# Pesticide Residue Analysis: How Far Have We Come – and Where Are We Going?

Renowned experts from around the globe discuss the trends, challenges – and techniques – that will dominate the future of food analysis.

**Thermo Fisher**
SCIENTIFIC

# We Are What We Eat

*As consumer awareness of food safety continues to rise, we speak with the analytical scientists at the forefront of food analysis and consider the cutting-edge tools pushing the limits of pesticide residue detection and quantitation.*

Over the past 18 months, I've been working with Thermo Fisher Scientific and had the pleasure of talking with many pesticide residue analysis experts. In that same 18 months, I've started to think more carefully about how the average consumer considers the old adage: "you are what you eat."

Traditionally, of course, the expression is linked to nutrition – but, as is proven by the rise in popularity of organic produce (and a rising interest in proof), consumers are becoming increasingly aware of what contaminants they are absorbing – and how those chemicals could impact on their (future) health or that of their children.

Protecting the world from harmful levels of pesticide residues in foods is an army of scientists. And it's fair to say that they do not have the easiest job in the world of analytical chemistry. On page 12, Kate Mastovska, Associate Scientific Director, Nutritional Chemistry and Food Safety at Covance Laboratories, calls it "The Never-Ending Challenge of Pesticide Analysis." I remember asking Kate why she felt the challenge was endless, and she gave me three ever-changing and compounding reasons – large numbers of analytes, low limits of detection, and a diversity of notoriously tricky matrices. In her article, she adds a fourth: the increasingly global nature of trade in the food industry – "Wider sourcing of raw materials (and distribution of products), unknown pesticide use in certain regions, and different regional regulatory landscapes all add extra complexity and scope," Kate adds.

Kate continues on to discuss her passion for increasing confidence in analytical results wherever possible – a trait admittedly shared by all the analytical scientists I have ever spoken to. In pesticide residue, false negatives – but also false positives – can have significant real-world implications; no one wants to declare food safe, if it is tainted by an old, banned pesticide or contains a new and approved pesticide at unacceptably high levels – it's a simple issue of safety. On the other hand, false positives could wrongly result in huge economic implications for those involved. And, as Kate also notes, "the quantification of a wrongly-identified compound is entirely pointless."

Increased confidence comes with more advanced tools. In the following pages of this compendium, our experts – including Amadeo Rodríguez Fernández-Alba, Fera Science's Mike Dickinson and Stuart Adams, and Microbac's Mohamed Hamad – describe their exploits with the latest cutting-edge solutions.

It's safe to say that the desire to measure everything, in everything, from everywhere is only going to increase – as is the diversity of food matrices. Fortunately, new technology platforms, such as high-resolution, accurate-mass MS, are rising to tomorrow's challenges.

**Rich Whitworth**
*Editor, The Analytical Scientist*

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# Food (Analysis) for Thought

**Driving the quality and scope of pesticide residue analysis forward is a constant and global endeavor. Is it time to embrace full scan, high resolution and accurate mass?**

*By Amadeo Rodríguez Fernández-Alba, Professor of Analytical Chemistry and Director of the Department of Chemistry and Physics, University of Almeria, Spain.*

Originally from Madrid, my deep interest in food analysis and control actually began when I came to Almeria 20 years ago. Almeria is the main producer and exporter of fruit and vegetables during Europe's winter; focusing on pesticides control made a great deal of sense to me as an analytical chemist. And over the years I've been fortunate enough to collaborate with a number of regional institutions and producers to ensure that Almeria is at the leading edge of pesticide analysis and control. We recognized that one of the most serious issues in pesticide control was unacceptable discrepancies in the results obtained by different laboratories – and that can have a big impact on trade. We began to focus on analytical quality control and method validation procedures for routine pesticide control laboratories.

## Harmonizing quality

It was also clear back then that a forum for knowledge exchange would help address those same challenges, and in 1996 the first European Pesticide Residue Workshop (EPRW) was held in Alkmaar, the Netherlands. I presented results from the procedures we had developed, which in some ways was the starting point for cooperation between the whole network of routine laboratories.

Another important step to get us where we are today came in 2006, when the European Commission's Directorate-General for Health and Consumer Affairs made an open call for four European Reference Laboratories (EURLs) for residues of pesticides. We became the EURL for fruits and vegetables (EURL-FV), and I've been its head ever since. We work together with the three other pesticide EURLs (cereals and feeding stuffs; foods of animal origin; and single residue methods).
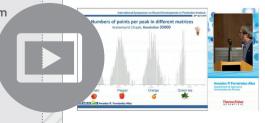
In a nutshell, our main duties are to harmonize results and improve the quality of the whole network. Today, I am proud to say that the European Union has the world's best network of routine laboratories for pesticide residue analysis, at least in my opinion.

## The role of technology

Much of my research is dedicated to the development and validation of new and improved analytical methods. Part of that responsibility means ensuring that National Reference Laboratories are kept up to speed on the latest advances in instrumentation, including mass spectrometry. New technology can have an impact on development of more appropriate or comprehensive methods, and ultimately improve the quality and equivalence of results between laboratories.

There have always been two main issues in our field: sensitivity and scope, both of which have grown in importance as international trade has increased. Go back 20 years, when I first joined this field, and the limit of quantitation (LOQ) was typically close to 1 mg/kg and the typical scope was 20-50 compounds in each run. Instrumentation in an average lab was a GC-single quad MS and LC with UV and florescence detection – and laboratories would spend half a day on very few samples. Today, laboratories must now routinely monitor hundreds of pesticides at very low detection limits – very rapidly. In terms of technology, it's a totally different world; today's instrumentation has risen to help analysts meet the challenges.

The most notable recent advancement in technology comes in the form of high resolution, accurate mass (HRAM) mass spectrometry, which I believe will play a big role in increasing scope and capacity. Introducing such technology for GC and LC into routine laboratories for pesticide residues is the next step, but obviously represents a significant change throughout our network and will take time to implement. We are very much involved in this process, and the instrument companies also have a role to play – and that includes making such technology affordable; after all, pesticide control laboratories, by their very nature, need high-throughput, broad scope, and cost-effective analytical methodologies.

## A new way to fish

My university is close to the sea, so you can see fisherman at work – sometimes fishing with a rod and line, sometimes with a net. I can draw an analogy to mass spectrometry. Line fishing is targeted – you select your line weight and appropriate bait to catch the right size and kind of fish, tossing away rogue catches. In triple-quad MS, we target selected ions using the quadrupole filter. Fishing with a net is a completely different approach – as is full-scan MS – as it captures all fish (or ions). With full-scan MS, the

software determines detectability, as the hardware collects all information, and that means that we have the opportunity to not only investigate thouasands of compounds of interest, but also to revisit data for retrospective analysis – something that is very useful in unusual cases or amidst food scandals. It's essentially a much more flexible analysis concept – and it really opens the door in terms of identification. When it comes to pesticide control, there are two important aspects: i) enforcement of regulations and ii) assessment of risk. And an increase in scope allows us to gain a better understanding of current and future risks.

When they were first introduced, full-scan HRAM instruments were considered complementary to triple-quad instrumentation in routine analysis – sensitivity was an issue, as was cost, so they were reserved for challenging samples. But over the last few years, the sensitivity of Orbitrap-based instruments has increased, software has become much more powerful, and cost is coming down. Such instrumentation is no longer simply complementary – rather they are viable contenders to be the workhorses of routine analysis.

New, more affordable technology, such as the Thermo Scientific Q Exactive Focus mass spectrometer, allows us to conduct routine analysis as we would with a triple quad instrument; there are no major differences in analytical performance in terms of sensitivity, reproducibility, and linearity. And though the analytical performance is similar, the advantages in selectivity are significant.

