The Column

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Cover Story

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Thrown Out Of Court

Knowledge gaps in method validation and forensic analysis

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Features

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The *LCGC* Blog: Forensics, Lawyers, and Method Validation — Surprising Knowledge Gaps

Kevin A. Schug, University of Texas Arlington, Texas, USA.

***LCGC* Blogger Kevin Schug has uncovered some surprising knowledge gaps in method validation and forensic analysis.**

Photo Credit: Ryan McGinnis/Getty Images

Recently I served as an expert witness in a case involving the detection of a cocaine metabolite, benzoylecgonine, in a defendant's urine using gas chromatography–mass spectrometry (GC–MS). This test was performed at a forensics laboratory following a reported positive test using a preceding immunoassay screen for drug metabolites. I will not relay any more details than this, because the problem in question dealt with an apparent lack of GC–MS method validation. For the analytical community, method validation in some form or another is a natural extension of best practice in the analytical laboratory. However, the notion of method validation, and many aspects of detailed forensics analysis, are not well understood by most lawyers and judges. I suppose that this might not be surprising to most, but it does present a serious knowledge gap that must be bridged in cases involving substance or alcohol abuse, if the associated case is to be properly litigated. In this particular case, I was called to testify on the basics of GC–MS, its complexities in analytical method development, and the necessity of method validation and verification. As part of my testimony, I was asked to write a very basic account on the importance of method validation. Below is the bulk of the text that I submitted for this purpose. I thought it might be interesting to relay in the *LCGC* Blog forum to raise awareness for others that such a knowledge gap does widely exist, and that it is vitally important for analytical scientists to be able to convey such concepts to the public community in fairly simple terms. At the end, I give a bit more about the problem associated with the case in question, which itself is fairly surprising.

Method validation, the comprehensive performance and documentation of measurements to verify a method is reliable and fit for purpose, is an essential component of any analytical chemical

measurement. The failure to appropriately validate and document a method makes it is impossible to prove the validity of the scientific test performed by that method. Such a result would be scientifically unacceptable.

There exist many degrees of rigour for method validation that depend on the value and use of an analytical measurement. On the one hand, simple "quick and dirty" feasibility measurements at the outset of a research project may only require simple system suitability requirements are met, and that a result can be reproduced. These may only be recorded in one's lab notebook and alone would not be appropriate for broad dissemination. On the other hand, unequivocal verification of drug purity for a marketed pharmaceutical, determination of the presence or absence of substances in a biological sample for the purpose of legal action, or dissemination of a new method in peer-reviewed literature require the highest degree of rigour in method validation to ensure the validity of the result. There are many aspects to the validation process, and there is a great deal of guidance available to ensure that proper steps are taken throughout the experimental design and performance of work.[1–3]

The components of method validation depend on the desired output of the method. At the outset, a method validation plan, which sets the parameters to be measured and the desired performance metrics, should be devised and documented. The parameters may vary generally depending on whether the method is meant to be quantitative (to determine the amount of a chemical compound in a sample) or qualitative (to identify or verify the presence of specific chemical compounds in a sample). There are metrics associated with each manipulation of the sample from acquisition through preparation and chemical analysis.

When a new instrument is installed in the laboratory, it must first meet manufacturer specifications for proper operation. It must be validated to provide the appropriate information for its intended purpose. The proper operation of the instrumentation must also be verified on the day it will be used to measure real samples (for example, casework samples), as well as when regular maintenance is performed. All of these activities should be carefully documented and verified correct by a laboratory manager. For some chemical analyses, the sample must first be treated in some way to remove interferences (for example, solid-phase extraction or liquid–liquid extraction are common sample preparation procedures) or to better

prepare the analytes for chemical analysis (for example, derivatization of analytes to improve chromatographic separation or MS detection). Each step in a chemical analysis adds variance, or error, to the method. The sum of variance for each step in a method equals the total variance of the method, and some steps are more prone to error than others. Therefore, each step must be carefully validated to understand and document its contribution to total error of the method, so that the overall reliability of the end result can be judged.

Quantitative analysis requires the documented validation of multiple figures of merit.[1,2] Required parameters include establishing the accuracy, precision, limit of detection, limit of quantification, and specificity of the method. A reliable calibration model (a correlation between analyte concentration and instrument response) must be established. The potential for interferences (from the sample matrix as well as from carryover) must be assessed. Throughout the analytical method, instances of sample loss must be evaluated. In some cases, where deemed necessary, stability of samples and robustness of the method should also be determined. The definition and precise procedure associated with each of the above parameters varies depending on the laboratory setting, the types of samples measured, the techniques used, and the intended use of the measured results. When a method is first established on a new instrument, all of these parameters should be performed and documented with the utmost rigour. As the method is used, the performance must be periodically verified (on a regular basis, in conjunction with instrumentation maintenance, and when real samples are analyzed) using appropriate subsets of the full validation procedures. All documentation should be curated, maintained, and made available when it is necessary to verify that these procedures have been properly implemented and that reliable performance has been proven.

In some cases, only qualitative verification of the presence of a substance in a chemical sample is desired (for example, by MS). Such an analysis may be triggered if a decision point from a previous measurement (for example, by immunoassay) was positive and needs to be verified. Several of the validation parameters mentioned above should still be determined and documented to ensure a reliable qualitative analysis.[2] This is most important when an analyte in a highly complex biological sample is to be scrutinized. Biological matrices

contain many small and large molecules with variable chemical character that can interfere with different measurements. Thus, specificity must be ensured by assessing potential interferences from exogenous constituents that may be in the sample and that may respond similarly to the analyte of interest. In addition, multiple samples of the biological fluid of interest from different sources should be measured to ensure that endogenous components, which might vary from sample to sample, do not interfere with the intended measurement.

Many of today's instruments are exceedingly sensitive. It is important to assess the limit of detection of an instrument for a given analyte, even as part of a qualitative analysis, as well as to determine carryover. Many instruments can measure into the parts-per-billion concentration range. If any residue of the analyte remains in the instrument or in a sample introduction device, then a false positive reading could result. It is important to verify that the level of the measured analyte is consistent with the decision point that prompted the qualitative analysis in question. Ideally, the qualitative confirmation by the complementary technique would also involve a quantitative analysis. This is often not mandated, but it would improve the reliability of the determination. That said, to reiterate, at a minimum, the qualitative analysis requires validation of specificity (interference and carryover determination) and the limit of detection of the method. Some guidance documents also suggest determination of precision in these cases.[3]

Many of the specifics associated with the recommended actions for validation can be significantly detailed in terms of procedures and expected performance levels when a particular analytical method is in question. It is important to remember that all steps of sampling handling and analyte determination require validation to ensure reproducible results. Similarly, validations and verifications are best carried out by the analyst who will also handle the real samples, and the documents generated should be approved by a laboratory manager. Overall, clear documentation is critical for all aspects of validation, verification, maintenance, methodological changes, and other factors that might influence a final reported result. Such information provides the confidence necessary to make decisions and draw conclusions about situations that are important for safety and well-being.

For the case in question, the qualitative determination of benzoylecgonine in

urine, the forensic laboratory had never performed a method validation. In 2007 when new GC–MS instruments were installed, they had simply made one injection of a benzoylecgonine standard to visually confirm an appropriate electron ionization spectrum was recorded. There were no attempts made to assess specificity in urine, limit of detection, carryover, or precision — throughout an approximately 7–8 year period. While my task was to explain why this was a problem to the prosecutors and judge, and to bridge the knowledge gap, I was most surprised that the forensic laboratory had not performed due diligence. Guidance on such method validations in this case was to come from ISO 17025, which clearly conveys recommended procedures for validation in this and other determinations. According to the information I was given, not only did the laboratory fail to follow these recommendations, the associated laboratory accrediting body charged to verify compliance and good laboratory practice failed to notice this inconsistency. I fear that this type of situation might be more widespread than we know. I do believe that many forensics laboratories operate at the highest standard of performance and validation. Yet, there are many forensics laboratories and the compliance and competence surely vary among them. If you couple the knowledge gap in communicating science to some lawyers and judges with a potential lack of quality lab results in some cases, it is a little scary to think of the number of decisions that may have been incorrectly rendered. Perhaps this is another justification that all citizens should be scientifically literate — an extra set of eyes to locate these potential inconsistencies would seem to be a good idea, especially if someone's money and livelihood are at stake. I think most people would agree that the system needs to be meticulously authenticated so that those who are guilty are found guilty, and those that are not, are not.

