# Introducing the AB SCIEX 6500 Series with IonDrive<sup>™</sup> Technology

Driving the power of innovation

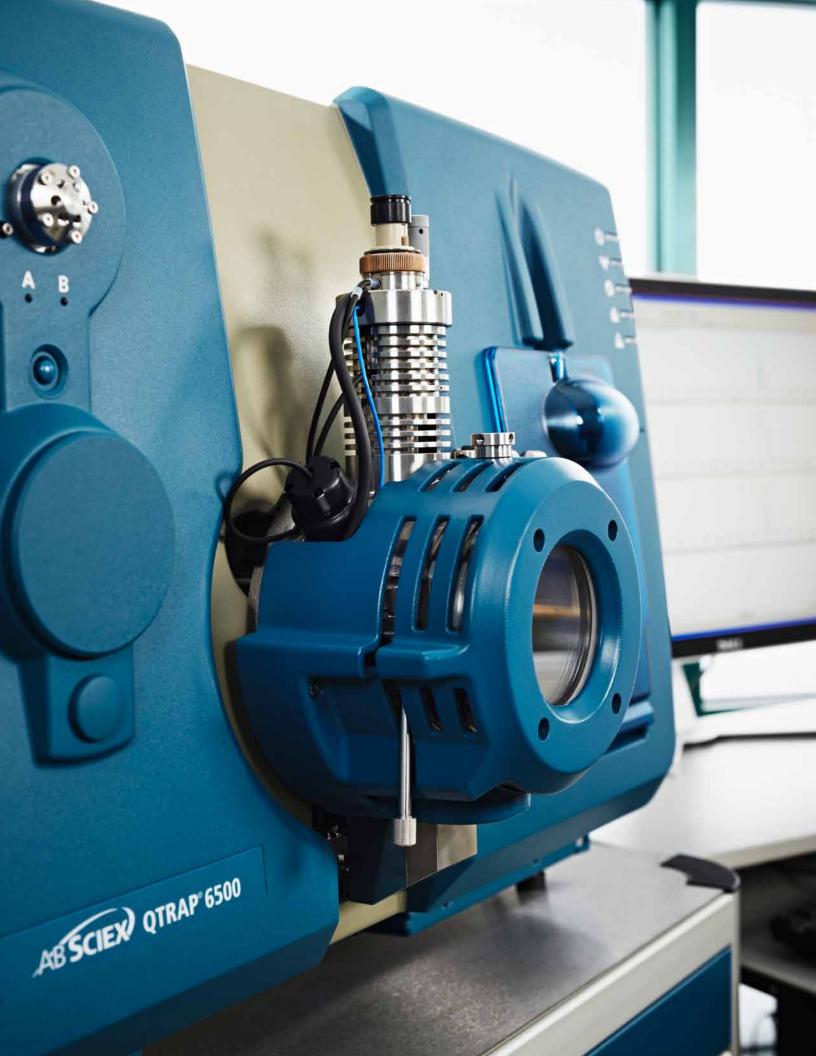
Eurofins teams up with AB SCIEX and Phenomenex

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**Masspectator** Issue 1





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# Introducing the AB SCIEX 6500 Series with IonDrive<sup>™</sup> Technology

Anthony Romanelli, Loren Olsen, Tom Biesenthal, Hesham Ghobarah and Johnny Cardenas



Over the past 30 years AB SCIEX has been delivering innovation in the field of mass spectrometry solutions. From the groundbreaking API III<sup>™</sup>, to the legendary API 4000<sup>™</sup> and on to the unique and versatile QTRAP<sup>®</sup> technology, the widely adopted TripleTOF<sup>®</sup> high-resolution-MS platforms, and the SelexION<sup>™</sup> differential ion mobility technology.

AB SCIEN QTRAP 6500

This legacy of innovative, intelligent instrument design has been based on 6 key principals that guide our research and development initiatives: sensitivity, selectivity, speed, simplicity stability and software (see article 'Mapping ideas to drive innovation on pg. 12). These guiding principals are our prime directives in instrument design. AB SCIEX continues its legacy of new mass spectrometry solutions.