One main advantage is that the identification capability is higher than triple-quad instruments, which is especially notable in dirty matrices with many endogenous compounds, such as tea or orange. In such complex samples, retention time and transition ratio overlaps can lead to false negative or false positive results. The production of false positives and negatives using accurate mass is much lower, because you're not working with nominal mass transitions; you have two or three ions at accurate mass. In a recent presentation (see sidebar), I offered a particularly good example, involving a false negative of linuron in coriander. A second major point is the overarching fact that information is not missed with full-scan MS – everything is collected by the instrument. Of course, advanced software is required to extract that information – but nothing is lost.

## Embracing change

The switch to full-scan HRAM instruments is not going to happen overnight – but I do believe that we've reached a tipping point in pesticide analysis. Comparable performance – and price – coupled with the advantages of full scan mode and accurate mass for identification make more widespread adoption almost inevitable.

I'd like to conclude by quickly thanking all of the National Reference and official laboratories in Europe for their past and continued cooperation. Four years ago, we conducted a proficiency test on screening methods and many laboratories have participated voluntarily. I am very proud of our network, which is very motivated to introduce new methods and technologies to increase analytical performance. And that makes my job a lot easier.

# Evaluating Q Exactive LC-MS

Amadeo Fernández-Alba presented at the 1st International Symposium on Recent Developments in Pesticide Analysis in Prague. You can view the full presentation online: http://tas.txp.to/0815/pesticide. Here, we present a brief summary.

Four main evaluation areas:
• Sensitivity
• Reproducibility
• Resolution
• Linearity

"In food analysis, quantification is a very critical issue. The results of our analysis can mean the exclusion of a consignment."

Evaluated four different commodities, representing a range of challenges:
• Tomato
• Pepper
• Green tea
• Orange

Considered a number of factors:
• Influence of resolution on detection
• Influence of resolution on peak shape
• Number of points per peak at different resolution

Pilot Study (full scan + MS/MS)
• 100 samples
• Over 180 pesticides
• Mass accuracy (full scan) <5 ppm
• Mass accuracy (MS/MS) <10 ppm
• Sensitivity = 0.01 mg/kg
• Linearity = no saturation
• Reproducibility + linearity < 20% + 10-500 ppb

Conclusions:
• Similar level of quantification to triple quad MS
• More robust identification; no false positives or negatives

# Marching Forward with Food Analysis

**The analytical aspect of the food industry has changed significantly over the last decade – mainly because of advances in technology. From pesticide residues to metabolomic approaches, the field is now more exciting than ever.**
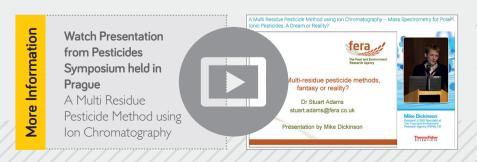
*By Mike Dickinson, Research LCMS Specialist, Fera Science Ltd., Sand Hutton, UK.*

I've been with Fera (in its various guises) for 12 years now. I joined for what I thought would be a single year of good experience after finishing my BSc in Biology at the University of York. But I never left – I was hooked. After two years, I'd made it into a research team, and was lucky enough to work with food analysis research scientist Richard Fussell (who recently joined the team at Thermo Fisher Scientific).

My time at Fera so far has given me an excellent and broad view of the field. In the early years I was involved in the application of emerging mass spectrometry techniques for the multi-residue determination of pesticides and veterinary medicines in food. I also looked at the fate and behavior of pesticides, veterinary medicines and pharmaceuticals in the environment. Twelve years isn't such a long time in terms of analytical chemistry, but during that time, mass spectrometry has advanced at a furious pace. In more recent years, high-resolution (HR)-MS systems started to appear in our laboratories and, as luck would have it, I began working on fate and behavior projects that could benefit from HR-MS. Now, I find myself working almost entirely in the world of HR-MS, including TOF, QTOF, and Orbitrap instruments. I lead metabolomics at Fera in Adrian Charlton's section and essentially bring LC- and GC-MS expertise alongside NMR-spectroscopy for biochemical profiling. We aim to exploit fully the complementary nature of the three approaches; NMR gives excellent reproducibility and you can quantify without analytical standards – but sensitivity can be an issue. LC- and GC-MS systems offer additional coverage, especially in terms of sensitivity. We're currently involved in a EU project called ABSTRESS (www.abstress.com), which is looking at dual stress in legumes. There have been many studies addressing resistance mechanisms in drought-stressed and disease-stressed plants individually, but with dual stress the biochemistry in the plant can be different. We're using metabolomics and transcriptomics to identify the hub genes that are crucial for dual stress resistance, working with 12 national and international partners in the EU, including a state-of-the-art plant phenotyping platform at INRA (the French National Institute for Agricultural Research). It's exciting work.

## The IC-MS story

In addition to cutting-edge research that addresses global food challenges, one of the big goals at Fera is to improve analytical throughput and efficiency. Back in 2008, Richard Fussell and I embarked on a project to try and combine single residue methods for polar ionic pesticides (PIPs), such as glyphosate, chlormequat, mepiquat, ethephon – the project continues today with Stuart Adams, Senior analytical chemist at Fera. I recently gave a lecture that summarizes the project and outcomes at the 1st International Symposium on Recent Developments in Pesticide Analysis called "IC-MS Multi-residue pesticide methods, fantasy or reality?" – the video is available here: http://tas.txp.to/0915/pesticide

In pesticide analysis, multi-residue methods are king; they enable higher throughput and increase laboratory efficiency. Over the years, analytical chemists have done a fantastic job of shoehorning a high number of pesticides into such multi-residue methods. Unfortunately, several stubborn stragglers refuse to be constrained – either they don't quite fit with the chromatography chemistry or the MS analysis is not ideal. Not only are PIPs difficult to separate, they need to be quantified and identified at low concentrations (for example, 0.1 µg/L in drinking water). And the truth is that even the single residue methods we do have are not particularly successful for certain compound-matrix combinations; for example, glyphosate at low µg/kg concentrations in maltodextrin products.

In fact, glyphosate was a big reason why we decided to investigate the potential of ion chromatography (IC) in pesticide residue analysis. Glyphosate is the most used pesticide throughout the world – people have been splashing it over their gardens for year (as an aside, renewed interest in its toxicity has been hitting the headlines recently). Glyphosate is also one of the most difficult compounds to analyze. Fortunately, it is amphoteric (can exist in

different ionic forms) and that triggered a question: why not use IC?

One hurdle was the perceived difficulty in hyphenating IC with MS; MS systems don't get along with salt mobile phases (potassium hydroxide, in our case). The introduction of robust and reliable membrane electrolytic suppressors to convert potassium hydroxide to water on exit from the column was a game changer. Nevertheless, we still had our concerns, and kicked off the project using a relatively old MS instrument, primarily to protect the expensive MS systems – against damage should the suppressor fail. Despite the antiquity of the MS system, we started getting really promising results for retention of glyphosate and glufosinate (another systemic herbicide) using our Dionex ICS-3000. More important were the findings of transformation products – AMPA (from glyphosate) and MPPA and N-Acetyl glufosinate (from glufosinate) – all of which are in the residue definitions for either food or water, or both. We had to load 5-ml samples into the system using an inline concentrator pre column; you can probably imagine the state of the MS source after a series of injections, given that we were not only concentrating our compounds of interest but also matrix co-exactractives. Maltodextrin products proved especially challenging.

### The pesticide lab of tomorrow

In terms of IC-MS for pesticide analysis, we've learnt a number of tricks along the way (for example, writing a script that shut down the pump in the case of a consistent offscale detector response,

which can be indicative of a suppressor failure emergency) – and we benefited from the work of Anastassiades et al., in particular the QuPPe method (1). Indeed, Stuart has continued to develop the method, extending it to other pesticides of interest as well as some other stragglers that don't fit particularly well into multi-residue methods. Notably, we've also moved onto much more advanced triple quadrupole MS systems and we now inject only 100 µl samples because of advances in sensitivity. The use of internal standards was also another big step forward, and I suspect another area where we can expect to see further benefits.

