MassSpectator

Issue 4

Improving residue testing with MRM^3

Increasing sensitivity and linear dynamic range for drug quantitation using the AB SCIEX QTRAP® 6500 LC/MS/MS System

University of Cambridge scientists are going to Xtremes on Mt. Everest

Ultra-fast amino acid analysis – maximizing speed, sample throughput and cost effectiveness

Lessons in food safety from an astounding 14 year old scientist

Insights to findings of hazardous disinfection by-products in swimming pools

AB SCIEX

Contents

Improving residue testing with MRM^3

Scientists at RIKILT Institute of Food Safety, Wageningen University Research Center, are continuing to look for new and innovative approaches to detect banned and registered chemical substances in foods and animal feeds. They typically target residues such as growth promoters, veterinary drugs, natural toxins, and pesticides, in a variety of matrices ranging from meat, milk, eggs, seafood, and honey to urine, hair, and other assorted forensic samples.

The goal of the residue testing team is to identify residues in our food supply and ensure that residue limits meet national and international regulations, while also scoping for zero tolerance, or forbidden residues, to assess the illegal use of particular banned substances in food farming and manufacturing practices.

The use of steroidal hormones in the food chain

Farmers all across the globe are required to raise an extensive quantity of food-producing animals to meet global food demands. In some cases, feedlots are given assorted steroidal hormones to promote faster growth, with the goal to improve the productivity of the feedlot. Some animals are administered testosterone surrogates, some estrogens or progestins, to enhance growth and fertility. Ultimately, the use of these substances is designed to make the animals more productive – either through gaining leaner muscle more rapidly or becoming more fertile to grow the brood.

Unfortunately, the use of these steroidal hormones results in the potential accumulation of these residues in the edible tissues of the animal, causing risk of human consumption. Hormone consumption can cause disruption in the natural hormonal balance in the human body, and consuming animal products laced with hormones can result in adverse health effects, particularly in children whose bodies are still developing and are more sensitive to hormone consumption.

Suggested side effects of excessive hormone consumption include premature sexual development, development of ovarian cysts in young girls, and auto-immune problems, among others. Due to these negative effects, many global food regulators monitor animal tissue, urine, or hair to identify the presence of steroidal hormones to help to enforce and regulate the use of these substances in food-producing animals.

Analysis of steroidal hormones in hair

Steroidal hormones are delivered to animals as synthetic esters (to improve the half-life of the compound in the animal and enhance its efficacy), and the routine testing of these synthetic steroidal esters is often a big challenge. The esters are rapidly hydrolyzed into substances that are endogenously present in urine, therefore scoping urine for the presence of these steroidal compounds is not always an effective approach to determine the illegal use of these residues. The team at RIKILT is addressing this by proposing an interesting alternative – to test for the intact steroidal esters in animal hair samples.

Not only is hair easy to collect and transport, but there is also a long traceability, and testing can be done long after slaughter. By analyzing for steroidal residues in hair, the steroids are directly detected, giving concrete evidence of the use of the steroids in the animal.

Unfortunately, hair can be a difficult matrix to test, with many matrix interferences that can cause challenges with the accurate analysis and quantitation of the residues of interest. Scientists at RIKILT are using their QTRAP® 5500 and QTRAP®

6500 systems with MRM³ workflows to overcome the matrix interferences produced by the hair to more accurately and selectively quantify low level steroidal esters in hair samples.

MRM vs. MRM³

MRM³ takes routine MRM detection to the next level. With MRM, the precursor ion is selected in Q1, fragmented in Q2, and the product ions are selected in Q3 and detected. In MRM³, rather than selecting the product ion in Q3, those product ions are accumulated in Q3 and fragmented further by collisional-induced dissociation. This results in fragmentation of the product ion, giving a third generation fragment that can be detected by the mass spec system. So, rather than having a unique precursor/product ion pair, you detect the precursor/product/sub-product ion triad, adding an additional dimension of selectivity that can improve signal-to-noise by reducing the impact of matrix interferences on the results. And, the MRM³ results can be used for quantitation, also resulting in improved accuracy and precision of the reported results.

Shown in Figure 1 is an example of the detection of testosterone acetate in hair using MRM (leftside) and MRM³ (rightside). It is clear that there is a huge matrix interference at the retention time of the compound of interest (5.23 minutes) in MRM mode, but that interference is significantly reduced when using MRM³ mode. This is just one way that RIKILT is using MRM³ on their QTRAP® systems to overcome matrix interferences and get better quantitative results for low level steroidal esters in hair samples.

Conclusions

Food testing scientists are always looking for new and creative approaches to accurately detect assorted residues that might make their way into our food supply. RIKILT is paving the way in food testing innovations, using QTRAP technology and advanced scan functions to improve selectivity for their residue analyses, reduce the interferences that occur from the matrix, and enable low level quantitation of banned and registered chemical substances used in food production.

Learn more about the unique work at RIKILT by viewing their webcast presentation, which also includes an overview of the QTRAP 6500 LC/MS/MS system to enable improved sensitivity and selectivity for residue testing in food and food-related matrices.

View the webcast

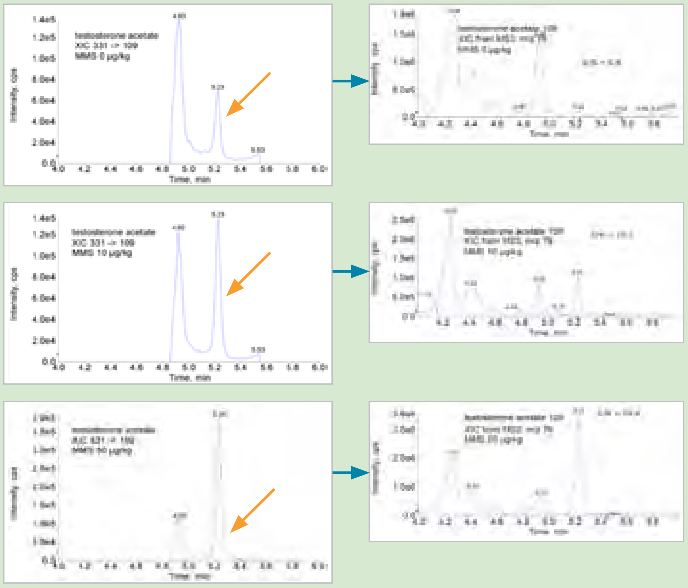
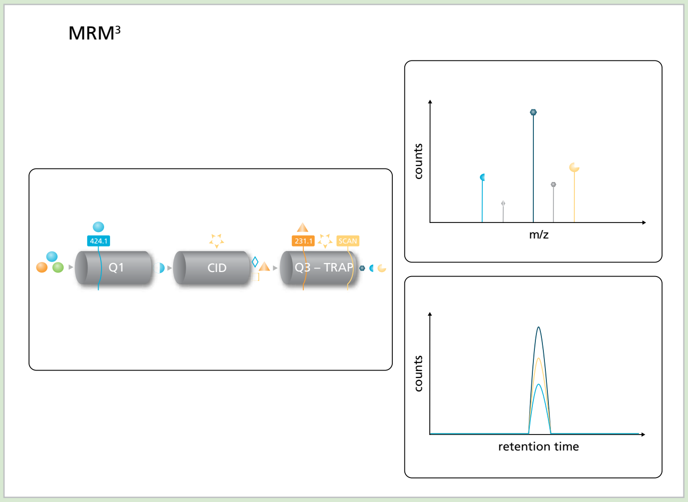


Figure 1: Results (courtesy of RIKILT) highlighting the extracted ion chromatogram (XIC) of testosterone acetate in hair samples using MRM (leftside) and MRM³ (rightside). A clear matrix interference peak is present in the MRM XIC at the retention time of testosterone acetate, an interference that is almost completely eliminated with the use of MRM³. This is just a single example of the benefits of MRM³ to overcome matrix interferences for better low level residue quantitation in challenging matrices.



