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The Column

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Tackling the Big Spill

Chromatography and the *Deepwater Horizon* project

Cover Story

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Photo Credit: Courtesy of Chris Reddy, Woods Hole Oceanographic Institution

Marine Pollution Analysis:
The *Deepwater Horizon* Oil Spill

Chris Reddy from the Woods Hole Oceanographic Institution (WHOI) spoke to Alasdair Matheson of *The Column* about the role of chromatography in the ongoing environmental analysis of the *Deepwater Horizon* oil spill, how GC×GC works in practice, and why this oil spill led to the return of thin layer chromatography (TLC) to his laboratory.

Photo courtesy of Chris Reddy, Woods Hole Oceanographic Institution

Q: Tell us about your group's involvement in the work at the *Deepwater Horizon* disaster site, which has attracted widespread attention in the media. What were the objectives?

A: In April of 2010, the *Deepwater Horizon* (DWH) drilling rig exploded and released approximately 200 million gallons of crude oil along with a large quantity of methane, ethane, and propane.

It was an ongoing spill for 87 days with oil residues that we continue to find along the Gulf beaches as recently as January 2014. We have studied — and continue to study — a wide range of research questions from determining the flow rate, analyzing how nature breaks down or "weathers" the oil, and fingerprinting it to confirm that oiled samples we have found have come from the DWH disaster.

Our field work has led us to collecting samples using a robot right where the oil was coming out of the pipe that you may have seen on TV at the time. We have walked many miles of the Gulf of Mexico coastline and even 300 or 400 miles away from the explosion. So we have gone from analyzing oil samples a foot away from the source of the spill to hundreds of miles away

I expect to be working at the site for the next 10 years, alongside some other oil spills and projects .

Q: One of the main techniques you used was comprehensive two-dimensional gas chromatography (GC×GC). Why did you decide to use a GC×GC method and what are the advantages of this technique compared to other methods?

A: My team has extensive experience in tackling some interesting research questions with GC×GC by studying numerous oil spills that have occurred as well as natural oil seeps. What makes GC×GC so powerful is that it has the capacity to resolve and detect many more compounds than with

traditional analytical techniques, such as gas chromatography with mass spectrometry (GC–MS).

Now, I want to be very clear here. A lot of people hear me say GC×GC can do more than GC–MS, and they immediately assume that GC×GC is a replacement to GC–MS, but it is not. It is just another tool in the laboratory that allows us to address some specific questions where a regular benchtop GC–MS cannot.

On the other hand, for polycyclic aromatic hydrocarbons (PAHs), I don't think GC×GC can do any better than a benchtop GC–MS can do and the GC–MS software is much, much more user-friendly. And so, in my lab, we don't quantify PAHs by GC×GC. There's no point. It's easier and faster to do so with a GC–MS.

One of the main factors that makes GC×GC very powerful, that I think a lot of people miss out on, is that when you look at a chromatogram in two-dimensional GC space you are not only just able to identify and measure compounds (many, many more compounds than with traditional techniques), but also can convert retention times in the first and second dimension to vapour pressure and water solubility.

If you're interested in the fate of oil there are two key questions you want to know: What is the vapour pressure of a compound (or what is the likelihood the compound will evaporate?); and what is the water solubility of the compound (what is the likelihood the compound will dissolve in water?)

Now if we use some newly developed algorithms, we can allocate how much of a compound evaporated versus how much dissolved in water. That is the real major leap in my mind: GC×GC allows us to discover where the compounds are going. It's beyond just making your Excel spreadsheet bigger and identifying many more compounds. It allows us to say where is this compound going, or where has it gone.

Q: Can you describe an interesting example of the use of chromatography on the *Deepwater Horizon* project using GC?

A: There was a question about some oil residues that were found on corals on the bottom of the seafloor, not too far from where the well blew out. It was possible that natural seeps around that area caused the oiling and not from the DWH. For understanding the impacts of the spill, it was critical that we could accurately fingerprint these oil residues on the coral. We were able to show — with much more confidence than what you could do with traditional tools — that that oil was from the Macondo well.

Q: Are there any particular difficulties you have to overcome when developing GC×GC methods?

A: I think that the reason that GC×GC is not more popular and has not fully matured, despite being around for decades, is that it is hard to get it up and running day in and day out. This is counter to your typical scientist who might start a job in a new laboratory, buy a benchtop GC–MS, and be obtaining publication-quality data the next day.

The cost of GC×GC with a flame ionization detector (FID) is comparable to a GC–MS, but it might take you months to achieve adequate and reproducible chromatograms sustainably.

Even then, the basic maintenance is a little tricky as you have a lot of potential for more leaks and so the learning curve is a lot steeper — what's more there aren't many manuals available on troubleshooting. This sounds negative, but I think it's just that the technology hasn't matured, and it's probably where GC–MS was in the late 1960s.

To me, maturity will come from more users contributing to the field with manufacturers developing better and easier to use hardware and software. The more users out there, the more everybody learns the tricks, and the more that it will allow us to pollinate so that it can be more user-friendly.

Q: So, you see it evolving and being easier to use as time progresses? Do you think it could be used for routine analysis in the future?

A: Absolutely. It would be in the best interest of the manufacturers to help it evolve faster, and I know they are working hard on this at the moment. I truly hope it become mainstream and not a boutique tool. I have been really lucky to have a colleague, Bob Nelson, work in my lab for the last 12 years and all he has done is GC×GC.

For routine analysis, the one thing that needs to evolve is the software to interpret the data. It's a case of "be careful what you wish for: You might get it!" You get a lot of resolved peaks and a lot of information, but the developments in hardware are way ahead of the software — data analysis is therefore not as easy as it is with a regular benchtop GC–MS to integrate peaks and such. The software exists but it needs to be refined and that's what I also tell potential users — give it time and look for ways to improve it. I think there is a lot of low-hanging fruit and a lot of talented people can make this technology even better.

Q: Is there anything you would like to add about the use of GC×GC in your group?

A: Now there's one more powerful thing with GC×GC that we use a lot is that we

can do algebraic operations, mathematical operations, on a whole chromatogram. So let's make-believe you have a sample of oil that was in a ship before it got spilled. Now we might collect a slick sample that was collected a few days later a couple of kilometres away. We can analyze each sample with the same exact method, align the chromatograms, and then subtract one from the other. We get a "difference" chromatogram. And that "difference" chromatogram can tell you across the whole gas chromatographic plane what compounds have already been lost to the environment.

Q: Any advice for chromatographers who are embarking on using GC×GC?

A: My advice is if you buy one of these instruments is to be patient and be willing to invest the time to get it going. You're not going to be able to return in a day but that doesn't mean that you can't. You just have to recognize that this is an instrument that sometimes can be a little counter-intuitive and has its own challenges, but there are no different challenges in other techniques that haven't gone into the mainstream. I think the best advice for a good analytical chemist is to give it a little bit more time and to tell their bosses to give them a little bit more time when they get the technology and they will reap the benefits. Patience is a virtue.