Fera has an upcoming collaborative research project with Thermo Fisher Scientific that will employ a state-of-the-art system – a Dionex ICS-5000 coupled to a TSQ Quantiva triple quadrupole mass spectrometer. It's a combination that promises to give us the highest possible levels of sensitivity, and also offers the potential to enter into the world of 2D-LC. The ability to use conventional reversed-phase chromatography on the first dimension column and IC on the other would certainly help separate some of the trickier compounds from the matrix. We could also run two columns in IC mode, using the second column as a concentrator before MS analysis. Stay tuned for further developments!

Looking to the future makes me think about how quickly things have changed. Walking through the laboratories at Fera is very different today than when I started just 12 years ago; there is less wet chemistry with fewer "hands

on" analysts, and the instrumentation is much more sophisticated, which has increased efficiency immensely. Technicians no longer need to spend hours concentrating samples because the equipment provides the sensitivity we require, especially with the revolution in triple quadrupole instruments.

We now have more than 35 MS systems that deal with routine analyses (the majority are LC-triple quads, with about five HR-MS instruments). And though the triple quadrupole instruments are very sensitive, reliable and ideal for quantification and targeted methods, it wouldn't surprise me if hybrid quadrupole high-resolution instruments start to take over at some point in the future. Instruments like the Q Exactive offer good sensitivity for targeted analysis alongside the potential for full-scan, high-resolution data acquisition.

Looking further into the future, there will no doubt be a continuation in the development of hardware, but software is likely to result in breakthroughs with the biggest impact in my opinion. Indeed, software companies and developers must focus on providing user-friendly, efficient, fully capable solutions. I also expect to see scaling down of instruments to drive towards portable equipment that can be operated by non-experts. We've already seen the beginnings of a revolution in NMR benchtop devices like the Picospin, so why not with MS instruments? The core technology may not be there quite yet, but it will come. Miniaturized Hybrid Quadrupole-Orbitrap anyone? I'll take two.

*Reference*

1.  M Anastassiades et al., "Quick method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPe Method, Version 8.1 www.eurl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf, 2015)

**More Information**

**Watch Short Interview with Mike Dickinson**

# A Taste of the Other Side

**When Richard Fussell still worked at the UK's Food and Environment Research Agency, he was the first customer to see the Thermo Scientific Q Exactive™ GC in action – well ahead of its official launch at ASMS 2015. The latest Orbitrap™ innovation made him wonder – not for the first time – if the grass was greener on the other side.**

Take us back to your pre-Thermo Fisher Scientific days...

I worked in government laboratories for a very long time before moving to Thermo Fisher Scientific – latterly at the Food and Environment Research Agency in York, UK, working on a diverse range of projects, spanning many research areas, techniques and applications. Throughout those years, I very often found myself working in close collaboration with different manufacturers, helping to guide new and emerging technologies. As an analytical scientist, I always found it very exciting to be involved in such developments, contributing to advances and progress in the instrumentation we used on a daily basis.

My entry into the world of analytical chemistry, which actually began in the 1970s, was a little unconventional. I come from a working-class family of electricians, carpenters, plumbers, and so on. I was never great (or perhaps interested enough) at school and when I left, I went into the building trade. I remember one particularly nasty day in winter when my van broke down and I was late for my own birthday party. The very next day, I applied for – and got – a job in a laboratory. From there, I moved into a government laboratory – who paid for my education up to MSc level, and the rest is history.

So much has changed since those early days. I remember when I first started doing chromatography, we used a hacksaw and a file to cut and polish stainless steel tubing when building our own LC systems...

Why jump the fence?

Over the years, I received quite a few tempting offers from instrument companies – even as far back as the 1980s. I was always intrigued by the prospect, but never quite attracted enough to make such a leap of faith. But when the recent opportunity to join the team at Thermo Fisher Scientific ahead of the launch of an exciting new addition to the portfolio came along, the timing seemed right. Why Thermo Fisher Scientific, specifically? I honestly believed that Orbitrap technology was the best in the field, so it seemed like the winning team.

And that was confirmed when I visited Austin, Texas, to see the pre-launched Q Exactive GC. I was amazed; the performance of the instrument was almost unbelievable. Aside from the technology, one of the things that really impressed me was how open they were. We had such great discussions – and it really felt invigorating to be involved. Furthermore, it was a really nice atmosphere, and it seemed to me that I could learn a lot – not just in terms of the technology, but other skills as well. When you've worked in a particular environment for a long time, you have to be careful that you don't get stale. Looking back, maybe I should have challenged myself at an even earlier stage, but that's just the way it worked out...

How has GC-MS changed?

I remember when GC-MS was first introduced into our laboratory (when it had finally become affordable enough). We started with GC-single-quadrupole MS, which had certain limitations but was the best we had at the time. And in the early 2000s, GC triple quadrupole MS systems came along, which added a lot of advantages, both in terms of the selectivity and the signal to noise we could obtain for pesticides residue analysis. We could suddenly analyze more pesticides in even more difficult matrices, just because of the extra selectivity.

But despite the advantages, I guess I wasn't alone in hoping for a full-scan acquisition technique that would allow us to capture as much information as possible. That is possible with single quadrupole instruments, but the problem is sensitivity – and the selectivity isn't great either.

It seems the Q Exactive GC was highly anticipated in your field?

Absolutely. GC Orbitrap technology takes us a big step forward by essentially combining the advantages of all techniques in one platform: much better sensitivity in full-scan acquisition mode, and better selectivity because we've got high resolution combined with high mass accuracy. Back in the days when we were using single quadrupole systems, I don't think anybody could have predicted we would get this far – that we would develop cutting-edge instrumentation to the point where it could become a routine technique. Certainly, concurrent developments in computer science and electronics have been crucial... The first computer I used in a laboratory was a ZX Spectrum, so to get to where we are now, there really have been quantum leaps on many levels.

## More Information

**Presentation from Pesticides Symposium held in Prague**
Options for Fast, Reliable Pesticide Residue Analysis in Food



Options for Fast, Reliable Pesticide Residue Analysis in Food by Triple Quadrupole GC-MS/MS

Richard Fussell[1]
*Vertical Marketing Manager, Food and Beverage*

Michael T. Heltmanski[2], Cristian Cojocariu[2], Victoria Bailey-Horne[2], Dominic Roberts[3] and Paul Silcock[3]
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**Richard Fussell**
Global Marketing Manager
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Thermo Fisher Scientific

**What makes Q Exactive GC so attractive for food analysis?**
You have to remember that the whole area of residues, contaminants, and food safety has changed dramatically over the years – and there are a lot of other changes going on at the moment. For example, interest in authenticity and food integrity is burgeoning – looking at the bigger picture is becoming increasingly important. Orbitrap technology not only gives us the capability to look at residues and contaminants, but allows us to tap into other aspects. A good example is the whisky profiling and characterization work described by Jana Hajšlová last month (tas.txp.tp/1015/jana).

**How quickly will it be adopted?**
It won't happen immediately, of course. Introduction of new technology is an evolutionary process. The bigger research laboratories are often the first adopters; they often want to investigate the potential of the technology – and also push extra development. The smaller labs will follow. Years ago, we were one of the first labs to use an LC-MS/MS method, and I remember giving a presentation on the multi-residue analysis of about 30 pesticides. People couldn't believe it could be a robust, routine technique – now everyone's using LC-MS/MS. It's hard to believe that the same won't happen with GC-HRAM technology. You can take your sample; do the quantification, the identification – and the screening – all in one single analytical run.

As with any new technique, affordability will be perhaps the biggest barrier. But that too will change. As Alexander Makarov notes on page 48, Orbitrap technology is constantly evolving, which increases the knowledge base and reduces cost. For example, on the LC side we now have the Thermo Scientific Q Exactive Focus, which is an Orbitrap-based instrument intended for routine implementation at a more competitive price.