At the recent 2012 ASMS meeting in Vancouver, Canada, AB SCIEX introduced the **new 6500 Series of mass spectrometers**, the next generation of ultra-sensitive LC/MS/MS platforms equipped Revolutionary multi-

component IonDrive<sup>™</sup> technology.

AB SCIEX adopted an intelligent approach to increasing limits of quantitation with the patented IonDrive<sup>™</sup> technology, introducing a series of system-wide technological advances that not only increases the number of ions produced but also enhances the mechanism for ion transmission and detection.

> Learn more about the new AB SCIEX 6500 Series by watching a brief technology over view video on the IonDrive™ technology.



FULLER FLA

#### IonDrive<sup>™</sup> Technology Produce more ions Transmit more ions Detect more ions

The IonDrive<sup>™</sup> technology is built into this system from the ionization source, to the ion-focusing region and through the detector. It's a holistic approach to intelligent mass spectrometry design that pushes the limits in LC/MS/MS sensitivity and allows scientists to achieve the lowest limits of quantitation in complex matrices.



Technology: Produce more ions with the IonDrive<sup>™</sup> Turbo V Source. Focus and transmit more ions with the IonDrive<sup>™</sup> QJet<sup>®</sup> Guide. Detect more ions with the IonDrive<sup>™</sup> HE Detector.

OTRAP<sup>®</sup> 6500 700 ag Alprazolam in plasma CV = 7.6% (n=3) 0.92 0.94 0.96 1.06 1.10 1.12 1.14 1.16 1.18 0.98 1.02 1.04 1.08

Achieve new limits of quantitation while maintain robustness and reproducibility

The next generation IonDrive<sup>™</sup> Turbo V source increases ion production using enhanced gas flow dynamics and optimized heater configurations while improving reliability, reproducibility and robustness. The patented IonDrive<sup>™</sup> improves ion containment and collisional focusing through a dual stage QJet design that captures and focuses ions more efficiently. Lastly, the new IonDrive™ High Energy Detector maintains the benefit of pulse counting fundamentals to achieve accurate

quantitation for low level signals while increasing linearity at higher count rates.

The IonDrive<sup>™</sup> technology empowers the new 6500 Series to deliver levels of sensitivity never before achieved in an

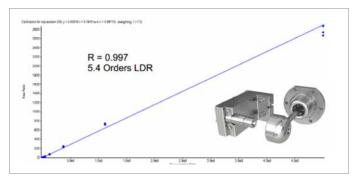
QJet guide technology

LC/MS/MS system. Analytes with very low plasma circulation levels such as inhalation, topical, and ophthalmic drugs stand to benefit the most from the performance levels of the 6500 Series.

#### Sensitivity

To evaluate the bioanalytical performance of the new 6500 Series, standard curves of a series of commonly studied analytes were prepared in protein precipitated plasma and in

neat solution. Alprazolam, a common short-acting anxiolytic compound used to treat anxiety disorders was one of the first compounds studied on the 6500 Series with IonDrive technology The system was able to achieve a lower limit of quantitation of 700 attograms (ag) of alprazolam in protein precipitated plasma with a CV of 7.6% for triplicate injections at the LOO.



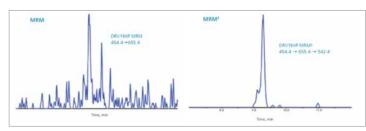
With the new IonDrive™ HE Detector, achieving 5-6 orders of linear dynamic range becomes routine, increasing throughput and efficiencies.