Q: Are there any actual other chromatographic techniques that you think are particularly interesting in the environmental analysis at the moment?

A: You know it's funny. One of the pieces of technology that we have found to be incredibly powerful is thin layer chromatography with a flame ionization detector (TLC–FID). This is an automated instrument that has been around for decades. It has been invaluable for looking at compounds that are not GC-amenable — compounds that are either thermally unstable or what we call too sticky to go through a gas chromatograph instrument.

This approach has become useful for the DWH project because we believe there has been a lot of biology or photochemistry that has oxidized some of the oil samples that makes them difficult to be analyzed by any type of GC, but that can be easily separated and quantified on a TLC–FID. So I think there's some irony that we have resurrected a piece of technology that was in my building and now there's a queue of scientists waiting to use it. In fact, we're thinking about buying another one. So there's an interesting spin on using something that's been around for a long

time that we really think has a lot of power. It's very easy and the curve is easy to get going. We have already published several papers using it. So it's an interesting end of the spectrum from a chromatographic perspective.

Q: It seems simplicity sometimes has a lot to offer.

A: Indeed. It's really funny because I only knew how to use it because my PhD advisor was formally trained in looking at lipids and he did a lot of work on pollution. His name is Jim Quinn. He's still alive and a great guy. He had a project going on where somebody was looking at lipid chemistry, and so when I was in the lab I got to see these people using it. When we started to think about how we might be able to elucidate what was going on in the *Deepwater Horizon* site I wondered if we could use TLC–FID.

We found one in our building and we have a lot of chemists and biochemists, so naturally somebody had one on a shelf. And lo and behold, we brought some rods and development tanks and we were in business.

Q: Interesting stuff! Your Institution does a lot of brilliant work. Is there anything you'd like to say about the role of separation science at your institute?

A: We do a wide range of chromatography in a relatively small building. We have about six investigators: Six professors in a relatively small building and I'd probably wager that we have maybe 25 mass spectrometers and probably 35 different types of chromatographs.

The analyses run the whole gamut from looking at methane to inspecting large molecular biopolymers that have much, much bigger molecular weights to any possible spectrum, potential usefulness, we have in our building. And it's a lot of fun. We've had a lot of people come to our lab and say "I've never seen so many chromatographs."

Q: So many professors in one laboratory!

A: Yeah, it's really amazing. To be honest with you, one of our biggest problems is

that we're about 100 kilometres away from Boston. Our power supply sometimes can have some issues so that we have a lot of problems making sure that our instruments can switch to emergency power. And then the other challenge that we face, and we're working on it, believe it or not, is the sheer amount of gas and liquid gases that we consume. Right now, we have been bringing in standard bottles and liquid nitrogen on tanks — to the point where our hallway is lined with tanks all over the place and it's a fire hazard.

We also only get a gas delivery on a Monday, Tuesday, and Friday. So if you are out of gas at four o'clock on a Friday afternoon and you're trying to batch up samples over the weekend, you can't run them.

That's a huge problem for us. And so we've been working with our administration so that we can have a permanent plumbed gas source so we would remove all these tanks. Actually, it's a lot cheaper and safer. And it will allow us to be more efficient. And so really in many respects, our two-biggest challenges in terms of day-to-day operations are good power and gas supply.

Q: How is this perceived from a cost perspective?

A: Good point. We have been working with our administration and the upfront costs are not cheap. We're in financially tough times. And for us to be able to say, "Look, the return on the investment in this plumbing will be in only in a couple of years or so and it's going to be safer" is important. We're not going to have so many bottles in the hallway. We're not going to be relying on all this traffic. Even these big diesel trucks have to deliver these tanks about 80 miles away from a service place and so we have a lot of traffic to get these tanks here and our building is small. So there are a lot of challenges, including getting these instruments working day in and day out with the bare necessities of electricity and gas — as well as the separation science.

Chris Reddy is a senior scientist at Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA. He is currently studying the short and long-term fate of oil seeping off the coast of Santa Barbara, California, and the Gulf of Mexico, World War II wrecks in the South Pacific, and spills that have occurred in 1969, 1974, 1996, and 2003 in New England, two that occurred in 2007 in San Francisco Bay and South Korea, the *Exxon Valdez*, the 2002 *Prestige* spill along the Spanish coastline, and the *Deepwater Horizon*.

According to a 2010 survey by Thomson Reuters, he is one of the top cited and published scientists studying oil spill effects, remediation methods, and petroleum microbiology. He has extensive experience with the *Deepwater Horizon*, including being the academic liaison at the Unified Area Command where he interacted and provided guidance to state, Federal, and BP officials.

He had led or participated in two major research cruises on the DWH, many small boat operations, overflights, and sampled the beaches of the Gulf from Pensacola, Florida, to Port Fourchon, Louisiana, countless times.

He received his BS in chemistry from Rhode Island College, Providence, Rhode Island, USA, and his PhD in chemical oceanography from the Graduate School of Oceanography at the University of Rhode Island (Narragansett, Rhode Island, USA).

E-mail: creddy@whoi.edu
Website: http://www.whoi.edu

News

Eurofins CEO Gilles Martin Appointed to Bruker Board of Directors

Gilles Martin, Chairman and Chief Executive Officer of the Eurofins Scientific Group (Luxembourg, Luxembourg), has been elected to the Bruker Board of Directors (Massachusetts, USA). Martin founded the original Eurofins Scientific Nantes food authenticity laboratory in 1988, and is a past President of the French Association of private analytical laboratories, and the North American Technical Committee for Juice and Juice Products.

www.bruker.com

Sigma-Aldrich in "Top 100 Most Sustainable Corporations in the World" List

Sigma-Aldrich (St Gallen, Switzerland) has been named in the "Global 100 Most Sustainable Corporations in the World". The list was announced at the World Economic Forum in Davos, Switzerland.

The *Global 100* is generated annually by Corporate Knights (Toronto, Canada). Twelve quantitative key performance indicators are used to score qualifying companies and range from energy and water consumption, to corporate tax strategy and employee compensation. Around 400 companies were shortlisted from global indices of mid and large cap stocks (approximately 4000 companies).

In 2010, Sigma-Aldrich implemented a number of sustainability goals that they are set to achieve by 2015, according to Sigma-Aldrich President and CEO Rakesh Sachdev.

www.sigma-aldrich.com

Milton Lee Wins *LCGC Europe Lifetime Achievement Award* 2014

Professor Milton L. Lee of Brigham Young University in the USA was presented with the 2014 *LCGC Europe Lifetime Achievement Award*. Alasdair Matheson, editor-in-chief of *LCGC Europe*, presented the award to Lee to a packed HTC-13 conference in Bruges, Belgium, for his outstanding achievements in hyphenated chromatographic techniques and for distinguished service to the international separation science community. Lee joins previous award winners including James Jorgenson (2006), Robert Smits (2008), Pat Sandra (2010), and Milos Novotny (2012).