**What about the future of food analysis?**
New instrumentation empowers people to do and look at things differently. It's already the case that labs are trying to combine different analyte classes in analytical methods; for example, looking at pesticide residues and mycotoxins in the same analysis. Traditionally, these areas have been separated; I suppose the laboratories become compartmentalized – constrained by the instrumentation and methods available.

I see a future trend where, for certain samples, you'll be able to look for multiple analyte classes in the same method, or perhaps test for pesticide residues at the same time as collecting data for characterization or authentication. Similarly, there is a growing interest in looking for environmental contaminants – I've looked at the uptake of pharmaceuticals in plants caused by the use of treated sewage effluent on land, for example. It's surprising how many pathways exist for contaminants to get into food. And let's not forget food contact materials – John Gilbert goes into much more detail on page 28, but it is yet another separate world of contaminant analysis. The real driver for moving in this direction is the capability of the instrumentation available.

Another trend I see developing is using full-scan instruments to detect markers to help food manufacturers ensure product consistency from a quality control point of view. With global food trade, raw ingredients come from many different sources and are difficult to track. The use of chemicals varies over the world – as do the potential routes of contamination. I believe food manufacturers will increasingly want to screen their raw ingredients to ensure that the whole finished product is consistent over time. They certainly don't want any surprises that would undermine consumer confidence.

**Do you feel like instrument manufactures are leading the charge?**
Many of the potential trends I've indicated above would really not be possible without HRAM technology – so it does appear that in some aspects, analytical laboratories are very much dependent on the development of new instruments to be able to move forward in new directions. Certainly, not everybody recognizes that fact, but even if you consider something as simple as the QuEChERS method, would it really have become so successful without the introduction of LC-MS/MS?

**And is the grass greener?**
I've seen a lot of changes over my career – and many of the big ones came from instrument manufacturers. I think that's one of the reasons I recently decided to make a pretty big change for myself when I joined Thermo Fisher Scientific. Luckily, people from my old world still talk to me, even though I've crossed over to the "other side". And that's important – I made some great friends over the years on the conference circuit and beyond. Now, I've been on both sides of the fence – and I consider myself a mediator of sorts. In my current role, I can make sure we are communicating effectively with our customers and perhaps facilitate the kinds of collaborations I enjoyed in my previous life. I'm very happy to be where I am at this exciting time, and as for whether the grass is greener – well, that would be telling...

**More Information**

Short Interview with Richard Fussell

Richard Fussell
Global Marketing Manager of Food and Beverage
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# The Never-Ending Challenge of Pesticide Analysis

**A growing target list, increasingly complex matrices, and the need for low limits of detection can make our field seem like an uphill treadmill. Here, I share my thoughts on some of the major challenges – and consider how new technology might help us push through the pain barrier.**

*By Kate Mastovska, Associate Scientific Director, Nutritional Chemistry and Food Safety, Covance Laboratories, Madison, WI, USA.*

Earlier this year, I had the pleasure of delivering the plenary lecture at the 1st International Symposium on Recent Developments in Pesticide Analysis in Prague, Czech Republic (watch the presentation online at: http://tas.txp.to/1115/Mastovska).

I wanted to provoke discussion, and so decided on a bold (perhaps even intimidating) title: ''New and Never-Ending Challenges for Pesticide Routine Testing Laboratories.'' Why do the challenges feel never-ending? Firstly, pesticide residue analysis must constantly react to three (ever-changing) compounding factors: large numbers of analytes, low limits of detection, and a diversity of matrices.

Moreover, the increasingly global nature of trade in the food industry adds to the mix. Wider sourcing of raw materials (and distribution of products), unknown pesticide use in certain regions, and different regional regulatory landscapes all add extra complexity and scope. At Covance, we are well aware of the global nature of the challenge and are focused on global harmonization. That means using the same robust methods, the same SOPs and quality systems – even the same laboratory information management systems – across the company, which is no mean feat.

From a regulatory point of view, even more challenges emerge. We know that there are different maximum residue limits and different compounds in use around the world, but pesticide residue analysis is more than just meeting the appropriate regional regulations. Global companies – and our clients – are increasingly interested in measuring everything, in everything, from everywhere – setting global specifications based on the strictest requirements in each case. Our target lists are growing...

For regulatory and contract labs, strange (and sometimes unknown matrices) are a regular occurrence – especially when it comes to botanicals and other supplements. And though analyzing an unknown sample for (known or unknown) pesticides is clearly an extreme case, it does highlight a challenge that will not go away: the matrix. Perhaps more importantly, it also highlights a trend; gone are the days when cereals, fruits and vegetables were the mainstay of analysis. The matrix challenge appears to be an ever-increasing circle that began with produce, grains and oils, and then

*"Sample number 1037593: dark green, sticky, strange scent."*

expanded to include specialized matrices, such as spices, tea, cocoa, and so on. Today, the circle has grown bigger still, with herbal drug mixtures, dietary supplements... The list continues – as does the complexity.

### Maintaining quality in the mayhem

In our labs, we use the SANCO guidelines for pesticides analysis both for validation and routine quality control as a minimum. The importance of quality control, particularly for difficult matrices, cannot be understated. In these difficult matrices, quantitation accuracy can represent a significant challenge, because unknown matrix effects can potentially affect sample preparation (recovery) and quantification (signal suppression/enhancement).

Clearly, in all walks of analytical life, identification of contaminants is of paramount importance. Just the presence of certain unexpected contaminants could have huge economic implications (and actually make quantification unnecessary in some cases). Conversely, the quantification of a wrongly-identified compound is entirely pointless.

In short, we need very high confidence in our results. For identification with MS/MS, SANCO/12571/2013 states that the minimum should be:

- ≥ 2 product ions
- ± 30 percent maximum relative tolerance for ion ratios.

and non-targeted – at least in my experience. From a holistic standpoint, you can consider the difference as two simple questions:

- Targeted: is compound X in the sample?
- Non-targeted: what is in the sample?

The reality is, of course, much more complex – and I believe that it is important to consider both data acquisition and data processing. If you are using analyte-specific conditions, then your data acquisition is targeted (for example, multiple/ single reaction monitoring, selected ion monitoring). If not, you are acquiring data through non-targeted means (for example, full-scan MS, all-ion fragmentation, data-independent MS/MS). However, when it comes to data processing, the complexity increases; after all, can't we process non-targeted data in a very targeted way? At this point, Rumsfeldian analogies are inevitable:

- Known knowns: targeted processing of targeted – or non-targeted – acquisition data, using analyte-specific conditions (retention time, MRM or selected ions) in the data processing method created with reference standards.
- Known unknowns: (non-)targeted processing of non-targeted acquisition data, using database/ library search (fragment match, structure correlation, accurate mass) to get presumptive identification.
- Unknown unknowns: non-targeted processing of non-targeted acquisition data, using chemometric (differential or statistical) analysis, followed by identification of compounds of interest. A little like trying to find a needle in the haystack.

But are we satisfied with minimum confidence? Notably, improved selectivity and identification confidence can be gained by developing methods that fully exploit the significant analyte overlap between GC-MS/MS and LC-MS/MS, using orthogonal selectivity as a means of confirmation. Another way of improving confidence in challenging matrices is by developing methods that closely evaluate multiple MS/MS transitions – not just the ones that offer sensitivity, but rather those that confer better selectivity.

Last but not least, the use of high-resolution accurate-mass (HRAM)-MS instruments, such as the Q Exactive™ systems, can increase confidence in compound identification by providing additional accurate mass information and thus increasing selectivity. And though right now we don't use such technology routinely for pesticide analysis, in difficult cases (where other techniques have failed to give us the confidence we need), we have found the selectivity of HRAM-MS analysis very useful. In other applications areas, for example, non-targeted analysis of adulterants, full-scan, accurate-mass, high-resolution data really comes into its own.

When we consider our ever-expanding list of compounds in our target list (right now, we are currently validating a method that looks at over 500 compounds), the ability of HRAM-MS systems to perform non-targeted analysis starts to look increasingly attractive.

