Next Generation GC-MS

Six experts consider the implications of disruptive technology in the fields of food analysis and metabolomics.

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A comprehensive understanding of samples has been out of reach for GC-MS users for too long. The Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system and the new Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system have changed all of that.

The Q Exactive GC Orbitrap GC-MS/MS system is here with the superior resolving power, mass accuracy and sensitivity that only Thermo Scientific™ Orbitrap™ technology can deliver. And the Exactive GC Orbitrap GC-MS system brings the power of high-resolution, accurate-mass (HR/AM) analysis into the routine environment for the first time.

Both systems allow scientists working in the fields of food safety, environmental, industrial, forensics and anti-doping to revolutionize their workflows by taking their analytical capability to the next level.

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Following a Story of Innovation

Back in 2013 – the year The Analytical Scientist launched – I interviewed Alexander Makarov to discover the secret to true innovation; what does it take to invent something as disruptive as Orbitrap technology? (Find some of the answers here: http://tas.txp.to/NextGenGCMS/Orbitrap).

Just two years later (on Orbitrap's 10th Anniversary) I had the opportunity to see how far Makarov's dream of an "Orbitrap in every lab" was coming along. The short answer? Very nicely, thanks to an important addition to the portfolio in 2015.

In the following compendium of articles and videos, Alexander Makarov is joined by esteemed analytical scientists from the fields of food analysis and metabolomics to discuss the impact of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system – the next logical step in the Orbitrap story.

So, what does high resolution, accurate mass, full-scan mass spectrometry bring to the gas chromatography party? Hans Mol, Jana Hajšlová, Richard Fussell, and Amadeo Fernández-Alba consider the value of such technology – especially with the launch of the new Thermo Scientific™ Exactive GC system, which brings the power of Orbitrap GC-MS into the routine environment. And Joshua Coon, Nicholas Kwiecien, and Karl Burgess reflect on the implications for the highly complex world of metabolomics, where advanced tools that can increase metabolite coverage are always highly anticipated.

Welcome to "Next Generation GC-MS."

Rich Whitworth
Editor, The Analytical Scientist

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Orbitrap: Ten Years Young

Coupling gas chromatography with Orbitrap™ technology wasn't easy, but the outcome – the introduction of the Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS system– represents a big step towards bringing full-scan, high-resolution, and accurate mass data into routine labs around the world. And my dream of an "Orbitrap in every lab" inches ever closer.

By Alexander Makarov, Director, Global Research Life Science Mass Spectrometry, Thermo Fisher Scientific, Germany.

We started thinking about GC-Orbitrap technology a long time ago – very soon after the dust had settled following the launch of the first commercial instrument at the June 2005 ASMS Conference in San Antonio, Texas – the LTQ Orbitrap tandem mass spectrometer. But back then it was clear that one or two second peaks were too narrow for the wide application of Orbitrap technology in GC. Nevertheless, Joshua Coon (a professor at the University of Wisconsin-Madison) expressed an interest and we initiated a research project to look at the potential. Originally, the project was simply a continuation of the mainstream work in his lab, which focused on electron transfer dissociation (ETD) for the LTQ Orbitrap instrument. At that time, ETD utilized anions that essentially came from the ion source of a GC quadrupole system, and so the connection was relatively straightforward. Indeed, the work resulted in the first rudimentary GC-Orbitrap system. The initial data proved that there was high potential, but also indicated some challenges.

Amelia Peterson (from the Coon Research Group) came to the Thermo Scientific research lab in Bremen, Germany to continue work on the GC-MS-LTQ Orbitrap instrument and, on her return to UW-Madison, presented several applications in high-impact journals. Although the projects were only exploratory in nature, they proved invaluable in allowing us to gauge interest in GC-Orbitrap technology – and a number of customers began asking for more information.

An essential confluence

In reality, we were not able to communicate any fixed date about GC-Orbitrap technology to our customers. We needed to fully assess what was needed in the market and put together an entire development team – and Orbitrap technology was still not ready for GC.

Over the next few years, information was gathered and the potential became clearer – but, more importantly, Orbitrap technology development continued. By 2011, we had increased the speed of the Orbitrap by a factor of four, by combining two innovations: i) enhanced Fourier transform algorithms, which doubled resolving power, and ii) the high-field "compact Orbitrap" (where an increase from 3.5 kV to 5 kV boosted frequency by 20 percent and the smaller trap provided a factor of 1.8 increase in speed.) Finally, we had an Orbitrap analyzer that was completely compatible with GC separations. At the same time, a talented development team became available in Austin, Texas, which could take on the not insignificant challenge of giving GC its first new mass analyzer in half a century.

Without these streams coming together, we could not have moved forward; the confluence of user demand, increased Orbitrap capability (in terms of speed, sensitivity, mass accuracy and selectivity), and the necessary resources gave us the critical mass we needed to begin in earnest. At which point, the ball started rolling very quickly.

What Orbitrap technology means for GC – and vice versa

At ASMS 2015, exactly 10 years after the introduction of the LTQ Orbitrap system, we launched the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system – an excellent way to celebrate Orbitrap's anniversary. What does the Q Exactive GC system offer the world of GC-MS? The real breakthrough is the combination of accurate mass with high sensitivity. Imagine a triangle of mass accuracy, sensitivity and speed – traditionally, optimization of mass accuracy comes at the sacrifice of the other two factors. Instruments that were not constrained by mass accuracy – triple quadrupoles, for example – were far ahead of the game in terms of sensitivity and speed. On the other hand, the only accurate mass instruments – time-of-flight systems – suffered from a severe compromise in other features. In other words, the size of the triangle is limiting. Orbitrap technology expands the triangle so drastically that we can now match the speed and sensitivity of triple quadrupoles, but at the same time provides high mass accuracy and resolution.

Since its launch, I've been pleased to see an extremely enthusiastic reception to the Q Exactive GC system from the community. People are excited to learn how their samples behave; we've already shared stories from Hans Mol in pesticide analysis, Karl Burgess in metabolomics, and Jana Hajšlová in food authentication in this article series – and I think there will be many more interesting stories to be told as the technology is adopted in labs around the world.

GC is an interesting addition to Orbitrap technology as it combines high resolution GC separation (with

its large peak capacity) with the high resolving power (and mass accuracy) of the Orbitrap mass analyzer. The combination allows us to look deeper into the volatile and semi-volatile end of the analytical spectrum than we have done before – and with high clarity. Moreover, classical GC-MS with electron ionization reduces the need for MS/MS analyses, making straightforward full scan a routine mode of operation, without losing vital fragment information.

Indeed, we were surprised how far simple full-scan MS analysis could take us, using a combination of spectral library matching (with the vast, commercially available nominal mass libraries) and high resolution-accurate mass filtering. Acquisition using MS/MS is still important, but is typically used with chemical ionization mode in the search for further structural information about a compound for higher level confirmation – or, of course, to help us build an understanding of a compound that is not known and does not appear in libraries. The point is that, even though the technology appears to be more complex, the high resolution and accurate mass gained actually make analyses simpler, reducing the

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need for tedious method development. I think that has surprised a number of experienced analysts.

Clearly, Orbitrap brings something very new to GC – but the innovation also means that our technology is stepping outside its more traditional setting in life science applications. For me personally that means a lot, because I believe that the combination of easy mass accuracy and sensitivity could benefit many other types of analysis – we just need to look further into where unique advantages can be gained.

We also learned a lot in the development process, for example, how to reduce or completely eliminate ion molecule reactions, which were not present in electrospray produced ions, but were visible for ions produced by electron impact ionization. And we have now adopted a modular approach – the Orbitrap is one module that can be combined with a number of different front-end modules (ion sources). And excitingly, we now have two product development lines – one for LC and one for GC. Though the Q Exactive GC system is an important milestone in Orbitrap history, rather than considering that it completes the story, I like to believe that it is the beginning, with more expansion ahead.

Orbitrap trajectory

I can foresee several different trajectories for Orbitrap technology; for example, analysis of aerosols and other ion sources. And we have even discussed the potential of sending Orbitrap technology into space with various agencies – Orbitrap in orbit! Certainly, we are keen to investigate any area where the combination of analytical qualities that Orbitrap technology provides can add real value – and that takes time. But where serious opportunities exist, we will be pushing the boundaries of what is possible.