Schematic of MRM³: In this approach, the precursor ion is selected in Q1 (424.1, blue circle), fragmented in Q2 (CID), and the product ion (231.1) is then dissociated again in the Q3 QTRAP. The fragments of the product ion are scanned and detected, giving more selectivity for the compound of interest to help to reduce the impact of matrix interferences for better low level residue quantitation.

Increasing sensitivity and linear dynamic range for drug quantitation

Using the AB SCIEX QTRAP® 6500 LC/MS/MS System

Charles River Laboratories continues to invest in the latest analytical technologies, enabling our scientists to provide clients with the most precise and accurate data possible, on time, every time. With the recent installation of three AB SCIEX QTRAP 6500 mass spectrometers in our Montreal bioanalytical laboratory, our scientists now have access to the highest level of LC/MS/MS performance for drug quantitation.

Specifications for the QTRAP 6500 promise up to 5 times greater sensitivity than its predecessor, the QTRAP 5500, and a 20-fold increase in detector dynamic range, the latter allowing drug quantitation greater than three orders of magnitude. Our scientists can leverage the sensitivity gains afforded by the QTRAP 6500 for achieving challenging detection limits when sample volume is limited, such as in dried blood spot and capillary microsampling studies, studies involving small rodents or neonatal patients, and matrices such as epithelial lining fluid (ELF), cerebrospinal fluid (CSF) and other tissues. Additional LC/MS/MS sensitivity is crucial when a drug exhibits poor bioavailability, demonstrates low mass spectral ionization efficiency and/or dissociation characteristics, and/or cannot be extracted with high recovery. With optimized scan speeds up to 20,000 Da/sec with polarity switching speeds of 20 msec, the QTRAP 6500 is perfectly suited for coupling with our ultra-high performance liquid chromatography (UHPLC) capabilities.

This article presents applications demonstrating the sensitivity and improved dynamic range of the QTRAP 6500, illustrating the versatility and robustness this platform affords the bioanalytical scientist.

Leveraging the sensitivity gains of the QTRAP 6500

Capillary microsampling

Capillary microsampling (CMS) is a technique for collecting small and exact volumes of biological matrices, most commonly blood, plasma or serum. In rodent toxicology studies, CMS offers the possibility for excluding satellite animals, thereby reducing and refining animal use, maximizing scientific value, increasing productivity and decreasing overall study cost. The low volume collected in CMS approaches makes repeated sampling feasible and, when coupled with the ability to harvest both plasma and serum from collected

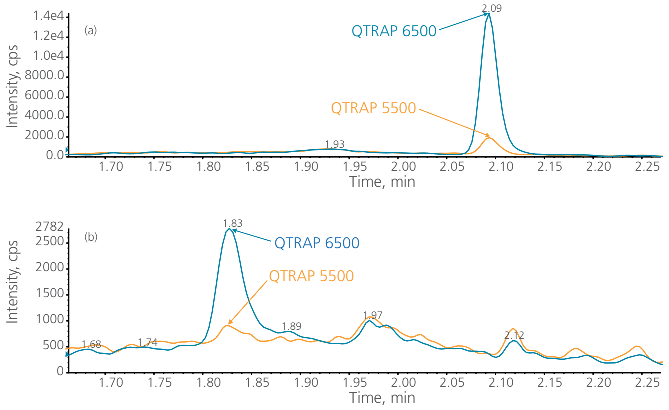


Figure 1: QTRAP 5500 versus 6500 LLOQ response comparison for the detection of (a) MDZ and (b) OH-MDZ. Rat whole blood was sampled in K_2EDTA hematocrit tubes (32 µl). Following centrifugation, hematocrit tubes were scored and plasma transferred to exact volume microcapillary tubes (8 µl) which were then sonicated in sodium bicarbonate (pH 9.7, 180 µl) containing the stable label internal standards [$^2H_3$]-MDZ and [$^2H_3$]-OH-MDZ. Buffered plasma displaced from the microcapillary tube was extracted by SLE with ~ 85% recovery using ethyl acetate. Extracts were chromatographed on a Waters Acquity HSS PFP column (2.1 x 100 mm; 1.8 µm) using a Shimadzu Nexera LC-30AD UHPLC system.

It's been almost ten months since the introduction of the AB SCIEX 6500 Series mass spectrometer which was unveiled in Vancouver, Canada during the 2012 ASMS conference.

In the months since, AB SCIEX has seen countless instances of the 6500 Series meeting and surpassing our customers' expectations for performance across sensitivity, selectivity and extended linear dynamic range.

The article below was written by Jeff Plomley, Principal Scientist at Charles River Laboratories in Montreal, Canada and serves as an unbiased scientific testament to the value delivered by the technological advances of the IonDrive™ technology on the 6500 Series.

Congratulations to Jeff Plomley and the bioanalytical team at Charles River Labs Montreal on their accomplishments.

Johnny Cardenas, Global Marketing Manager, Pharma/CRO

whole blood, PK profiles can be generated from three different matrices originating from a single donor. However, low sample volume dictates the development of highly sensitive methodologies, as illustrated in Figure 1, which compares the QTRAP 5500 and 6500 responses for an extracted LLOQ of 10 pg/ml (40 fg on-column) for midazolam (MDZ) and hydroxymidazolam (OH-MDZ) from 8 µl of plasma.

To access the full article, **click here**

Poor ionization efficiency, MRM selectivity and a 25 pg/ml LLOQ

In this example, our research group was faced with a novel compound lacking functional groups which could be directly ionized. Parent ions could only be generated indirectly via transient adduction with formate followed by in-source dissociation to furnish [M-H]-. Although a linear process as a function of concentration in the presence of formic acid containing mobile phase, ionization efficiency was poor in comparison to compounds containing basic or acidic functionalities. While analyte recovery from rat urine was quantitative, selectivity could only be obtained using the least sensitive MRM transition of two candidate dissociative channels whose response differed ten-fold. As shown in Figure 2, the QTRAP 5500 failed to meet precision acceptance criteria at the LLOQ (1.25 pg on-column), whereas the six-fold increase in signal-to-noise ratio (s/n) afforded by the QTRAP 6500 for the same extracted sample set readily met regulatory criteria.