#### **Dynamic Range**

With the IonDrive<sup>™</sup> High Energy Detector technology, the 6500 Series delivers significant sensitivity improvements and the dynamic range required to cope with the increased ion flux entering the mass analyzer. With the IonDrive<sup>™</sup> High Energy Detector, the 6500 Series is able to achieve a linear range of 5.4 orders of magnitude. Using alprazolam as a test analyte, the 6500 Series can reach an upper limit of quantitation of 500 pg on column with 95% accuracy and a CV of 3.8% for triplicate injections. It delivers up to a 20X expansion in ion counting capacity by delivering a wider dynamic range and higher sensitivity.

#### Selectivity & Sensitivity

With a dramatic increase in sensitivity, matrix interference can become limiting factor in achieving the desired LOQ. Isobaric interferences and high baseline can pose serious challenges despite best efforts with extensive chromatographic separations.



The MRM<sup>3</sup> workflow can often provide higher specificity and therefore better LLOQs in complex matrices over MRM workflow alone, as shown here for the heavy labeled peptide DRVYIHP in digested human plasma.

In situations where co-eluting interferences make standard MRM quantitation difficult, the 6500 Series offers two alternative approaches to increasing selectivity and reducing interferences: MRM<sup>3</sup> and differential ion mobility

#### **MRM**<sup>3</sup>

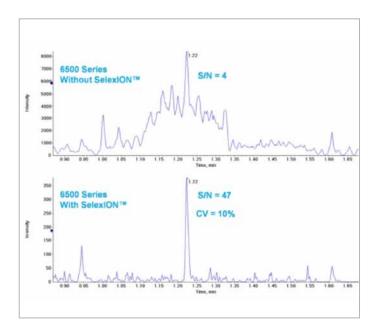
A unique approach to increasing quantitative selectivity for the analyte of interest is to monitor the MS/MS/MS (MRM<sup>3</sup>) fragmentation pattern rather than just the MS/MS (MRM<sup>2</sup>) fragmentation pattern of traditional MRM scans. The MRM<sup>3</sup> delivers a fast, easy-to-use approach to improving selectivity, reducing high baseline, and increasing throughput that reduces the need for time consuming sample preparation techniques to eliminate co-eluting interferences.

As can be seen in this heavy-labeled peptide DRVYIHP in digested human plasma example, the MRM<sup>3</sup> approach offers higher specificity and therefore, better LOQ's in complex matrices over standard MRM workflows

#### **Differential Ion Mobility**

For the second approach to increasing selectivity we examine the analysis of salmeterol, a commonly prescribed drug for the treatment of chronic obstructive pulmonary disease. The sub pg/ml LOQ objective is difficult if not

impossible to reach in rat plasma due to matrix interferences – despite the added sensitivity of the 6500 Series. However, by leveraging the **SelexION™ technology** from AB SCIEX, it was possible to use differential ion mobility for additional orthogonal selectivity. This lowered the isobaric interferences substantially permitting the 1.4fg on-column concentration to be easily detected exhibiting a 10X improvement in signal to noise.



Achieving real improvements in LOQ's for bioanalysis requires both sensitivity and selectivity. When SelexION Technology is combined with the 6500 series, it is possible to reduce isobaric interferences and increase signal to noise ratios for challenging bioanalytical problems such as Salmeterol in plasma.

### AB SCIEX 6500 Series mass spectrometer with IonDrive Technology

- Up to 10X greater sensitivity over the 5500
- Up to 20X increase in detector dynamic range
- Redesigned interface for enhanced robustness
- Enhanced QTRAP<sup>®</sup> technology option
- Increased mass range, 5 2000 m/z

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ABSCIEN QTRAP 6500

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# Redefining protein quantitation with breakthrough MS/MS<sup>ALL</sup> with SWATH<sup>™</sup> Acquisition

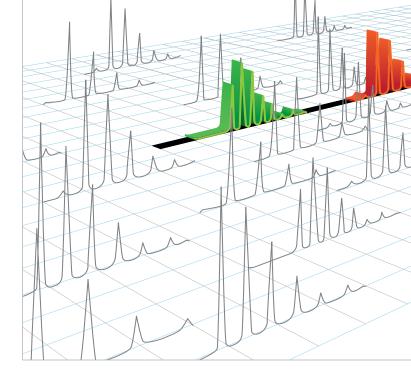
Ruedi Aebersold, ETH Zurich, and Mark Cafazzo, AB SCIEX, Framingham USA

MS/MS<sup>ALL</sup> with SWATH<sup>™</sup> Acquisition provides the highest levels of multiplexing for comprehensive quantitative proteomics. Previously, scientists have used discovery strategies on powerful mass spectrometers to identify large numbers of proteins in a sample along with targeted techniques to quantify proteins using reference proteome maps by Multiple Reaction Monitoring (MRM).

