's High Value Manufacturing Catapult; a group of manufacturing research centers established to provide companies with world-class facilities, technologies and skills. CPI offers support to regional, national and international businesses and academic partners to help them drive forward novel technologies. The center actively collaborates with its partners, using its extensive technical capabilities to reduce the challenges of bringing innovative products to market.

CPI – a member of the UK's High Value Manufacturing Catapult with sites across Northern England and Scotland – was established to promote the research, development and commercialization of novel ideas through collaborations with scientists and engineers from numerous fields. Partially funded by the UK government, CPI was set up to reduce the risk, time and cost associated with transforming innovative concepts into marketable products for small- to medium-sized enterprises, academic partners and large corporations.

CPI acts as a catalyst, bringing together academia, businesses, government and investors across diverse market sectors – from electronics to pharmaceuticals and much more – with the goal of strengthening the scope and capabilities of its customers to support effective innovation. Through its Biologics Business Unit, CPI provides expertise in upstream, downstream and analytical stages of development, including in-process and final product analytics. Louise Taylor, a Team Leader in Downstream Processing at CPI's

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National Biologics Manufacturing Centre in Darlington, explained: "At CPI, we essentially reduce the risk of innovation by investing in state-of-the-art technologies and capabilities so that our partners don't have to. We use our expertise to quickly develop and optimize processing methods for our partners, so that products are not in development for long periods of time. Alongside this, we provide technical support and help with funding applications. As a Catapult Centre, we have strong links with Innovate UK and other public sector partners that we work with collaboratively."

Vicky Smith, a Principal Scientist in the Analytical Team at Darlington, took up the story: "To develop processes quickly, we need reliable analytical techniques that offer high sample throughput. We started using SCIEX equipment about two years ago, initially procuring an X500B QTOF system and then adding a CESI 8000 Plus ESI-MS system to couple with one of our other MS systems, and have found that these instruments provide the accuracy and versatility that we need to perform multiple diverse biopharmaceutical projects."

Vicky continued: "The X500B QTOF system was installed for a large CR&D project, to check that the continuous downstream purification process that we were developing generated the correct product throughout numerous cycles. We analyzed targeted host cell proteins (HCPs) to monitor how well they were removed; we had a list of HPCs known to be immunogenic and were able to accurately observe whether or not they were present. This gave us a clear indication of the reproducibility of our purification technique over multiple processing stages and cycles, and the confidence that we could provide the reliability our partners needed. The X500B QTOF system has an extremely intuitive user interface and simple workflows, and was accessible by a large proportion of our team – including those without previous experience in mass spectrometry – which accelerated our sample throughput. We saved our protocols so that anyone could come along, enter a workflow and hit go – it was that easy."

"We later procured a CESI 8000 Plus capillary electrophoresis (CE) system to expand our capabilities and analytical capacity," Vicky added. "It's a versatile platform that we have adopted for many projects, and it is now heavily used for a range of RNA and DNA analyses. A combination of UV- and laser-induced fluorescence (LIF) detection gives us the specificity we need to identify key proteins for biopharmaceutical research. The system is very user friendly, but SCIEX is always on hand to offer troubleshooting advice, review our data or assist in method development if necessary."

