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Novel Methods for the Analysis of Chemical Residues in Food

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INTRODUCTION

The North American Chemical Residue Workshop (NACRW) continues to be an important venue for scientists to collaborate about new tools and technologies for analyzing pesticide and chemical residue levels in food and agricultural samples. For the third consecutive year, the NACRW's organizers have collaborated with the *LCGC* editorial team to share with our readers some highlights from the 2018 presentations.

First, Anton Kaufmann of the official food control authority of the Kanton of Zurich in Switzerland spoke with *LCGC* about the use of high-resolution mass spectrometry (HRMS) in routine analyses and his newly developed methods for the determination of biogenic amines and nitrofurans using liquid chromatography (LC)–HRMS.

Brian Eitzer of the Connecticut Agricultural Experiment Station also sat down with *LCGC* ahead of the NACRW meeting to describe his work analyzing levels of neonicotinoid insecticides in pollen and nectar in an effort to asses whether they pose a risk to honey bees and other pollinators.

Last, Pearse McCarron from the National Research Council of Canada discusses the complexities involved in algal toxins analysis, and how his team is using LC–HRMS methods for such studies.

As the field of pesticide and chemical residue analysis in food continues to evolve, it is clear that analytical scientists are up to the challenge of updating, fine-tuning, and developing new methods and techniques in tandem with such growth.

Using High-Resolution Mass Spectrometry for Routine Food Analysis

Interview with Anton Kaufmann

In the past, most routine food analysis, such as for pesticide and veterinary drug residues, has been done using liquid or gas chromatography with triple-quadrupole mass spectrometry, with the use of high-resolution mass spectrometry (HRMS) being reserved for investigative work and new research. Recently, however, more food laboratories have been considering the advantages of using HRMS for routine analyses. Anton Kaufmann, of the official food control authority of the Kanton of Zurich in Switzerland, leads a group that focuses on veterinary drug residue analysis, in a laboratory that is transtioning to using exclusively high-resolution instruments. He recently spoke to us about his thinking on the topic and about some of the methods he has developed.

You developed an easy and fast method for the determination of biogenic amines in fish using LC combined with high-resolution mass spectrometry (HRMS) (1).

Why is it important to measure biogenic amines in food?

Many consumers show increased sensitivity to the presence of biogenic amines (histamines) in food. This reaction may only be manifested by a general feeling of discomfort, but for particularly sensitive people, it can even lead to hospital admission. Therefore, our laboratory occasionally receives remaining food samples which have been consumed by admitted patients, to aid the diagnosis and therapeutic process.

Why was a new method needed?

Most often, only a single sample has to be analyzed and it must be prioritized. Such urgent samples interrupt other planned analyses and even analysis series (sequences) that we are in the middle of running. So, we were interested in having a very simple method (no derivatization required) that can be run on our old and therefore less busy single-stage orbital trap instrument.

Your approach uses an ion-pairing agent. Why did you take this approach? How did you avoid some of the problems that are sometimes encountered when using ion-pairing agents?

I am aware that ion pairing has a questionable reputation. Yet, I think that the problems regarding the prolonged rinsing time of the stationary phase are only relevant for long alkali chain ion pair agents and methods that use quaternary amines. As mentioned previously, we may only analyze a single sample (including a standard as well) and then rinse the system to have it ready for other analytical methods. So if ion-pairing agents were as bad as their reputation is, we would not squeeze the biogenic amine method between two series of analyses based on non-ion pairing chromatographic separations.

What results were you able to achieve with this method?

Often, many samples are negative. Yet there are a few samples that show really high levels of histamine, at concentrations that are indeed capable of explaining the symptoms experienced by the patient. For us, however, the most important thing is the fact that we can produce reliable results within a significantly shorter period of time. The method shows a higher selectivity and therefore a higher sensitivity than the previous LC–UV and fluorescence–based method. But even more attractive is the virtual absence of sample preparation. The high sensitivity of the instrument permits the injection of highly diluted samples. This not only prolongs column lifetime and stabilizes retention time, but it also leads to negligible related signal suppression effects related to the electrospray interface.