SWATH<sup>™</sup> Acquisition allows users to acquire quantitative MS and MS/MS data on all peptides in a sample, while simultaneously capturing qualitative information that can be used to confirm ID or structure. This technique generates a complete digital archive of all features of a sample that can be repeatedly re-interrogated without the need to re-acquire data.

To create a complete digital map of the sample proteome, SWATH Acquisition generates quantitative MS/MS data through rapid systematic repeated scanning. SWATH is a unique and effective technique due to its wide dynamic range and high sensitivity (Figure 3 top right). It provides the ability to retrospectively interrogate samples *in silico*, which allows direct data comparison for subsequent experiments to address new hypotheses.

Data acquisition: obtaining a complete proteome map Using an AB SCIEX TripleTOF® 5600 System is used for data acquisition, this new technique acquires high resolution, accurate mass MS/MS data for all precursor ions in a sample (that are within a user-defined, retention time vs m/z window), by rapidly and systematically sweeping through the total mass range in windows of 25 m/z as peaks are eluted from the chromatography system. The choice of the 25 m/z



windows and 100 ms retention time results in approximately 10 measurement points over the chromatographic peak for each peptide, allowing for reconstruction of the quantitative curves in a manner similar to MRM. The outcome of the measurement is a permanent digital record of quantitative MS/MS data for the entire sample (Figure 1).

#### Data search

The digital proteome maps obtained with SWATH Acquisition cannot be analyzed with conventional database search engines. The data are too complicated, and most search engines would be unable to de-convolute the fragment ions and assign them to the correct precursor ions. With SWATH Acquisition, the data are represented so that an MRM-like approach can be applied for peptide analysis (Figure 2 top right). The fragment ions for the peptides of interest are extracted from a library and the signals are aligned and compared to the experimental results (Figure 2 bottom left). The search requires the coordinate of each peptide, including Q1 precursor ion mass, fragment ion mass, retention, time and the intensity of the signals for relevant transitions.

#### Ground-breaking discoveries

With a unique mix of high throughput, specificity, sensitivity, accuracy, and reproducibility, SWATH Acquisition is a breakthrough mass spectrometry technique. In summary, it creates a permanent digital record of an analysis and provides comprehensive quantitative analysis with qualitative confirmation – a combination that we expect will lead to ground-breaking discoveries in systems biology and other areas of science.