Lee told *LCGC*: "It was a great honour to receive the Lifetime Achievement Award in Bruges. It caused me to reflect on the many exciting times I have enjoyed over the years working with talented graduate students, postdocs, and colleagues. In fact, I feel additional enthusiasm now to see if I can still contribute more in the future to the field of separation science!"

Lee has mentored more than 71 graduate students and 26 postdoctoral researchers since joining Brigham Young University in 1976. He has been actively involved in the transfer of technology from the laboratory to industry, is listed as co-founder of three companies set-up to commercialize instrumentation, and is listed as co-author on 20 issued patents. His current research interests lie in the field of monolithic column technology for capillary liquid chromatography and instrumentation for field sampling and hand-portable gas chromatography–mass spectrometry.

www.chem.byu.edu/faculty/milton-l-lee/

Make sure to check out
***LCGC TV* for an upcoming interview with Milton Lee at HTC-13.**

LC–MS–MS Method Developed to Detect Synthetic Opioid

US scientists have developed a new method for an emerging synthetic opioid associated with increased health risks and deaths.

Scientists from the Center for Drug Detection and Response, a biomedical research consortium, have developed a method using liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) for the detection of acetyl fentanyl in human urine. The study published in the journal *Analytical Chemistry* outlines a new confirmation procedure that can be performed by toxicology laboratories.[1] Acetyl fentanyl has recently been linked to over 50 fatalities in the states of Rhode Island and Pennsylvania in the USA.[1]

In May 2013, the state of Rhode Island Department of Health confirmed to the Center for Disease Control and Prevention (CDC) that a synthetic opioid acetyl fentanyl was implicated in a number of illicit drug overdose cases.[2] The compound has never been approved for human use and is not available on prescription. It is the one of the latest synthetic compounds to come to the forefront of toxicological testing, as a so-called "designer drug".

Amy Patton, who is a chemist at the Arkansas Department of Health, Public Health Laboratory in the USA and lead author of the paper, told *The Column* that the compound "may be linked to increasing morbidity reports supposedly involving a dangerous form of heroin". Patton said: "Recent reports from Pennsylvania and Rhode Island indicate that synthetic opiates may be gaining popularity within the designer drug markets. The method we report is the latest method toxicology laboratories may want to adopt as they work to keep pace with what seems to be a never-ending supply of designer drugs."

When faced with an unsuspected acetyl fentanyl death, toxicological laboratories may potentially be perplexed when enzyme-linked immunosorbent assay (ELISA) screening positively identify fentanyl, but gas chromatography–mass spectrometry (GC–MS) confirmatory tests do not. The team of collaborating scientists and medical practitioners set out to develop a validated method to detect acetyl fentanyl and the predicted human metabolite, acetyl norfentanyl. An LC–MS–MS approach with deuterium labelled internal standards was performed to separate and detect the two compounds in spiked urine samples. As part of the sample preparation, urine samples were hydrolyzed as opoids are commonly conjugated to glucuronic acid before excretion.

The major challenge of the analysis was the development of analytical standards and authentic labelled internal standards required for analyzing trace levels of acetyl fentanyl, according to Patton. She said: "Before this work, these standards were not available and no metabolic investigations had been performed with acetyl fentanyl. Here we report both the parent drug and the predicted human metabolite acetyl norfentanyl." She further added a caution to laboratories performing analyses to control for carry-over, commenting "the drugs seem to be 'sticky' and carry-over can become problematic when evaluating highly concentrated samples".

Patton told *The Column* that this study is a small part of a much bigger effort to continually update regulations of synthetic drugs. She said: "I work with a large group of extremely talented scientists, and we are all committed to protecting public health and preparing our state infrastructure for meeting the scientific and legal challenges posed by designer drugs. Members of this group represent several institutions including the Colleges of Pharmacy and Medicine at the University of Arkansas for Medical Sciences (UAMS), Arkansas Children's Hospital (ACH), Arkansas Department of Health (ADH), and Arkansas State Crime Laboratory." — B.D.

Bethany Degg, Assistant Editor,
bdegg@advanstar.com
www.chromatographyonline.com

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3. Designer Cannabinoids, *The Column* **9**(22), 18 (2013) http://www.chromatographyonline.com/lcgc/article/articleDetail.jsp?id=830872&sk=64b8a33e8ec5404b71ede27855c95ab4

Photo Credit: hidesy/Getty Images

Photo Credit: George Marks/Getty Images

To Validate or Not to Validate

Incognito investigates if changing the carrier gas really requires a full method revalidation.

I just googled "chromatography method revalidation" and of the first 20 or so legitimate hits, only four specifically mentioned revalidation of (GC) methods. Maybe this is to be expected as the top hits mostly relate to validation in a pharmaceutical context, where GC is typically used much less than high performance liquid chromatography (HPLC).

It's not just the pharmaceutical industry that validates methods, and HPLC methods are not the only methods that need to be changed or updated occasionally. The helium crisis has abated somewhat, but helium still isn't a long term proposition for chromatographers. I would go as far as to say that it's probably not even a medium term option from about 2016 onwards when considering its price and availability in North America and Continental Europe. We are going to have to make the move to a different carrier gas, so ask yourself the question I have asked (and have been asked) numerous times over the past year: "Does changing carrier gas require a full method revalidation?" My automatic response is yes, it has to, doesn't it? Am I the only one who sees train headlamps rapidly approaching down the track? Am I the only one preparing for the inevitable by scheduling many hours of revalidation work into the planner?

Here lies a problem that applies across all of the regulated chromatography industry. Ask the question "Does this change require a method revalidation?" and the stock response will be "That is a question to be settled between your QA /regulatory department and your regulator". This nicely avoids anything remotely controversial or opinionated!

There are over 200 United States Pharmacopeia (USP) GC methods and the allowed changes to GC methods, as defined by the USP[1] and European Pharmacopeia (EP)[2], are summarized in Table 1. Can anyone spot any reference to changing the carrier gas? Is that a

Table 1: Allowable changes to gas chromatographic methods as defined by the United States and European Pharmacopeia.

	USP	EP
Column length	±70%	±70%
Column internal diameter	±50%	±50%
Particle size	-50%, SST must pass	-50%, no increase
Film thickness	-50 to +100%	-50 to +100%
Flow rate	±50%	±50%
Oven temperature	±10 %	±10 %
Injection volume	May be decreased (if LOD and repeatability OK)	May be decreased (if LOD and repeatability OK)

no? Good, we are all paying attention, lets continue.