I would consider Orbitrap game-changing or even disruptive technology – especially now that we've entered into the world of GC with the Q Exactive GC system – but I don't think all other MS technology will (or should) retire just yet. If we look back at the history of mass spectrometry, even some of the earliest examples of hyphenated analyzers, such as magnetic sector instruments, are still leading in those areas where they confer a distinct advantage – and there are probably more magnetic sector instruments produced today than 30 years ago. Yes, we will see expansion and contraction of market share, but each will retain its own niche – and it really depends how attractive those niches are. Certainly, LC and GC applications are growing rapidly, with thousands of instruments worldwide, so this area often gets all the limelight – and here I expect Orbitrap technology to continue expanding at a higher rate than other analyzers. Why? Because it is fundamentally simple technology; it uses three electrodes with one voltage and its data system is a conventional PC. As a result, it has the potential to be competitive to quadrupole instruments in terms of investment.

We're not quite there yet – after all, we are working at the edge of what humanity can provide in terms of electrode accuracy and electronics stability – but the simplification trend has already begun; for example, if you consider the evolution from LTQ Orbitrap with five turbomolecular pumps to Q Exactive with two, you can see the tendency to use acquired knowledge and advances to decrease complexity and increase accessibility. Another example is the introduction of the Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer – specifically for heavy workloads in environmental and food safety – at a price that is comparable with high-end triple quadrupoles. In other words, Orbitrap is on a continually shifting pathway – and I hope that will continue for years to come.

In the end, the simplicity of the Orbitrap analyzer's design will be key to the future simplification of the technology – at that point, my dream of an "Orbitrap in every lab" starts to sound realistic.

Video interview with Alexander Makarov: tas.txp.to/1015/MakarovGC
To find out more: thermoscientific.com/QExactiveGC

The Power of High Resolution Accurate Mass Using Orbitrap Based GC-MS

By Cristian Cojocariu, Dominic Roberts, and Paul Silcock, Thermo Fisher Scientific, Runcorn, UK.

The Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer is a benchtop instrument, designed to bring the power of Orbitrap high-resolution, accurate-mass (HR/AM) to gas chromatographic (GC) separations.

Obtaining mass spectrometer (MS) measurements with high mass accuracy is essential to providing the required selectivity in complex matrices and to increasing the confidence in compound identification and confirmation. For the former, obtaining a consistently high mass accuracy allows the use of very narrow mass extraction windows, taking full advantage of the instrument's mass resolving power. For the latter, measuring the mass of a chemical with sufficient accuracy allows the chemist to predict the elemental composition and isotopic ratios to help identify the chemical structure of the substance.

The results of this work demonstrate that, using the high resolution of the Q Exactive GC mass spectrometer, excellent mass accuracy is always available to the user, across a wide concentration range and in the presence of a complex chemical background.

Selectivity through accurate mass

With Q Exactive GC technology, one can achieve selective detection of target analytes in complex matrices through the use of very high resolving power that delivers sub-ppm mass accuracy. This capability is demonstrated in Figure 1, where a leek sample extract (spiked with several pesticides at the 10 ng/g level) was acquired in full scan at 60k resolving power (FWHM at *m/z* 200). Extracting the exact mass for iprodione (*m/z* 314.0094) with a ±5 ppm mass tolerance window enables generation of a highly selective extracted ion chromatogram (XIC) with reduced chemical interferences from the leek matrix background. In contrast, a nominal mass extraction window (±3,184 ppm), simulating unit-mass resolution acquisition, will not provide enough selectivity to detect this pesticide (Figure 1).

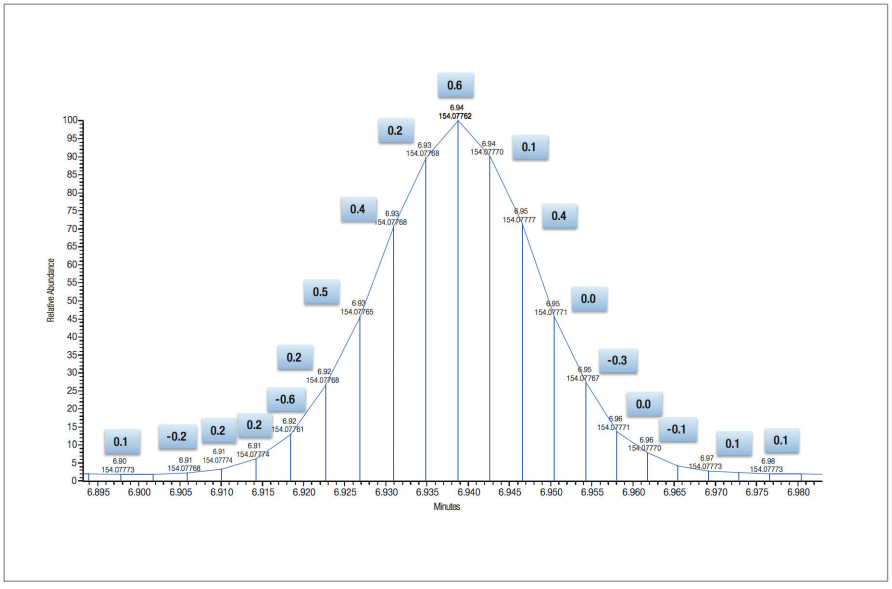


Figure 2. Scan-to-scan mass accuracy of 0.5 (RMS) of biphenyl identified in a carrot sample at 10 ng/g level. Data acquired at 60k (FWHM at *m/z* 200) resolution with >18 scans/peak (peak width 4 sec).

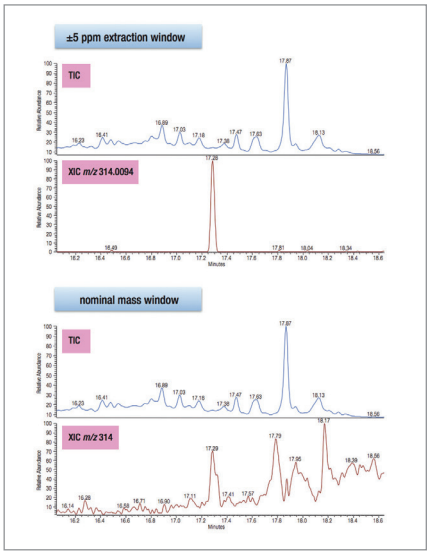


Figure 1. Full scan accurate mass selectivity demonstrated for iprodione at 10 ng/g in a leek sample. Accurate mass measurements enable confident detection (±5 ppm) (top), whereas at nominal mass acquisitions this pesticide is not detected (±3,184 ppm) (bottom).

Consistent sub-ppm mass accuracy across peak profile

The Q Exactive GC system provides routine stable mass accuracy of <1 ppm (internally calibrated) across the entire chromatographic peak, from the scans measured at the inflection points to the apex scans (Figure 2).

Read the full application note: http://tas.txp.to/NextGenGCMS/one

Reinventing GC-MS

At ASMS 2015, GC-Orbitrap™ technology was unleashed onto an expectant analytical community. Here's the backstory.

By Joshua Coon and Nicholas Kwiecien, Department of Chemistry, University of Wisconsin-Madison, USA.

Historically, The Coon Research Group has been focused on protein analysis with mass spectrometry. More recently, we've been interested in small molecule work in the field of metabolomics. It's pretty clear that quantifying small molecules can give a better correlation with biological phenotype than work further upstream. Moreover, until very recently, it was an area in serious need of new technology – and that's where our interest in coupling gas chromatography with Orbitrap technology started. As a group, we're very driven by new technology and its application to problems – especially when there's such a fundamental gap. Sure, you can already detect these small molecules pretty effectively with mass spectrometry, but more often than not, you can't understand their chemical formula. And it's very hard to go from signals in a spectrum to biological function, if you don't know what the molecule is... How can we identify these structures? Well, GC coupled with Orbitrap and its accurate mass capability seemed to be a great starting point to solve this problem.