## What do targeted and non-targeted really mean?

There appears to be a slight lack of consensus on the meaning of targeted

The never-ending challenges in pesticide analysis are driven by a growing target list and a growing number of increasingly complex matrices.

**The realities of non-targeted analysis**

Having defined non-targeted analysis, we are now in a position to consider the challenges, which I hinted at earlier with the term "analyte-specific conditions." When we think about non-targeted analysis, we typically focus on the mass spectrometry aspect. But in my presentation in Prague, I told the sad (but poetic) story of "Ten Little Pesticides," where only one lonely pesticide was identified in non-targeted analysis. My point was: how do we know that all analytes of interest even make it to the data processing step? In other words, all steps of the analytical workflow (extraction, cleanup, separation, ionization, detection, identification) could lead to loss of analytes of interest. The real challenge here? Optimizing non-targeted methods and establishing adequate quality control for those methods.

Despite that warning about non-targeted approaches, let us not be too quick to dismiss the power of HRAM-MS in addressing some of the broader challenges in pesticide analysis. HRAM-MS has utility across the full spectrum of users, which includes academia, pesticide R&D labs, government, the food industry,

*"I can see a point in the future where we can conduct both targeted analysis and non-targeted screening on a single platform."*

and contract testing laboratories. We can break that down more simply into two areas: research and routine.

In research, HRAM-MS is clearly useful for discovery and identification of new metabolites, for fate studies for new pesticides, or for the identification of unexpected/illegal pesticides. For routine use, I believe HRAM-MS is well suited as a complementary tool to targeted analysis of pesticides for comprehensive testing or – especially in the commercial world –

for the development of risk-based target lists for customized food-safety testing programs. Indeed, we are launching two non-targeted methods that we feel meet our clients' needs.
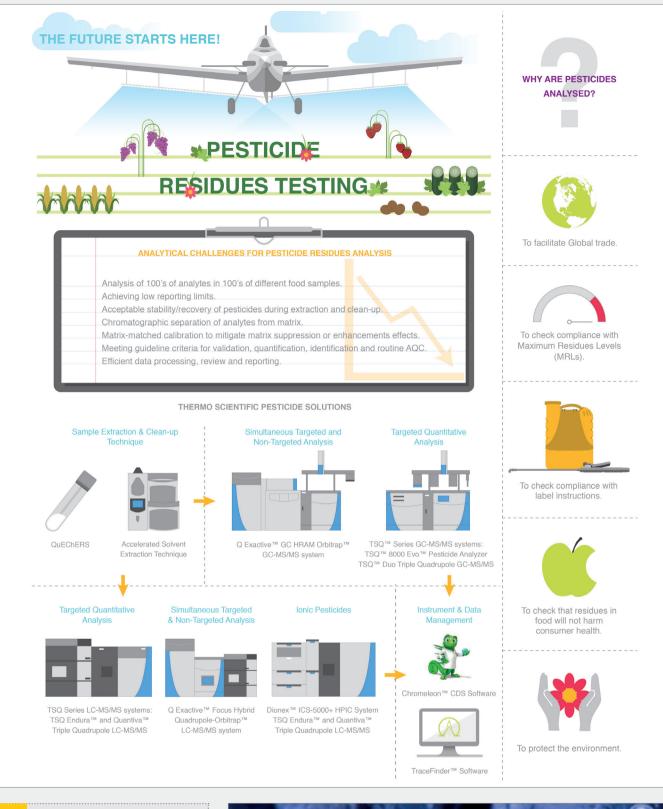
What is potentially powerful in both areas is the ability to retrospectively interrogate data, which could be particularly interesting when considering emerging contaminants or investigating whether a new problem is in fact a new problem at all.

**A single platform?**

As the sensitivity of HRAM-MS instruments increases, I can see a point in the future where we can conduct both targeted analysis and non-targeted screening on a single platform – a very attractive proposition. In fact, for less complex matrices, we are probably pretty close to that point already. But...

Implementing new technology involves a great deal of effort for accredited routine labs (new method development, validation of all aspects), so I suspect that many laboratories will continue to use triple-quad instruments for quite some time. Nevertheless, there's certainly a real buzz about non-targeted analysis at conferences – the introduction of GC to the Orbitrap™ portfolio will probably add to that buzz. Right now, I get the sense that non-targeted data acquisition (with its potential to speed up method development) followed by streamlined and targeted processing of that data is a good midpoint between the old and the new for routine labs (we don't need or want every sample to be a research project!). Data processing is an ongoing challenge, but it seems that the software is fast catching up with the hardware.

In five or ten years' time, who knows how far we will have traveled on our treadmill?

# THE FUTURE STARTS HERE!

# PESTICIDE RESIDUES TESTING

## ANALYTICAL CHALLENGES FOR PESTICIDE RESIDUES ANALYSIS

Analysis of 100's of analytes in 100's of different food samples.
Achieving low reporting limits.
Acceptable stability/recovery of pesticides during extraction and clean-up.
Chromatographic separation of analytes from matrix.
Matrix-matched calibration to mitigate matrix suppression or enhancements effects.
Meeting guideline criteria for validation, quantification, identification and routine AQC.
Efficient data processing, review and reporting.

## THERMO SCIENTIFIC PESTICIDE SOLUTIONS

**Sample Extraction & Clean-up Technique**

QuEChERS

Accelerated Solvent Extraction Technique

**Simultaneous Targeted and Non-Targeted Analysis**

Q Exactive™ GC HRAM Orbitrap™ GC-MS/MS system

**Targeted Quantitative Analysis**

TSQ™ Series GC-MS/MS systems:
TSQ™ 8000 Evo™ Pesticide Analyzer
TSQ™ Duo Triple Quadrupole GC-MS/MS

**Targeted Quantitative Analysis**

TSQ Series LC-MS/MS systems:
TSQ Endura™ and Quantiva™
Triple Quadrupole LC-MS/MS

**Simultaneous Targeted & Non-Targeted Analysis**

Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ LC-MS/MS system

**Ionic Pesticides**

Dionex™ ICS-5000+ HPIC System
TSQ Endura™ and Quantiva™
Triple Quadrupole LC-MS/MS

**Instrument & Data Management**

Chromeleon™ CDS Software

TraceFinder™ Software

## WHY ARE PESTICIDES ANALYSED?

To facilitate Global trade.

To check compliance with Maximum Residues Levels (MRLs).

To check compliance with label instructions.

To check that residues in food will not harm consumer health.

To protect the environment.

# Introducing the Pesticide Explorer Collection

**Simplified workflows to support pesticide analysis from start to finish.**

Conscious of the increasing demands placed on routine pesticide control laboratories, Thermo Fisher Scientific has developed the Pesticide Explorer Collection, comprising four complete solutions that cover all levels of pesticide analysis. Here, we share details of the first: the Triple Quadrupole "Standard Quan" solution.

The standard quantitation configuration – just like its stablemates – includes all the workflow components needed, from consumables and hardware through to software and built-in instrument and data processing methods. Dipankar Ghosh (Director, Enviro & Food Safety, LSMS, at Thermo Fisher Scientific) says, "The Pesticides Explorer Standard Quantitation configuration is designed to meet the complete needs of high throughput laboratories running routine targeted quantitation of pesticides. It provides the analyst the complete tools from sample preparation and analytical methodologies to reporting templates to achieve the desired results fast."

Pre-configured and pre-tested to get you up-and-running as soon as possible, the standard quantitation solution features a TSQ Endura triple quadrupole mass spectrometer to ensure compliance against regulated levels of detection in a routine environment.