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3. United Nations Office on Drugs and Crime, Guidance for the Validation of Analytical Methodology and Calibration of Equipment Used for Testing of Illicit Drugs in Seized Materials and Biological Specimens, New York, 2009, pp. 1–67.

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News

Chang Gung University, Taiwan, Joins Waters Centers of Innovation Program

The Taipei's Chang Gung University (CGU) Healthy Aging Research Center has joined the Waters Centers of Innovation (COI) Program as a partner, making it the first in Taiwan to do so. Led by Dr. Daniel Tsun-Yee Chiu, Dean of Research & Development, and director of CGU's Healthy Aging Research Center, the Center focuses on investigating the mechanisms of disease, particularly in the elderly. Chiu has a special interest in the effects of oxidative stress on cellular function and establishing reference laboratories to assess oxidative stress and antioxidant capacity in health and in diseases. He is currently the president of the Society of Free Radical Research-Asia and he has published over 140 scientific articles in this area. **www.waters.com**

Thermo Fisher Scientific Proteomics Facility for Disease Target Discovery Opens at Gladstone Institutes

The Thermo Fisher Scientific Proteomics Facility for Disease Target Discovery opened at the Gladstone Institutes (California, USA) on 24 June, as part of a collaboration between Thermo Fisher, Gladstone, the University of California, San Francisco (UCSF), and QB3, to accelerate targeted proteomics research using advanced mass spectrometry techniques. The facility is directed by Nevan J. Krogan, PhD, a senior investigator at the Gladstone Institutes, a professor of cellular and molecular pharmacology at UCSF, and director of the UCSF division of the life science research institute and startup accelerator QB3. The facility will provide scientists from Gladstone, UCSF, and QB3 with state-of-the-art mass spectrometry technologies to characterize protein dynamics in complex biological systems. **www.thermofisher.com**

Diagnosing Diabetes Using Amino Acid Profiling of Hair

Profiling amino acids in scalp hair could aid the diagnosis of type 2 diabetes, according to a new study published in the journal *Analytical Chemistry*.[1] Researchers performed gas chromatography–mass spectrometry (GC–MS) on hair samples taken from diagnosed patients and healthy patients to show that type 2 diabetes alters the amino acid composition of hair's main building block, keratin, and that this biomarker could aid diagnosis in the clinic.

There are two types of diabetes, type 1 and type 2. Type 1 diabetes usually develops in childhood because of an underproduction of insulin, whereas type 2 diabetes develops because of insulin resistance in later life. According to the paper, because of this insulin resistance, amino acid update by cells is reduced resulting in a variation in freely circulating amino acids available for hair growth. Hair sampling is widely used in forensic investigations because it is noninvasive and hair samples can be stored for longer periods of time than blood or urine. The reason for this is that hair follicles in the scalp source freely circulating amino acids from the bloodstream, therefore newly grown hair reflects changes in these freely circulating amino acids over a period of time. Peter de B. Harrington from the University of Ohio told *The Column*: "The pilot study[2] was an initial investigation if we could use amino acid profiling of hair to identify individuals for forensic investigative leads. From the survey results, we had a large collection of diabetic individuals, so we decided to see if we could identify this disease state from the amino acid profiles in hair."

To investigate whether scalp hair contained biomarkers, scalp hair samples were taken from 27 people in Jordan — 15 were diagnosed with diabetes mellitus type 2. Hair samples were hydrolyzed using hydrochloric acid followed by amino acid derivatization using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and subsequently analyzed by GC–MS. The nonessential amino acids Gly, Glu, and Ile were more abundant in the hair of diagnosed patients compared to control subjects.

Harrington told *The Column*: "There are two key phases for future work: find a faster and gentler method for digesting the hair and freeing the amino acids — we could only reliable observe 14/20, so being able to characterize all 20 would improve the informing power — [and] increase the size of the study so we have more individuals and validate the diabetic condition with blood tests." — B.D.

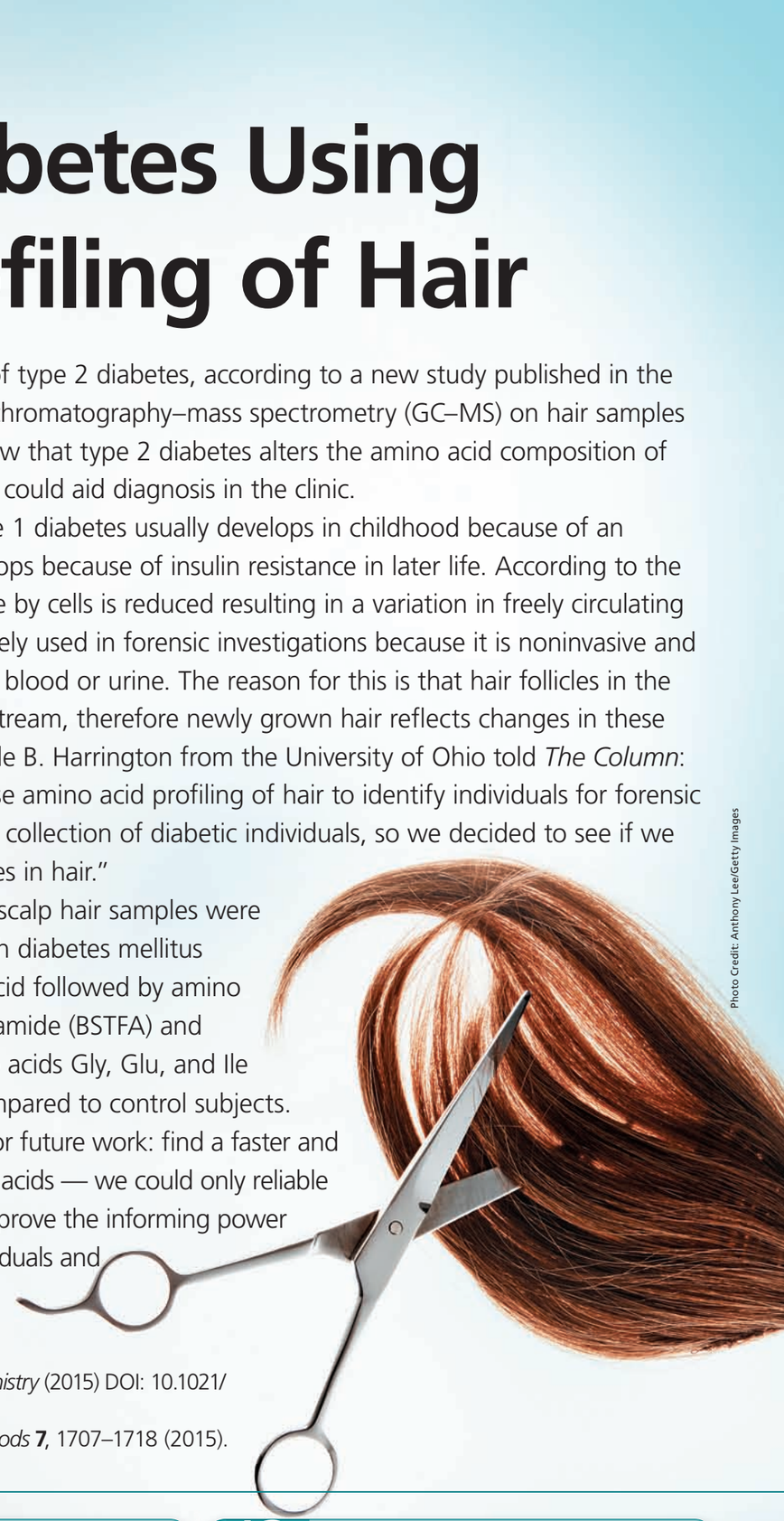

Photo Credit: Anthony Lee/Getty Images

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1. A.H.B. Rashaid, P.B. Harrington, and Glen P. Jackson, *Analytical Chemistry* (2015) DOI: 10.1021/acs.analchem.5b00460
2. A.H.B. Rashaid, P.B. Harrington, and Glen P. Jackson, *Analytical Methods* **7**, 1707–1718 (2015).