"In the absence of a positive histamine finding, HRMS permits you to look at other compounds as well."

This method uses HRMS. For routine analysis, particularly for quantitative work, it is more common to use triple-quadrupole instruments. Why do you recommend HRMS for this analysis? Do you think many labs performing this type of analysis will have access to HRMS instruments?

Well, this analysis certainly could also be done by tandem quadrupole mass spectrometry. Yet, in the absence of a positive histamine finding, HRMS permits you to look at other compounds as well. Food legislation currently only limits the presence of histamine. Yet, it is known that other biogenic amines can produce similar symptoms. So, in the case of a negative histamine finding, by using HRMS we have the chance to look for other biogenic amines or even other compounds. Most of our samples are fish (tuna). Hence, we can compare the investigated tuna sample against precisely measured tuna samples

and find significant deviations between the HRMS traces.

We are aware than most labs do not have such instruments available. Yet in our case, the oldest available HRMS instrument has been used for this kind of work. HRMS is becoming increasingly accepted in food safety labs. Such instruments are initially used for high-end applications. Yet, such instruments will certainly age and finally be replaced by more advanced instruments. I see a huge potential for using such "old" instruments for more basic applications, such as the analysis of biogenic amines, dyes, conserving agents, and so on.

You developed a method for the determination of nitrofuran and chloramphenicol residues using UHPLC coupled to HRMS. Your sample preparation approach for this method involves derivatization followed by liquid–liquid extraction and reversed-phase–solid-phase extraction. Why did you choose this approach?

It is basically the need to obtain sufficient sensitivity. Sufficient sensitivity is only obtainable if we have sufficient selectivity. This can be partially achieved by a proper clean-up, but also by selective (Q-HRMS) detection. The fact that the method still requires a derivatization step is perhaps the ugly part of the method. Yet, the small and polar analytes cannot be sufficiently separated and detected in their underivatized form. On the other hand, the method includes two compound groups (nitrofurans and phenicols) that previously had to be extracted and analyzed by two different analytical methods. Now, these compounds can be analyzed with a single method.

Why was high-resolution MS needed for this method?

It is the availability of these instruments in our lab. We are currently using four HRMS instruments. There is only a single, rather old tandem quadrupole instrument left. We plan to retire that instrument next year. Therefore, going forward, all methods (we primarily analyze veterinary drug residues) will be analyzed by HRMS instruments.

In addition, the unit mass isolation followed by the HRMS detection of product ions gives us increased selectivity. This is visible by having cleaner trace level baselines than these obtainable with unit mass resolving tandem quadrupoles.

What challenges did you face in optimizing the HRMS detection for this analysis?

Our lab has been using HRMS for some 14 years, so we are very familiar with this technology. Initially, HRMS was not really a routine methodology. For us, HRMS has been something like a love affair. You are attracted to the technology as a whole, but see and criticize certain aspects. Therefore, we have written a number of technical HRMS papers that clearly pointed to these issues. These critiques were not always well received by the instrument companies. I think it was one high-

light of my career as analytical chemist, when a representative of a leading HRMS company told me that they came up with a new HRMS instrument to address a limitation that I had previously published in a peer-reviewed paper. In the meantime, most of the pressing problems with HRMS have become history. There can be issues that HRMS cannot tolerate very heavy matrices and produces insufficient sensitivity in the full scan mode. I think, however, that those issues are much less relevant when you have access to modern time-of-flight (TOF) or orbital trap technology. In case of high sensitivity or selectivity requirements, we analyze the critical compounds in targeted Q-HRMS modes, while the less critical compounds are quantified using the more universal full scan mode.

"Method development is greatly aided with HRMS. We see the whole spectrum. This helps us to improve and select the proper sample clean-up steps."

What does this method achieve that previous methods could not?