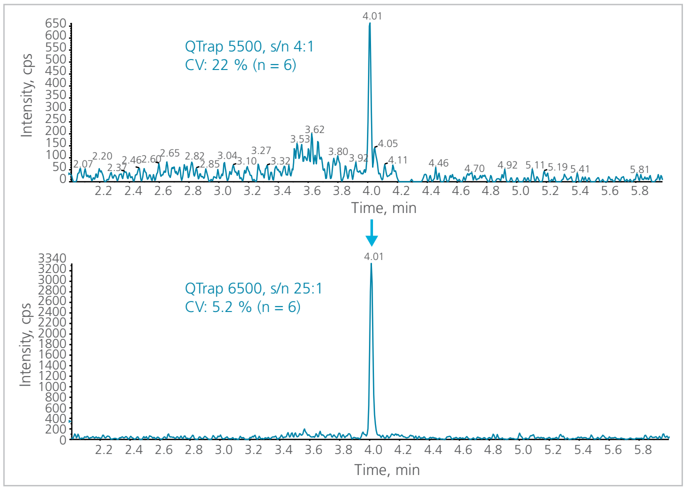


Figure 2: QTRAP 5500 and 6500 response comparison demonstrating improved LLOQ precision with increased response factor. Extracts were chromatographed on a Waters Acquity HSS C18 column (2.1 x 100 mm; 1.7µm) using a Shimadzu Nexera LC-30AD UHPLC system.

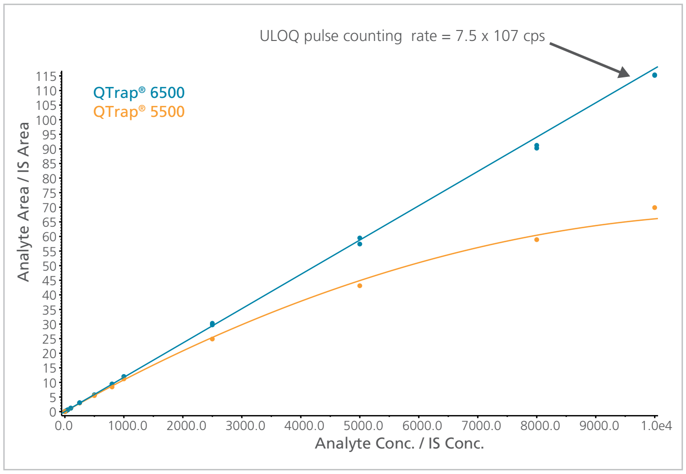


Figure 3: QTRAP 5500 vs. 6500 calibration curves for imatinib extracted from plasma spanning four orders of magnitude (1.00 – 10,000 ng/ml). LLOQ responses were equalized between platforms by varying the injection volume. Deviation from linearity was noted for the QTRAP 5500 at concentrations > 1,000 ng/ml with detector saturation preventing accurate back-calculation of the ULOQ and high QC samples (8,000 ng/ml). In contrast, a linear regression weighted 1/x2 could be fitted to the QTRAP 6500 response, furnishing a correlation co-efficient of 0.9996 with precision and accuracy data meeting all acceptance criteria (Table 1 and 2).

Theoretical Concentration (ng/ml)	Calculated Concentration (ng/ml)	Accuracy (%)
1.00	1.03	103
2.50	2.47	98.7
5.00	5.00	100
10.0	9.47	94.7
25.0	25.0	100
50.0	53.3	106
100	102	102
250	260	104
500	491	98.3
1000	1020	102
2500	2570	103
5000	5060	101
8000	7680	96.0
10000	9810	98.1

Table 1: Back-calculated calibrant accuracy for the extraction of imatinib from plasma

Theoretical Concentration (ng/ml)	Mean Calculated Concentration (ng/ml)	Mean Accuracy (%)	CV (%)
1.00	0.99	99.8	6.0
2.50	2.50	100	4.8
100	98.5	98.5	2.5
8000	7561	94.5	1.6

Table 2: QC precision and accuracy from six different control donors at four concentration levels extracted from plasma

Extending linear dynamic range

In a preclinical investigation involving tolerability and toxicity of new formulations of imatinib, it was necessary to develop an LC/MS/MS plasma method capable of supporting a concentration range spanning four orders of magnitude (1.0 - 10,000 ng/ml). Previously, such a dynamic range would challenge the saturation limit of the pulse counting detector in the QTRAP® 5500, resulting in problematic curve fitting. Therefore, ideal concentration ranges were often truncated to practical limitations, with repeats a necessity for samples whose response exceed the upper limit of quantitation (ULOQ). However, with the introduction of the Ion Drive™ Detector in the QTRAP 6500, ultra-fast pulse counting (10^8 cps) with a higher saturation point is feasible, without loss of low-end sensitivity. As outlined in Figure 3, the QTRAP 6500 demonstrated a linear response for imatinib extracted over four orders of magnitude, without saturation at the ULOQ, thus allowing a complete PK profile without the need for sample repeats (Figure 4). Had the assay been limited to three quantifiable orders of magnitude, ~ 50% of the toxicology samples collected over the course of the study would have required costly repeat extraction and analysis due to response > ULOQ.

The carryover challenge

With continuing increases in mass spectrometric sensitivity and the ability to quantitate over larger concentration ranges, autosampler carryover becomes a formidable challenge for compounds exhibiting adsorption to materials used in the injection flow path (e.g., needle, needle seal, rotor seal, etc). Carryover can impact the precision and accuracy of an analytical batch (particularly low concentration samples), can lead to inaccurate sample data and can cause incurred sample reanalysis failure. In regulated bioanalysis, carryover should be ≤ 20% of the LLOQ, and is determined by the drug response in a blank sample following a ULOQ injection. Recognizing the importance of eliminating carryover, Charles River Laboratories Montreal has paired the top-performing Shimadzu Sil-30AC autosampler with our Shimadzu Nexera LC-30AD UHPLC chromatography pumps. Equipped with Pt-coated needle and peristaltic pump, external, internal and injection port rinses can be configured using a combination of solvents, all controlled by Analyst® v1.6 Software (AB SCIEX) for GLP compliance.

The advanced rinsing capabilities of the Shimadzu Sil-30AC autosampler were recently exemplified in troubleshooting an assay transferred from another laboratory for the determination of methotrexate in plasma (10 µM ULOQ). The provided method required the injection of reagent blank between samples in order to mitigate autosampler carryover. As a first approach to reducing carryover, the extract dilution factor and injection volume were carefully titrated to provide a robust LLOQ response while minimizing the injected oncolumn amount of methotrexate. This resulted in 35% carryover when using an external needle rinse only, a modality reflective of the previous generation of Shimadzu autosampler (Figure 5). However, all carryover could be eliminated when performing a combination of internal needle rinses with three different solutions, an active external needle rinse (wash solution is replaced while the needle remains immersed in the rinse port and an injection port rinse Figure 6).

Conclusion

With the sensitivity gains and expanded dynamic range of the AB SCIEX QTRAP 6500 LC/MS/MS System as a key component in our bioanalytical toolkit, Charles River Laboratories is able to support our customers' most challenging bioanalytical projects, now and into the future.

Article prepared by Jeff Plomley and Mohamed Makhloufi. For further information on this topic, please do not hesitate to contact Jeffry Plomley at **jeffry.plomley@crl.com**

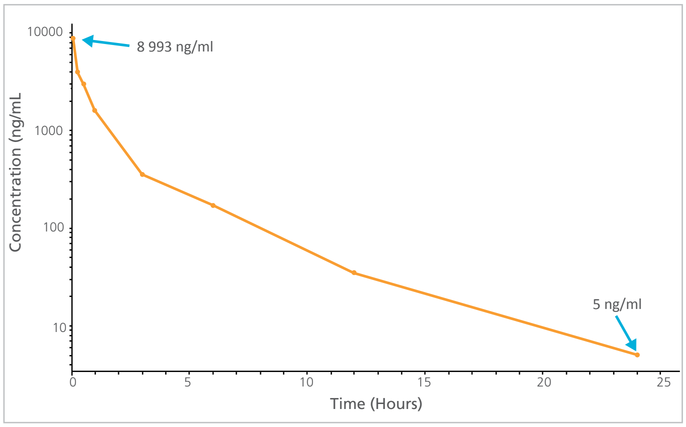


Figure 4: Pharmacokinetic profile derived from plasma for imatinib infused over a 15-minute period, illustrating the benefit of four quantifiable orders of magnitude.