Improving Sanitation with the Help of GC–MS

In lower-income countries there is a lack of sanitary toilet facilities, resulting in the contamination of open water sources with human waste. One of the issues reported is that the available toilet systems are poorly maintained and smell, meaning that people often avoid them altogether. As part of a wider project funded by the Bill & Melinda Gates Foundation to develop sustainable next-generation toilets for lower-income countries (the "Reinvent the Toilet Challenge"), researchers have applied gas chromatography–mass spectrometry (GC–MS) to profile the malodour compounds in the headspace of latrines in Africa and India.

It is estimated that around 2.5 billion people worldwide do not have access to clean sanitation facilities, which means that sewage is poorly controlled and can enter the environment, encouraging the spread of disease. One of the issues with toilet facilities that discourages people from using them can be malodours. Researchers from the perfume and flavours company Firmenich (Geneva, Switzerland) profiled the headspace around latrines in Africa and India to identify compounds responsible with the aim of eliminating them with future designs.

Corresponding author Christian Starkenmann told *The Column*: "The Bill & Melinda Gates Foundation try to help developing countries to have a decent sanitations facilities. But who wants to build latrines and maintain them for free? Therefore they would like to design a long-term business model. One possible aspect of this business model is to have people pay when they go to the toilets. These toilets must be nice and clean. If the toilet is nice and clean but stinks, what is the point to pay to use them?"

Because odourant molecules are at low concentrations in the gaseous phase, the team developed a method with two sample concentration steps. The first step was to accumulate volatiles in buffered water and then load onto a solid-phase extraction cartridge in the field for subsequent extraction and analysis of analytes back in the laboratory using GC–MS with olfactory detection (GC–MS–O). The aim was to detect analytes of interest — hydrogen sulphide, methyl mercaptan, butyric acid, p-cresole, indole, and skatole — at levels below the odour detection threshold (ODT). The method was then applied to analyze the headspace samples taken from pit latrines in Ahmadabad, India; Nairobi, Kenya; and Durban, South Africa.

According to Starkenmann, many available sensors just give an indication of compound concentrations, while the human nose is much more sensitive. He said: "Many sensors are available on the market but they just give a rough idea of the concentration of critical odourant compounds. For example they cannot differentiate between methyl mercaptan, which smells of sewage, with a very low odour threshold, compared to hydrogen sulphide, which smells of rotten eggs. This method will be very useful for people who try to understand the concentration critical odourant in the headspace at very low level." — B.D.

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Photo Credit: Kathy Konkle/Getty Images

LCGC TV Highlights

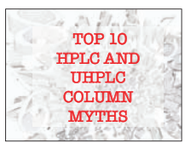
***LCGC* TV: Metabolic Fingerprinting Using GC–MS**
In this new video from *LCGC* TV, Christian Wachsmuth from the University of Regensburg in Germany, compares the performance of different ionization methods for GC–MS in metabolic fingerprinting. He also goes on to talk about how GC–MS could be applied in a clinical setting and what developments are needed for this to happen.
Watch Here>>

***LCGC* TV: Factors to Consider when Developing a 2D LC Method**
Comprehensive liquid chromatography (LC) is a multidimensional technique that has the potential to increase peak capacity resolution when separating complex mixtures, especially in food analysis. Luigi Mondello from the University of Messina, Italy, gives his advice for chromatographers who want to develop 2D LC methods. **Watch Here>>**

Peaks of the Week

The *LCGC* Blog: Eight Steps to Better Results from Solid-Phase Extraction — If you use SPE in your work, then most likely it's very important to the success of your applications, and its proper implementation will be key to the performance of your analyses. In an effort to help you develop a better understanding of the technique, *LCGC* blogger Tony Taylor offers eight steps to solid-phase extraction success. **Read Here>>**

Interview: Miniaturizing Biomarker Detection — The development of novel microfluidic systems opens up new opportunities to quantify clinically relevant biomolecules to further the understanding and diagnosis of disease. The 2015 recipient of the AES Mid-Career Award, Adam T. Woolley, from Brigham Young University, Utah, USA, is working in this area to develop novel and sophisticated integrated microfluidic systems for enhanced biomarker quantitation and quantification. He recently spoke to *LCGC* about this work. **Read Here>>**



The Top 10 HPLC and UHPLC Column Myths: Slideshow — *Webster's New Collegiate Dictionary* defines a myth as "an ill-founded belief held uncritically, especially by an interested group". Could that group be misinformed chromatographers? Find out by clicking through the slideshow here.

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News In Brief

Markes International Expands UK Operations

Markes International is expanding its UK headquarters in Llantrisant, near Cardiff, Wales, with the acquisition of another building. The new building, which is adjacent to the company's existing manufacturing facility and technical centre, comprises nearly 3000 square feet of office space, which initially will be allocated to the company's expanding engineering operations.
www.markes.com

TLC×GC Analysis of Fatty Acid Composition

Researchers have coupled comprehensive thin-layer chromatography (TLC) with gas chromatography (GC) using headspace sampling for the analysis of fatty acid composition. According to the study, the technique is a viable alternative to high performance liquid chromatography (HPLC) coupled with GC.
DOI: 10.1016/j.chroma.2015.05.011

Novel Food Wrapping to Eliminate Odours

Using nanocellulose-zeolite composite films in food packaging could eliminate the problem of smelly foods. Scientists developed films capable of trapping volatile thiol-based odours and confirmed elimination of odours using headspace solid-phase microextraction (SPME) coupled to gas chromatography–mass spectrometry (GC–MS).
DOI: 10.1021/acsami.5b02252

Photo Credit: fatmayilmaz/Getty Images

How to Get a Job in Analytical Science

Incognito offers some careers advice to aspiring separation scientists.

I am currently hiring analytical scientists from bench analysts to more senior management — and a lot of the CVs that I'm seeing aren't really doing it for me at all.

There are certain things I personally like to see on a CV to attract my attention. I want to see your qualifications, who you have worked for previously, and a brief description of your previous job roles. For the more qualified of you, I want to see your publications and presentations; and for the even more senior your management experience and so on.

I also want to to see what sets you apart from the rest. This generally involves situations in which you solved a problem, took an alternative approach, gained valuable experience, or showed a willingness to further understand your work. These needn't be long-winded stories but some illustrative examples from past CVs include:

- I can maintain and repair high performance liquid chromatography (HPLC) equipment to the point where I know when to call an engineer without costing you money.
- My personal "right first time" metric on gas chromatography–mass spectrometry (GC–MS) is 92% and I have a spreadsheet to prove it!
- I act as an adjudicator in the company method transfer resolution panel and can spot when someone is lying or covering up.
- I know enough to clean a source but not enough to understand why it works — and I'm looking to change this as quickly as possible.
- I am a GC method developer. Not an "on-paper" method developer which, when you start to question my approach, you will find out I know very little, but one who can develop robust and fit for purpose methods that can actually be used to support your business.
- I have a solid background in analytical chemistry, which at a basic level means I can pour anything into pretty much anything without spilling something. I know how annoying it is to leave a balance in a dirty condition, and therefore I don't and I can make HPLC and MS equipment sing and dance like Fred Astaire (look him up if you are too young to remember who he is)!
- I can examine a method SOP and tell you where I think the issues might be. I can then

confirm that I was right by implementing that terrible method; I can also make it better if that's what you'd like.
- I won't be the guy that bothers you with questions on why a method doesn't work; I'll be the guy who comes to tell you why the method doesn't work.
- I drove an improvement scheme, which reduced our department overheads by one-third last year by remodelling our instrument ultilization. Sorry — I should have said I actually introduced a system, which worked better than a spreadsheet designed by an accountant.

I don't have the means to check these out without actually meeting the person and discussing their claims — oh wait a minute, that's an interview, right? Am I saying quirky statements get you an interview — well, yes, I guess I am. Even if it's so I can bust your claims wide open!