Seize the gap

Clearly, there is a big difference between recognizing a gap and attempting to fill it. But fortuitously in the mid 2000s, we worked on a separate development project in collaboration with Thermo Fisher Scientific on electron transfer dissociation (ETD) for the Orbitrap, and we all recognized that it would be relatively straightforward to use that test system to try GC on an Orbitrap. The first 'Frankenstein's' system certainly wasn't practical, but it gave us data. In fact, it worked so well that another collaborative project was initiated to further investigate the potential. The short version of the story is that those initial efforts sparked Thermo Fisher Scientific's development cycle (led on the R&D side by Brody Guckenberger and Scott Quarmby) for the commercial instrument that was released at ASMS 2015: the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system.

Of course, going from a proof-of-concept system to commercial instrument is in no way straightforward. And a big – often overlooked – part of the journey involves leveraging informatics. That's where Nicholas (Nick) Kwiecien stepped up to the plate. We were generating a lot of data – and if you knew what you were analyzing, you could get the right

answers. But how do you go backwards? Nick expressed interest in trying to figure it out and came up with some outstanding ideas on how to leverage accurate mass to get back to structure.

For the past 50 years or so, people have been using GC-MS systems equipped with unit resolution mass analyzers – and that means there are a lot of great resources out there in terms of mass spectra repositories. The big question became: how can we leverage those resources? The answer led us to an innovative algorithm call high-resolution filtering (HRF), which is incorporated into the data processing software for the new instrument. HRF is uniquely enabled by the mass accuracy provided by Orbitrap technology and allows us to search existing reference databases with our acquired spectra in the same way people have been doing for many years. But because we have such precise accurate mass, we can annotate every single peak in a spectrum using a simple combinatorial process. We take combinations of atoms from putatively identified molecules and map those forward to peaks. The approach was extremely discriminatory against false positives, and should really increase the throughput of mapping unknowns back to structure.

Taking GC-Orbitrap for a spin

We've taken on a large number of proteomics studies – thousands of different cell lines or hundreds of tissue samples – to try to understand how protein abundance varies from sample to sample. Now, we can complement all of those experiments with deep and high-quality metabolome profiles generated by the Q Exactive GC system.

Our first acquisition of a 1200 sample set showed that the correlation between the metabolome and proteome profiles is remarkably close. It turns out that it's much easier and faster to collect metabolome profiles GC-Orbitrap technology than it is to do proteomics. Given very large sample sets, we envision that our group – and many others – are likely to perform broad metabolome work to discover the most meaningful population subsets ahead of further work in the proteomics space.

With high quality data for both the proteome and the metabolome, you can investigate a small molecule with raised abundance and match it to the upregulated enzyme responsible. Such studies really allow you to understand function across the whole pathway at multiple molecular planes – from small molecule to protein.

"With high quality data for both the proteome and the metabolome, you can investigate a small molecule with raised abundance and match it to the upregulated enzyme responsible."

Monitoring reactions

The folks at ASMS 2015 that we spoke to seemed very interested in acquiring the technology; you can almost hear them thinking how they can integrate GC-Orbitrap technology into their work. And certainly there have been lots of questions. Perhaps more interestingly, people who have not traditionally done metabolomic work (certainly, not in the way that we have done) appear to be seriously tempted by the possibilities. Indeed, there is a distinct air of surprise surrounding some of the corresponding proteome and metabolome results we've been able to show – especially at the scale we've worked on.

In our own lab there have been moments of surprise too. Frankly, we were quite shocked by how well the new instrument worked right out of the box. We'd been using the proof-of-concept system, which was not really capable of the sample throughput needed for our large-scale studies. So when we set up the new instrument and realized that the crew at Thermo Fisher Scientific had taken the GC-Orbitrap concept to a completely different level. The Q Exactive GC system was a real surprise – in a very good way. Suddenly, we had the throughput to match the quality of the data.

People also seem really excited about the capability of the software tools mentioned earlier that are included with the instrument. I think our most fundamental contribution (besides providing a motivating force for instrument development) is offering the solution to deal with the data. I guess that sort of capability is on everyone's wish list – but previously we didn't have the right data to permit those kinds of algorithms. Now, we do.

Beyond metabolomics

Our group is very excited about the instrument's ability to map unknowns. But there are a lot of areas where scientists want to look for compounds that they already know – in pesticides and sports doping, for example. If you know what you're looking for, the system still offers many benefits. The accurate mass really boosts sensitivity,

because you can pick out targets from chemical noise. It means you can achieve the level of sensitivity for target analysis that is approximately the same as the most sensitive GC instrument – the triple quad. But (and it's a big but) you can cover all the ions in the spectrum. Where sensitivity coupled with full scan capability is highly sought after, GC-Orbitrap technology will be of great interest.

From an informatics point of view, the fact that the data is so remarkably reproducible is also a pretty big deal. For our largest scale project to date, we had to cope with data files that were collected 45 days apart – but the runs looked the same. Such reproducibility really helps you gain access to meaningful results much faster – and it also facilitates the writing of custom code to analyze your data.

10th Anniversary

At ASMS 2015, Orbitrap celebrated its 10th birthday. Where will GC-Orbitrap technology be at its own party in 2025? Well, you can bet that the instrument will continue to improve over the next 10 years – that's just the trajectory of Orbitrap technology. At the same time, we're rapidly going to get a handle on unknown mapping and quantitation. Assigning identifications to unknowns is the current bottleneck in metabolomics (and a lot of other small molecule analyses) – and that's simply got to change. Accurate mass will allow people to go beyond current spectral libraries – and who knows how far software will have come by then? In terms of scale, today we're running 1000 samples and that's considered impressive. In 10 years, people won't be shocked by numbers 10 or 20 times bigger. And at that scale, you can almost force discovery.

As the technology rolls out, it's very likely that it will be used in areas that we cannot even envisage right now. Even talking to people at ASMS this year, exciting new ideas are already pouring forth; it's clear that once you introduce powerful new technology, the sky is the limit.

Video interview with Joshua Coon: tas.txp.to/0615/JoshuaCoon
To find out more: thermoscientific.com/HRAMGCMS

Cutting-Edge Metabolomics

As new technology platforms push us to the limits of what's possible, the metabolomics community is closing in on the future of the field: routine and rapid quantitative analysis.

By Karl Burgess, Head of Metabolomics, Glasgow Polyomics, University of Glasgow, Scotland.

Believe it or not, I started out as an undergraduate in computer science and cybernetics. Unfortunately, the world of robotics involved a lot more mathematics than I expected. And so after a year of computer programming, I switched to pathobiology. But I never lost my interest in computers and programming, and that has been invaluable as I've progressed through my career; during my postgrad days I moved into bioinformatics and molecular modeling, which brought my two halves together. I soon realized that I wanted more time in the lab, which led me to do a research-based masters degree in biological and biomedical science.

I ended up in the proteomics lab at the University of Glasgow in a world where robotics, wet-lab work, biology and computer happily co-existed. I'd found my calling – at least for a while. Using mass spectrometry coupled to computational techniques that make sense out of the biological data is where my broad interests now lie. In many cases, it's not about creating new algorithms, it's about processing data and presenting them in a useable format that biologists can understand.