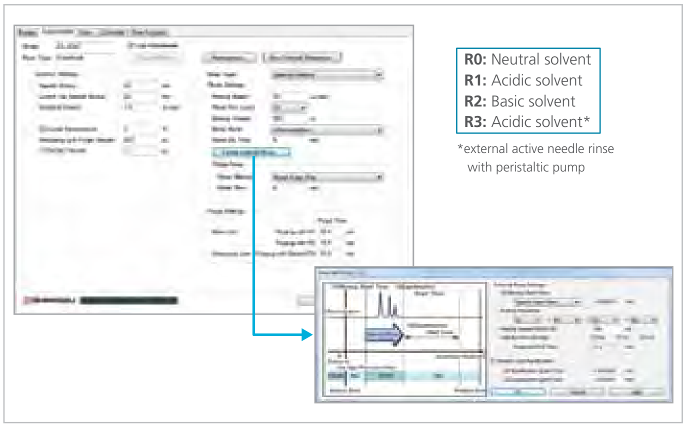


Figure 6: Shimadzu Sil-30AC autosampler rinse sequence controlled by Analyst® Software v1.6.

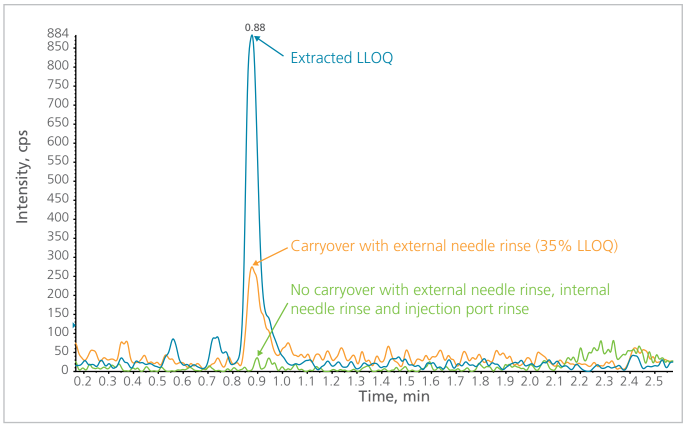


Figure 5: Carryover profile for methotrexate as a function of rinsing procedure using a Shimadzu Sil-30AC autosampler. Rinse solutions included acetonitrile/water, methanol/water/acetone/formic acid and methanol/dimethyl sulfoxide/formic acid.

University of Cambridge scientists are going to Xtremes on Mt. Everest

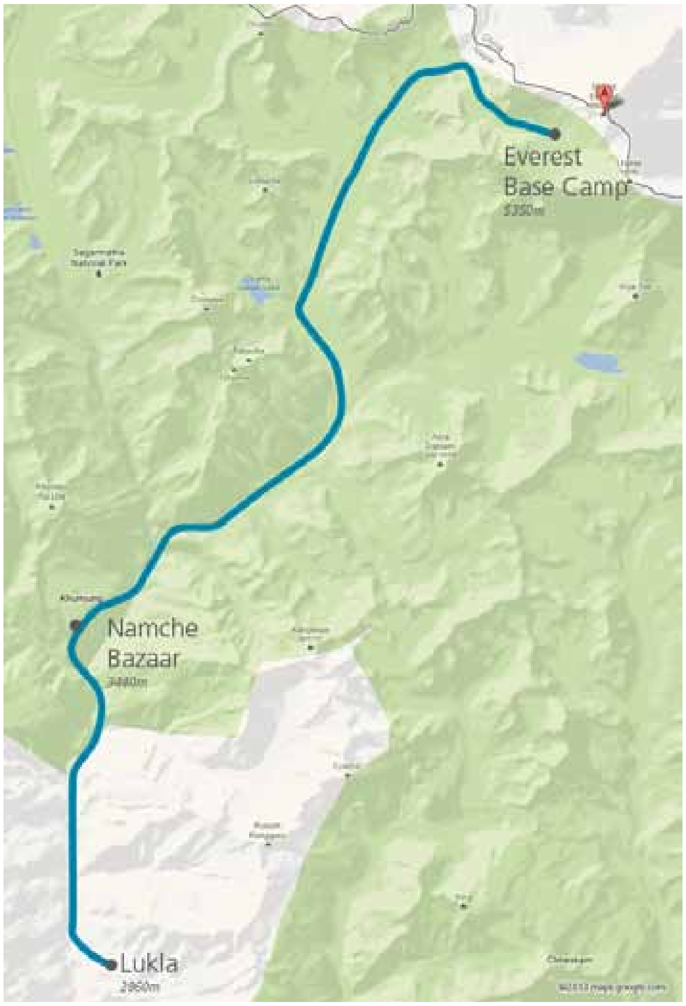
University of Cambridge (UK) scientists Drs. Julian Griffin and Andrew Murray, as well as Ph.D. student Tom Ashmore recently embarked on the Xtreme Everest 2 expedition; where researchers will use themselves as guinea pigs for medical science at the Mt. Everest Base Camp.

The expedition aims to understand how the human body is affected by low oxygen – called hypoxia – to help to find new treatments for intensive care patients. Xtreme Everest is a not for profit organisation, led by doctors and scientists from UCL, University of Southampton, U.K. and Duke University in the United States.

“This is going to be a unique opportunity both scientifically and personally to study human physiology in these extreme conditions,” said Dr Julian Griffin, Group Leader of Lipid Profiling and Signalling at MRC Human Nutrition Research and the University Of Cambridge Department of Biochemistry before the team departed from London.

“While the concentration of oxygen at Everest Base Camp drops to half its normal level at sea level, we know the body adapts very well to this. If we could understand better how this is done we could have new targets and therapies for a variety of major human diseases.”

Dr. Griffin and his colleagues, as well as more than 130 scientists, nurses, and medical doctors from around the UK, travelled to Kathmandu on March 16, then to Everest Base Camp at 5,350m above sea level to monitor how a group of healthy volunteers naturally adapt to low oxygen concentrations.



Upon his return, Dr Griffin will also be studying samples taken during the expedition to better understand how the body metabolizes lipids under hypoxic conditions. Blood samples and muscle tissue biopsies will be used in a number of metabolomics and lipidomics experiments designed to help the researchers to better understand how protein function and, more specifically, branch chain amino acid metabolism are impacted by low oxygen concentrations. This work is scheduled to be conducted on AB SCIEX LC/MS/MS systems.

During Xtreme Everest many individuals will enter negative energy balance and start to use their body's fat reserves – the opposite of what happens in obese people. "We are particularly interested in how the body switches between food reserves at low oxygen levels, as this is important for people in intensive care and other situations where oxygen delivery may be impaired," said Dr Griffin.