Using MS/MS for a multiresidue method requires the definition and maintenance of retention time windows. Adjusting such windows is time consuming and is normally done late in the afternoon when everybody wants to go home. More often than not, a chromatographic peak is located within a retention time window, but slowly drifts away within a prolonged sample series. It is a bad discovery the next morning to realize that one or several peaks have drifted out of the redefined windows. This does not happen with full-scan HRMS. I appreciate that I can inject and ask questions after the completion of the acquisition. Frequently, a positive finding leads to new questions. Is a related drug present as well? Do I see metabolites or degradation products?

Last but not least, method development is greatly aided with HRMS. Most importantly, we see the whole spectrum. This helps us to improve and select the proper sample clean-up steps.

We recently developed a method for steroids in animal-based food by HRMS. The sensitive detection of steroids requires the use of virtually unbuffered mobile phases. Our analytes had stable retention times, yet the retention times of most matrix compounds (as seen in the full scan) varied from sample to sample. Hence, some abundant matrix peaks were coeluted with analyte peaks in some of the samples. This led to significant irreproducible signal suppression issues. Using HRMS, we identified these intensive matrix compounds as long-chain fatty acids. Thus, the clean-up (the pH of the liquid–liquid extraction step) was changed to remove these interfering compounds. This solved the signal suppression issue. Most likely, we would not have been able to recognize and solve this

problem if we had used a tandem quadrupole instrument.

What are your next steps in your food analysis work?

As mentioned, we will migrate all our remaining tandem quadrupole methods to HRMS. But this should be done in an intelligent way. Whenever possible, we try to reduce the number of methods by putting compounds from different methods into a new HRMS method. Frequently, this is less a technical than an organizational issue. It is possible to analyze fish or fish products for residues of veterinary drugs and at the same time to look for undeclared preservatives and illegally added dyes. Yet, within a big food safety institution, there may be a lab that is responsible for vet drugs, another group that handles preservatives, and a third and a fourth that are responsible for dyes and pesticides. Therefore, not everybody will be equally happy when such "multi-multi" methods are going to be implemented.

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Anton Kaufmann is currently employed at the official food control authority of the Kanton of Zurich in Switzerland and is responsible for a group that focuses on veterinary drug residue analysis. He has some 30 years experience in food analysis, including work involving instrumental analysis of wine including the application of chemometric techniques to elucidate the geographical origin of wine. His current activity focuses on multiresidue methods for veterinary drugs in animal tissues. This includes state-of-the-art technology like ultrahigh-pressure liquid chromatography (UHPLC) and high-resolution mass spectrometry (time-of-flight and orbital trap) as well as ion mobility. In addition to developing new analytical methods, he has investigated technical aspects related to LC–MS, such as signal suppression, ionization phenomena, structural elucidation by HRMS, and alternative method validation approaches. Mr. Kaufmann is the author of some 60 peer-reviewed scientific papers and four book chapters.

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Assessing Pesticide Transfer to Pollen and Nectar

Interview with Brian Eitzer

A major concern with the use of pesticides, particularly systemic pesticides such as neonicotinoids, is whether these compounds are transferred to pollen and nectar at levels that might pose risk to pollinators such as honey bees. Brian Eitzer of the Connecticut Agricultural Experiment Station has conducted research on this topic. He recently spoke to us about this work.

You conducted a study to assess whether the use of neonicotinoid insecticides in model plants grown in nurseries poses a risk to pollinators like honey bees (1). Why did you embark on this study?

The ornamental horticultural industry is important to the State of Connecticut. The interest in the plight of pollinators has led to public demands that nurseries produce pollinator-safe plants. At the same time, however, the industry must produce plants that are free from insect infestations. Systemic pesticides such as neonicotinoids can protect all parts of the plant and can be compatible with integrated pest management, but these pesticides could also translocate to pollen and nectar, which would then be accessible to honey bees. We therefore thought it would be important to understand what the actual concentrations of pesticides were in these matrices (nectar and pollen) when they are applied under normal horticultural practices. Use patterns for these insecticides leading to concentrations lower than those known to cause harm might still be continued, while those practices leading to concentrations higher than known risk thresholds could be discouraged.