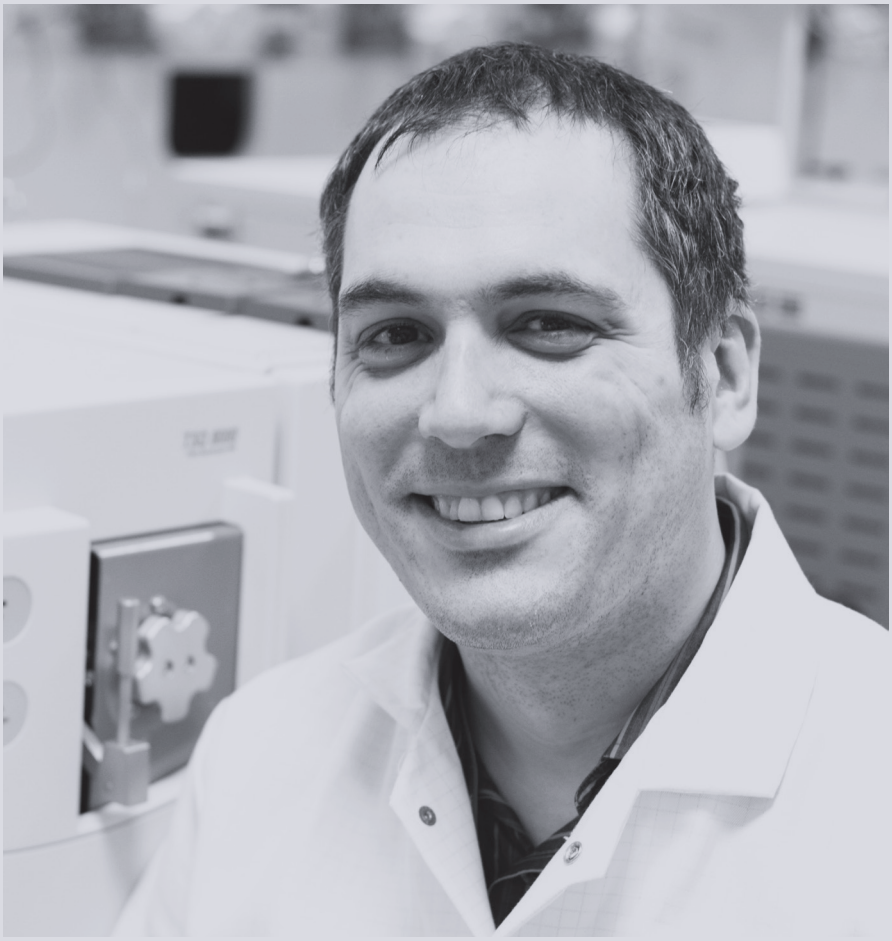
Orbitrap temptation

So, why the shift from proteomics to metabolomics? One of the reasons was to get my hands on an Orbitrap instrument to be honest... I actually started out uncomfortable with the idea of metabolomics – it's a completely different ball game. In proteomics, we could use Mascot to provide a probabilistic score for a given protein based on the mass spectra. You can use a cut-off system and, much like a court of law, you end up with an innocent or guilty verdict on the identity. In metabolomics, we were working entirely on mass and retention time – it's a very binary way of working and felt quite limiting; it was a "yes" or "no" answer to identification without knowing how

certain you were in either case. Now, we're building fragmentation libraries and the requirements for supporting metadata in studies are increasing all the time.

Indeed, metabolomics is now very rigorous – and it's been a big learning curve for me in terms of quality control. Excellent reproducibility is key; dozens of replicates may be necessary to get the statistical quality for quantitation. And that's the point where clinicians start to become very interested – robust, quantitative data on biomarker-style molecular relationships they are used to working with.

I did most of my PhD work on a relatively fast-scanning but pretty low-resolution ion trap instrument. When I first got an Orbitrap instrument (an XL), I was showing my boss the data at 100,000 resolution, and he actually thought it was centroided – I had to zoom in about 20 times before I could demonstrate the reality of the peak widths. It was a really great moment! I've also done some work on high-resolution QTOFs, but stability of mass accuracy was a problem. The Orbitrap has always been rock solid in that regard. In fact, when we bought our ex-demo XL, it had been boxed up in the demo lab, left in a crate for three months, unboxed outside the building and bumped up a rough slope into the lab. After pumping the instrument down we found that it was still within 3ppm...



Metabolomics today

Heading up metabolomics at Glasgow Polyomics means that I get to work on some really diverse projects – all sorts of crazy samples. Indeed, the whole facility is geared up to apply state-of-the-art technologies to investigate biological systems by combining multi-level, multi-omics datasets.

As an example, we've had a lot of success partnering with Matt Dalby's group on the analysis of stem cell differentiation and interaction with surfaces. With Matt, we've got some fantastic collaborations (Nikolaj Gadegaard and others, who make nanopatterned materials) where we explore how different nanostructures promote different kinds of differentiation. Obviously, if differentiation occurs, there are lots of complex modifications to the metabolome. Tracking these changes over the course of differentiation on different surfaces is enormously powerful.

I'm now trying to tie up my interests in infectious disease with the surface attachment work in the area of bacterial biofilms. Infection of medical implants is a really significant problem, especially with antimicrobial resistance increasing. We're looking into novel antimicrobials that modify biofilm formation with endogenous metabolites and repurposed drugs. I've got a great collaborator: Gordon Ramage, who works in the Dental School and has been analyzing multispecies biofilms for many years. With his expert clinical microbiology knowledge, and the three PhD students we've got on the project, we're now starting to get some interesting results.

On the software side, we're working on probabilistic annotation of metabolites from data using a Bayesian clustering approach. This is part of the drive towards providing a meaningful probabilistic analysis of identification. In many ways, it's a first step towards creating a framework in which we can slot multiple measures of physicochemical

properties to determine the likelihood of a particular ID.

GC-Orbitrap joins the party

We've already put GC-Orbitrap technology to the test in a really cool project called 'the way of all flesh' with Richard Burchmore, which is essentially analyzing the decomposition process of dead bodies. Time of death is really tricky to work out once liver temperature has dropped to ambient. And so, the search is on for biomarkers of death, using metabolomics and proteomics. First, we let a big piece of steak decompose over a 12-day period, taking MS datasets as time went by. We got some very interesting leads in terms of amino acid biomarkers.

Whilst at Thermo Fisher Scientific in Runcorn, UK, we were able to move onto rat models. First of all, the data reproduced the work we'd done on LC-MS previously, but the added resolution and the presence of the NIST libraries allowed us to distinguish things like sugar isomers that we have difficulty with on our untargeted LC-MS method. In fact, the software on the GC-Orbitrap system allows us to automate metabolite identification using enhanced spectral deconvolution, NIST library candidate searching and accurate mass filtering. Sensitivity was phenomenal; with a 1 µL injection we were overloading the system, so we had to move to split injections.

In the final stage of the project, we managed to acquire samples over various time periods from a body farm (or more correctly, a forensic anthropology research facility) in Texas. We are gearing up to run the human work on the freshly installed GC-Orbitrap system in our lab right now – exciting stuff. We're hoping that GC-Orbitrap technology can deliver better coverage of the biomarkers we've discovered, as well as the opportunity to perform good quantitative measurements.

We presented all of our findings at the 11th International Conference of the Metabolomics Society in San Francisco Bay Area in June 2015.

In the near future, I'm also looking forward to doing a lot of biofilm work on the instrument. I actually started this research area as it provided a platform for pushing metabolomics innovation, but once you've got your own bit of biology to investigate, it all gets quite exciting. High-resolution separations and mass accuracy are really key to analysis of biofilms.

Moreover, the GC-Orbitrap enables untargeted metabolomics because it provides accurate mass full scan data rather than targeted transitions, as you would get on something like a triple quad. The array of quorum sensing molecules that bacteria use to communicate with each other triggering, for example, biofilm adherence and dispersal, are very diverse, and not yet well characterized. An untargeted approach gives us the potential to identify new compounds; accurate mass EI fragments allow us to characterize them. Additionally, high GC resolution allows us to separate isomeric compounds and, with some extra chemistry, even chiral compounds, which are extremely important in bacterial signaling and peptidoglycan synthesis.

In metabolomics, we're essentially looking for everything. Therefore, access to NIST libraries is enormously powerful as it allows us to make unexpected discoveries in a non-targeted fashion. Targeted metabolomics by definition narrows the field.

Metabolomics of 2025

In my view, GC-HRMS is fast approaching the point of being the ultimate metabolomics platform. And LC-MS is catching up rapidly. In 10 years, I predict that metabolomics will be easy (!) You'll buy an instrument and a set method, and advanced software will do the work for you. In an ideal world, we'll have contributed heavily to the development of that software. We've got quite a few publications in software and algorithm development for MS, and they're beginning to coalesce into one single web-based platform. Once again, it's about providing people with useful, interesting data. I would say software is the biggest challenge right now; the hardware tools we need are here.