To learn more about the Xtreme Everest 2 Expedition, follow the team's posts on the AB SCIEX Facebook page: **www.facebook.com/absciex**

For more information about AB SCIEX solutions for metabolomics and lipidomics, visit our web site: **www.absciex.com**

Ultra-fast amino acid analysis–maximizing speed, sample throughput and cost effectiveness of established assays

Microflow chromatography with column diameters ≤1mm is an exciting approach for sensitive, high-throughput LC/MS/MS within the clinical research arena. High-throughput workflows, such as the analysis of amino acids from physiological fluids, require fast, high performance separations, as well as low carryover and fast injection-to-injection cycle times. Such performance is particularly critical within the field of amino acid analysis, due to the large number of structurally similar, isobaric compounds present which can demand extended chromatography in order to achieve the required separation for quantitation.

The Eksigent ekspert™ microLC 200 System is a dedicated microflow UHPLC system that has been designed with these considerations in mind, for optimal performance in the microflow regime, including a new autosampler injection system with modifications for very small volume sample handling with minimal sample waste as well as very low carryover.

Robust and simple sample preparation using aTRAQ™ technology

Sample preparation for amino acid analysis using the ekspert microLC 200 system is identical to the approach used for the application running with conventional chromatography. No modifications or additional steps are needed.

Current dedicated amino acid analyzer systems have long run times, are prone to interferences from buffers, matrices, and other co-eluting amino acids, and can be difficult to maintain. An LC/MS/MS method for the analysis of amino acids in physiological fluids using amine-reactive isotope-coded tags (SCIEX aTRAQ reagents) has been established. Because only compounds with the same mass require chromatographic separation, the analysis time can be significantly reduced versus traditional methods. The combination of LC/MS/MS with aTRAQ reagents also provides better sensitivity (therefore lower detection limits), a wider dynamic range, and the ability to use labeled internal standards for more accurate and robust quantitation.

Separation of the isobaric amino acids is critical and one of the reasons that a long conventional column is used in the traditional aTRAQ kit. In developing the microflow LC application care was taken to ensure the separation was maintained for these compounds.

Conclusions

The use of the reduced diameter columns allows significantly reduced flow rates and injection volumes, reducing sample consumption, instrument downtime and cost of ownership. The Eksigent ekspert microLC 200 shows potential for the analysis of amino acids in physiological fluids using the aTRAQ kits. The improvements to the method given by the use of microflow LC allow the quantitation of 45 amino acids in samples within 8 minutes, including column re-equilibration time, compared to 18 minutes using conventional HPLC. Microflow LC allows dramatic savings in solvent consumption and hence cost of ownership. Sample preparation remains unchanged and assay performance, in terms of sensitivity based on injection volume comparisons, is greatly improved.

To access the full technical note, **click here**

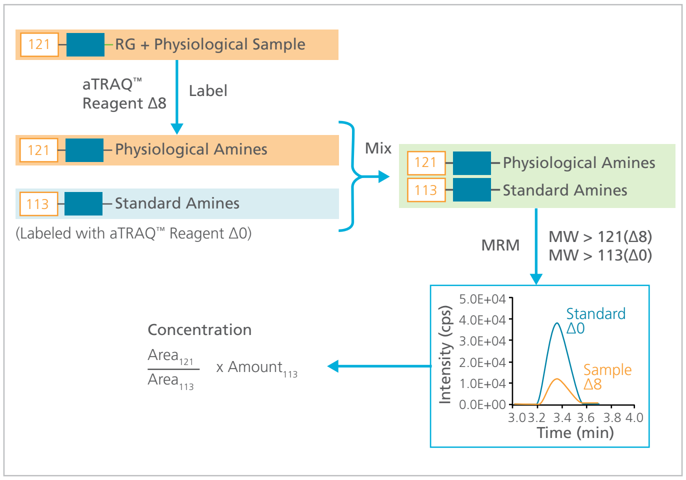


Figure 1: Illustration of SCIEX aTRAQ™ reagent labeling. The sample is labeled with the Δ8 reagent and the internal standard (IS) is pre- labeled with the Δ0 reagent. The sample and IS are mixed and injected for LC/MS/MS analysis. Quantification of the individual amino acids is calculated using a ratio of the sample to internal standard peak areas.

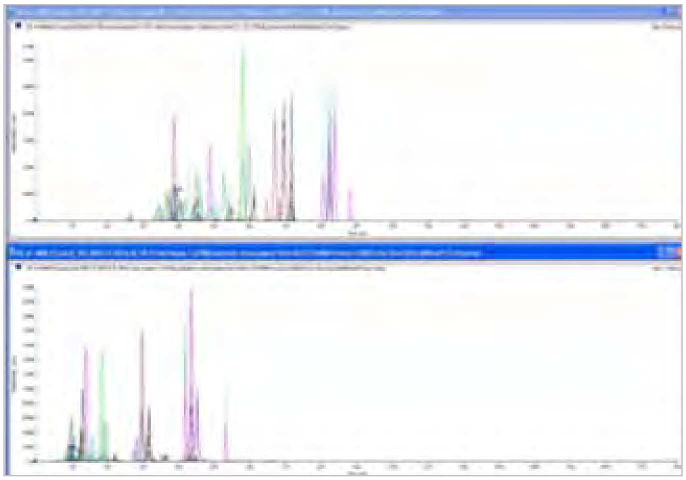


Figure 2: Comparison of conventional (top) and microflow LC (bottom) for aTRAQ™ analysis. Both chromatograms are scaled to the runtime of conventional LC (18 min)

The new MasterView™ Software

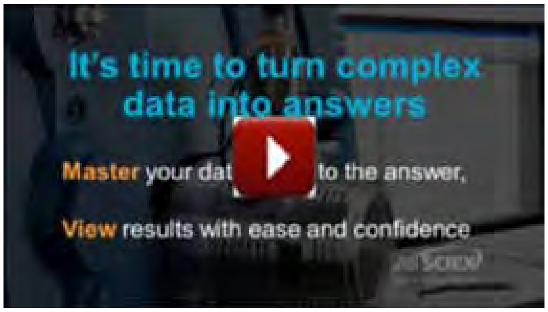
Master your data to get to the answer, view results with ease and confidence.

Mass spectrometers, including high resolution accurate mass instruments, produce a significant amount of complicated data. And, turning those data into results and decisions is a big challenge.

The new MasterView™ Software can help, simplifying compound identification, quantitation, and data review from complex LC/MS/MS data files, so you can quickly answer the question – "What is in my sample?"

What makes MasterView Software unique?

- Targeted list analysis with less than five clicks to results
  Watch the video to see how!



- User friendly interface with colored symbols for more efficient data review, accessible to users of all levels of expertise – reduced training needed
  Watch the video to learn more!

- Sample-control comparison to remove irrelevant, endogenous peaks or for quick comparison to a control standard
  Watch the video to learn more!