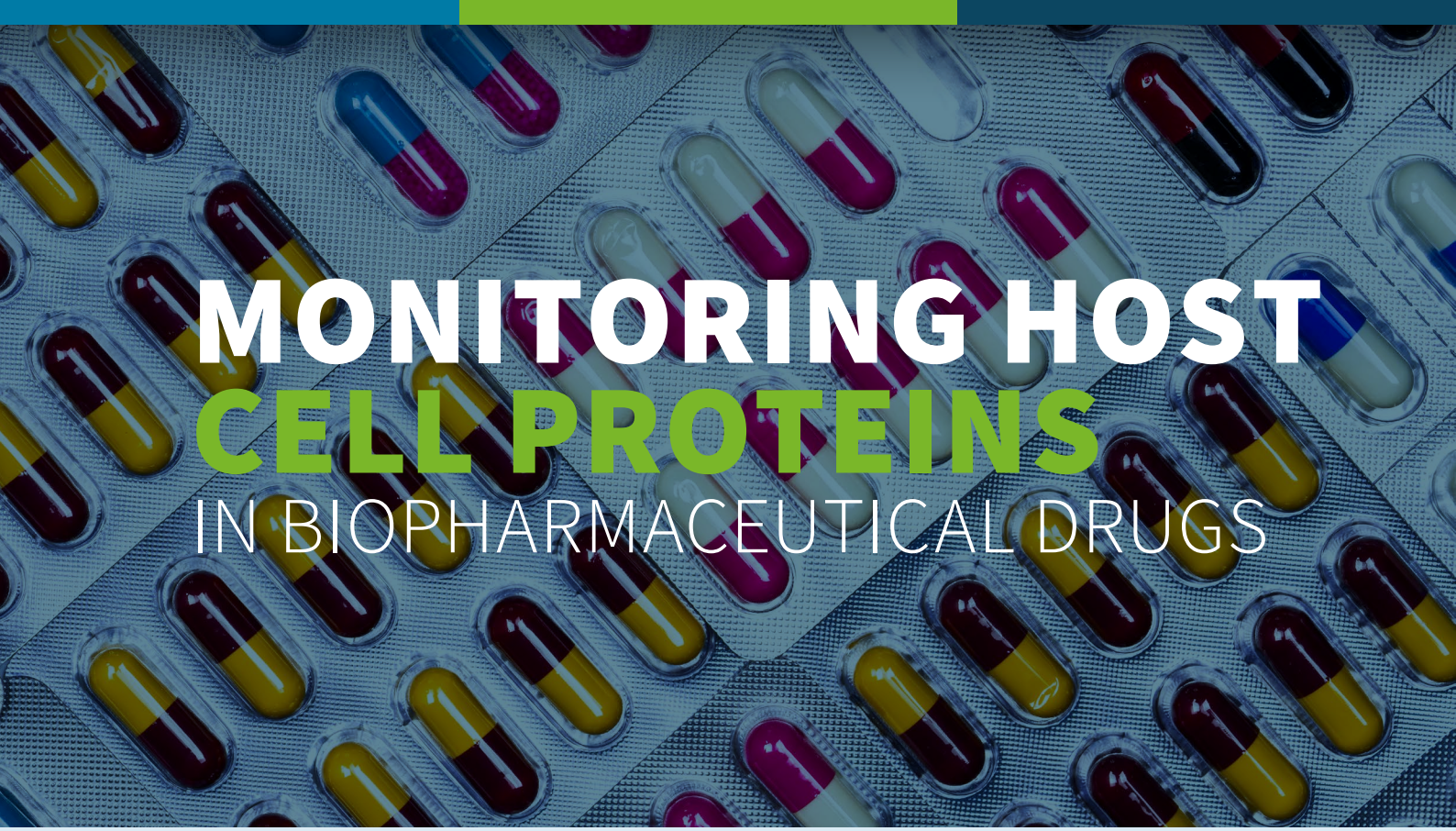
"SCIEX's team of dedicated specialists in CE and mass spectrometry delivered comprehensive training on how to optimize the use of our systems. The support from SCIEX has been valuable, and its online university has provided us with the training and assistance that we have needed – even in lockdown. This gives us access to free online resources, including training and webinars, that cover a range of topics and abilities so that there is something for everyone. Learning the basics of mass spectrometry, and the technical details of each SCIEX machine, gives us the freedom to troubleshoot and expand our own capabilities," Louise concluded.

To understand more about how CPI supports its partners, visit **https://www.uk-cpi.com**

To find out more about CE systems from SCIEX, visit **https://sciex.com/products/capillary-electrophoresis**

To read more about the X500B QTOF system, visit **https://sciex.com/products/mass-spectrometers/qtof-systems/x-series-qtof-systems**

"THE SUPPORT FROM SCIEX HAS BEEN VALUABLE, AND ITS ONLINE UNIVERSITY HAS PROVIDED US WITH THE TRAINING AND ASSISTANCE THAT WE HAVE NEEDED – EVEN IN LOCKDOWN."