I like to hear what you do in your spare time; however, spending time with your family, doing sports, reading, or outdoor activities are a little passé. I'd like to hear the quirky things about you. Again here are some example that have caught my attention:
- (While I was working for an International Coatings company) I have a vested interest in the quality of decorative coatings, as my Father is an interior decorator.
- I like to prove mathematical formulae (that was the only thing written in the box headed "Tell us about Yourself").
- In my spare time I'm a cat trainer for a well-known local street theatre group (domestic feline — not large and scary).
- I'm an end-of-life companion with my local hospice team.
- I design and build machines for automation around the home (please stop thinking of Wallace and Gromit!)
- I'm an international pool champion.
- I'm an ex-professional footballer.
- Working in an analytical laboratory is merely my first step on the career ladder to becoming an international scientific super-genius.

Again, I'm not saying you should lie or mislead anyone because this will not do your job prospects any good, but something a little out of the ordinary always catches the eye.

Now let us take a second to consider the recruitment programme from my side. How on earth do I assess if you have what it takes from a short interview? Obviously I can ask you a bunch of questions that get progressively more difficult until I reduce you to a quivering wreck, but I'm not sure if this works well. I asked one candidate a while ago why they were laughing after I asked a tricky question — "I feel my sense of humour is all I have left" came the reply.

I can also give you a questionnaire to complete, which helps reduce the nervousness of the interview situation. However, I've seen some really dumb questions in these tests and, unless very carefully designed, they can be almost meaningless. Here are just a few illustrative examples:
- Which process is considered as an important approach in the field of analytical science?
- What are the various functions of the process of spectroscopy?
- What are the modes of quantification in related substances tests?
- What is the difference between the silica used for TLC and that used for HPLC?
- What is a baseline?

Presentations can also be difficult. I have a friend who prepared a talk entitled "The Role of Serendipity in Analytical Method Development" for a job interview; in introducing the ethos of the laboratory, the manager explained in some detail the rigorous nature of the method development process and how "automated" and decision tree driven their approach was. It's always very difficult for a candidate to pitch the perfect presentation and is very time consuming for both parties.

My favourite (and I think most informative) interview task is to take someone into the laboratory and get them to make up an HPLC eluent and adjust the pH with the commensurate recording of data. If it's a GC job then we make up some standard solutions. Of course I ask questions as I go and we will discuss various aspects of the analytical techniques but these simple operations can reveal much about a person. I do this no matter what level in the company the person will be working — even if their role is laboratory management and they will ostensibly be office based.

So — what's the take home?
- Be quirky but in the right measure — it helps to differentiate your CV.
- Only write statements that you can back up with knowledge, facts, or skills.
- Be prepared to undertake practical work.
- Most important of all — look like you really want to work in analytical chemistry and not just be another pair of hands that is being thrown in to provide resources. I know this is cynical but I see it so often these days.
- And lastly, have the ambition to become an international scientific super-genius. It got our friend a plum job within the organization I was working for at the time. Don't knock it until you've tried it!

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Solid-Phase Microextraction in Clinical Diagnostics

Researchers at the University of Waterloo in Canada are collaborating with clinicians at Toronto General Hospital to develop preclinical and clinical applications of solid-phase microextraction (SPME). Bethany Degg of *The Column* spoke to Barbara Bojko from the team to find out more.

Photo Credit: TEK IMAGE/SCIENCE PHOTO LIBRARY/Getty Images

Q. What are your main research interests at present?

A: My professional background is in clinical laboratory diagnostics, and so my research interests have always centred on clinical applications. I am currently a member of Professor Pawliszyn's research group, the inventor of solid-phase microextraction (SPME). My main investigations are focused on new clinical application of this technology and the one that caught my attention was *in vivo* tissue analysis.

Q. What are the advantages of solid-phase microextraction (SPME) over other sampling methods in bioanalysis?

A: First of all, we need to remember that a single technique cannot be a panacea and solve all the problems we face in bioanalysis. As Professor Pawliszyn always emphasizes: "SPME is another tool in the toolbox" and we should know where and how to use it.

From my perspective, the most important feature of SPME that makes it a superior technique for clinical and especially *in vivo* analysis is its minimal invasiveness. As mentioned a number of times in our articles, there are a very limited number of analytical approaches that permit such studies and standard methods based on biopsy are too invasive to be used in applications requiring repeated analysis.

The other advantage is that we are very flexible in terms of the chemistry of the coatings, so we can cover a very wide range of analytes or we can use a selective coating to target specific analytes (for example, a drug or biomarker). We can also provide fully quantitative results, which allow us to use SPME for applications such as pharmacokinetics, or we can evaluate compound distribution in the organ(s) when using several probes simultaneously without causing damage to the tissues. In addition, when coupling to mass spectrometry (MS) we very rarely see the matrix effect.

Q. You recently developed a low invasive *in vivo* tissue sampling method for monitoring biomarkers and drugs during surgery. How did this come about?

A: We have been developing low invasive simple tools (SPME devices) based on

approximately 100 micrometre diameter flexible wires coated by a thin layer of sorbent material with biocompatible morphology and applying it to a range of applications, including environmental, food, and pharmaceutical analysis. We have determined that this approach could also be effective in the clinical environment because this wire can be placed into living tissue to sample small molecules that can be removed and transferred to analytical instrument for characterization. Historically, the platform of choice for SPME was gas chromatography (GC) or gas chromatography coupled with mass spectrometry (GC–MS) because the method was mainly used for sampling volatile compounds. Nowadays, especially for the clinical and pharmaceutical applications, we use liquid chromatography–mass spectrometry (LC–MS) systems to analyze non- and semi-volatiles in biological matrices.

We've been collaborating with a number of clinicians from different specializations for the past few years, which has resulted in many interesting applications of SPME. Tissue analysis is particularly important because standard sample preparation protocols are very tedious and require collection of the biopsy, which makes them highly invasive when it comes to analysis of living systems. This particular application of SPME was used to evaluate organs for transplantation, and was a perfect case for us to demonstrate unique features of the method, such as low invasiveness and the possibility of repetitive analysis, thus its applicability to monitoring changes of biochemical profile of grafts in time.[1] Our collaborators from Toronto General Hospital work on novel methods for organ preservation, which permits extending their lifetime and improves their performance, and aims to increase the pool of organs available for transplantation. Our joint research gives us a chance to better understand changes occurring in the organs during their preservation and to select the ones with the lowest risk of rejection.

Surprisingly, current evaluation of the organs, which could potentially be used for transplantation, is very limited, based mainly on general and non-specific tests and visual assessment. Therefore, information about the entire biochemistry of the grafts will definitely improve this process and will allow specific biomarkers to be selected; these could be determined within a few minutes, and allow clinicians to make an immediate decision on the usability of the organ, or its condition and level of damage.

Q. What were your main conclusions from this study?

A: Initially, part of our work was focused on optimizing the sampling procedure and validating the practical aspects of the intervention because, although it is minimally invasive, it is still direct sampling of a living system. However, after receiving positive feedback from the surgeons regarding the sampling step itself we were ready to evaluate the analytical part of the studies. In this instance this meant determining what compounds we are able to extract, what type and size of the probes we should use, what time of exposure is optimum for our studies, and so on.[2] In the end, we used an optimized protocol for analysis of a few pig models. We were able to extract analytes with a wide polarity range, starting from amino acids, sugars up to lipids, which are involved in many metabolic pathways. We have already characterized the molecules we may capture with the coating we used here in more detail in several other matrices, and the information can be found in our previous papers.[3–7]

Our results demonstrated that we are able to monitor differences between "healthy" and "injured" organs as well as identify progressing changes during the time of preservation of the organs and, subsequently, after their transplantation and reperfusion. For example, we found the difference in the profile of some compounds involved in Krebs cycle when we compared liver subjected to warm ischemia and control liver. The changes suggested impairment of enzymes in the injured livers. The "nice" finding was that monitoring the metabolism of one of the drugs routinely used during this medical procedure showed inhibition of the metabolism of the parent drug, which confirmed the assumption regarding the enzyme function made based on the metabolomics study. Of course, finding the selective biomarkers and drawing the clinically relevant conclusions requires performing studies on a large number of individuals — we have to first learn how to walk before we start running!