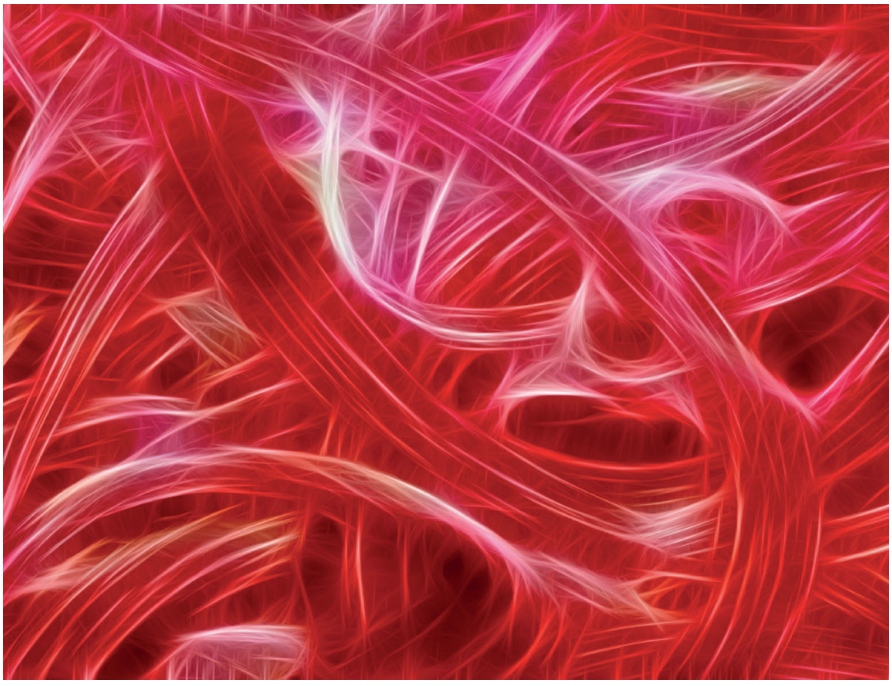
As far as GC-Orbitrap technology goes, I'm deliberately trying to keep my acquisition a bit of a secret (this article won't help). The people I have told are extremely excited about the prospect of running samples and, candidly, I don't want a never-ending backlog just yet.

Even if I'm 10 percent more confident in the data, it's really important – and in reality, it's a lot more than that because I can provide compound matches to fragment patterns in percentage terms and then use accurate mass to really drill down into specific fragments. To put it simply, GC-Orbitrap technology gives us extra confidence. And confidence is an extremely important asset in our field.

Video interview with Karl Burgess: tas.txp.to/0515/KarlBurgess
To find out more: thermoscientific.com/HRAMGCMS

Untargeted Metabolomics Using Orbitrap-Based GC-MS

By Stefan Weidt, Bogusia Pesko, Cristian Cojocariu, Paul Silcock, Richard J. Burchmore, and Karl Burgess.



Metabolomics aims to characterize and quantify the complete small molecule complement, or metabolome, of a biological system. The metabolome consists of a diverse mixture of small molecules, including amino acids, sugars and phosphosugars, and biogenic amines and lipids. Untargeted metabolomics is exceptionally challenging due to the requirement to both identify and quantify hundreds of different compounds with limited a priori knowledge of the metabolites. It is, therefore, advantageous to use a detection system that is not only capable of sensitive detection of specific molecules in an untargeted way, but can also provide accurate mass information for confident confirmation and structural elucidation of unknowns.

Gas chromatography-mass spectrometry (GC-MS) is routinely used for metabolomics applications due to its inherent advantages, especially its chromatographic resolution, reproducibility, peak capacity, and convenient spectral libraries. GC provides excellent chromatographic separation capability for biomarker discovery using untargeted metabolomics, but has previously been hampered by the lack of high-end mass spectrometry support providing the dynamic range, accurate mass, and scan rate sufficient to analyze very complex samples, such as mammalian muscle tissue. The polar nature of the majority of central metabolites means that derivatization must be performed to allow effective volatilization and ensure good chromatography. High sample throughput and advanced automation is required for metabolomic analysis, especially for clinical metabolomics.

This work demonstrates the application of a complete untargeted metabolomics workflow using a novel Thermo Scientific™ Orbitrap™ MS-based GC to detect biomarkers for time of death in a rat model. Estimation of postmortem interval (PMI) is one of the most critical, yet difficult, tasks in forensic investigation, particularly after the cadaver has equilibrated to the ambient environmental temperature. Current methods to determine PMI are inaccurate and primarily based on visual inspection of the body. A laboratory-based method, using a robust biomarker for PMI, would assist forensic investigation.

This GC-MS configuration using an Orbitrap-based detector enables ultra-high mass resolution, sub-ppm mass accuracy, a large dynamic range, and a scan rate commensurate with the efficient quantitative analysis of highly complex metabolomic samples. The high resolution, mass accuracy, and scan speed is critical for consistent data deconvolution to permit the detection of species from overlapping TIC peaks, allowing for an untargeted metabolomics pipeline. Accurate mass electron ionization (EI) fragment patterns are also suitable for matching against the widely available NIST and Wiley libraries for tentative compound identification, while providing accurate mass for more in-depth characterization.

Read the full application note: http://tas.txp.to/NextGenGCMS/two

Stefan Weidt, Bogusia Pesko, Richard J. Burchmore, and Karl Burgess are based at Glasgow Polyomics, University of Glasgow, Glasgow, UK. Cristian Cojocariu and Paul Silcock are based at Thermo Fisher Scientific, Runcorn, UK.

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 Orbitrap GC-MS/MS 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 Thermo Scientific™ Q Exactive™ GC Orbitrap™ system. 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 pesticide 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 system 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.

What makes the Q Exactive GC system 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 on page 20.

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 4, 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 Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, 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, 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 manufacturers 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...

Video interview with Richard Fussell: tas.txp.to/1015/Fussell
To find out more: thermoscientific.com/QExactiveGC

The Frontier of Gas Chromatography

When I was asked to evaluate a brand-new instrument with disruptive potential in my field, I did not spend long thinking about the answer. Here, I share a little background and my first impressions.

By Hans Mol, Group leader Natural Toxins and Pesticides, RIKILT Wageningen UR.

I've been working for nine years at RIKILT-Wageningen UR in the Netherlands, predominantly working with the government on aspects of food and feed safety. For that reason, we are always interested in evaluating new instruments and techniques that can address the current – and future – challenges facing us. As such, I was very pleased to have a pre-production version of the much-anticipated GC-Orbitrap sitting on my lab bench...

Looking back, I've often been fortunate in finding myself at the cutting edge of GC.

My analytical journey really began when I did my masters project in Udo Brinkman's group at the Free University in Amsterdam – he was well-known and respected, and my interest grew. I continued onto a PhD at the Technical University in Eindhoven under professor Carel Cramers – a couple of years behind Hans-Gerd Janssen (who actually supervised my PhD). My research was very much focused on large volume injections (for residue analysis), using programmed temperature vaporizing (PTV) injectors. PTV is commonplace now, but this was in the early 1990s – and it was somewhat disruptive technology back then, competing with retention gap, on-column-type of large volume injections from other groups. We were pretty sure early on that, in routine applications for food and environmental samples, PTV would become the industry standard.

I finished my PhD in 1995 and continued on as a post-doctoral research working on GC coupled to both MS and an atomic emission detector (AED). I then worked for about 10 years for a contract research organization offering analytical services for food, (agro) chemical and pharmaceutical industry. Importantly, we did a lot of method development work on LC-MS, GC-MS – and myriad other techniques – and I gained a great deal of experience. And that brings me to RIKILT.

There have been many technological advances over the past 20 years or so. The availability of LC-MS for food and environmental analysis was a huge milestone. When I started, the field was very GC oriented. If compounds were not amenable to GC, we would use derivatization to make them amenable. LC was a last resort in some ways – until the commercialization of electrospray ionization. As the instruments became increasingly affordable (they were already in use in big pharma with its big budget) – they changed the field.

Another step change was the introduction of high-resolution MS (HRMS) techniques (time-of-flight (TOF) or Orbitrap instruments) to LC-MS; indeed, in certain applications these are now replacing triple quadrupole instruments.

But what about similar progress in GC? Much of the effort from instrument suppliers seemed to be focused on LC (remember the pharmaceutical industry's big budget?) and GC – despite its utility in persistent organic pollutants and pesticides – was left behind. Until now.

I expect the new GC-Orbitrap instrument will count itself among the aforementioned milestones and redress the imbalance!