MONITORING HOST CELL PROTEINS IN BIOPHARMACEUTICAL DRUGS

Yosuke Ikeda, Quality Development Department at Chugai Pharma Manufacturing Co., Ltd.

Biopharmaceuticals are increasing their share of the pharmaceutical market. Unlike traditional small molecule drugs, which are commonly synthesized by chemical means, protein biopharmaceuticals result from recombinant expression in bacterial, yeast and mammalian cells. However, as they are produced in a biological system, they are co-expressed with host cell proteins (HCPs). HCPs can cause immunogenicity in patients or reduce the potency, stability or overall effectiveness of a drug, and so it is important that they are monitored and removed during downstream processing. Optimizing the downstream processing steps to reduce HCPs to a level where they are acceptable to regulatory authorities is a critical step in biopharmaceutical development.

It can be a challenge to detect and measure HCPs due to the generally low quantities produced relative to the recombinant protein. ELISA is the gold standard, as it offers high sensitivity and high throughput. However, these assays do not detect all HCPs present in a sample, and only detect and quantify HCPs as a whole population, not on an individual basis. Mass spectrometry is becoming recognized as an alternative and complementary technology for HCP characterization, as it can provide both qualitative and quantitative information on individual HCPs.

Chugai Pharma Manufacturing Co., Ltd. is a Japanese company specializing in the production of biopharmaceutical drugs, including antibody, highly active synthetic and investigational drugs. These are produced by Chugai Pharmaceutical Co., Ltd. and Roche Group. Mr. Yosuke Ikeda is part of the Quality Development Department, where his group is involved in the evaluation of investigational drugs. He explained: “We are working on the general analysis of the biopharmaceuticals, evaluating the manufacturing process and analyzing the impurities to ensure the quality of the products. We also work on method development for impurity clearance.”

Related to RUO-MKT-19-13639-A

"We use a number of techniques in the laboratory, including ELISA, PCR, HPLC and LC-MS/MS. For the last five years, we have been using LC-MS for HCP analysis. We found with our previous MS system that we were getting too much variability with results – we would get different readouts from the same samples if we measured them two or three times – and so we wanted to invest in a new platform that would provide more consistency," Mr. Ikeda continued. "SCIEX instruments featuring SWATH acquisition enable data-independent scanning for HCP detection, and so we conducted a demo measurement of our samples on one of these systems. We found that the instrument was user-friendly and provided consistent data measurements. As a result of the demonstration, we invested in a TripleTOF 6600+ system, as it provided the sensitivity we needed and offered a good analysis time per test. I'm not a specialist in LC-MS, but the equipment and software were easy to use."

Mr. Ikeda was supported in the set-up and implementation of the system by the team at SCIEX. He noted: "Although there were some challenges immediately after the operation started, we were soon obtaining the stable results needed to help with process development. I was very satisfied with the support and service we received from SCIEX, because of the immediate response we received whenever we needed advice. They continue to provide support if we need guidance on how to analyze or develop the method."

"I'M NOT A SPECIALIST IN LC-MS, BUT THE EQUIPMENT AND SOFTWARE WERE EASY TO USE."

The group is now routinely using the TripleTOF 6600+ system for HCP analysis. Mr. Ikeda concluded: "We are currently using the TripleTOF 6600+ system to monitor impurities from drug substances produced by a Chinese hamster ovary cell line. We use both SWATH acquisition and MRM modes for qualitative and semi-quantitative analysis of HCPs. However, I think this instrument has a wide range of uses, since it can also be used for characterization of biopharmaceutical drugs, and I could see it being useful for QC testing in the future."