## Standardized sample prep and separations

Irrespective of the depth of analysis, accurate results are essential. To that end, all configurations of the Pesticide Explorer Collection include the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) sample preparation reagent kit and HPLC columns, both of which facilitate more accurate pesticide determinations in high moisture samples. QuEChERS is rapidly becoming the method of choice in food sample preparation and clean-up because of its high recoveries, time-savings, and simplicity. Moreover, QuEChERS generates minimal solvent waste but retains the power to cover a wide pesticide range, including polar and pH-sensitive compounds.

Regarding column choice, Mike Oliver (Product Manager, Sample Preparation and Accucore LC Products) says, "Pesticide analysis requires the separation of highly complex samples. In order to quantify and qualify accurately and provide confidence in analysis, highly reproducible and robust separations are required. To meet this challenge, the Pesticide Explorer Collection contains Thermo Scientific Accucore solid core HPLC columns, which deliver greater separation efficiencies in combination with robust formats."

## Software that works with you

Pre-configured methods are simple to access on the included USB drive and can be easily set up and adapted in just a few steps.

Compounds can be selected from the database to automatically create the instrument and processing method. But flexibility allows you to upload, create or modify pre-configured methods with SRM transitions and retention times with ease.

Once the optimized data acquisition has been completed, the color flagging features in the bundled TraceFinder software enable you to quickly review data. The final step? The generation of high-quality standard or custom reports that turn your data into results.

Ed George, Senior Application Scientist in Environmental and Food Safety at Thermo Fisher Scientific, was heavily involved in the development of the Pesticide Explorer Collection, and believes the solutions reflect the constant drive for reproducible and robust results in pesticide control. George highlights the key goal of the Standard Quan solution: "The package for the TSQ Endura includes proven multi-class pesticide methods with compound databases and consumables to help you save time."

**More Information**

**Pesticide Explorer Collection**
The Pesticide Explorer Collection provides start-to-finish workflows for pesticide analysis.

**More Information**

**Download Now:**
Pesticide Explorer Brochure

# Raising the Bar for Routine Analysis

**The Pesticide Explorer Collection comprises four complete workflows that meet the challenges of modern pesticide residue analysis. High-resolution, accurate mass measurements – courtesy of Orbitrap™ technology – represent the ultimate solutions for laboratories that want to take routine analysis to the next level.**

We previously introduced the Pesticide Explorer Collection and shared details of the "Standard Quantitation" (see tas.txp.to/1215/standardquan) and "Premium Quantitation" packages (see tas.txp.to/1215/premiumquan). In the final article, we introduce the "HRAM Quantitation" and "HRAM Screening and Quantitation" solutions, both of which benefit from the analytical power of the Thermo Scientific™ Q Exactive™ Focus MS system.

## Definitive quantitation

The Orbitrap-based "HRAM Quantitation" configuration uses the Thermo Scientific UltiMate™ 3000 LC system as the separation platform – as do all Pesticide Explorer Collection solutions – but differentiates itself with high-resolution, accurate mass analysis – a unique capability that enables quantitation without compromise in sensitivity, accuracy, precision, and linear dynamic range. When it comes to the complex matrices often encountered in food analysis, high resolving power is particularly useful because it overcomes the masking effects of isobaric interferences, allowing detection of pesticides at very low concentrations.

As with all packages of the Pesticide Explorer Collection, HRAM Quantitation comes with all the workflow components needed, from consumables (including the QuEChERS sample preparation reagent kit and HPLC columns), essential hardware and software. The complete package facilitates method development and ultimately enables fast, accurate and cost effective routine pesticide determinations.. Indeed, pre-configured instrument methods for targeted quantitation enable you to start acquiring data with a lot less time and effort – one of the main focal points during development of the collection, according to Dipankar Ghosh, Global Director for Environmental, Food Safety & Industrial Markets at Thermo Fisher Scientific.

When it comes to data analysis, the addition of the HRAM Spectral Fragmentation Library (fully integrated and searchable using TraceFinder™ software) – with over 2,600 compounds and more than 15,000 spectra – gives you the ability to identify compounds with speed and confidence.

## Adding non-targeted screening to the mix

The high-resolution accurate-mass MS/MS spectral library is also key for "HRAM Screening and Quantitation" as it also facilitates screening of non-targeted compounds. But in the ultimate Pesticide Explorer package, it is joined by two other powerful pieces of software: Thermo Scientific Compound Discoverer™ and SIEVE™. Compound Discoverer includes an extensive set of tools to ensure confident compound identification and structural elucidation. And SIEVE enables label-free, semi-quantitative differential analysis of complex LC-MS datasets, allowing you to reproducibly identify components with statistically significant inter-sample differences.

Naturally, it's not all about the software; the HRAM Screening and Quantitation package also fully exploits the power of the Q Exactive Focus system, which allows targeted quantitation and non-targeted screening from a single dataset. With MS/MS HRAM analysis, no sample-specific method optimization is necessary, and the risk of missing important non-targeted compounds is greatly reduced. Once the data has been acquired, it can be reanalyzed retrospectively without the need for sample reinjection.
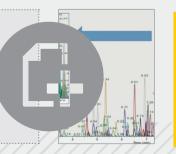
Ghosh notes the upcoming nature of non-targeted methods, "Though our triple quadrupole MS-based solutions excel in targeted pesticide analysis, the Q Exactive Focus unlocks the door to unknown screening in routine environments, using the power of Orbitrap technology. This is of increasing importance given the globalized nature of the food industry. And, of course, this capability extends well beyond pesticides."

And as Kate Mastovska stated in a recent article on the never-ending challenges of pesticide analysis (visit http://tas.txp.to/1215/Mastovska), "When we consider our ever-expanding list of compounds in our target list (right now, we are currently validating a method that looks at over 500 compounds), the ability of HRAM-MS systems to perform non-targeted analysis starts to look increasingly attractive."
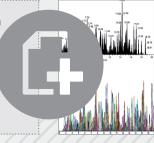
*For more information on the Pesticide Explorer Collection, visit:*
*http://tas.txp.to/1215/explorer*

**More Information**

Fast Screening and Quantification of Pesticide Residues Using a Comprehensive LC-MS Solution:



**More Information**

Increased Productivity in Pesticide Residue Analysis – Quantifying 440 Pesticides Following China GB 2763-2014

# Taking Analytical Control

**Then & Now, with Katie Banaszewski, Method Development Scientist III at Now Foods, Bloomingdale, Illinois, USA.**

Then: one cloudy day in 2014...
We're a dietary supplement company and we're naturally very conscious about the safety of our products, so it's tough having to rely on contract analytical laboratories for our pesticide residue analysis. Our samples are highly complex (multivitamin products can have 40 compounds!) – and we simply don't have the instrumentation needed to perform such analyses. Unfortunately, I don't feel like we're fully in control of our data and I also don't know exactly what kinds of methods are being used by the contract lab. I know the contract lab will be doing their best – we chose them carefully. Nevertheless, it makes me feel a little uneasy – especially, given the finicky nature of pesticide residue analysis in botanical matrices (by far the most difficult analysis there is!). We want to continue to be the best, so we need to look at other options.

Bringing pesticide residue analysis in house makes great sense, but we need to make sure we invest in the right instrumentation. Of course, sufficiently sensitive hardware is important, but we also need software that is powerful enough to help us process the data efficiently; analyst time is valuable to us! Importantly, we also need flexibility; we may want to use the system for other kinds of analyses at a later date.

It's a competitive market out there for analytical systems; we need to do some solid research...



Katarzyna "Katie" Banaszewski (left) takes the new instrument for a spin.

Now: May 3, 2016
Having reviewed the systems on the market, we finally decided on the Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS. There are a couple of points that made this particular system stand out from the crowd. We are especially impressed with the versatility of the system; though pesticide residue analysis was a primary driver, we also needed to be able to accommodate other kinds of analysis; for example, headspace analysis for residual solvents or analysis of essential oils. Moreover, we needed the ability to make that switch quickly, avoiding downtime. Another appealing aspect was the ability to remove the source without breaking vacuum, which also allows us to maximize uptime of the instrument.