Q. What developments are needed for the sampling technique to be used in the clinic?

A: We have demonstrated that an "acupuncture-sized" flexible wire coated with appropriate sorbent is a suitable medium to extract biomarkers from the living tissue. Now we need to make an easy-to-handle tool for medical personnel. The more applications we develop and the more experience we gain in this field the more ideas we will have on what should be improved to meet the expectations of clinicians: the future users of the method. Currently, we are working on a new tissue sampler, which could be easily used by the surgeon during the medical intervention.

Also, we still push the limits of the size of the device to minimize the invasiveness of the sampling. Of course, such miniaturization of the devices results in a lower recovery of the extracted compounds, thus more sensitive instruments needs to be used for the detection. However, today's technologies meet this expectation, making the microextraction methods great alternatives to standard approaches. The manufacturers of the analytical platforms tune the sensitivity and decrease the amount of sample needed for the analysis and this is all in favour of "micro" methods. Moreover, current trends lead towards direct introduction of samples/extracts to detectors such as mass spectrometers with no prior chromatographic separation and SPME is a perfect tool to assist in such a coupling. Such a hyphenated rapid diagnostic tool can provide immediate, precise, accurate and reproducible results.[8]

Q. How do you see this technology developing?

A: I'm just hoping that our work can be translated to the use of this technology globally. It is difficult to introduce a novel analytical approach for daily use because it not only involves practical issues, such as going through the regulatory processes and financial involvement of medical institutions to set up a new method in their facilities, but also overcoming some mental barriers of the everyday users: the physicians or technicians who simply get used to standard approaches and most of them don't see the need of replacing "good enough with better". But on the other hand, we are looking for the niche in clinical analysis where SPME could fit and so far the response we have received from our collaborators is promising, so maybe we can make a case and SPME will become an established technology in the clinical environment.

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Advances in Sample Preparation: Removing Phospholipids from Biological Samples

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Solid-phase extraction (SPE) has long been used for sample preparation — and is often chosen over liquid–liquid extraction (LLE) — because it can efficiently clean up and concentrate analytes of interest without the use of the more toxic organic solvents. This article describes the properties and use of a new SPE sorbent that removes phospholipids from biological samples, and illustrates the benefits of this approach with practical examples.

Reversed-phase solid-phase extraction (SPE) has long been a staple of bioanalytical and environmental analysis because it is relatively simple to perform.[1–3] A sample is loaded on to a reversed-phase sorbent (often in a primarily aqueous medium), and, because the compounds of interest are generally hydrophobic, they bind to the sorbent preferentially over the aqueous media. The sorbent is then rinsed or washed with an aqueous solution containing a low percentage of water miscible organic solvent (such as methanol) to remove salts, polar matrix compounds, and other interfering components. Finally, the analytes of interest are eluted from the sorbent with an organic solvent, at which point they can be either analyzed directly, or dried down and reconstituted in an appropriate solution. There are myriad variations on this technique. By customizing sample pretreatment, pH, and the percentage and nature of the organic solvents used for washing and elution, SPE can be an extremely versatile, specific, and powerful technique for extracting and concentrating a variety of analytes from complex matrices.

Early reversed-phase SPE sorbents consisted of inorganic base-particles; for

example, silica bonded with a hydrophobic phase such as C_{18} hydrocarbon chains. Silica-based SPE sorbents can be modified in a number of ways, such as changing the length or nature of the bonded phase (for example, C_8 versus C_{18}; cyanopropyl or diol versus hydrocarbon). More recently introduced reversed-phase stationary phases are polymeric in nature, often consisting of polymerized divinylbenzene that can be modified on the surface or incorporate additional monomers to improve performance or enhance the retention of specific chemical classes.

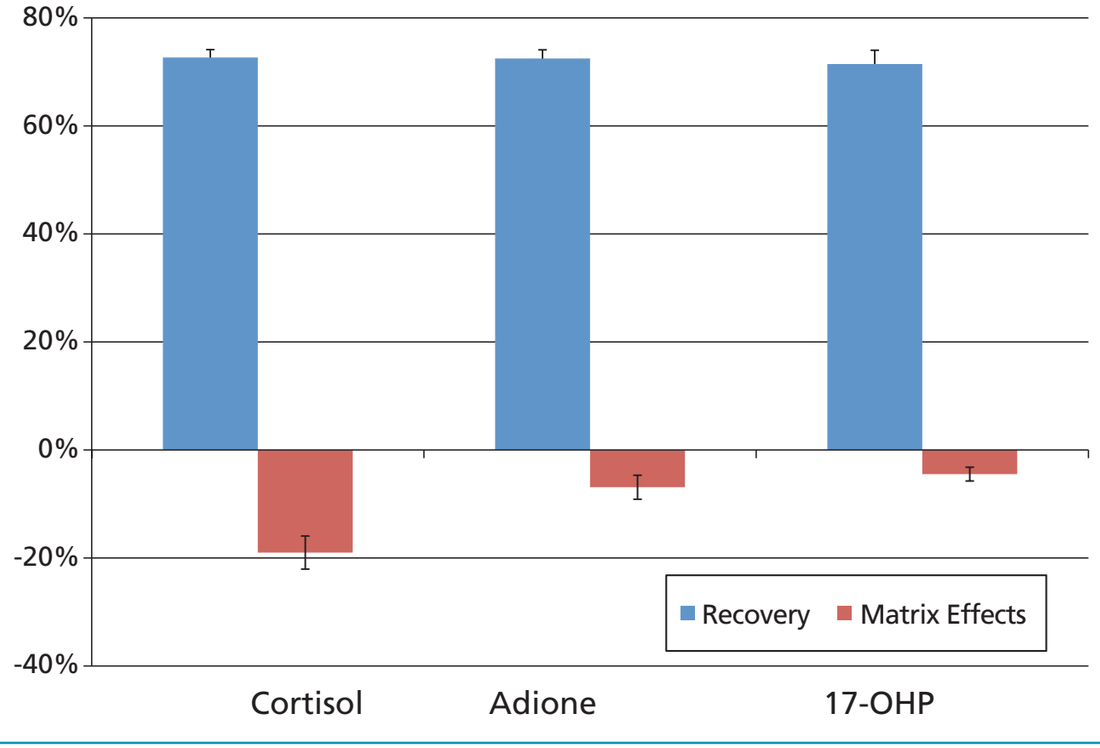
Despite the development of highly specific extraction methods, one common challenge is the presence of phospholipids in many biological samples. These components of the lipid bilayer of cell membranes include phosphatidylcholines, lysophosphatidylcholines, phosphatidylinositols, and sphingomyelines, to name a few.[4] Phospholipids can contribute significantly to adverse matrix effects in liquid chromatography–mass spectrometry (LC–MS) analyses.[5–7] This can become an even more significant issue with food safety applications, using matrices such as meat or milk, in which up to 15–20% of the wet weight of the sample is composed of fats and up to 3% consists of phospholipids. In addition to the adverse effects these components have on electrospray ionization (ESI), the accumulation of phospholipids on LC columns and ionization sources can lead to reduced column lifetimes and more frequent MS maintenance.

A new reversed-phase SPE sorbent has now been developed that combines the "catch and release" properties of reversed-phase SPE with the ability to simultaneously remove phospholipids from biological samples. It also shares many properties with other polymeric, reversed-phase sorbents including water wettability, enabling the direct loading of aqueous samples without the need to condition or equilibrate the sorbent. This approach can save time and simplifies the sample preparation workflow, but also eliminates the risk of analyte retention loss during the extraction process, should an individual cartridge or well within a plate dry out during the conditioning or equilibration steps. This article describes the performance of this novel sorbent, and provides specific analytical examples.

Materials and Methods

Oasis PRiME HLB cartridges and plates (Waters Corporation) were used for all solid-phase extractions. ACQUITY/Xevo UPLC–MS–MS systems were from Waters Corporation. All solvents (acetonitrile, methanol, isopropanol) were Optima grade and were obtained from Fisher.

Figure 1: Recovery and matrix effects from the extraction of corticosteroids from plasma. Adione and 17-OHP refer to androstenedione and 17α-OH progesterone, respectively. Samples were extracted as described in the Materials and Methods section. (N = 4).