To find out more about Chugai Pharmaceutical Co., Ltd., visit **https://www.chugai-pharm.co.jp/english/index.html**

To find out more about the SCIEX TripleTOF 6600+ system, visit **https://sciex.com/products/mass-spectrometers/qtof-systems/tripletof-systems/tripletof-6600plus-system**

SCIEX Triple Quad 7500 system

The SCIEX 7500 system heralds a new era of sensitivity and LC-MS/MS innovation. Offering the highest level of sensitivity of all the SCIEX systems, it enables low level quantification of crude and complex samples to further research, with a linear dynamic range of up to six orders of magnitude.

The innovative SCIEX 7500 system enables testing of more samples, offering measurable gains in productivity and the detection of trace compounds that could not previously be seen. The OptiFlow Pro ion source allows users to simplify sample preparation and inject less sample. Its modular source design combines the reliability and efficiency of the legendary Turbo V ion source with the E Lens probe – which boosts the transition of crucial ions into the mass spectrometer – and enables easy switching from high to low flow and between ESI and APCI. A larger orifice allows more ions to enter the mass spectrometer and the unique design of the D Jet ion guide retains and captures more of the ions from the ESI spray plume, increasing assay sensitivity to allow characterization of analytes that were once masked by matrices.

The SCIEX 7500 system is powered by SCIEX OS software, with traditional software features and functions complemented by visual aids and components to support quick, accurate and confident data review.

For more information, visit **https://sciex.com/products/mass-spectrometers/triple-quad-systems/triple-quad-7500-system**

ZenoTOF 7600 system

The ZenoTOF 7600 system is the latest innovation from SCIEX. It combines the power of Zeno trap pulsing with EAD (electron activated dissociation) to offer a step-change in fragmentation technology and unparalleled MS/MS sensitivity. This set-up enables researchers to uncover previously inaccessible structural information and enhance the limits of quantification.

EAD technology allows fine tuning of the electron energy within an experiment, enabling multiple fragmentation mechanisms that can be applied to large molecules, peptides, lipids and small molecules, while Zeno trap ushers in a new era of sensitivity for accurate mass. Ions are accumulated in the Zeno trap before being pulsed rapidly into the TOF, enabling detection of up to twentyfold more ions. This allows more useful MS/MS information to be collected, including lower abundance species that were previously undetectable. Zeno MRM^{HR} unlocks new levels of sensitivity, while SWATH acquisition supports higher throughput proteomics.

For more information, visit **https://sciex.com/products/mass-spectrometers/qtof-systems/zenotof-7600-system**

Echo MS system

The Echo MS system takes advantage of industry-leading quantitative MS technology to deliver a highly quantitative and sensitive solution that will redefine current and future high throughput workflows. This novel Acoustic Ejection Mass Spectrometry (AEMS) system delivers speed, scale and reproducibility with unyielding data quality.

The Echo MS system was uniquely built for high throughput screening workflows, offering consistent precision and reproducibility in challenging matrices to deliver unparalleled standards of quantification. It enables direct analysis of compounds up to one sample per second, breaking through the bottleneck of conventional LC-MS/MS. This will help to transform project timelines from weeks to a matter of days, without compromising on the precise, information-rich data that allows faster decision-making.

For more information, visit **https://sciex.com/products/integrated-solutions/Echo-ms**

SCIEX OS software

SCIEX OS software is mass spectrometer software designed for the modern laboratory, offering powerful algorithms and automation to enable users to make informed decisions more quickly. Its remarkable quantitative usability, efficiency and integrity will help to transform and streamline LC-MS/MS workflows.

The user-friendly SCIEX OS software offers an integrated experience across all SCIEX mass spectrometers, from QTOF to triple quadrupole to QTRAP systems, so operators can switch between systems with ease. Users can rapidly get up to speed with this easy-to-learn software, increasing productivity and consistently delivering high quality and actionable data from mass spectrometry workflows for maximum efficiency. The accuracy, precision and consistency of the software ensures that data quality is never compromised at any stage of the workflow, batch after batch. Laboratories also benefit from the flexibility to tailor and deploy security policies to meet their specific needs, with tools such as electronic audit trails, electronic signatures and detailed security configurations to support GxP and 21 CFR Part 11 regulations.

For more information, visit **https://sciex.com/products/software/sciex-os-software**

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.

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