Why did you choose sunflower and swamp milkweed as model plants for the study?

One of the difficulties in understanding the movement of these chemicals into pollen and nectar is that plants produce very little of these matrixes, and these insecticides can have effects on insects

when present at low parts per billion concentrations. In order to obtain sufficient pollen or nectar to analyze the residue concentrations, we needed close to one gram per sample. Sunflower and milkweed produce unusually large quantities of pollen and nectar, respectively, and so they were appropriate model plants to use in our study. It is unfortunate that the same species of plants that make it possible to conduct these studies would be the same plants that would be inadvisable to treat with systemic insecticides—due to the unusual amount of nectar or pollen that they produce.

"It was important that we use very sensitive methods so that we would be able to observe sample concentrations in the low ppb range in samples that were less than a gram in size."

You used a factorial design for this experiment, addressing the choice of insecticide, application methods, application rate, and application timing before bloom. Can you explain briefly how the study was structured and what that study design enabled you to assess?

We knew that the collection of samples of reasonable size for analytical analysis was going to be difficult. We therefore wanted to maximize the information that could be obtained from each sample. The factorial design enabled us to study three insecticides, two different application methods, three different application rates, and five application timings. This experimental design allowed us not only to look at each of these factors in isolation, but also to investigate whether there would be any important interactions among them. Factorial designs also have a property called "hidden replication" that permits greater precision in statistical analysis.

Were there any challenges in developing or identifying suitable sample preparation and analytical methods for the study?

A primary challenge in this work is the size of the sample that is available. As food pesticide residue chemists, we are used to homogenizing large amounts of sample and then taking subsamples for duplicates or spiking. In this work, that could not be done. Collecting a gram of sample could require hours of time and we needed multiple samples to assess different practices. Therefore, it was important that we use very sensitive methods so that we would be able to observe sample concentrations in the low ppb range in samples that were less than a gram in size. With some modifications to a QuEChERS (quick, easy, cheap, effective, rugged, and safe) protocol, both enzyme-linked immunosorbent assay

(ELISA) and liquid chromatography with tandem mass spectrometry (LC–MS/MS) had sufficient sensitivity.

ELISA is much cheaper than LC–MS/MS, but unable to distinguish between parent compounds of the insecticides and their metabolites. What approach did you take for incorporating ELISA into the study?

For this particular study, we wanted to use ELISA so that we could increase the number of samples analyzed (for better statistics) while keeping down the cost of the study. We were able to overcome some of the limitations of the ELISA procedures by splitting the samples and analyzing a portion of the samples by the more selective LC–MS/MS procedure and then using the results on the split samples to optimally dilute and calibrate the ELISA-only samples.

Which neonicotinoid insecticides produced the highest levels of residues in pollen and nectar?

The application rates for the three insecticides were not identical because our use of each was set by their label directions, which differs from one insecticide to the next. As would be expected, lower application rates led to lower observed concentrations. Overall, the concentrations of the three insecticides were on the same order of magnitude in the milkweed nectar, while in sunflower pollen the dinotefuran was higher and imidacloprid was lower. It should be noted that the observed concentrations in milkweed nectar were in a range that could be toxic to pollinators, indicating that these compounds should not be used with these plants.

What did the study find in terms of which application regime produced the highest levels of residues in pollen and nectar?

We typically found lower concentrations in the pollen and nectar following spray applications as compared to drench applications.

In an earlier study, you measured pesticide levels in pollen and developed a Pollen Hazard Quotient to assess the risk posed by the pesticide levels to pollinators (2). What is the Pollen Hazard Quotient, and how is it calculated?