GC-Orbitrap technology lands

Back in mid-2015 (at the time of writing), one of the first GC-Orbitrap instruments was installed in my lab. Ahead of installation, the space we created raised a few eyebrows with certain visitors (other instruments had to be relocated). Anticipation was high, so keeping the installation a secret was not easy.

Before the installation, we had the opportunity to see the instrument at Thermo Fisher Scientific's operations in Runcorn, UK, and it looked very promising, so we had high expectations – as did my colleagues, who have been forming a relatively orderly line, samples in hand, ever since! Over the months that followed, we've been putting the instrument through its paces.

The main challenge in my particular field is the sheer number of pesticides of interest – around 1400. The question is relatively simple: "are there any pesticides in this sample, and if so are they above the maximum residue limit (MRL)?" For targeted analysis, you can use a triple quadrupole MS system, but you're limited in terms of scope, because you are only measuring pre-defined compounds. If you want to look for something new or different, you need to go back to your sample and re-run the analysis.

"The main challenge in my particular field is the sheer number of pesticides of interest – around 1400."

Conversely, with full-scan methods, you inject your extract, measure the compounds of interest but have the option to look back into the raw data for other analytes. Moreover, the number of compounds that can be measured in a single run is much higher than a triple quadrupole. Using a dedicated triple-quad method, you can routinely target

100-150 compounds (instruments have improved here as well – shorter dwell times potentially allow a slightly higher number to be squeezed into a given method). But with full-scan analysis, you measure everything – and there are 700-800 pesticides that are amenable to GC. That's a gain we are excited about.

From a method development point of view, there are also advantages to full-scan analysis because the conditions can be quite generic. In fact, there's little optimization needed at this stage – that's addressed in the data handling. In contrast, in GC-triple quad methods, you have to set acquisition windows and if you want to add compounds you need to optimize the transitions for each of those compounds. In simple terms, it takes more time.

Hands on – first impressions

In terms of resolution, the GC-Orbitrap is clearly a major step forward, outperforming everything on the market. And so in Runcorn, we were more interested in assessing sensitivity and selectivity. We ran a calibration curve in a more difficult matrix (a leek sample) and were impressed by the sensitivity, which was actually better than the triple quadrupole instrument in our lab. However, our instrument is previous generation, so the next question was, how does it compare with the current generation of triple quads? Fortunately, we were able to perform that experiment in Thermo's lab, which had the two set ups side by side. For the analytes tested, comparable results were obtained.

Maintaining sensitivity while adding the full-scan capability (and the advantages that come with it) is a big plus point. Selectivity is equally important but, to be honest, I think that's much more difficult judge – we need to run more samples and look at more analytes to form a fuller picture on how HRMS compares with MS/MS, which also has limitations, especially in terms of electron ionization (fragments of fragments become less and less specific after all).

Complex samples, such as food supplements, are perfect to test the true capability of GC-Orbitrap. Feed ingredients are also very complex (essentially they are manufactured from any food industry output that holds nutritional value but which cannot be used for anything else). Traditionally, such samples present real challenges in terms of detection limits, demanding more attention and time on sample preparation and method development. Broadly speaking, the GC-Orbitrap will help; we can use fewer methods because of the selectivity, and the sensitivity will allow us to reduce injection volumes (from around 5µl down to 1µl) or to use less concentrated samples. By introducing fewer co-extractants in this way, we can reduce deterioration in GC performance.

One of my colleagues works on forensic-style analysis and has expressed particular interest in the GC-Orbitrap. The samples in these 'cold cases' are 'suspect' but we don't know why – has something toxic been added at some point in the supply chain? Alternatively, there may be a dead animal and a big question mark. Different procedures apply in this field because the analysis needs to be as unbiased as possible. Samples must be screened and then cross-referenced against very large NIST libraries to find a match. Alternatively, comparative analysis against known reference products can be useful to assess which samples are deviating from 'normal' by overlaying profiles and identifying suspicious peaks. Up to now, this type of work is being done with comprehensive GC (GC×GC) with a nominal mass (low-resolution) MS system. We are very interested in the potential of doing the same analysis using one-dimensional GC coupled with high-resolution (Orbitrap) MS.

Surveying a changing landscape

I'm not one to make sweeping predictions, but I expect that targeted methods with triple quads will be phased out as time goes on. Full scan instruments are just as capable – and even if you don't get sufficient selectivity, with Q-Orbitrap or Q-TOF you have the ability to do MS/MS as well. At a certain point, the question will become: why do I still need a triple quadrupole instrument? I can only think of one reason: its highly stable quantitative performance – and that's another area I am very interested in exploring with the Orbitrap.

Will the transition from triple quadrupole methods happen overnight – or in five years? Well, even if the instrument far exceeds all our expectations, there will be a considerable lag in wider adoption. After all, our lab is working at the cutting-edge – we're much quicker to evaluate and embrace the great and the good. In more routine analysis, extra time will be required for general acceptance – and established procedures must be challenged and changed. After all, the GC-Orbitrap is something very new and different indeed.

Video interview with Hans Mol: tas.txp.to/0415/HansMol
To find out more: thermoscientific.com/HRAMGCMS

High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry

By Dominic Roberts, Hans Mol, Marc Tienstra, Cristian Cojocariu, and Paul Silcock.

Laboratories are under ever-increasing pressure to screen samples for pesticides in a single analysis, with a fast turnaround time and at a competitive cost. Most existing laboratories rely on targeted analytical approaches using both gas chromatography and liquid chromatography coupled to mass spectrometry instrumentation. These techniques cover the wide range of chemical classes that need to be monitored and at the required levels of sensitivity and selectivity. However, they are limited to only those compounds in the target list, which are usually selected based on the residue definition and legislation requirements to demonstrate that the food is fit for consumption. These techniques require careful optimization of acquisition parameters for each compound and the monitoring of acquisition time windows to ensure detection of the analyte.

To increase the scope of the analysis, chemical screening methods using high-resolution, full-scan mass spectrometry have received significant attention in recent years. These methods use non-targeted acquisition, in which a generic full scan acquisition is run, followed by targeted data processing of a list of compounds within a database.

Although data interrogation is performed against a list of target compounds, retrospective data analysis is possible in order to identify new compounds that were not screened for at the time of acquisition. For this approach to be used in routine analysis, screening data processing software needs to be fast and accurate enough to detect residues at low concentrations with an acceptably low level of false negative results, as described in the European Union guidelines. There is no recommendation for the number of false positives, but it is necessary for routine laboratories to keep this number as low as possible to minimize the time required for additional investigation.

The majority of samples that pass through a laboratory are compliant with the legislation. Therefore, it is efficient to quickly screen compliant samples from those that are suspected to be contaminated. Following an initial screen, the suspect positive samples are reanalyzed using a second confirmatory method (e.g., GC-MS/MS) to confirm suspect positives and to accurately determine the concentration of the pesticide present. The confirmatory analysis contains a complete calibration series in an appropriate matrix that is not included in the screening analysis.

In this study, we evaluate the performance of the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer (MS) for the accurate screening of GC-amenable pesticides. The Q Exactive GC Orbitrap MS provides high mass resolving power up to 120,000 (*m/z* 200) full width half maxima (FWHM) to facilitate highly accurate mass measurements and to enable confident discrimination of co-eluting and isobaric compounds in complex samples. Fast scan speeds and a high intrascan dynamic range (>5000) facilitate the detection of trace compounds in the presence of high matrix components.

Read the full application note: http://tas.txp.to/NextGenGCMS/three

Hans Mol and Marc Tienstra are based at RIKILT – Wageningen UR, Wageningen, The Netherlands. Dominic Roberts, Cristian Cojocariu, and Paul Silcock are based at Thermo Fisher Scientific, Runcorn, UK.