I remember when we were first considering our investment, good software was also high on our priority list. We are now using the Chromeleon™ 7.2 Chromatography Data System, which I would say is currently the most powerful software for navigating MS data. The fact that the qualitative and quantitative capabilities are integrated is fabulous. Plus, our analysts find it easy to use – they were very excited to start working with the new system.

Now that we've moved testing in house, we are in control of every aspect of our trace analysis program – and that means we're also in control of the data, and in a better position to defend that data. Before, we could only trust the contract labs to do the best they could with our very difficult matrix sets.

We've always had very talented science teams, and now I feel like our laboratories are world class to match. And as a company, we can fully focus on the safety of our products. The dietary supplement industry is dynamic and vibrant – and our customers demand quality supplements. To stay ahead of the game, it's clear that we need the most advanced technologies out there.

**Thermo Fisher**
SCIENTIFIC

# High Hopes for High Resolution

**Then & Now, with Amadeo Fernández-Alba, Professor at the University of Almeria, Spain.**

Then: one sunny day in 2006...
Ten years ago, we started working with accurate mass, high-resolution mass spectrometry (MS). It was one of the first times such an instrument — a liquid chromatography-time-of-flight (TOF) MS system — had been introduced into a routine laboratory for pesticide residue analysis. I have to say, it was really exciting to see how we could detect and identify a compound simply by inputting its molecular weight or identify new compounds by comparing molecular weights with a database — a great prospect for food safety, as we could detect banned pesticides for which there were no analytical standards. I was truly enthusiastic about the new capabilities.

Unfortunately, every silver lining has a cloud... Credible quantitation on the new system was not possible in many cases — and in pesticide residue analysis, reliable quantification is essential. As a consequence, our conclusion on that time was that accurate-mass, high-resolution MS could only really become a complementary technique (to triple-quadrupole instruments) in food safety analysis; for example, when we had only one transition on the triple-quads for specific compounds or if there was a very strong co-elution of matrix with an isobaric transition.

We had a new tool — but it wasn't quite the revolution I was hoping for. There was a dark side!

Now: June 2, 2016
Over the last ten years, the situation has changed and technology has improved tremendously — and improvements to system software have also been pivotal. Today, good sensitivity, good linearity and good reproducibility — coupled to incredible resolution and excellent mass accuracy (the Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer provides a resolving power of 70,000 at m/z 200 in



full-scan mode and 1 ppm mass accuracy) mean that HRAM platforms have developed from a complementary technique to the technique of choice. And that's not a statement I make lightly. I am sure we will see more incremental improvements in the future, but we've already reached the point where identification capabilities are higher in HRAM instruments, and where quantitation is comparable for pesticide residues in food. It's true that the sensitivity can be a little lower than the newest triple quadrupole systems

— but it is high enough. And after thousands of samples, I can state that the robustness is excellent.

In addition, new identification options are open to laboratories: we can now analyze samples in a retrospective way to detect, identify and quantify new unexpected compounds — even without analytical standards.

In reality, the requirement for HRAM MS systems (LC or GC) will depend on the objectives of each lab. But as labs disappear and those that remain become bigger, we can expect that the scope of the analytical challenge (which covers hundreds of different commodities) will only grow. Moreover, an increasing number of target compounds (and an awareness of untargeted contaminants) in increasingly complex matrices is a clear trend; being able to efficiently cope in this new world will become a real differentiator for routine labs.

Right now, I would guess that around 10 percent of labs in my field have adopted HRAM technology. But in 5–10 years, I believe that HRAM-MS will be highly popular, perhaps even outnumbering triple-quadrupole instruments. New concepts always take time to catch on — and for Orbitrap technology, GC was the missing link; laboratories were perhaps wary of switching over to a new concept of analysis for LC but not for GC. With the introduction of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system, the situation has changed.

Our primary driver is to protect consumers, so we must always strive to achieve the best possible pesticide residue control in food. The advanced capability of HRAM-MS systems, such as those based on Orbitrap technology, represent a very important step in that direction.

# Breaking New Ground with IC-MS

### Then & Now, with Stuart Adams, Higher Analytical Chemist at Fera Science Ltd, York, UK.

Then: a bright but chilly day in 2007…

About nine years ago, we were having problems with the analysis of glyphosate and glufosinate. We'd been using derivatization prior to GC-MS/MS, but too many repeats of samples told us we needed a new solution. We got in touch with Dionex to assess whether ion chromatography (IC)-MS could help reduce the amount of time spent in the laboratory and produce more reliable results. That first conversation kicked off a collaboration with Dionex, who supplied an ICS-3000; we provided a mid-range mass spectrometer with a few upgrades. Sensitivity was always going to be an issue, so we sought inventive ways to get the most out of the system. We came up with the idea of using inline concentrators for sample cleanup. But if you inject as much as 4700 μL of extract onto a system, you add an awful lot of background matrix, so we needed to flush the concentrator with water to remove the non-ionic components of the matrix before bringing it inline with the rest of the system.

For years, we worked with this solution. But it wasn't without its own challenges. We had to use two sets of control software (and therefore two PCs), so errors occasionally but inevitably crept into the sequences, meaning that the IC and MS systems were not always synchronized. We fudged around those problems by running the two control systems on the same PC, but we were still a little uneasy.

We spent the first couple of years — the "honeymoon period" — understanding what preventative maintenance was necessary to keep the IC-MS system running as smoothly as was possible. In fact, all of our systems go through weekly preventative maintenance — something that we've found to be a real time-saver in the long run. For our IC-MS system, the most important task was re-conditioning the columns each week.

There was always a certain "home-built" feel to the system – after all, we were one of the first labs working at this particular frontier. Nevertheless, the benefits were also clear; the number of repeated runs dropped dramatically. Essentially, we'd moved on from analysis that was very difficult using any other technique to much improved analysis on an albeit slightly cranky system. It also allowed us to expand our analytical services; the scope of IC-MS was not limited to glyphosate and glufosinate. A third compound – ethephon – appeared; I remember running the first batch of grapes for the Pesticide Residue Committee Survey and finding an MRL exceeding sample. We'd not done such analysis before, so we weren't sure what to expect – but from that point on, we regularly found ethephon in grapes…

Now: June 8, 2016

Where we are today is very different. Sample injection volumes have dropped from 4700 μL of extract to 100 μL of 10-fold-diluted extract (so 10μl in reality) – less is more! A stark and pertinent difference between "then and now" is how much the technology has advanced. Our Thermo Scientific™ Dionex™ ICS-5000™ is paired with a TSQ Quantiva™ MS system, both of which are controlled with a single software platform, TraceFinder™. Not only is the system easier to use but it is also much more reliable. In other words, we've progressed from the initial excitement of getting our first system to (mostly) work to the excitement of using a system that works the way we want it to out of the box. Columns have also become much more efficient in the intervening years, which allows us to get better peak shapes. And the TSQ Quantiva has got a special low-mass tuning solution – perfectly suited to our compounds of interest in IC-MS.

The scope of IC-MS analysis has also increased with chlorate, perchlorate, and phosphonic acid, all of which have become very topical. Rather than using an LC-MS system with uncertainty about the retention mechanism, we've got a tool that's designed specifically for anionic compounds. It's another robust tool in our toolbox that allows us to step away from the constraints of other techniques when we need to.

Nine years ago, we were certainly an early adopter of IC-MS for pesticide residue analysis. Today, I get the sense that IC-MS is being embraced by an increasing number of organizations in our field and beyond. And now that we've got a reliable system – and experienced staff – we certainly sell the technique internally.

When I started at Fera, there were a lot of single-residue methods. Over the years, such methods are diminishing as compounds are getting slotted into multi-residue methods. IC-MS fits into that evolution with its ability to target a suite of 40-50 analytes. We have developed and validated methods for anionic pesticides and going forward we hope to work with Thermo Fisher Scientific to evaluate cationic pesticides.