Extraction and Analysis of Corticosteroids from Plasma:

Sample Preparation: Samples were prepared as follows: Plasma samples of 150 µL were precipitated with 300 µL of a solution of 4:1 methanol:89 g/L $ZnSO_4$ and then centrifuged. A volume of 300 µL of the resulting supernatant was then added to 900 µL of 4% H_3PO_4 and aspirated to fully mix the sample. The resulting pretreated sample was then directly applied to the wells of a micro elution plate. All wells of the SPE plate were then washed with 2 × 200 µL aliquots of 25% methanol. The samples were then eluted with 2 × 25 µL aliquots of 90:10 acetonitrile: methanol and diluted with 25 µL of water. 7.5 µL was injected onto the UHPLC–MS–MS system.

Extraction and Analysis of Synthetic Cannabinoids and Metabolites from Whole Blood:

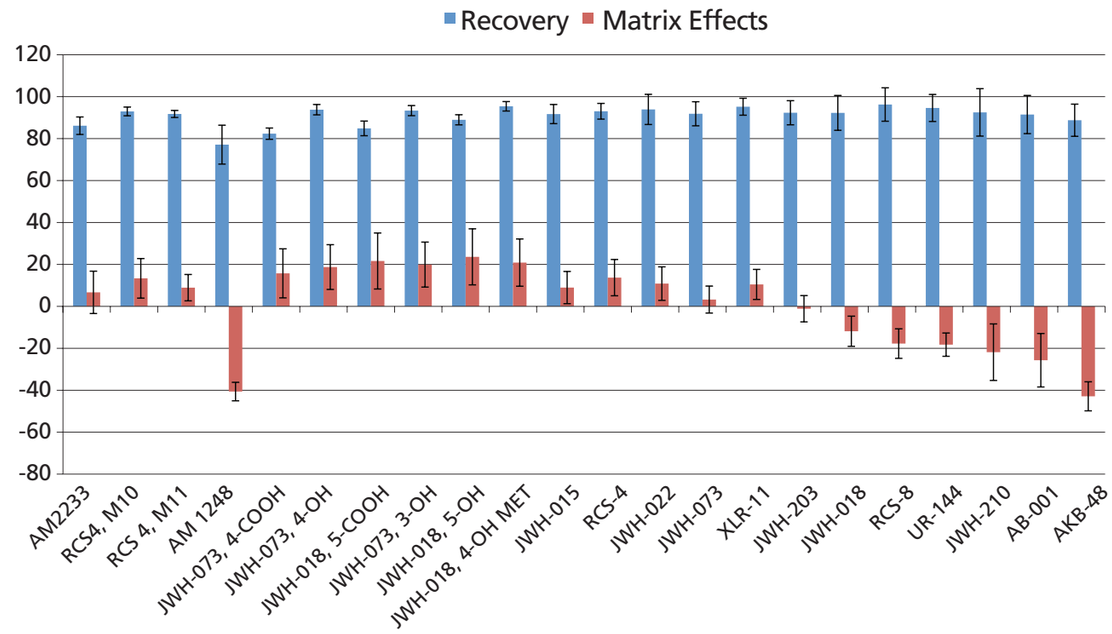
Sample Preparation: Samples were extracted using the novel sorbent in 30 mg plate format. A 100-µL solution of 0.1 M zinc sulphate/0.1 M ammonium acetate was added to 100 µL of whole blood, and vortexed for 5 s to lyse the cells. All samples were then precipitated by adding 400 µL acetonitrile. The entire sample was vortexed for 10 s and centrifuged. The supernatant was then diluted with 1.2 mL water and directly loaded on to the 30 mg plate. All wells were then washed with 2 × 500 µL 25:75 methanol:water, and eluted with 2 × 500 µL 90/10 acetonitrile/methanol. The eluate was then evaporated under nitrogen and reconstituted with 100 µL 30% acetonitrile, and 5 µL was injected onto the UHPLC–MS–MS system.

Analysis of Veterinary Drugs in Milk:

Sample Extraction: 4 mL of 0.2% formic acid (FA) in acetonitrile was added to 1 mL of milk and thoroughly mixed. Samples were centrifuged for 5 min at 10,000 rpm. Aliquots of the supernatant were used for SPE cleanup.

Solid-Phase Extraction (SPE) Cleanup: 3 cc (60 mg) SPE cartridges were prepared by conditioning with 3 mL 0.2% formic acid in acetonitrile. (Note: This conditioning step is

Figure 2: Recovery and matrix effects from the extraction of synthetic cannabinoids and metabolites from whole blood. Samples were extracted as described in the Materials and Methods section. (N = 4). Average recoveries were 91%. The average magnitude of matrix effects was 17%.



only required if gravity loading is desired. This step is *not* necessary if a sample is processed with minimal vacuum). The supernatant was passed through the cartridge and collected, then evaporated to dryness under a gentle nitrogen stream. After reconstitution in 1 mL of 5% methanol in water, the samples were filtered and transferred to a vial for UHPLC–MS–MS analysis.

Analysis of Veterinary Drugs in Salmon: *Sample Extraction:* A sample of 2.5 g of homogenized tissue was placed in a 50 mL centrifuge tube. A solution of 10 mL 80:20 acetonitrile:water with 0.2% formic acid was added, and the samples were vortexed for 30 s, agitated on a mechanical shaker for 30 min and then centrifuged at 12,000 rpm for 5 min.

Solid-Phase Extraction (SPE) Cleanup: The resulting 0.5 mL supernatant was directly loaded onto the SPE cartridge (3 cc, 60 mg) and drawn through the sorbent under a gentle vacuum. No conditioning or equilibration was performed. The 300 µL eluate was then diluted with 600 µL of 10 mM NH_4COOH (pH 4.5) and analyzed by UHPLC–MS–MS.

Results and Discussion

Extraction of Corticosteroids from Plasma: Figure 1 illustrates the recovery and matrix effects seen for corticosteroids after extraction from plasma. Extraction recoveries ranged from 72–73% with %RSDs under 5%. The matrix effect for cortisol was -19%, indicating minor ion suppression, and was minimal for the other compounds. The average matrix effect for all three compounds was -10.1%. Standard deviations for matrix effects were 3.1% or less. This method was also assessed for accuracy and precision. R^2 values for all three analytes were 0.99 or greater with mean percentage deviations of <10% for all calibration points. Quality control results are listed in Table 1. All results had accuracies within 10% of target values and %CVs less than 7%.

Extraction of Synthetic Cannabinoids from Whole Blood: A different bioanalytical matrix and method were evaluated by extracting a panel of synthetic cannabinoids and metabolites from whole blood. These compounds have proliferated

Table 1: Quality control results from extracted plasma samples. ($N = 6$ for each compound at all three levels).