When bees collect pollen, they collect from many different plants. These plants can be treated with a variety of pesticides. It is, therefore, common to see multiple pesticide residues in a bee-collected pollen sample and the residues can vary widely in concentration. The pesticides also vary in their toxicity. The Pollen Hazard Quotient is a way of combining the pesticide concentration data with the toxicity data. This is done for a pollen sample by dividing the concentration observed for each pesticide by the honeybee oral LD_{50} (the dose lethal to 50% of adult worker honey bees) for that pesticide and then

summing across all the pesticides observed in a sample. We can thus compare a pollen sample with a high concentration but low toxicity pesticide with one that has low concentrations but high toxicity. Note that these calculations in a pollen sample are first approximations as they do not account for any synergistic or antagonistic effects of particular pesticide combinations.

Were the levels of neonicotinoids found in the plant nectar and pollen in your later study (1) at levels that would be understood to be dangerous for pollinators? Did you use your previously developed hazard quotient to measure that risk?

In the later study, we did not use the Pollen Hazard Quotient to assess risk as we were examining individual residues in actual plant pollen and nectar (not bee-collected pollen). We did, however, assess the potential risk by looking at the concentrations observed and comparing the observed levels to EPA threshold levels for these compounds. We did find that for the milkweed nectar that concentrations observed could exceed those thresholds.

What recommendations do you have for the use of these insecticides by nurseries and in agricultural fields?

The simplest messages align with common sense: If plants are being marketed for their use by pollinators (for example, milkweed is often grown as a host plant for monarch butterfly larvae; later, the nectar is fed upon by many pollinators), then don't treat these plants with systemic insecticides! This is especially true for ornamental plants that produce large quantities of nectar that are highly attractive to pollinators. We observed systemic insecticides readily being transported into nectar at concentrations that would be expected to be toxic to bees. Certain uses, such as foliar applications several weeks before bloom to plants that are not particularly attractive to pollinators, would not be of great concern. Generally speaking, the quantity of nectar reward found in the flowers will be an indicator of the risk posed by treating the plant with systemic insecticides. The nectar rewards (and risk) can be assessed by how many pollinators visit the flowers. We didn't assess agronomic crops, but other groups have studied the risk to pollinators from various of these agricultural crops.

"We observed systemic insecticides readily being transported into nectar at concentrations that would be expected to be toxic to bees."

What is your next step in this work?

We are continuing our work at ornamental nurseries in two different ways. First, we continue to study model plants to understand how much of different pesticides get into the pollen and nectar of plants. We are also using honey bees to collect pollen at nurseries. These bulk pollen samples are analyzed, typically now by LC with high resolution mass spectrometry (LC–HRMS). Those samples with the highest hazard quotient then have the pollen samples sorted by color: As many as 10 to 20 different colored pellets can be seen in a single bulk sample. These samples are then split with a small portion sent out for palynological analysis, to determine the plant species the pollen was collected from, while the remainder is re-analyzed by LC–HRMS. These analyses allow us to determine which particular plant–pesticide combination is the most hazardous to the pollinators.

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Brian Eitzer, PhD, obtained a B.S with a double major in chemistry and environmental science at the University of Wisconsin–Green Bay. He spent the next year as an analyst in the Quality Assurance department of Sandoz Pharmaceuticals. He received his PhD in Analytical Chemistry from Indiana University in 1989 where he studied the atmospheric transport of dioxins. Since 1989 he has been with the Connecticut Agricultural Experiment Station, where he currently holds the rank of Scientist. He is an expert in the analysis of organic contaminants in a wide variety of matrixes using liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry. In addition analyzing official state samples, he conducts research on the use of mass spectrometry to determine toxins in foods, and has collaborated on research into the role of pesticides in pollinator declines.