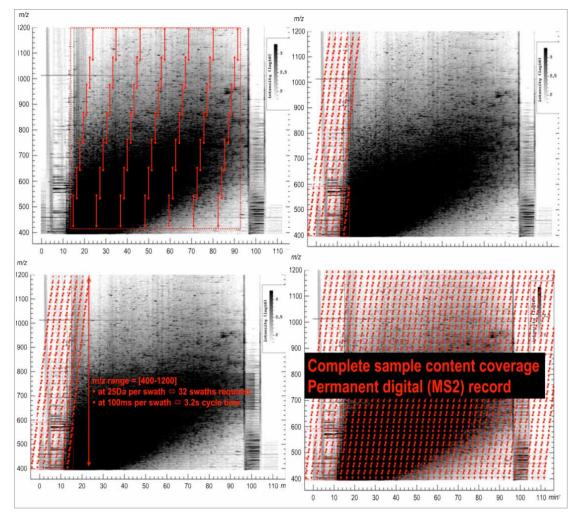


Figure 1: SWATH Data Acquisition

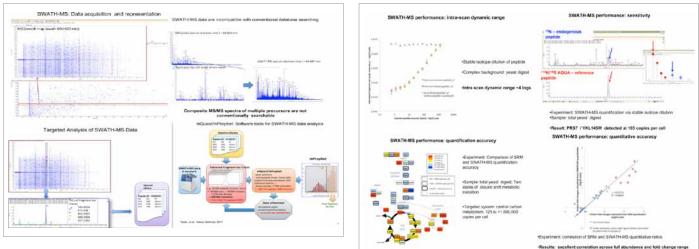


Figure 2: SWATH Data Representation(top), Targeted Data Analysis (bottom left), Data Analysis Using Software (bottom right)



#### References

- 1. MRMAtlas database, ISB Home, http://www.mrmatlas.org/original.php
- 2. GL Andrews, BL Simons, JB Young, AM Hawkridge, DC Muddiman, Performance Characteristics of a New Hybrid Quadrupole Time-of-Flight Tandem Mass Spectrometer (TripleTOF 5600), Anal Chem, 2011, 83, 5442-5446

# Quantification of ethylglucuronide (EtG), a marker for chronic excessive alcohol consumption

Dr Stephen Lock (AB SCIEX, Warrington, UK), Dr Simon Elliott (ROAR Forensics, Malvern, UK) and Dr Eleanor I. Miller (ROAR Forensics, Malvern, UK)

Ethanol (alcohol) is converted to acetaldehyde and subsequently to acetic acid by liver enzymes (Figure 1). The traditional approach to assess the extent of alcohol consumption is through blood and urine analysis but unless the blood or urine samples are stored in appropriate specimen containers with preservatives such as fluoride oxalate, ethanol can be produced as an artifact of bacterial fermentation post collection.<sup>1</sup> This can result in elevated and hence inaccurate ethanol concentrations in these samples, which can potentially affect the interpretation of any results obtained.

#### Introduction

An advantage of the use of EtG in hair is that it is not subject to the effect of bacteria and also offers the opportunity to extend the window of detection (up to 6 months) compared to hours or days in blood and urine.<sup>2</sup> As ethanol metabolism is independent of dose, blood and urine concentrations can vary widely between individuals. However with hair analysis the use of a 30 pg/mg cut off, as proposed by the Society of Hair Testing (SoHT), can be applied as standard.<sup>3</sup>

#### Method

An LC/MS/MS method to detect EtG in hair has been developed using a solid phase extraction step for sample clean-up and leveraging the sensitivity achievable on the **QTRAP® 5500 System**. For the detection of EtG,

LC/MS/MS has the advantage over gas chromatography mass spectrometry (GC/MS) in that it detects the native EtG species, which does not have to be derivatized [e.g. into a trimethylsilyl (TMS) derivative] prior to analysis.<sup>4</sup>



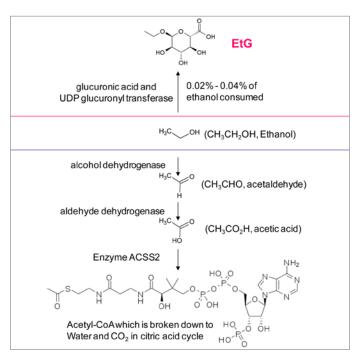


Figure 1: Metabolism of ethanol in the liver

As can be seen in Figure 3, EtG elutes early in the HPLC run so the latter high flow part of the gradient is present to clean and re-equilibrate the HPLC column prior to the next injection. The signal-to-noise shown in Figure 3, shows that EtG is easily detected at low pg/mg concentrations in hair. When a calibration line of spiked hair extracts is analyzed the response is also shown to be linear over the range tested.



In this method, several transitions for EtG were detected, but it was found that product ions of 75 and 85 seemed to be optimal for the samples run. Additional transitions for the internal standard were also acquired, but again, the 226-75 transition was the strongest with the least amount of matrix interference and was therefore used for quantification.