There is one additional parameter for which "allowable changes" are not defined by the EP but which are defined as follows by the USP: "Oven Temperature Programme (GC): Adjustment of temperatures is permitted as stated above. When the specified temperature must be maintained or when the temperature must be changed from one value to another, an adjustment of up to ±20% is permitted."

Do you know what this means? Has any reader ever asked the USP what this means? We presume that it means ±20% of the temperature programme rate value, for example, 20 °C/min has an allowable range of 16–24 °C/min. But who knows! I may just have opened a very large can of worms for myself!

Time for some chromatography. It's my personal empirical experience that changing carrier gases can lead to changes in selectivity, which is what I really worry about when optimizing chromatographic methods. I know that the caveat to the "allowable" changes in the Pharmacopeial methods is that system suitability tests defined in method monographs must be passed — fine. What happens if peaks invert and resolution of the peak pair actually increases but you end up measuring the wrong thing! GC analytes with widely differing polarities on a temperature can do some very strange things when switching from helium to hydrogen carrier, which is why I get a little nervous relying on System Suitability Test criteria when changing parameters that can have an effect on chromatographic selectivity.

When changing from helium to hydrogen one typically enters one of two scenarios: a) Just switch the gas and whatever else you need to do and get on with the work of looking at a chromatogram that is identical to the one before; or b) take the opportunity to optimize the method and see how we might improve throughput by using reduced column dimensions or increased carrier linear velocity.

Either way substantial changes have to be made to the methods, including to the temperature programme in both cases as well as to the carrier gas flow rates when changing gases, and to the column dimensions in the latter case. My concern is that the changes may fall within the so-called "allowable limits" and that the guidelines might be misinterpreted and assumed to be compliant. Or, even worse, the validation status of methods when switching gases may not even be considered. My personal recommendation is that you revalidate your method when switching carrier gas. This is where we get into the full versus partial revalidation argument and although I'm tempted to refer you to your QA or regulatory department, I won't. Revalidate the method in full. Seems quite clear cut, right? Wrong.

Let's take a look at some of the wording from USP method 467 for Residual Solvents, Procedures A, B, and C (widely used in some of the most heavily regulated industries): "The carrier gas is nitrogen or helium with a linear velocity of about 35 cm per second, and a split ratio of 1:5."[3]

Wow, so we can use either carrier gas as long as the resolution between the acetonitrile and methylene chloride peaks is greater than one, and the signal-to-noise ratio for the 1,1,1–trichloroethane peak exceeds a certain value? Surely not?

Does this indicate that changing carrier gas does not always require a method revalidation? Will nitrogen really have the efficiency at 35 cm/s over the range 40 °C to 240 °C to give a suitable separation?

Well, the answer is that I don't know as I haven't tried it but it does raise a very good point. I know that many of the methods in my own laboratory are

"over-resolved". This means that I could easily lose some resolution before the quality of the analysis is compromised. Therefore, instead of switching to hydrogen, would it be possible to move to nitrogen as a carrier? Many of us may already have a nitrogen supply of the required purity (if we don't we can fit gas scrubbers). The method changes required are much less radical than when switching to hydrogen, as the density of nitrogen and helium are much more comparable.

OK, so the flow rates that we operate at with helium may result in working beyond the Van Deemter minimum of nitrogen carrier, but will this really compromise our analyses? However, just because either carrier is possible does not mean that one could, or should, make a choice based on ease of use. Has anyone validated that the results are fit for purpose using either carrier gas at 35 cm/s, or that there are no appreciable selectivity changes? I'd be very interested to hear if that was you, or if you are successfully using nitrogen as the carrier for your USP 467 analysis.

I thought I had reached a steadfast position regarding the transition of methods in our laboratory. Starting with the most used, gradually switch methods over time to hydrogen carrier gas. Avoid switching methods that use mass spectrometric detection for now — this will be the subject of an upcoming Incognito column. Take the opportunity to optimize the method and improve throughput. And then revalidate.

I bet I'm not alone in this general strategy, but now that I have had time to reflect, perhaps this is too much? Perhaps I should switch to nitrogen for some methods — and for my USP 467 residual solvents methods, I may not need to revalidate at all…….right?

References

1. General Chapter <621> "Chromatography," in *United States Pharmacopeia 36–National Formulary 31* (United States Pharmacopeial Convention, Rockville, MD, USA, 2012).
2. European Pharmacopeia 8, Section 2.2.46, (2014).
3. General Chapter <467> "Residual Solvents," in *United States Pharmacopeia 36–National Formulary 31* (United States Pharmacopeial Convention, Rockville, MD, USA, 2012).

Contact author: **Incognito**
E-mail: **incognito@thecolumn.eu.com**

Characterization of Allergens in Cosmetics by GC×GC–TOF-MS With Variable-Energy Electron Ionization

Stephen Smith, Laura McGregor, and David Barden, Markes International, Gwaun Elai Medi-Science Campus, Llantrisant, Wales, UK.

The detection and quantitation of allergens in complex cosmetics extracts using two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS) is described. In particular, variable-energy electron ionization is shown to enhance both the sensitivity and selectivity of analyses by generating mass spectra containing structurally significant fragment ions with an improved molecular ion signal.

Photo Credit: Tetra Images/Getty Images

In 2003 a European Union Directive restricting the use of allergenic compounds in fragrances was released.[1] The directive named a total of 27 allergens that should be labelled if present at over 100 ppm in "wash-off" products, such as shower gels, or more than 10 ppm in "leave-on" products, such as perfumes. To comply with this directive, accurate detection and quantitation methods are required, and two-dimensional gas chromatography–time-of-flight mass spectrometry (GC×GC–TOF-MS) is well-suited. The coupling of two columns of different stationary phases minimizes the requirement for sample preparation steps that can introduce error into the analytical process.

Despite this, the identification of individual compounds in complex samples remains challenging when multiple compounds in a chemical class have similar spectra, or weak molecular ions. This can be addressed with the use of soft ionization to reduce the degree of ion fragmentation, but this can be cumbersome to implement.

In this article, the use of GC×GC–TOF-MS to identify allergens is described. A recently developed variable-energy ion source technology is demonstrated to enable efficient electron ionization at lower energies, resulting in improved compound identification and detection limits.

Figure 1: Close-up view of the GC×GC–TOF-MS contour plot of the allergens standard. The numerical labels refer to the compound identities in Table 1.