High Hopes for High Resolution

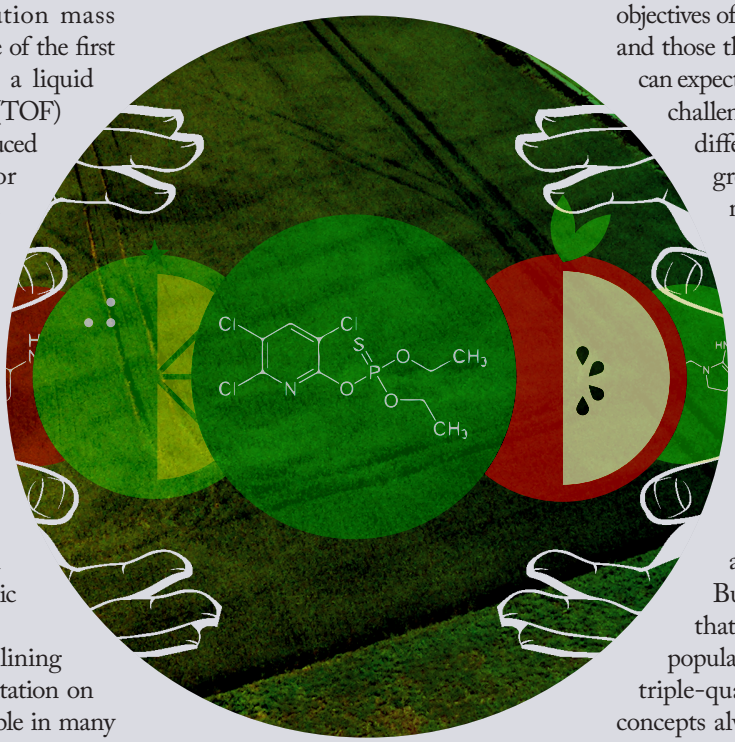
Then & Now, with Amadeo Fernández-Alba, Professor at the University of Almeria, Spain.

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 Thermo Scientific™ 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.

Routine Quantitative Method of Analysis for Pesticides using GC Orbitrap Mass Spectrometry in accordance with SANTE/11945/2015 Guidelines

By Dominic Roberts, Samanta Uclés Duque, Amadeo Fernández-Alba and Paul Silcock

The international trade in food commodities has enabled a wide variety of fruits and vegetables to be made available year round. However, this also creates a challenge for food safety regulators who seek to ensure a safe food supply chain, particularly with regard to the potentially hundreds of different pesticides in use across the globe. The European Union (EU) has some of the most stringent pesticide residue regulations. In 2008, it implemented regulation EC No. 396/20051, which sets default maximum residue levels (MRLs) at 10 µg/Kg for all pesticide/commodity combinations for which no substantive MRL had been set. Further to this, in 2009, the pesticide safety review EU 91/414/EEC2 led to the approval of approximately 250 pesticides and effectively set the permissible level for all other pesticides to the default limit (10 µg/Kg).

Recently, at the beginning of 2016, the latest version of the SANTE/11945/2015 guidance document on analytical quality control and validation procedures for pesticide residues in food and feed took effect. This document describes the method validation and analytical quality control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure. It is intended for use by Official control laboratories in Europe, but in practice it is used by pesticide laboratories worldwide. Implementation of the stringent requirements present a major challenge to testing laboratories who seek to provide an accurate and cost competitive services.

Pesticide residue testing requires detection using both liquid and gas chromatographic techniques typically coupled with triple quadrupole mass spectrometers. These analytical techniques can cover the range of compounds that need to be monitored with the required sensitivity and selectivity. However, they are limited to detecting pesticides that are measured at the time of acquisition and require careful method optimization and management to ensure selected ion monitoring windows remain viable. In recent years, high-resolution Orbitrap mass spectrometry has provided an alternative to MS/MS techniques with additional analytical advantages.

With high-resolution mass spectrometry (HRMS), the default acquisition mode is untargeted (full-scan), making it simple to manage and potentially allows for an unlimited number of pesticides to be monitored in a single injection. In addition to this, full-scan data analysis provides access to supplementary identification points, such as spectral matching, and enables retrospective interrogation of samples to additionally search for emerging pesticides or other contaminants that were not considered at the time of acquisition.

In this study, the quantitative performance of the Thermo Scientific™ Exactive GC Orbitrap™ mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in fruits and vegetables following SANTE/11945/2015 guidelines using full scan acquisition. The Exactive GC-MS system provides routine high-mass resolving power up to 60,000 (*m/z* 200) full width at half maximum (FWHM) with scan speeds suitable for GC peaks to facilitate the detection of trace compounds in the presence of high matrix components.

Results in brief

- 99.3 percent of the pesticide/matrix combinations were detected below the MRL with excellent linearity and meeting the required performance criteria. Importantly, the scope of the analysis is increased by acquisition in full-scan with targeted data processing with a compound database.
- Acquisition at 60,000 FWHM resolution dramatically reduces matrix interferences and increases confidence in results when screening for pesticides in complex sample matrices. Consistent sub ppm mass accuracy was achieved for all compounds over a wide concentration range ensuring that compounds are detected with confidence at low and high concentration levels.
- Repeated injections of a tomato matrix at 10 µg/Kg showed that the system is able to maintain a consistent level of performance over an extended period of time as is demanded by a routine testing laboratory.

Conclusion

The results of the study demonstrate that the Thermo Scientific Exactive GC Orbitrap high-resolution mass spectrometer, in combination with TraceFinder software, is a high performance analytical system that delivers robust and sensitive performance for routine pesticide analysis in fruits and vegetables in complete accordance with the SANTE guidance document.

Read the full application note: http://tas.txp.to/NextGenGCMS/five

Attaining Accurate Authentication

As fraudsters become increasingly knowledgeable and adept, authentication of food and beverages becomes more and more challenging. I was keen to see if GC-Orbitrap technology represented a new tool in the defense of food safety and quality – and Scotland's centuries-old whisky industry.

By Jana Hajšlová, Professor and Laboratory Head, Department of Food Chemistry and Analysis, University of Chemistry and Technology, Prague, Czech Republic.

My father graduated from the same university as me – the Institute of Chemical Technology Prague – and specialized in inorganic chemistry, so it wasn't too difficult to decide how I wanted my career to develop. But my father had set the academic bar very high; he was a guru in several weighty fields, including semiconductor research, and also worked for the United Nations on geological research projects. I decided to take a different route through chemistry and joined the faculty of food and biochemical technology. In the beginning, my father was a little disappointed by my choice as he considered it "university cooking", but it didn't take him long to realize that food chemistry and analysis was an exciting and cutting-edge field. Indeed, food analysis presents some of the most complicated matrices, which makes trace analysis very challenging at times. I too realized that I'd made an excellent choice and never regretted it.

Bitten by the technology bug

In the early days, I remember using gas chromatography instruments manufactured in Czechoslovakia; currency issues and availability prevented us from exploring imported options. The instruments were complex with many buttons and functions, but worked very well. More importantly, they allowed me to discover a great fondness for separation science – and technology. Even back then, I was doing sensory analysis on GC by removing the FID on repeat experiments and inhaling the scents from the peaks. Later, I moved more firmly into food safety because environmental issues were beginning to drive the industry towards change. I remember using a single chromatograph (funding was still challenging) connected to four selective detectors and an electronic printer; it was high technology at the time and very exciting. I knew I always wanted to be at the cutting-edge in terms of analytical instrumentation.

In the mid-1980s, I did a couple of years as visiting scientist at the Free University of Amsterdam working on very advanced techniques under two renowned chromatographers: Roland Frei and Udo Brinkman (who was head of the Royal Netherlands Chemical Society). Michel Nielen (now at RIKILT Wageningen UR) was my peer and remains my good friend and colleague. We are co-chairing the 7th International Symposium on Recent Advances in Food Analysis (RAFA 2015, www.rafa2015.eu) in November.

When I returned to the Institute in Prague, we started working on many more international collaborations and advanced instrumentation was more readily available. Our strategy was to focus on advances in mass spectrometry – something we continue to do today. We have a huge interest in assessing novel instruments and techniques from all the major companies. When I was asked to evaluate GC-Orbitrap technology ahead of its launch at ASMS 2015, I of course responded positively.

GC-Orbitrap technology – a true novelty

The pace of technological innovation has been startling, but the analytical challenges have also changed tremendously; the two aspects are part of the same cycle. Over the years, technology, such as automated sample injection and the sensitivity increase delivered by triple quadrupole MS (in both GC and LC), have constantly strived to answer the

analytical questions of the moment. I was telling my students recently that the current challenges in food analysis are most likely to be addressed by high-resolution MS (HR-MS), which offers so many advantages compared with unit resolution MS/MS.