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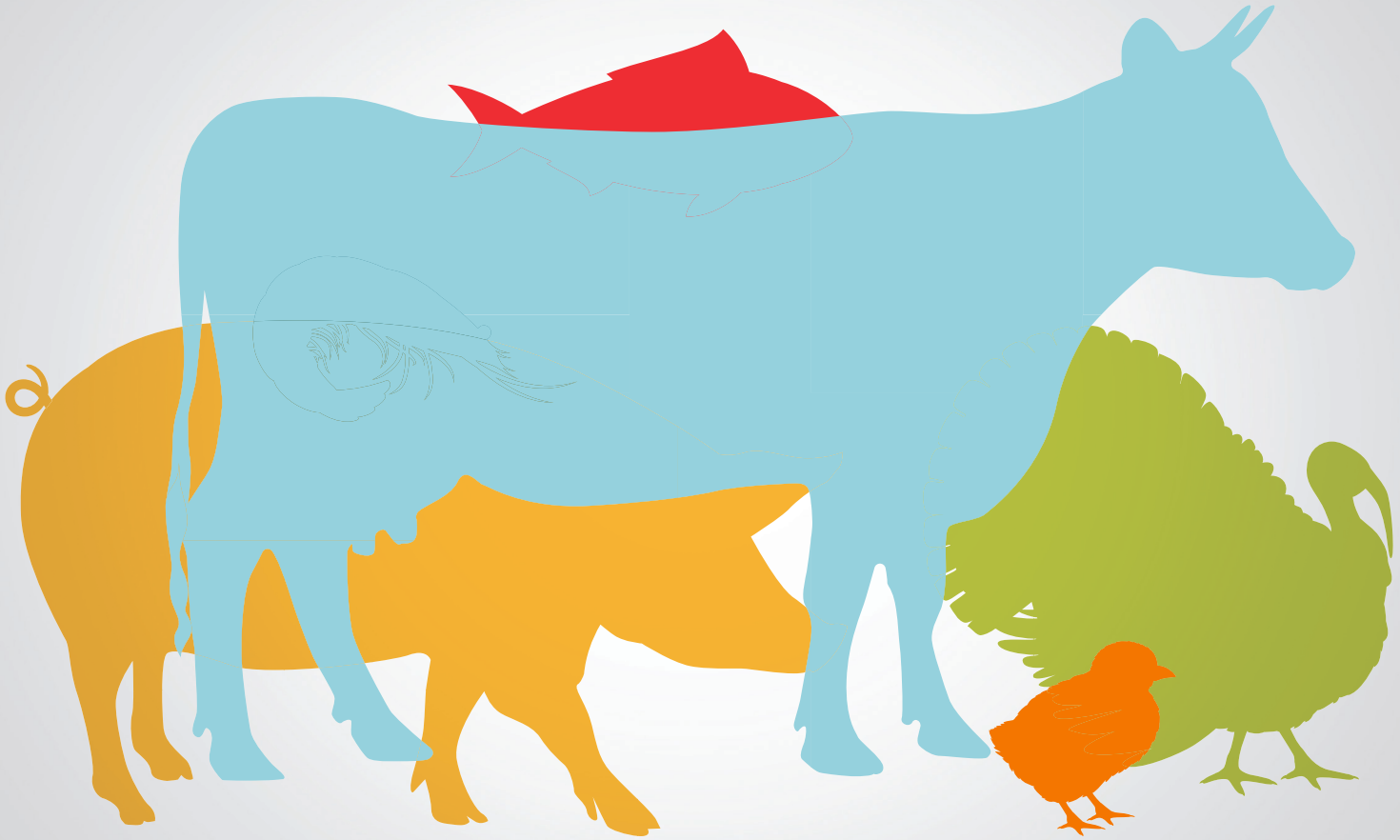

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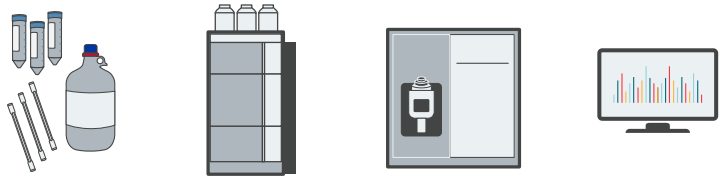