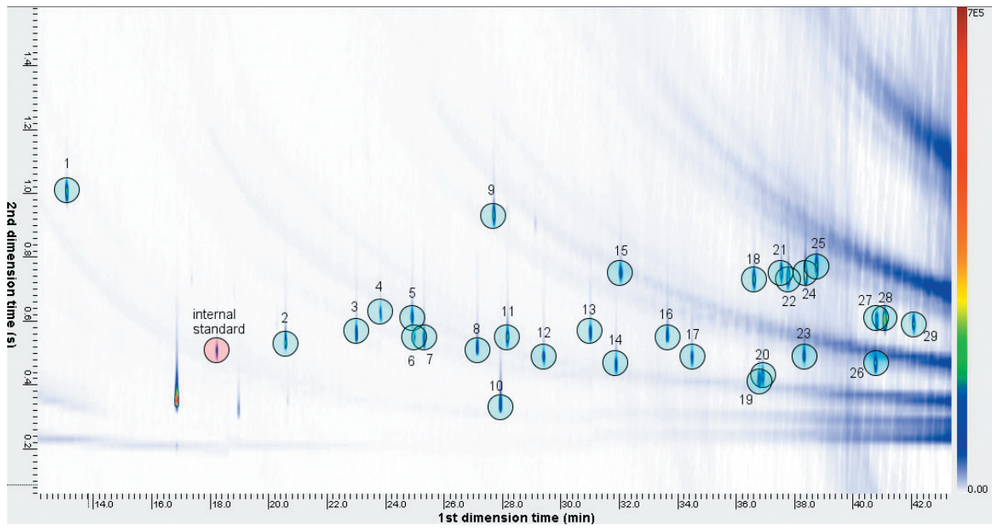
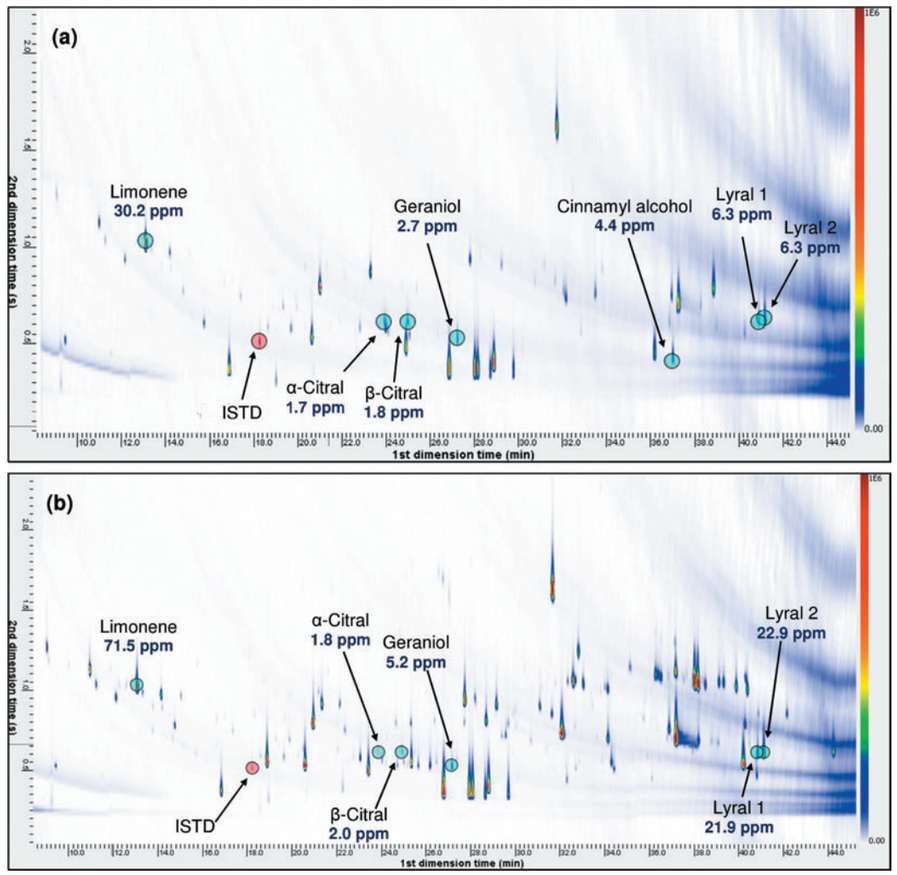


Figure 2: Quantified allergens in the (a) cream and (b) perfume extracts.



Methods

A series of calibration standards containing allergenic compounds were prepared in acetone, ranging in concentration from 0.2 ppm to 10 ppm. Two cosmetics extracts were also prepared, one from a cream and the other from a perfume. Further details of the calibration are available from the authors.

GC Conditions: The analysis was performed on a GC instrument (7890A, Agilent Technologies). Injector: Split/splitless injector; Liner: 4.0 mm i.d. liner, 1 µL injection; Carrier gas: Helium, constant flow at 1.0 mL/min; Mode: Split 10:1; Inlet temperature: 230 °C; Septum purge: On, 3 mL/min.

2D Column Set: 1st dimension: 30 m × 0.25 mm, 0.25 µm SolGelWax (SGE Analytical Science); 2nd dimension: 2.6 m × 0.1 mm, 0.1 µm DB1 (Agilent Technologies); Modulation loop: As for 2nd dimension; Column set: Equivalent pneumatic impedance to 35 m × 0.18 mm.