- Reduced risks of false positive results and more confident peak identification with MS/MS and library databases (Figure 2)
- Get answers faster with automatic formula finder and library searching
- Comprehensive structural elucidation tools with quick link to ChemSpider (Figure 3)
- Transition into MultiQuant™ Software for comprehensive quantitation in just a few clicks
- Retain data, not samples, for future sample interrogation and reduced retesting

Feel good about your results – Proof in the MasterView™ Software / TripleTOF® workflows

The AB SCIEX demo lab in Shanghai received 2 proficiency test samples and were tasked with identifying what pesticide compounds were present in those samples (Figure 4). Utilizing TripleTOF non-targeted data acquisition with targeted data processing using MasterView Software, they were able to correctly identify 43 of 44 pesticides across the 2 food samples. Their results indicated only one false negative result for Ethoxyquin, which was attributed to the low QuEChERS recovery for that compound, and zero false positive results.

This study indicated that that with TripleTOF data acquisition and MasterView data processing, routine testing labs can feel good about knowing that they will be able to find compounds or contaminants of concern in their samples, and the smart data processing software can enable them to get to those answers fast and efficiently.

Learn more about MasterView Software at **www.absciex.com/MasterView**.

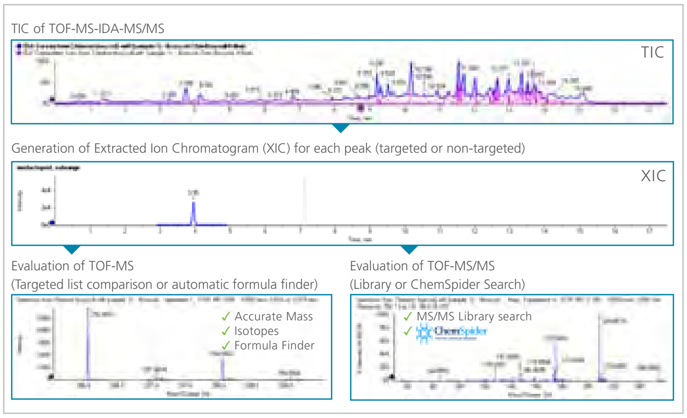


Figure 1: Leverage the hardware advantage of TripleTOF® technology for quality data and combine with MasterView™ Software for effective data processing of all targeted and non-targeted peaks in your unknown samples.

Figure 2: The ability to acquire quality accurate mass MS and MS/MS information on all peaks detected in your unknown samples is fundamental to ensuring proper compound identification. In this case, a false positive result for Prometon was avoided by using MS/MS identification.

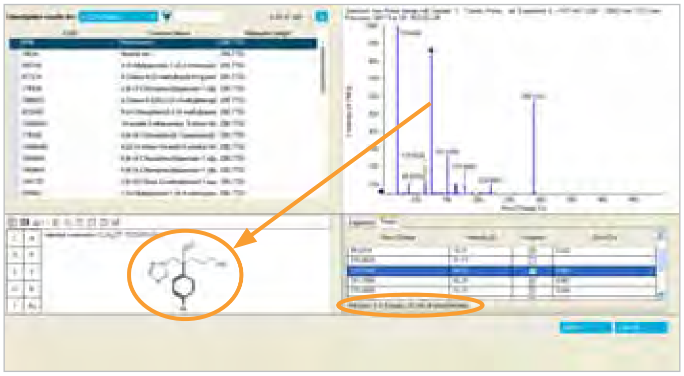


Figure 3: With the ChemSpider search and automatic MS/MS interpretation, ChemSpider hits are automatically compared with acquired MS/MS spectra for more confident identification of unknown peaks.

Figure 4: The results for the leek proficiency testing sample showed the correct identification of 22 out of 22 compounds present in the leek sample, with no false positive or false negative findings, results that give us confidence that these workflows can correctly answer the question, “What is in my sample?”

The youngest AB SCIEX user

Lessons in food safety from an astounding 14 year old scientist

Liesl Krone has proven that food safety is a topic of interest to consumers of all ages. Liesl, an 8th grader at Acton Middle School in Granbury, Texas, US, entered her research project titled "What Pesticides Have You Eaten Today?" in the 62nd Fort Worth Regional Science Fair earlier this spring, placing second and advancing to the Exxon Mobile Texas Science and Engineering Fair that was held in San Antonio, Texas, in March.

Liesl became interested in food safety after hearing discussions of food quality in the news. She loves fruit and was curious if she was eating any of the contaminants that were mentioned in the news reports. Like any curious young scientist, she decided to evaluate her favorite fruits, test them for pesticides, and investigate if washing or peeling the fruits are effective in removing pesticides.

Liesl's study presented some very interesting findings, including the detection of a pesticide on grapes that is not allowed for use in the US (the grapes were interestingly noted as a product of Peru), and that washing grapes with water did not reduce their pesticide content. Liesl also learned that in the case of oranges and bananas, the majority of the pesticides are found in the peels (although she did detect some pesticides in the pulp as well), so consuming contaminants can easily be avoided for fruits that must be peeled. Her insightful conclusion: "It is still better to eat fruit than junk food but maybe we need to eat fewer grapes!"

Liesl's project was supported through instrument time at Analytical Food Labs in Grand Prairie, Texas, QuEChERS sample preparation chemistry products provided by Restek, and scientific input from Andre Schreiber at AB SCIEX.

Figure 1: fruit separated into individual containers

Figure 2: homogenizing fruit with blender

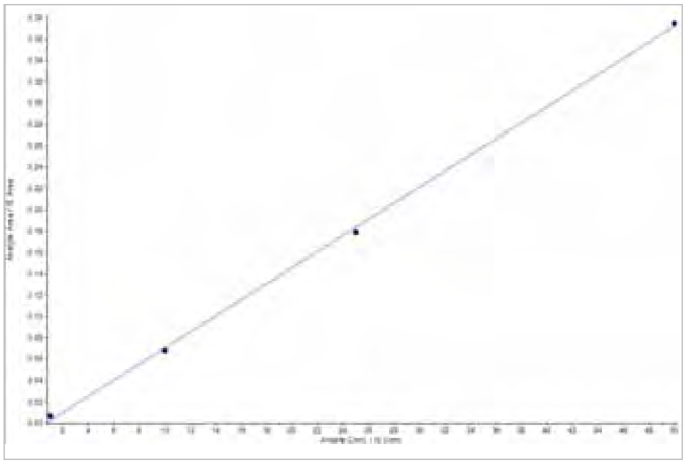
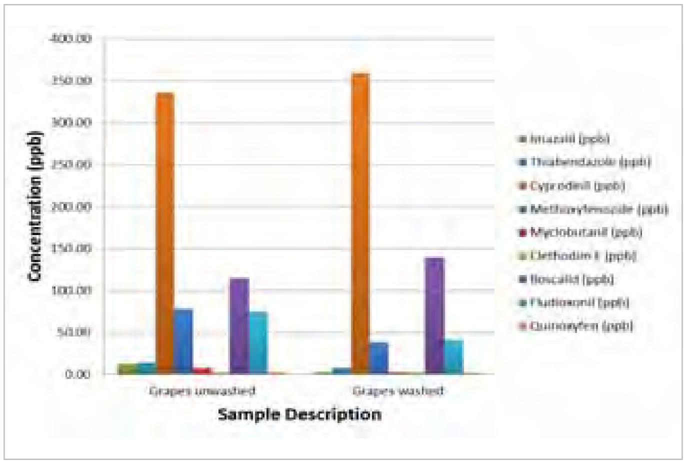