In the past, I've worked with medium resolution time-of-flight (TOF) instruments with a maximum resolving power of about 10,000 FWHM, and then moved onto improved TOFs with about 30,000 FWHM. Orbitrap technology coupled to LC was a real breakthrough, offering resolution up to 60,000 FWHM with high mass accuracy – and further developments increased resolving power in some variants up to 450,000 FWHM (at *m/z* 200).

Today, Orbitrap is available for GC instrumentation in the Q Exactive GC system, which is yet another key advance. I consider myself impartial when it comes to technology, but I can say that GC-Orbitrap technology offers several real benefits. I was particularly impressed with the linearity range of the instrument, which is a limitation of TOF instruments. In 'fingerprinting' style studies, relative ratios of responses for features are also diagnostic, so linearity plays a very important role. In our studies, we saw good linearity over six or seven orders of magnitude.

For me, two challenging areas stand out as real opportunities for Orbitrap technology to differentiate itself from triple-quadrupole instrumentation. The first is non-targeted screening, where you wish to confirm whether or not a sample is contaminated with unknown compounds – mycotoxins or other natural toxins - using LC-Orbitrap, for example. Here, the combination of full scan and accurate mass is unparalleled,

as discussed in my lecture 'Effective Food Safety Control: Pesticide Residues and More within a Single Run' at the 1st International Symposium on Recent Developments in Pesticide Analysis (you can watch the video here: http://tas.txp.to/0915/janapresents). The second area is food authentication, which I believe is even more challenging. Traditionally, several markers have been used to answer questions of authentication, but with little in-depth knowledge of the matrices and other potential clues. Comprehensive MS fingerprinting using full-scan HRAM data coupled with advanced chemometrics can offer surprising insights into authenticity and classification of samples – something that was not previously possible in a single analytical run.

Whiskey or Whisky?

When I tested the Q Exactive GC system ahead of its launch, I was keen to benchmark it in three main areas: linearity, sensitivity and selectivity. But more than that, I wanted to assess its potential in the aforementioned area of food authenticity, which is why we focused on several whisky samples in addition to pesticide analysis. I was quite surprised to find that many compounds were identified automatically in both sets of samples, which proved to me that the deconvolution function was working well.

Analyzing the very important food commodity that is whisky seemed like a good idea given the fact I was in the UK. In particular, we were interested to see if we could authenticate whiskies in terms of age, geographical origin, brand and raw materials by building up databases and statistical models from samples of known origin. The end game is to use the data and models generated to assess unknown samples using HRAM fingerprints to gain a probability of authenticity. In our early work with GC-Orbitrap technology, we were fine tuning the method and found that ethyl acetate extraction gave us a good signature in terms of the compounds derived from the oak casks used in the aging process for whisky. As I hinted earlier, I was especially impressed with the linearity across major and minor compounds and the ability to identify ions that could be used to discriminate between whiskies.

"We were interested to see if we could authenticate whiskies in terms of age, geographical origin, brand and raw materials ."

A growing wish list of recent advances

Having spent time with GC-Orbitrap technology, what is my conclusion? Well, the Q Exactive GC system is on my wish list! Especially as we have plans to establish a center of excellence in food and nutritional science – and that means we need great instrumentation. GC-Orbitrap technology represents the current pinnacle of innovation in that space right now, and would complete my collection – after all, I already have four TOF instruments, including a GC×GC-TOF-MS system.

Over the next few weeks, Michel Nielen and I – along with the rest of the team – will be conducting the strict selection process of oral abstracts for RAFA 2015. We started the conference 14 years ago to place an emphasis on excellence – and, as the name indicates, recent advances in the field – the two aspects that drive our selection process. Notably, we made a decision right from the beginning to separate presentations from independent (academic or industry) scientists and instrument company researchers – though certainly not in terms of quality. Richard Fussell is a perfect example of a quality scientist who will command attention and respect on both sides of the divide. Indeed, vendor lunchtime seminars are always packed and I am sure we will learn more about the Q Exactive GC system this November. I will also be very interested to see if anyone will independently present work based on their experience with GC-Orbitrap technology – I'm quite confident we will...

When I was invited to Thermo Fisher Scientific's laboratory in Runcorn, UK, to test drive GC-Orbitrap technology, I was very curious to learn what added value or extra features it could offer. I can say that it certainly fills a gap – especially in metabolomic style approaches. I also suspect it will have a disruptive impact on certain areas of the mass spectrometry market. My independent advice? Take Orbitrap technology for a spin and decide for yourself.

Video interview with Jana Hajšlová: tas.txp.to/0915/Jana
To find out more: thermoscientific.com/QExactiveGC

Chemical Profiling of Whiskies Using Orbitrap GC-MS

By Dominic Roberts, Jana Hajslova, Michal Stupák, Jana Pulkrabova, Richard Fussell, and Paul Silcock.

Whisky is a premium distilled spirit beverage produced using long-established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, has high commercial value, and can be economically important in the regions of the world where it is produced and consumed. As such, it is essential that whisky producers are able to obtain an accurate and comprehensive chemical profile that is characteristic of their individual product.

This work aims to demonstrate the application of a complete untargeted chemometric workflow using the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap™ to detect and identify chemical components in whisky. This proof-of-concept study also shows the process of identifying chemical differences in whiskies of different origins.

In brief, nine whisky samples from different regions and distilleries were extracted into ethyl acetate. The extracts, including a pool, were analyzed in all experiments using a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap™ mass spectrometer. Data was acquired and processed using the Thermo Scientific™ TraceFinder™ 3.3 software and Thermo Scientific™ SIEVE™ 2.2 software (for full method and results, see full application note).

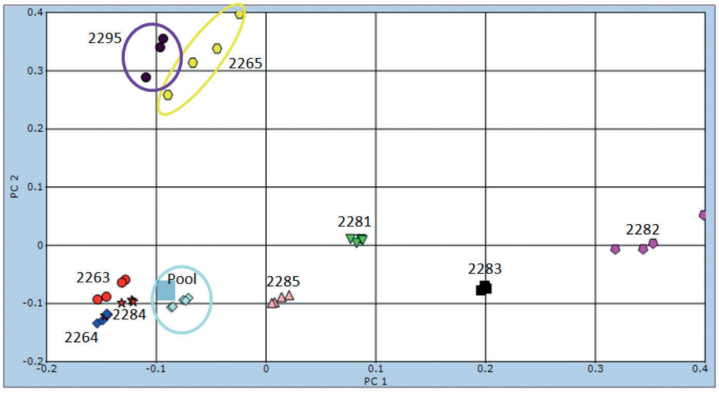


Figure 1. Principal component model of the nine whisky samples with four replicate injections of each. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different from the other samples, but show some similarities to each other.

Discovering differences

The complete data set, including all nine samples, pooled sample, and replicates, was processed in SIEVE 2.2 for component extraction and statistical analysis. This software initially performed a peak alignment to correct for any retention time variation across the batch, followed by peak detection, and finally statistical analysis. The results of this analysis are shown in Figure 2, which shows a principal component analysis (PCA) of all the samples and replicates.

Conclusions

- Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Q Exactive GC system an ideal platform for chemical profiling of complex samples.
- The consistent sub-1-ppm mass accuracy, in combination with excellent sensitivity, makes for confident identification of all components.
- SIEVE 2.2 and TraceFinder 3.3 software allowed for a fast and comprehensive characterization of the whisky samples, isolating and identifying compounds with confidence. A larger number of samples are required to draw clear conclusions on a particular whisky profile.
- The EI and PCI data obtained was used for tentative compound identification against commercial libraries. Where no library match was made the mass accuracy allowed for elemental compositions to be proposed with a high degree of confidence. Proposed identifications can be quickly confirmed or eliminated based on accurate mass of fragments.

Read the full application note: http://tas.txp.to/NextGenGCMS/four

Dominic Roberts, Richard Fussell and Paul Silcock are based at Thermo Fisher Scienti¬fic, Runcorn, UK. Jana Hajslova, Michal Stupák, and Jana Pulkrabova are based at the Institute of Chemical Technology, Prague, Czech Republic.

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