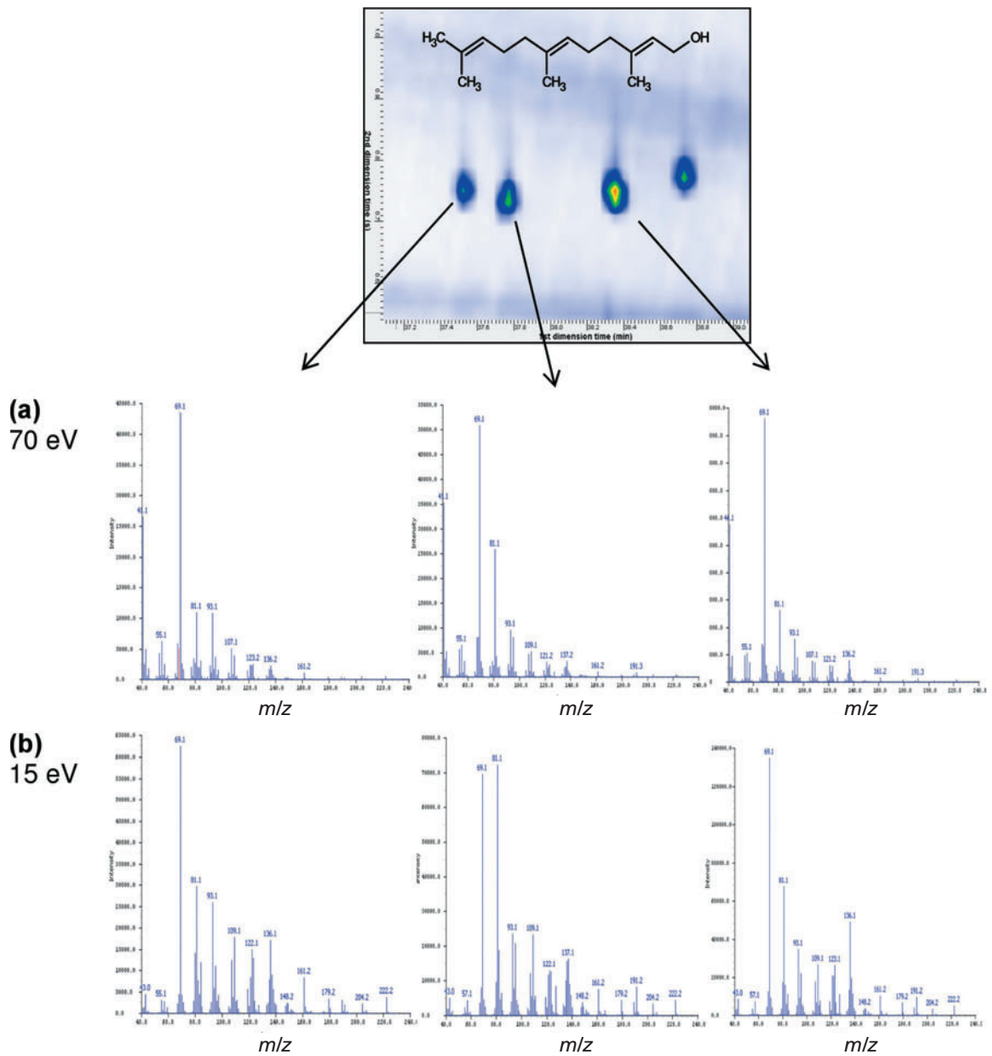
Temperature Programme: Main oven: 40 °C (1.0 min), 6 °C/min to 150 °C, 4 °C/min to 250 °C (20 min); Secondary oven: No offset; Hot jet: 140 °C (1.0 min), 6 °C/min to 210 °C, 4 °C/min to 250 °C; Cold jet: Dewar fill: high, 45%; low, 35%; Modulation period: 2.5 s, hot-jet pulse 350 ms; Total run time: 64 min.

MS Conditions: A BenchTOF-Select instrument (Markes International) was used for analysis. Filament voltage: 1.8 V; Ion source: 250 °C; Transfer line: 280 °C; Mass range: 40–600 amu; Data rate: 50 Hz with 200 spectra per data point. Image processing software: GC Image, LLC.

Results and Discussion

Compound Separation and Identification: A calibration series consisting of four standard solutions was

Figure 3: Spectra of three farnesol isomers at (a) 70 eV and (b) 15 eV. The greater degree of difference between the latter spectra aids identification.



analyzed using the conditions described. Separation of all 29 target compounds was achieved with the reverse-phase (polar–apolar) column set (Figure 1).

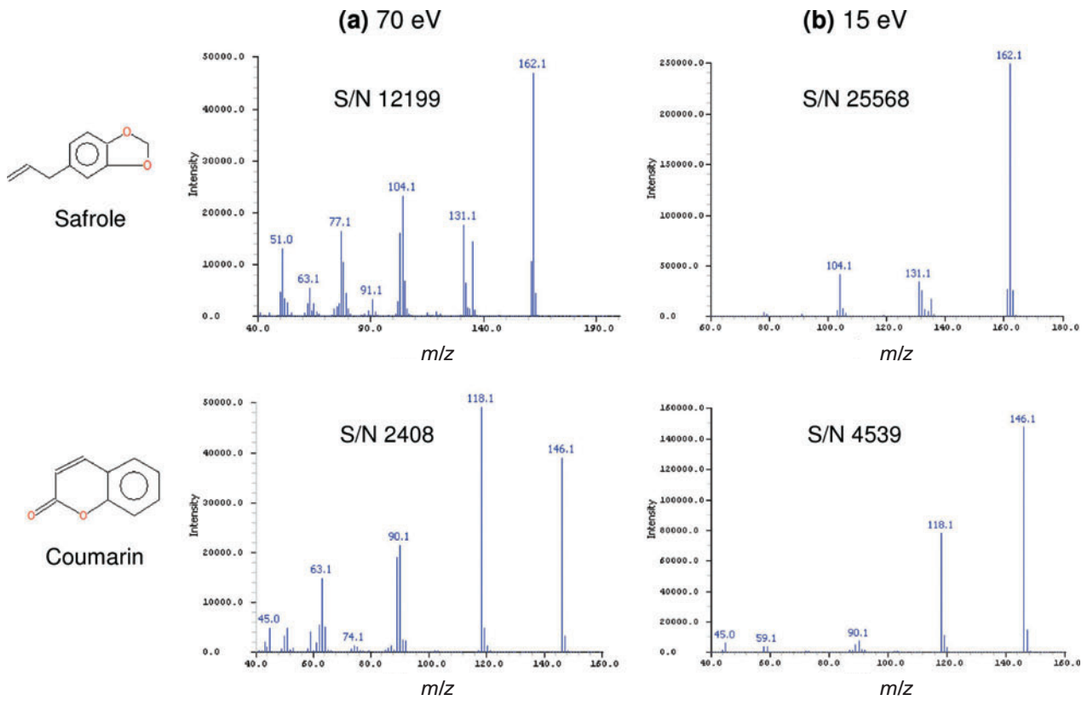
Spectral comparisons against the National Institute of Standards and Technology (NIST) database were performed for all allergens in the calibration mixture — this was possible because of the ability of the system to deliver "classical" EI spectra, with no mass discrimination. Retention times and library match results for all 29 target compounds are summarized in Table 1.

Quantitation of Target Compounds: A template file containing qualifier expressions that had been prepared by the image processing software was used to automatically identify allergens in all samples, allowing fast processing of results. The calibration curves obtained were then used to quantify allergens present in the cream and perfume samples. Contour plots of the cream and perfume extracts are provided in Figure 2, with identifications and quantitative results annotated.

The cosmetics extracts analyzed contained fairly low background matrix because robust sample preparation techniques were used. However, it can be seen that the GC×GC–TOF-MS method provided the high degree of chromatographic resolution necessary to separate target compounds and potential interferences.

Variable-Energy Electron Ionization: Isomeric compounds commonly have

Figure 4: Spectra of safrole and coumarin at (a) 70 eV and (b) 15 eV. The reduced fragmentation at lower energies leads to a significant improvement in signal-to-noise ratios.



similar mass spectra at 70 eV, making precise identification of individual isomers near impossible without the time-consuming analysis of standards to obtain retention time qualification.

However, the ability to generate electron ionization (EI) spectra at lower energies without a loss of sensitivity provides an extra dimension of information that allows the unambiguous identification of specific isomers. This is clearly demonstrated for the farnesol isomers in Figure 3.