Figure 5: Representative Calibration Curve



Graph 1: Grape Results

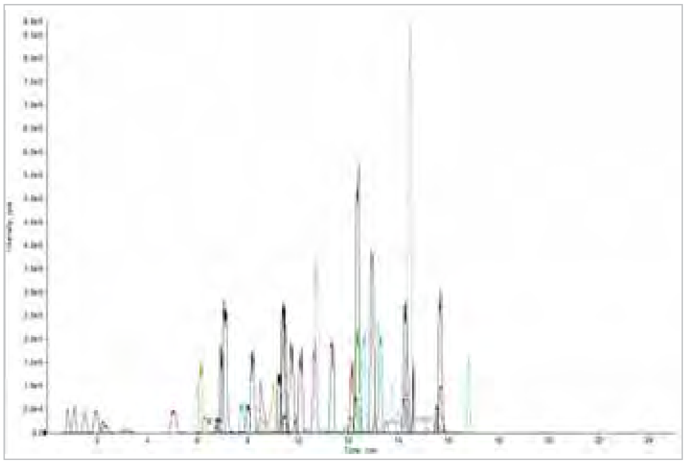
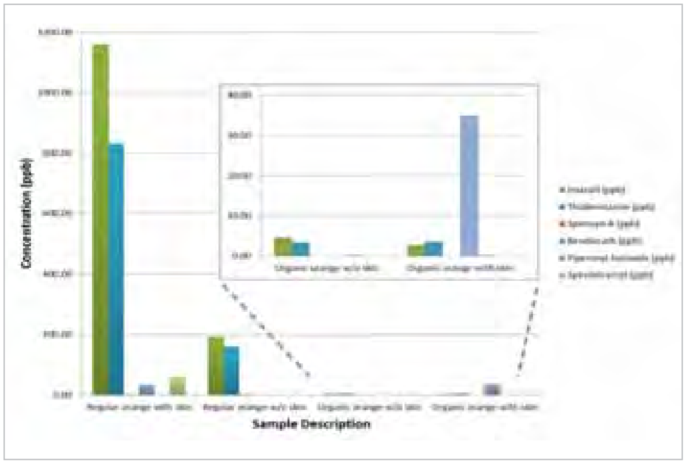
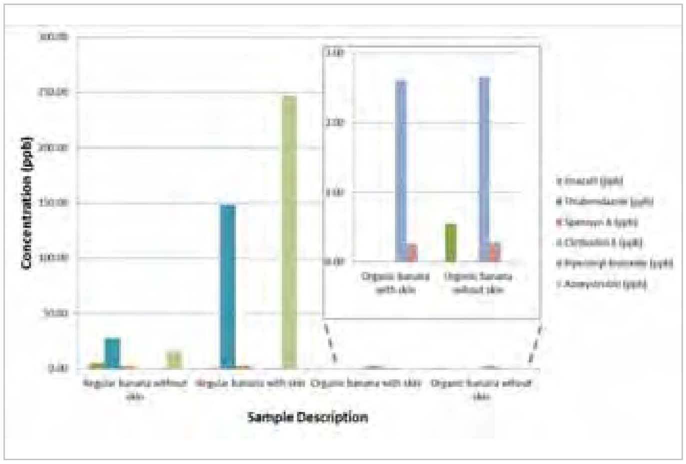


Figure 6: Mass Spectrum Example



Graph 2: Orange Results



Graph 3: Banana Results

At the state science fair, she was nominated to compete in the Broadcom Masters Competition, a national science competition for 6th through 8th grade students, and she was encouraged to do a continuation project for the competition next year. We are excited (and nervous!) to see what new findings Liesl may uncover in our food in her next project!

In July, Liesl presented her work at the 50th annual Florida Pesticide Residue Workshop / North American Chemical Residue Workshop (NACRW) in St. Pete Beach, Florida. At the meeting, she was able to present her studies to Steve Lehotay of the USDA and Michelangelo Anastassiades of CVUA Stuttgart, who were the originators of the QuEChERS sample preparation approach that Liesl used in her experiments. Liesl made a great impression on the scientists at NACRW, and she earned the best-liked poster award voted on by the conference attendees.

Congrats to Liesl on a great project and for going on record as the youngest AB SCIEX user!

Mass spectrometer gives one more reason for taking shower before swimming!

Insights to findings of hazardous disinfection by-products in swimming pools

Researchers: *Wei Wang, Yichao Qian, Jessica M. Boyd, Minghuo Wu, Steve E. Hrudey, Haiying Du, Jinhua Li, Birget Moe, Claire F. McGuigan, Shengwen Shen, and Xing-Fang Li*
University of Alberta (Edmonton, Alberta, Canada) & Jilin University (Changchun, Jilin Province, China)

Researchers at the University of Alberta recently published some insightful findings of what is lingering in the waters of one of summer's favorite pastimes. Wei Wang, and her teammates in the Division of Analytical and Environmental Toxicology in Edmonton, Alberta, Canada embarked on a study using the AB SCIEX API 5000™ LC/MS/MS System to quantify halobenzoquinones (HBQs), a class of disinfection by-products (DBPs) of health relevance, in swimming pool waters.

Halobenzoquinones and their significance

It is essential for drinking water to undergo some disinfection process to inactivate pathogenic microbes and prevent water-borne diseases. Common disinfection processes include chlorination, chloramination, ozonation, and UV. While these processes function effectively to remove microbial risks, these disinfecting agents can also react with the natural organic matter that might be present in the water, resulting in the creation of disinfection by-products (DBPs).

There are numerous types of DBPs that can be formed, depending on the composition of the organic matter in the water and the treatment process used, and significant scientific research has been focused on identifying and understanding DBPs that are toxicologically significant. Halobenzoquinones (HBQs) are one of five DBP classes that have been predicted to have toxicological importance. In a recent study published by Du, Li, and co-workers, the cytotoxicity of 4 HBQ compounds was evaluated (1). The research team identified that HBQs are, in fact, cytotoxic to T24 bladder cancer cells, with cytotoxicity impacted by oxidative stress. The comprehensive study produced significant results for identifying the toxicological significance of HBQ compounds as well as beginning to formulate the cellular mechanism of that toxicity.

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Swimming pool waters and HBQs – the connection

The formation of assorted disinfection by-products in swimming pools is a complex chemical process. Swimming pool waters usually contain significantly more organic matter than regular tap water (consider cosmetics, lotions, and sunscreens), and may also contain other contaminants (urine or sweat), among a variety of other residues. Swimming pools very commonly utilize chlorine as the primary means of disinfection, and the chlorine dose applied in swimming pools is usually higher than the residual chlorine in regular tap water to control waterborne disease. UV irradiation may also be used as secondary disinfection treatment. So, the variety of DBPs that could be present in swimming pool waters can be vast.