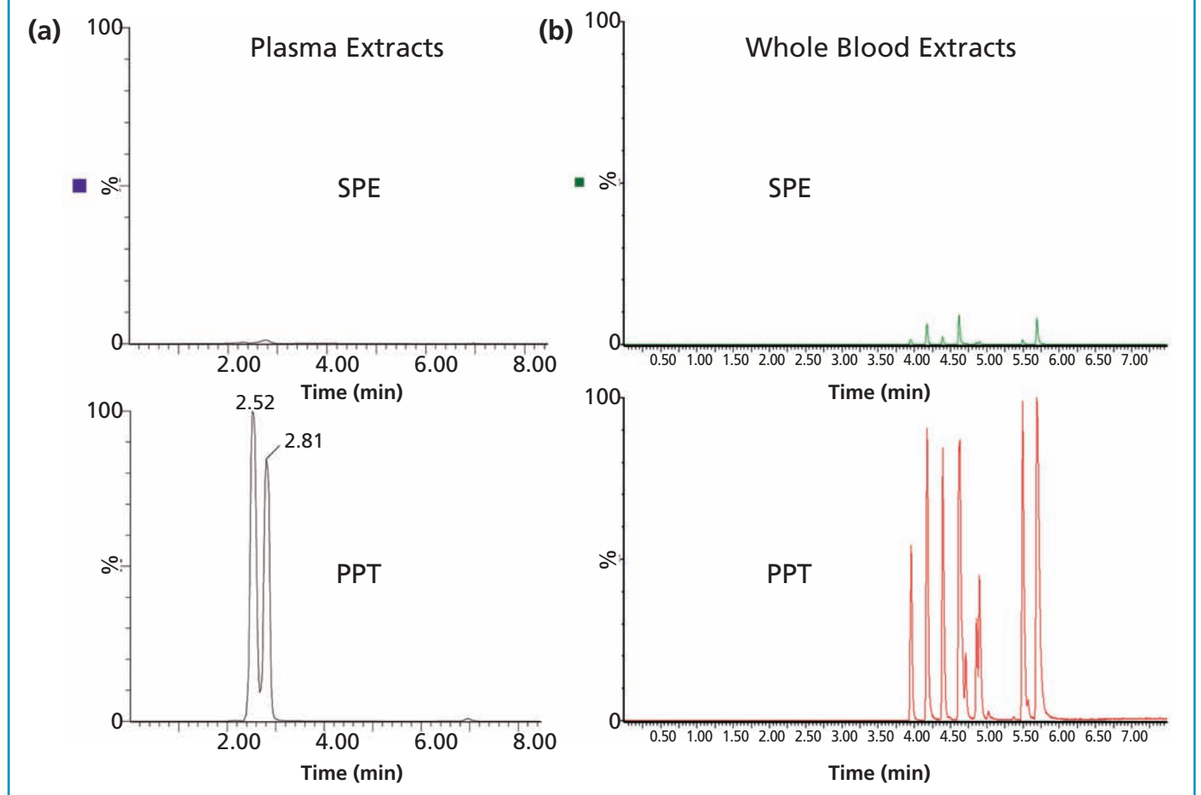
	Accuracy									
	Androstenedione			17α-OH progesterone				Cortisol		
QC Level (ng/mL)	Mean (%)	S.D. (%)	CV (%)	Mean (%)	S.D. (%)	CV (%)	QC Level (ng/mL)	Mean (%)	S.D. (%)	CV (%)
0.15	94.3	5.4	5.7	93.7	6.1	6.5	**3**	92.3	4.9	5.4
1.5	95.0	3.4	3.6	92.3	4.7	5.6	**30**	94.8	2.9	3.0
15	95.4	5.3	5.5	93.7	6.1	6.5	**300**	94.9	5.7	6.0
Mean	**94.9**			**92.6**			**Mean**	**94.0**		

drastically over recent years and represent a rapidly growing issue for medical examiners and forensic toxicology laboratories.[8] This panel represents several classes of these compounds including adamantoylindoles, napthoylindoles, phenylacetyl indoles, and tetramethylcyclopropylindoles. Major hydroxylated and carboxylated metabolites of JWH-073 and JWH-018 were also included because these compounds are rapidly metabolized *in vivo*.[9–11] Whole blood samples were prepared as described in the materials and methods section. Figure 2 summarizes the recovery and matrix effects seen across this entire panel. The average recovery was 91% with only a single analyte under 80%. Once again, these results were consistent, with %RSDs ranging from 2.2–12.2% and averaging 5.8%.

Figure 3 demonstrates the removal of endogenous phospholipids from the plasma and whole blood samples described above. Comparing the peak areas of remaining phospholipids revealed that >95% were removed from both plasma and whole blood samples.

Direct evidence of the impact of phospholipid removal was seen with the synthetic cannabinoid JWH 203. Under these conditions, this compound co-elutes with the putative phospholipid lyso-phosphatidylcholine 18:0 (LPC 524) at 5.7 min.[4] This corresponds to the final phospholipid peak in Figure 3(b). Figure 2 shows that matrix effects are minimal for JWH-203 using the phospholipid-removing sorbent. However, when the synthetic cannabinoid, JWH 203, was extracted from whole blood using a polymeric reversed-phase sorbent that does not remove phospholipids, ion suppression was increased to -94%, demonstrating the direct and deleterious effect that phospholipids can have in bioanalysis.

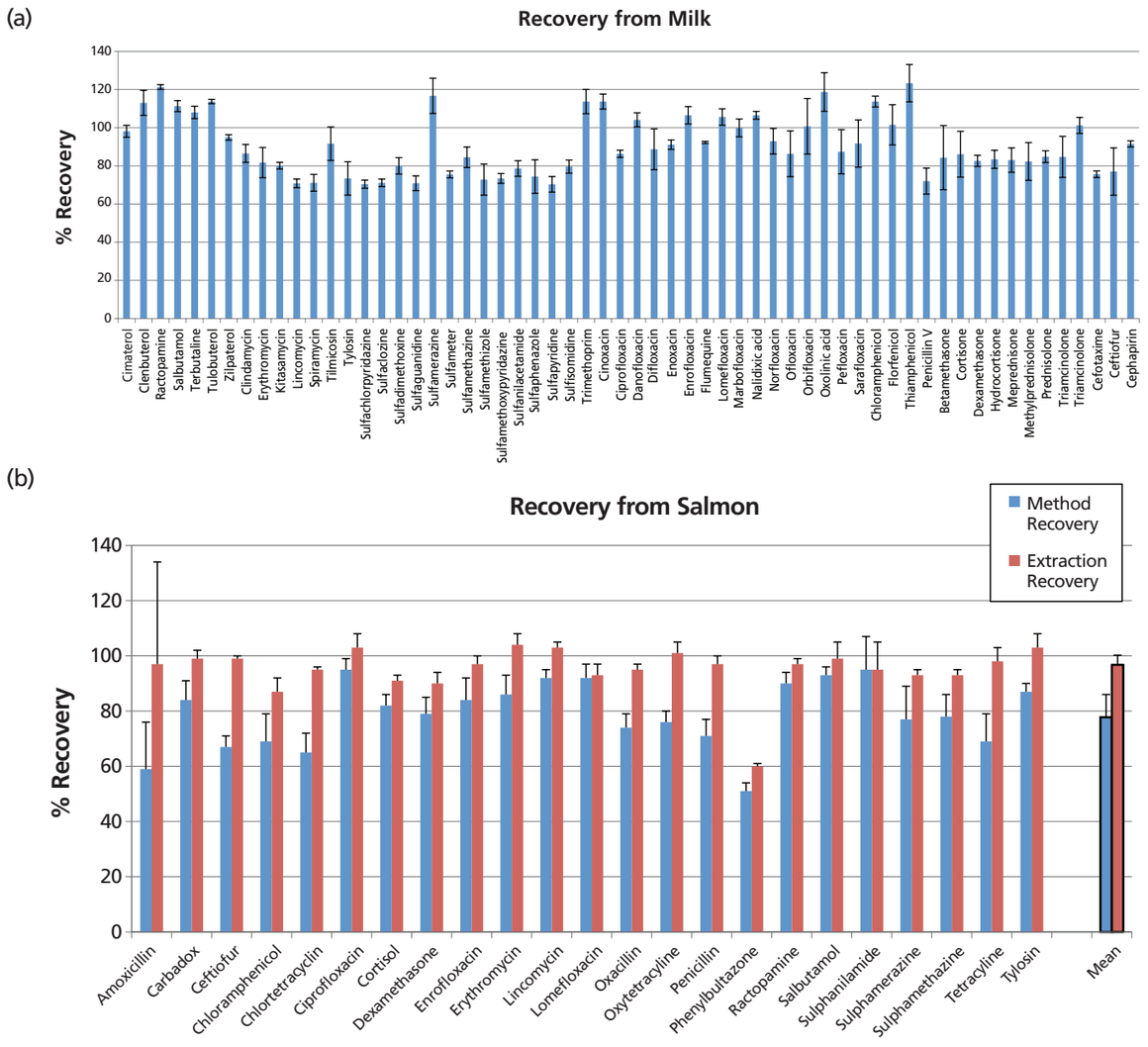
Figure 3: Residual phospholipids in plasma and whole blood extracts. (a): Residual phospholipids in plasma samples extracted with cartridges (top) and identical samples processed by simple protein precipitation (bottom). Compared to PPT, SPE eliminated >97% of phospholipids from plasma samples. (b). Residual phospholipids in whole blood samples. Compared to PPT, SPE eliminated >95% of phospholipids from whole blood samples.



Other polymeric reversed-phase sorbents showed an almost identical correlation between PL 524 abundance and ion suppression for this compound, revealing a direct and quantifiable correlation between the presence of phospholipids and ion suppression.