This is particularly useful in GC×GC–TOF-MS analyses, where the added sensitivity and selectivity delivered by low-energy electron ionization complement the highly structured separation space occupied by extremely complex samples.

The stronger molecular ion and reduced fragmentation at lower ionization

Table 1: Summary of identification results for all target allergens in the 10 ppm calibration standard.

No.	Compound	RT 1 (min)	RT 2 (s)	NIST Match factor
1	Limonene	13.08	1.00	925
2	Linalool	20.63	0.52	915
3	Methyl 2-octynoate	23.08	0.54	934
4	β-Citral	23.92	0.60	892
5	α-Citral	25.00	0.60	896
6	1,2-Dibromobenzene	25.04	0.54	913
7	Citronellol	25.42	0.54	914
8	Geraniol	27.08	0.50	925
9	α-Isomethyl ionone	27.79	0.92	931
10	Benzyl alcohol	28.04	0.34	932
11	Safrole	28.25	0.54	916
12	Hydroxycitronellal	29.50	0.48	897
13	Methyl eugenol	31.13	0.56	895
14	Cinnamaldehyde	32.00	0.44	937
15	Lilial	32.13	0.72	889
16	Majantol	33.75	0.52	894
17	Eugenol	34.58	0.48	914
18	Amylcinnamaldehyde	36.71	0.72	906
19	*p*-Anisyl alcohol	36.88	0.42	914
20	Cinnamyl alcohol	37.00	0.42	891
21	Farnesol 1	37.63	0.74	937
22	Farnesol 2	37.88	0.72	916
23	Isoeugenol	38.42	0.48	911
24	Farnesol 3	38.46	0.74	915
25	Hexylcinnamaldehyde	38.83	0.78	876
26	Coumarin	40.88	0.48	878
27	Lyral 1	40.92	0.60	894
28	Lyral 2	41.17	0.60	832
29	Amylcinnamic alcohol	42.17	0.60	865

energies also improved signal-to-noise ratios and therefore detection limits, as shown in Figure 4 for safrole and coumarin.

Conclusion

This article has shown that GC×GC–TOF-MS can provide a high degree of compound separation, sensitivity, and spectral quality in the analysis of allergens and other compounds in cosmetics extracts.

The value of variable-energy ionization technology to generate complementary spectra for enhanced compound identification is also demonstrated, along with an indication of the improved sensitivity and selectivity that this can offer.

Reference

1. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.

Stephen Smith studied in Bristol, UK, for both his B.Sc. and Ph.D., which he obtained in 2008 on innovative work profiling volatile organic compounds for disease diagnosis. Following post-doctoral positions at the University of the West of England and Bristol University, Steve joined Markes International as a senior applications specialist for thermal desorption and TOF-MS in 2011, where he now specializes in GC×GC–TOF-MS.

Laura McGregor received her M.Sc. in chemistry from the University of St Andrews, UK. She completed a further M.Sc. in forensic science at the University of Strathclyde, UK, followed by a Ph.D. in environmental forensics. Laura's research interests include the chemical fingerprinting of environmental contamination using advanced techniques such as GC×GC–TOF-MS. In 2013 she joined Markes International as a sales support specialist for their TOF-MS products.

David Barden is a technical copywriter at Markes International, having joined the company in 2011. David studied natural sciences at the University of Cambridge, UK, and remained there for his Ph.D. in organic chemistry, which he received in 2003. A placement at the European Journals Department of Wiley-VCH, Weinheim, Germany, was followed by seven years as a technical editor for various scientific journals at the Royal Society of Chemistry, Cambridge, UK.

E-mail: **enquiries@markes.com**
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ISC 2014:
The Sound of Chromatography

Wolfgang Buchberger[1], Michael Laemmerhofer[2], and Wolfgang Lindner[3], [1]Institute of Analytical Chemistry, Johannes Kepler University, Linz, Austria, [2]Institute of Pharmaceutical Sciences, University of Tuebingen, Tuebingen, Germany, [3]Institute of Analytical Chemistry, University of Vienna, Vienna, Austria.

The hills will be alive with the sound of chromatography when the 30th International Symposium on Chromatography (ISC 2014) takes place from 14–18 September 2014 in Salzburg, Austria.

As Mozart's birthplace, Salzburg, Austria, is well-known for its connections to classical music. However, on 14–18 September it will become host to the **30th International Symposium on Chromatography (ISC 2014)** attracting chromatographers from around the world. ISC 2014 is a major liquid chromatography/gas chromatography (LC/GC) conference that was first held in 1956 in London, and since then has been held biannually in various European cities.

Chromatography in its various forms has become an eminently powerful analytical technique that is now indispensable for combating challenges in fields such as metabolomics, proteomics, pharmaceutical analysis, food control, environmental analysis, clinical chemistry, and doping control. Hyphenation with sophisticated mass spectrometric detection has led to the possibility of identification of unknown analytes with quantitation limits in a range that was beyond reach only a few years ago. Gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) technologies now represent a gold standard for analysis methodologies.

Progress in chromatography will always be an interplay between academic research performed at universities and the production of sophisticated and reliable instrumentation by manufacturers. Some may remember that sub-2 µm particles were investigated decades ago as stationary phases for LC, but dedicated ultrahigh-pressure liquid chromatography (UHPLC) instrumentation only became commercially available in 2005. Monolith-type stationary phases and core–shell particles have been developed highlighting the continuous advancements in column and instrument technology

Photo Credit: Allan Baxter/Getty Images

that are occurring. Similarly, supercritical fluid chromatography (SFC) has been the focus of research for many years, but its acceptance by chromatographers was quite slow until recently when a new generation of SFC instruments has begun to promote a renaissance of this technique.

ISC 2014 will be a forum for discussing fundamentals, applications, and the latest in instrumentation developments. The internet is an immense source of information for all fields of research, but identifying the most relevant information in this environment has become tricky. Personal communication and networking are required more so than before to keep the impetus. One should try to know the faces behind the names of authors of scientific papers. Personal communication is a prerequisite for progress, as is reflected in the motto of **ISC 2014 "Communicating Separation Science for the Future".**

A broad knowledge of separation science is required to become an expert and to judge the best option for tackling and solving a given analytical problem. As well as modern LC and GC instrumentation, one should not forget capillary electrophoresis (CE and CE–MS), which can be a more appropriate approach. The selection of the most suitable analysis methodology depends on the analytical challenge at hand including consideration of the very crucial step of sample pre-treatment.

ISC 2014 will cover all aspects of modern separation science, including hyphenation with MS–MS instrumentation, and will, therefore, go beyond the scope of some other conferences that are dedicated to single fields of chromatography. ISC 2014 will provide a "one-stop shop" for analytical chemists and attendants to see a complete picture of current progress. It will bring together a wide range of international scientists from both academia and industry so that we can learn the most up-to-date information about theory and practice from each other to accelerate progress in our field.