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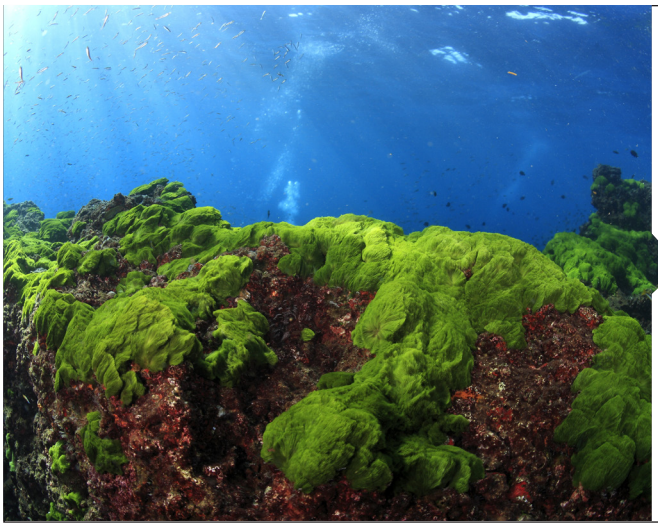

Advancing LC–MS Analysis of Marine Toxins

Interview with Pearse McCarron

In spite of recent advances in the study of marine algal toxins, we need to learn much more about the factors involved in harmful algal blooms, the range of toxins either accumulated or metabolized by marine organisms such as shellfish, and methods for accurate detection and measurement. Pearse McCarron, his team at the National Research Council of Canada, and various collaborators, are actively engaged in this work, advancing liquid chromatography–mass spectrometry (LC–MS) methods, pursuing increased use of high-resolution MS for such studies, and optimizing the combination of targeted and nontargeted screening. He recently spoke to us about this work.

You studied the relative molar response (RMR) of lipophilic marine algal toxins in LC–MS (1). Why did you undertake this study?

The analysis of algal toxins presents many challenges as a result of the diversity and complexity of chemical structures that exist, low regulatory limits for many of these toxins in seafood, and a variety of sample matrices that must be analyzed. The Biotoxin Metrology team at the National Research Council of Canada has an active research program covering activities such as algal identification and culture, natural product chemistry, analytical method development, and reference material production.

Accurate quantitation by LC–MS usually requires calibration solution reference materials for each individual analog being measured, but because of the broad range of toxin analogs known, it has not been possible to date to produce standards for all compounds. This becomes even more challenging if untargeted methods are being used. Therefore, we conducted these experiments to determine the feasibility of using reference materials for closely related analogs to calibrate LC–MS systems. This included an examination of general method parameters that can have an effect on relative molar responses. I was pleased to do this work in collaboration with our colleagues at IFREMER in

France, who also have a very active research program in the toxins field.

You mentioned that experimental conditions can affect the RMR factors in LC–MS for marine toxins. What did you find with respect to chromatographic conditions and mass spectrometry settings?

We examined a variety of method parameters including chromatographic elution conditions (such as gradient elution versus isocratic elution) and MS acquisition modes (such as single ion monitoring versus selected reaction monitoring). From a relative molar response point of view, we found that chromatographic conditions did not have a significant impact for most of the toxins studied, in particular that minor to moderate changes in mobile phase composition didn't have a major influence on ionization efficiencies. However, the choice of MS acquisition mode had a more significant bearing on results for certain toxin classes, and specifically for analogs from different subclasses of cyclic imines. These differences in response are reasonable when we consider that structural variations for some of the analogs could impact fragmentation, which would result in quantitative differences between toxin analogs when using selected reaction monitoring. Depending on the method application, for example fully quantitative measurements for regulatory testing versus semiquantitative analysis for screening work, the differences in response factors may be acceptable. In this work, we found relative molar responses in the range of 0.5 to 2.0 for most toxins, with the exception of the cyclic imines mentioned.

Does knowledge of RMRs provide any value for high-resolution MS approaches in the analysis of algal toxins?