#### Results

The results show that the use of the very sensitive QTRAP<sup>®</sup> 5500 system allows for detection of EtG in the pg/mg range and therefore an assessment of alcohol consumption from extracts of hair, with excellent linearity over the range tested.

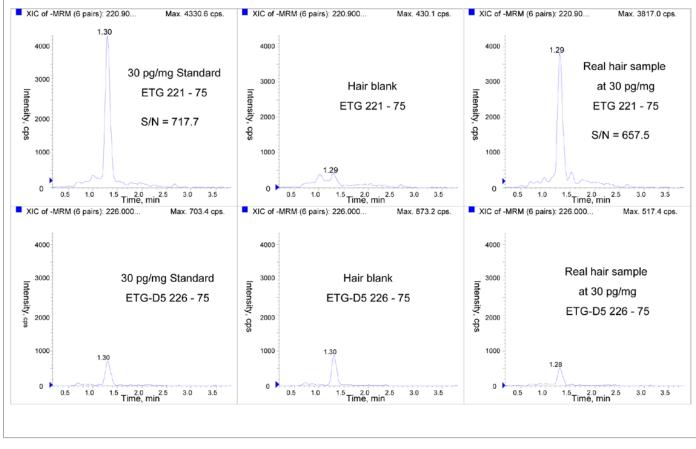


Figure 3: Extracted ion chromatograms of hair samples. The top row represents EtG traces and the bottom row the corresponded EtG-D5 internal standard chromatograms.

#### References

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- 2. Dahl, H., Stephanson, N., Beck, O. and Helander, A. (2002). Journal of Analytical Toxicology 26, 201–204.
- 3. Society of Hair Testing Recommendations. "Consensus of the Society of Hair Testing on hair testing for chronic excessive alcohol consumption 2011", www.soht.org.
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### Eurofins teams up with AB SCIEX and Phenomenex

Eurofins Central Analytical Laboratories, Metairie, LA, USA









Eurofins Scientific is the world leader in food and pharmaceutical product testing and environmental laboratory services as well as agroscience, genomics, and central laboratory services. Eurofins has been a trusted partner of AB SCIEX for years, serving as an innovator in food safety testing applications using mass spectrometry and advanced analytical techniques.

#### Response to crisis

Eurofins has a strong history of setting the benchmark for food safety testing across the globe through both innovation and crisis response. In 2007, Eurofins quickly responded to the melamine crisis, establishing accurate and reliable methods to analyze for the contaminant. Today, they continue to actively and aggressively test a variety of consumer products for melamine.

In 2010, big news surrounding the Deep Water Horizon oil spill spurred the team at Eurofins in Metairie, LA, to develop a series of methods for the accurate quantitation of PAH and aliphatic hydrocarbon compounds in seafood samples. The responsive efforts of Eurofins Central Analytical Laboratories

> following this crisis led to better awareness of the contamination risk of the local seafood, which was essential to the well-being of the local seafood industry.

#### Setting the standard

Since the Deep Water Horizon incident, the Eurofins method development team continues to develop innovative analytical methods for food and environmental safety. All types of food and consumer products require contamination analysis. As a result, contract testing labs are often tasked with analyzing difficult





matrices such as dietary supplements, essential oils, feeds, and spices. Success requires special attention to sample prep, LC conditions, and MS analysis for success.

#### New powerhouse collaboration

- To support Eurofins' mission
- to create better, more robust

analytical methods for food safety testing – and on the heels of the strategic partnership between

AB SCIEX and Phenomenex – we are embarking on a new collaboration. The collaboration combines the expertise of Phenologix, the full-service analytical support lab within Phenomenex, the method development scientists at Eurofins Central Analytical Laboratories in Metairie, LA, and the AB SCIEX mass spectrometry experts to create a method development team like no other.