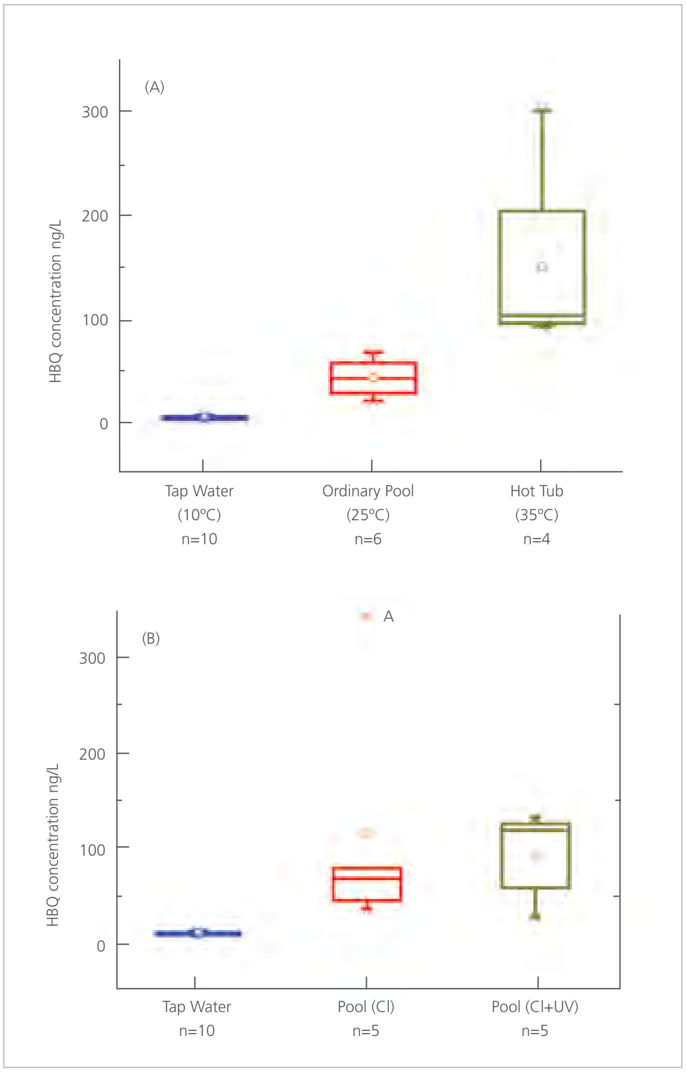

Figure 1: Study results showing the total HBQ concentration (in ng/L) for tap water compared to two different pool water temperatures (A) or two different pool water disinfection procedures (B). Results show an relevant increase in HBQ formation as temperature and disinfection processes are increased.

Study overview

Wang and co-workers sought to identify and quantify assorted HBQ compounds in swimming pool waters, with a goal to better understand how those compounds are formed as well as the potential human health risks associated with exposure to these HBQ compounds (2).

The team utilized solid-phase extraction (SPE) to extract HBQ residues from swimming pool water samples, with additional washes to remove excess salinity or other matrix residues. Samples were analyzed using LC/MS/MS, with LC separation performed using a Phenomenex Luna C18(2) column and MS analysis using an API 5000™ triple quadrupole system using negative electrospray ionization (ESI) mode and with multiple reaction monitoring (MRM).

Several sample types were evaluated in the study, including

- Swimming pool water treated with chlorine or chlorine + UV irradiation
- Diluted urine samples (to identify HBQ compounds that might form directly from urine)
- Water containing personal care products (to identify HBQ compounds that might form directly from assorted lotions and sunscreens)
- Tap water. Eight HBQ compounds were evaluated in the study

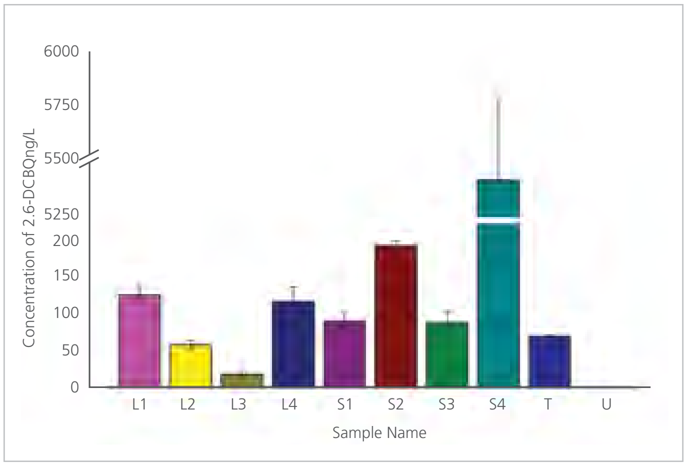

Figure 2: Study results showing the concentration of one HBQ compound (2,6-DCBQ) in a variety of swimming pool samples laced with lotions (samples L1, L2, L3, L4), fortified with sunscreens (samples S1, S2, S3, S3), or spiked with urine (sample U), and compared to the levels present in input tap water (sample T). Results indicate that 2,6-DCBQ formation increases due to the presence of lotions and sunscreens, although levels vary depending on the formulation of the personal care product.

Results and findings

Of all HBQ compounds studied, 2,6-DCBQ (2,6-dichloro-1,4-benzoquinone) had the most elevated concentration in pool waters relative to tap water (increased by a factor ranging from 5 to 100-fold), and 3 other HBQ compounds were detected in the pool waters but not detected in the tap water. The team then investigated what factors led to the increase of these HBQ compounds in the pool waters, looking into effects of water temperature and chlorine dose. The results showed that higher water temperatures and increased chlorine content both contributed to an increase in the presence of HBQ residues, particularly 2,6-DCBQ.

The team followed up with an investigation of what caused the increased concentration of HBQs in the swimming pool waters, evaluating whether the formation occurred from biological fluids (i.e. urine) or from personal care products (i.e. sunscreens and lotions). The results showed that lotions and sunscreens introduced by swimmers could increase the concentration of HBQs in the swimming pool waters, but it is dependent on the type of lotion or sunscreen present (most likely due to differences in formulations and active ingredients in different products). Urine did not seem to serve as a precursor to the formation of HBQs.

Conclusions

This study using the API 5000™ triple quadrupole system to identify and quantify HBQ compounds in swimming pool waters was very insightful in showing that multiple HBQ residues of toxicological significance do form in swimming pools as a result of the reaction between the disinfection agents used in the pool and the organic residues introduced by swimmers. What is the relevance of all of this? Epidemiological studies have indicated a potential association of consumption of chlorinated water with an increased risk of bladder cancer. The results showing the cytotoxicity induced by HBQs at micromolar concentrations to T24 bladder cancer cells could potentially be the first steps in understanding the toxicity of these compounds. While cytotoxicity testing is done at micromolar concentrations, HBQs in treated water are at ng/L levels. The effects of long term exposure to the trace HBQs are unclear. Therefore, additional research is certainly warranted. However, one thing is clear – enjoy your time at the pool this summer, but you must take a shower before jumping into the pools – the mass spectrometer can tell! Keep the swimming pool clean for your health!

Read more about this incredible work

1. Cytotoxicity and Oxidative Damage Induced by Halobenzoquinones to T24 Bladder Cancer Cells
   Environ Sci Technol. 2013, 47 (6), 2823-30.
   Haiying Du,†‡ Jinhua Li,† Birget Moe,† Claire F. McGuigan,† Shengwen Shen,† and Xing-Fang Li*†
   †Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, 10-102 Clinical Sciences Building, Edmonton, Alberta, Canada
   ‡Department of Toxicology, School of Public Health, Jilin University, Changchun, Jilin Province, China
2. Halobenzoquinones in Swimming Pool Waters and Their Formation from Personal Care Products
   Environ. Sci. Technol., 2013, 47 (7), 3275–3282.
   Wei Wang, Yichao Qian, Jessica M. Boyd, Minghuo Wu, Steve E. Hrudey, and Xing-Fang Li
   Division of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

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