Yes. There is significant interest and quite a noticeable trend towards high-resolution MS analysis in the broad field of trace level analysis because of the many advantages it offers, including increased confidence in the identity of compounds detected, the potential of untargeted screening, and the ability to retroactively analyze data for previously unknown compounds. The situation is no different in the case of algal toxin analysis. The potential of high-resolution MS is very exciting, however current limitations include issues relating to data management and processing, as well as the limited availability of reference materials for the broad range of toxin analogs that could potentially be detected by high-resolution MS. Knowledge of RMRs for known toxin analogs will allow analysts to make more informed decisions regarding the quantitation of related analogs for which standards are currently not available. This will increase the utility of high-resolution MS going forward and provide added confidence in data produced.

Your team recently carried out a study screening for cyclic imines (CIs) and paralytic shellfish toxins (PSTs) in the genus *Alexandrium* (dinoflagellate marine plankton) that involved developing new methods (2). What

approach would you recommend for this type of analysis elsewhere?

We are consistently learning more about the range of harmful algae species that exist, and about the algal toxins biosynthesized by these interesting organisms. Recent work has shown that some strains of *Alexandrium* are capable of producing different compound classes including paralytic shellfish toxins and cyclic imines. Therefore, we wanted to establish methods for screening for the full range of these toxins in a series of algal strains curated at the National Research Council. For this, we used a combination of targeted and untargeted LC–MS methods. The targeted methods facilitated the identification and quantitation of a suite of known toxin analogs, while the untargeted approaches based on high-resolution MS allowed us to confirm the known compounds while tentatively identifying a number of structural analogs that have not previously been reported. This comprehensive approach is important considering the increased occurrence of algal toxins worldwide, and will prove valuable in making researchers and regulators alike as aware as possible of the presence of toxins that might present a risk to local seafood industries and human health.

What were you able to achieve with your methods?

The methods were applied to a series of *Alexandrium* strains collected in Atlantic Canadian waters. The results showed a number of distinct profiles and varying concentrations of cyclic imines and paralytic shellfish toxins in the strains studied. It also highlighted the presence of new toxin analogs that have not previously been reported. In addition to demonstrating the utility of the screening approach implemented in this work, the results were also valuable in helping us identify strains of algae that will be useful for bulk culturing in support of reference material production, and also serves as an indication of the toxin analog profiles that might occur in seafood harvested from this region in the event of future *Alexandrium* blooms. The hope is that these targeted and untargeted approaches might be applied more broadly to provide necessary information on the complexity of *Alexandrium* toxin profiles worldwide, which is important for the implementation of toxin monitoring programs in developed and developing regions alike.

What are the next steps in your work on toxins produced by Alexandrium and other harmful algae species?

There has been significant progress over the last number of years in the field of algal toxin analysis. This progress was initially driven by the need to move away from traditional bioassay-based testing programs to approaches based on more rigorous chemical analytical and bioanalytical methods. However, there is a still a major effort required to fully understand

the factors that drive the occurrence of harmful algal blooms, and to provide the knowledge necessary to establish measurement protocols for the complex toxin profiles produced biosynthetically by the algal themselves and as result of metabolism of these toxins in shellfish.

Our team at the National Research Council (NRC) is committed to ongoing advancement of measurement capabilities in this area. In addition to algal and natural product chemistry research, we are putting a heavy focus on developing improved methods for monitoring toxins and increasing the availability of high-quality reference materials for validation and ongoing quality control in toxin analysis. From a method point of view, we have a particular interest in improving methodologies for both sample preparation and toxin detection. This will consider novel chemical approaches in sample preparation and a variety of separation techniques including liquid chromatography and capillary electrophoresis, to develop overall methods that are suited to quantitation of toxins from polar and nonpolar classes of toxins. Our activity in reference material production and certification is strongly aligned with NRC's role as the National Metrology Institute for Canada, and we are dedicated to providing both calibration solutions and matrix reference materials for a broad range of toxin analogs. We also have a significant interest in furthering method and reference material availability for the analysis of freshwater algal toxins such as microcystins, as these compounds represent an increasing threat to the security of freshwater supplies in various parts of the world.

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