It's clear that we all want to test for more compounds with less effort – and in 5–10 years' time, I suspect we'll be working on unknown screening, which will complement our targeted analyte approach. We are also evaluating the Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for other analyses, and hopefully IC-Orbitrap MS, especially given that, as Amadeo Fernandez-Alba noted last month, high-resolution accurate-mass MS systems are likely to become more dominant in the future. As analytical chemists, we don't want to be tied to a list, waiting for a problem – we want to be able to identify upcoming problems and trends. And for that, we need the right tools for the job.

# Hitting LOQs and Confirming Hits with GC-HRMS

**Then & Now, with Nuria Cortés-Francisco, Emerging Contaminants and Mass Spectrometry Specialist at the Laboratori Agència de Salut Pública of Barcelona, Spain.**



Then: only two years ago...
I started working with the LASPB (Public Health Agency of Barcelona Laboratory) even before I joined officially two years ago. I got the opportunity because of my experience in high-resolution mass spectrometry – specifically Orbitrap-based MS systems; it was the focus of my PhD at CSIC (the Spanish National Research Council) and I've been using Orbitrap technology ever since. We are an official control lab and we analyze more than 35,000 (mainly food) samples per year at LASPB – so we're pretty busy; in fact, we have an 'open accreditation scope', which means we're obliged to analyze any food commodity that a client sends to us – including requests for new analytes. The lab here introduced LC-Orbitrap systems five years ago, which are particularly useful for confirmation or to troubleshoot problematic analyses, but GC-Orbitrap was unfortunately unavailable.

We were facing two main problems in GC-MS analysis. First, we found it challenging to reach very low limits of quantitation (LOQ) for some emerging compounds – polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants. In 2014, the European Commission requested that such compounds be monitored, with LOQ recommendations. Limited to a triple-quadrupole MS system, we had to work hard on sample preparation to concentrate the analytes of interest – if your instrument can't catch the standard, you've got no hope in the matrix... We managed to hit

the LOQs for all PBDEs except one – the notoriously tricky BDE-209. It's a big molecule, which causes column difficulties but also sensitivity problems; sensitivity of triple quad instruments drops off significantly at higher molecular masses.

The second challenge in our GC-MS applications was in pesticide analysis – not because that is particularly challenging, but because, unlike LC-MS where we had Orbitrap-based systems, we didn't have a confirmatory analytical method or an alternative technology for challenging matrices/interferences when it came to GC. Having confidence in our analytical results is extremely important, because they can have legal and financial implications; we must avoid false positives or false negatives!

Now: September 8, 2016
Now that we have the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system, we still use the triple quadrupole instruments we did before for pesticide analysis – but if we get any doubtful or strange results, we've got advanced technology to dig deeper; GC-MS has finally caught up with LC-MS in that regard. The strategy with high-resolution MS is different to triple-quad methods, so we had to work hard on it. Analysis may be done in full scan, and certain parameters must be set. We developed a database that includes all the retention times, exact masses, and confirmatory ions – and from the database you can quickly set up a method for a confirmatory analysis. In

fact, the work led to a poster, which received an award at the 2016 European Pesticide Residue Workshop held in Cyprus – a proud moment! I can also say, with some satisfaction, that I recently applied our protocol with great success when addressing an alert for propargite in oranges. We were pretty confident that most of the samples analyzed with the triple-quad method were negative for propargite, but one sample was not so clear. Was it actually positive? I quickly set up the method for our Exactive GC using our new database, and sure enough – we had a positive.

As for the PBDEs, we're easily reaching all the LOQs now – including pesky BDE-209! The Exactive GC has good sensitivity and isn't deterred by higher masses, and the high resolution allows us to pick out all of the interferences, so we're obtaining beautifully clean chromatograms with very defined peaks. By creating a simple method with selected ion monitoring (SIM) windows, we can monitor not only the main peak but also the isotopic pattern – and we know for sure if we have a positive hit.

The upshot is that we now have the analytical confidence in the method to extend the analysis to other food commodities, beyond fish and seafood. On that note, I might add that we pushed the instrument really hard when it was installed – running 200 samples of salmon, tuna, and other fatty fish in the first couple of weeks. The instrument never missed a beat. We need such robust instruments.

# Exploring Pesticides Without Bugbears

**Then & Now, with Mohamed Hamad, Director, Food Chemistry & Nutrition, Microbac Laboratories, Pennsylvania, USA.**

## Then: 2009

I've worked in several fields over the years, first as a professor, then in the petrochemical and pharmaceutical industries – and even the United States Equestrian Federation. During that time I've used many different analytical platforms from most of the major manufacturers. In particular, I have extensive experience in mass spectrometry and know what I need (and want) in a system – and what I don't. One bugbear of mine has always been the cleaning of the ion transfer tube. It typically used to take several hours and resulted in the instrument being down for most of a day. I'd rather not think too much about the time wasted on this menial task! Looking back, down time in general has plagued certain MS systems, which is a significant inconvenience – especially in routine environments where meeting deadlines is key.

In 2014, I joined Microbac Laboratories, which runs one of the world's most diversified commercial laboratory networks, serving a client base of thousands. Essentially, we're responsible for delivering information that businesses

need to survive, so dependable results are a cornerstone of our company. Just after I joined, we experienced a significant expansion to the business and needed to increase our LC-MS capability for a range of analyses – including routine pesticide screening – and started looking for the best solutions available.

The big challenge in pesticide residue analysis is setting up a good, robust method, which includes setting up the right transitions for all the compounds to minimize interferences. We wanted a system that we could get up and running as soon as possible – preferably a total solution.

## Now: 2016

In 2015, we made the decision to purchase the standard quantitation solution from the Thermo Scientific™ Pesticide Explorer Collection. Our particular kit includes the Thermo Scientific TSQ

Endura™ Triple Quadrupole MS and the Thermo Scientific Vanquish™ UHPLC. We've been using the system for about a year now, so we've had enough time to assess various aspects. And I can honestly say I am impressed.

First of all, you get a lot of value for money, which is a consideration that doesn't always get discussed. The competitive price belies the fact that you walk away with a complete workflow – everything you need from sample preparation to data analysis for quantitation of nearly 300 pesticides (we use the short method). And having a method ready to roll straight out of the box really makes life easier for the analysts. It's a real workhorse that enables us to reduce our time to results, which is also good for our customers – in fact, we've been able to halve our turnaround time. Equally importantly, we have confidence in those results.
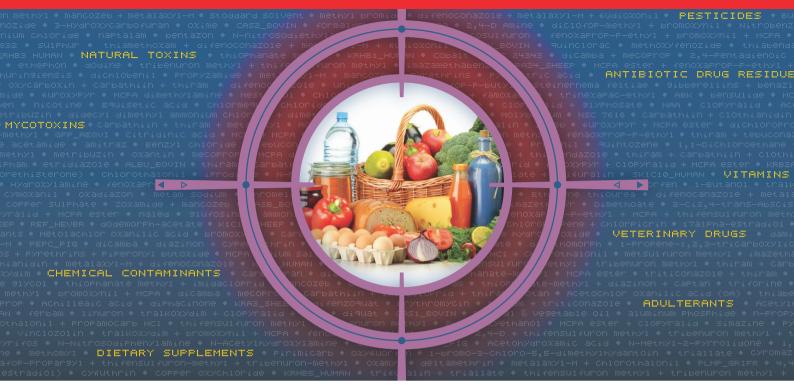
Another key point for us is the absence of carry over, which can be challenging with certain systems. The low levels of maintenance required in general (and the ease with which maintenance can be conducted) result in additional cost savings. Over the year we've had our Pesticide Explorer solution, we've experienced no problems that have resulted in down time. Given that we have the system running 24/7, that's not just good – it's surprising!

But what do I really love about the Endura? The fact that you can take out the ion transfer tube, clean it, and put it back in 10 minutes – all without breaking the vacuum. My bugbear has finally been vanquished!

**Thermo Fisher**
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