#### **Current focus**

This year, Eurofins will work with the teams at Phenologix and AB SCIEX to assemble new multi-residue analytical methods for contaminant analysis in some of their most challenging matrices. This collaboration will focus on:

- Sample preparation protocols that can eliminate interfering matrix components while still offering good recoveries of the analytes of interest
- HPLC method development for optimal peak shapes and separation of a variety of compounds covering multiple classes of residues, including different antibiotic drug residue classes, mycotoxins, pesticides, and others
- Mass spectrometry optimization for multi-residue analyses in dirty matrices

Specifically, Eurofins will be incorporating the new QTRAP<sup>®</sup> 4500 system into their laboratory to perform simultaneous quantitative analysis of contaminants in these tough matrices while also performing full MS/MS scanning for reliable confirmation of positives.

Eurofins is just one of many of our valued customers who are pushing the limits and working to improve the world we live in. We are excited for a successful and continued collaboration.

### Driving the power of innovation

AB SCIEX's R&D team goes out into the field to work closely with customers on a regular basis. That gives them the opportunity to see what mass spectrometry users are doing and what problems they are trying to solve.

"Customers may have a solution in their mind, but they don't know what is going on behind the scenes in research and development at AB SCIEX," says Lock. "We bring customers into the inner circles to discuss how we can combine our ideas and plans with what they are thinking."

#### The Six S's

The R&D team maps all new ideas to the following six "S's" that drive the company's new product development:

- Sensitivity
- Selectivity
- Speed
- Simplicity
- Stability
- Software

#### Sensitivity, selectivity and speed

From the beginning, AB SCIEX has excelled with the first two "S's": sensitivity and selectivity, followed by the addition of speed as the third "S" – all of which are performanceoriented. These factors alone were sufficient when mass spectrometry was basically a research tool.

#### Simplicity

However, as mass spectrometry has evolved into the mainstream of analytical science, including food safety testing, environmental analysis, forensic toxicology, vitamin D analysis, steroid analysis, other factors become critical. To respond to this need, R&D adopted simplicity as the fourth "S."



"In R&D at AB SCIEX, we think in terms of windows of accessible performance," says Lock. "The technology can be mind-blowing, but it also has to be accessible for a wide range of customers."

#### **Stability**

The fifth "S" is stability. Even though AB SCIEX has an industry leading service and support organization in the mass spectrometry market, many customers want fewer service calls related to issues with the instruments.

R&D has taken on this challenge in a big way, designing new products that are optimized for long-term stability. Maintenance then becomes a preventative action, and the service engineer works with the customers on a more consultative basis to maximize the use of the stable, robust and reliable machines in the labs.

#### Software

The company is also investing heavily in the development of software – the sixth 'S' – in order to, as Lock puts it, "capture the growing value of software as part of the total equation for instrument functions." Better software can help improve the interpretation of data and reduce the bottleneck of processing data.

"We may be working on a new feature or functionality that would solve a problem that may be beguiling mass spectrometry users today. After we share what we are planning, we often hear customers say in amazement, 'You can do that?'. But it's because we go way out of our way to understand the mind of the customer and what we can do that will really add value."

### The new workhorse in Mass Spectrometry. View the AB SCIEX 4500 Series video



"Ideas for new innovation and breakthroughs often come from unexpected places," says Chris Lock, Senior Director of Mass Spectrometry Research at AB SCIEX. "It starts with understanding the mind of the customer."

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# Worldwide events

For a complete listing of conferences and tradeshows AB SCIEX will be presenting at or for more details of our seminars and workshops visit **www.absciex.com/events** 



#### IMSC 2012, The 19th International Mass Spectrometry Conference September 15 -21, Koyoto International Conference Centre, Kyoto, Japan.

Held for the first time outside of Europe – Kyoto is not only renowned as former capital and culture heart of Japan, but also for its place at the cutting edge of mass spectrometry research. The scientific program will reflect the latest trends in fundamental and applied mass spectrometry and related disciplines. AB SCIEX and Eksigent invite you to attend our events at IMSC 2012. Discover the latest news on AB SCIEX mass spectrometry technology and meet with your peers from all over the world to learn how you can push the limits of your research beyond your current capabilities.