The abstract submission deadline is 30 April 2014 for oral presentations, and 31 May 2014 for posters.

Attendees at ISC 2014 will enjoy an exceptional scientific meeting in the unique and beautiful cultural surrounds of Salzburg!

E-mail: **i.kaehler@cmi.at**
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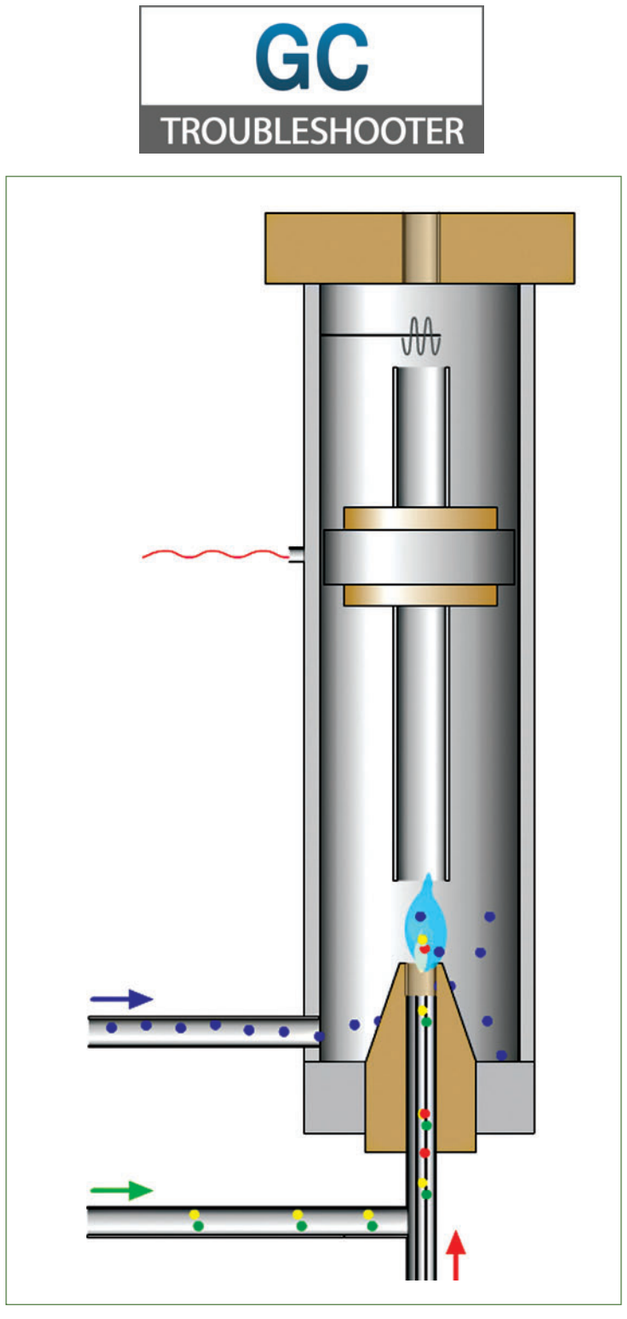

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The Open University, Milton Keynes, UK
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GC Troubleshooting and Maintenance
12 March 2014
Hilton Reading, Drake Way, Reading, UK
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Gas Chromatography: Fundamentals, Troubleshooting, and Method Development
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Practical and Applied Gas Chromatography
4–5 December 2014
Houston, Texas, USA
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HPLC/LC–MS

LC–MS for the Chromatographer
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HPLC Troubleshooting and Maintenance
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Website: http://www.chromacademy.com/Preparative_HPLC_Essential_Guide.asp?tpm=1_2

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METHOD VALIDATION

How to Develop Validated HPLC Methods: Rational Design with Practical Statistics and Troubleshooting
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Temple University, Philadelphia, Pennsylvania, USA
Website: http://www.crawfordscientific.com/training-online-calendar.asp

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GPC

GPC Academy
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PolyRMC Facilities at Tulane University, New Orleans, Louisiana, USA
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Website: http://www.wyatt.com/training/training/light-scattering-training.html

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11–14 March 2014
Warminster, Pennsylvania, USA
Website: http://www.spscientific.com/ProteinHandsOn/

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Congress Centre, Riva del Garda, Italy
Organizers: Luigi Mondello, Chromaleont, University of Messina, Italy
Tel: +39 334 3612788
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6–9 July 2014
8th International Conference on Breath Research & Cancer Diagnosis
Torun, Poland
Organizers: Nicolaus Copernicius University, Polish Chemical Society, and the Committee of Analytical Chemistry PAS
Tel: +48 56 6114753
E-mail: conference@breath2014.pl
Website: www.breath2014.pl

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31 August–5 September 2014
Dioxin 2014
Hotel Meliá Castilla, Madrid, Spain
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Tel: +34 91 400 93 84
E-mail: dioxin2014@mci-group.com
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ISC 2014 — 30th International Symposium on Chromatography
Salzburg Congress Center, Salzburg, Austria
Organizer: Austrian Society of Analytical Chemistry (ASAC)
Tel: +43 (0)512 575 600
E-mail: i.kaehler@cmi.at
Website: www.isc2014.at

Contact Information

Europe

Group Publisher
Michael J. Tessalone
mtessalone@advanstar.com

Sales Manager
Lindsay Jones
ljones@advanstar.com

Sales Executive
Liz Mclean
emclean@advanstar.com

Sales Operations Assistant
Sarah Darcy
sdarcy@advanstar.com

Editor-in-Chief
Alasdair Matheson
amatheson@advanstar.com

Managing Editor
Kate Mosford
kmosford@advanstar.com

Assistant Editor
Bethany Degg
bdegg@advanstar.com

Advanstar Communications
Bridgegate Pavilions, 4A
Chester Business Park,
Wrexham Road,
Chester, CH4 9QH, UK
Tel: +44 (0) 1244 629 300
Fax: +44 (0) 1244 678 00

North America

Group Publisher
Michael J. Tessalone
mtessalone@advanstar.com

Associate Publisher
Edward Fantuzzi
efantuzzi@advanstar.com

East Coast Sales Manager
Stephanie Shaffer
sshaffer@advanstar.com

Editorial Director, Analytical Sciences
Laura Bush
lbush@advanstar.com

Group Technical Editor
Stephen A. Bown
sbrown@advanstar.com

Managing Editor
Megan Evans
mevans@advanstar.com

Administation and Sales Offices
Woodbridge Corporate Plaza,
485 Route 1 South,
Building F, First floor, Iselin,
NJ 08830, USA
Tel: +1 732 596 0276
Fax: +1 732 225 0211

Corporate Office,
641 Lexington Ave., 8th Floor,
New York, NY 10022-4503, USA

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