Join our luncheon seminars to explore our new best-in-class solutions for a broad range of applications. Every day, from Monday to Thursday, from 12:20 to 1:30pm.

Save the date for our users meeting on Saturday, September 15th

http://www.absciex.com/events/conferences/imsc-2012



#### HET Instrument 2012

HET Instrument is the European marketplace for providers from Industrial Electronics, Industrial Automation and Laboratory Technology. The trade show is one event comprising three trade shows, each with its own exhibition program.

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#### **EBF** Barcelona

Over 400 participants contribute to the success of the EBF Open Symposia in Barcelona. Building further on this event, EBF announces the 5th Open Symposium which theme is "Old Battles, New Horizons". Again, EBF will be bringing a versatile and diverse program that combines regulated bioanalysis themes with technological and scientific developments for small and large molecule bioanalysis.

Come and meet us at our booth! More info coming soon about our AB SCIEX Workshop. **Register here** 

Dates: November 14-16, 2012 Place: Barcelona, Spain

# Why getting up close and personal with your lab speaks volumes



In today's world of technology with phone, email, the internet and online chat at our fingertips there's still nothing quite beats the personal touch. At AB SCIEX we aim to be a trusted partner to our customers. No two labs are the same, and it's having that insight and knowledge of your lab that makes all the difference.

#### World-class support

Whether it's your ion source, autosampler, or application, our service engineers can help get you back up and running quickly – because they know you can't afford downtime. Our application chemists can help streamline your sample preparation and eliminate manual steps. They can also help you develop methods for fast implementation, scale up for higher throughput, or utilize iMethod<sup>™</sup> Applications to get up and running fast. If it's experience with LC/MS or an application you need our training specialists can customize a program specific to your lab.

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Yet it isn't just the experts that you see that makes a difference. It's also the network and the expertise that supports those professionals. From the regional technical specialist teams to our groundbreaking R & D scientists, worldwide manufacturing, and global parts supply chain, it's all about having the answers you need at your fingertips.

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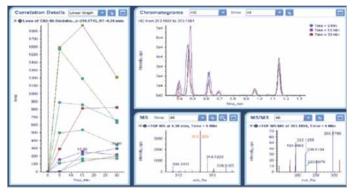


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Now you can give our software a test drive – at your own convenience, without leaving your office, and without having to undergo lengthy trial software installations. With the click of a mouse, you can log into the AB SCIEX cloud computer and begin exploring some of the latest software modules offered by AB SCIEX, including:



#### MetabolitePilot<sup>™</sup> Software Qual-Quant Workspace for Met ID Data

Leverage AB SCIEX's software Cloud and experience the advantages of MetabolitePilot<sup>™</sup> Software qual-quant workspace. See how the multi-sample correlation functionality allows users to correlate metabolites across multiple samples and species by, generating clearance plots, and overlaying analog chromatograms for MS and MS/MS data.

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If you would take a few minutes of your time to tell us about your research we'd very much appreciate it. Everyone who completes the survey will be entered into a prize draw\* to win 1 of 2 Electronic Tablet Devices!!

### TAKE SURVEY

\*It is mandatory that participants have reached the age of majority. Valid email address is mandatory for delivery of prize, if applicable.

\*NO PURCHASE NECESSARY TO ENTER OR WIN. Sweepstakes opens on September 20, 2012 at 10:30 a.m. PST and closes on December 31, 2012 at 12:00 p.m. PST. Void where prohibited by law. This promotion is available to residents of the US, Canada, Europe (excluding Quebec (Canada), Italy, Sweden, Norway, and Belgium) and all countries where not prohibited by law who have reached the age of majority at time of entry. Two (2) prizes are available to be won, consisting of one (1) electronic tablet each valued at approximately \$500. Odds of winning depend on the number of eligible entries received. **Official Rules available online by clicking here**. Sponsor: AB Sciex LLC.

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