Two food safety applications were also assessed using this new sorbent, both pass through cleanup methods for multi-residue veterinary drugs in milk and salmon. In these cases, phospholipids can be present in substantially higher concentrations than in blood or plasma and have the potential

Figure 4: (a) Recoveries of veterinary drugs from milk samples following protein precipitation and pass through SPE cleanup ($N = 5$). The recoveries represent total method recovery (PPT and SPE). Actual loss from SPE was less than 5% for all compounds. (b) Recoveries of veterinary drugs from salmon samples following protein precipitation and pass through SPE cleanup ($N = 5$). The recoveries represent total method recovery (PPT and SPE). Method recovery refers to the extraction efficiency of the entire method. Extraction recovery refers to the extraction efficiency of the pass-through SPE procedure.



to create even greater problems with matrix effects and system cleanliness.

Multiresidue Cleanup of Whole Milk: Whole milk is a complicated matrix, containing large amounts of proteins, fats, and phospholipids that can interfere with the detection and quantification of target analytes. It is essential to isolate the compounds of interest in milk from lipids and proteins, just as one does in other matrices. Milk samples were prepared as described above and passed through 3 cc (60 mg) SPE cartridges to remove fats, phospholipids, proteins, and other matrix components. The results from this extraction are shown in Figure 4(a). These 60 compounds represent nine different classes of veterinary drugs, including sulphonamindes, fluoroquinolones, β-agonists, macrolides, glucocorticoids, amphenicols, β-lactams, cephalosporins, and tetracyclines. Recoveries ranged from 70–120% with an average recovery of 91%. Recoveries were reproducible, with %RSDs ranging from 1–18% with a mean of 7.4%. As with the bioanalytical methods, this method removed >90% of phospholipids from milk samples.

Multiresidue Cleanup of Salmon: The final example involves the extraction of veterinary drugs from salmon. Like the whole milk example, this represents a pass through method designed to trap fats, phospholipids, and other matrix components from precipitated supernatants.

Figure 4(b) shows recovery results of the pass through method used for salmon. Both method recovery (blue) and extraction efficiency (red) are shown. The method recovery data refers to the efficiency of the entire method, including precipitation and pass through SPE using 3 cc (60 mg) cartridges. The method recovery was quite good, ranging from 51% to 95% with an average of 79%. The pass through recovery data only represents the extraction efficiency of the sorbent and shows that the average recovery for this panel of compounds was 97% with an average %RSD of 3.5%. Once again, assessment of remaining phospholipds revealed that this pass through SPE method removed >90% of phospholipids from the sample compared to the total phospholipids remaining after SPE cleanup with a traditional C_{18} sorbent.

Conclusion

This article reviews the results obtained with a reversed-phase sorbent that is water wettable and can eliminate phospholipids and many fats from biological samples. Four different examples are shown that demonstrate performance in a variety of matrices, including plasma, whole blood,

milk, and salmon. These examples also represent a variety of different compounds, including steroids, synthetic cannabinoids, and veterinary drugs. Despite the variety of matrices and target compounds, use of this sorbent resulted in high and reproducible recoveries with relatively low matrix effects. Both SPE methods used the same elution solvent: 90/10 acetonitrile/methanol. Both pass through methods used supernatants containing about 80% acetonitrile. Phospholipids were nearly eliminated when compared to protein precipitation or other revered-phase SPE techniques. This led, in one particular example, to the elimination of 94% ion suppression for a compound that co-eluted with a large phospholipid peak.

In summary, water-wettability, and the ability to nearly eliminate residual phospholipids represent an improvement in reversed-phase SPE technology that can help scientists to improve their analytical methods. The examples described here can be used as a guide for similar matrices.

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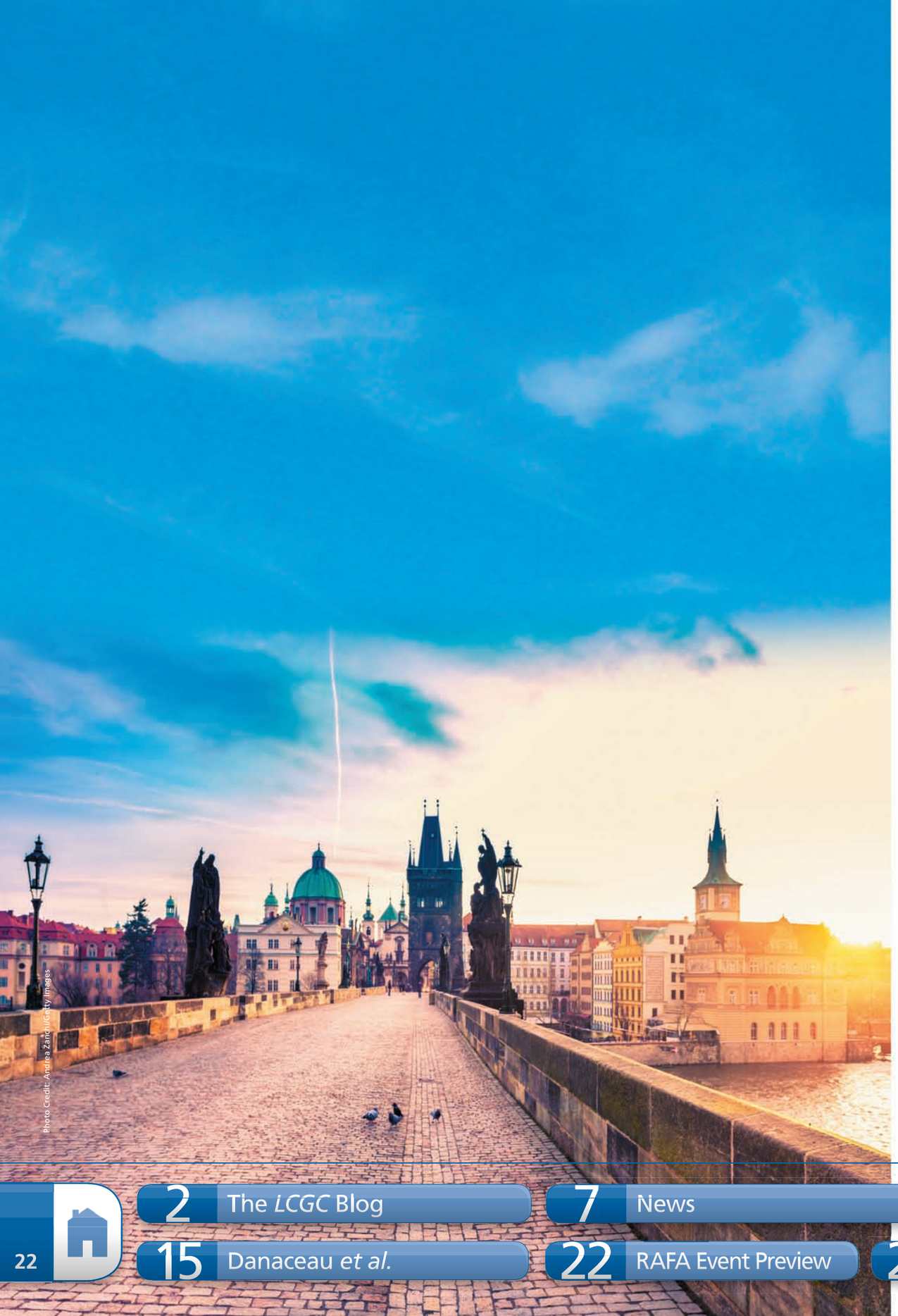

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7th International Symposium on Recent Advances in Food Analysis (RAFA 2015)

The 7th International Symposium on Recent Advances in Food Analysis (RAFA 2015) will take place at the Clarion Congress Hotel, Prague, Czech Republic, on 3–6 November 2015.

The 7th International Symposium on Recent Advances in Food Analysis (RAFA 2015) will provide an overview of the current state-of-the-art on analytical and bioanalytical food quality, safety control strategies, and introduce the challenges for novel approaches in this field. The programme will be tailored to provide networking opportunities as well as exploring the latest results from the food analysis community. Presentations will be given by leading scientists through keynote lectures and contributed oral and poster presentations. The following areas will be covered:

- **Food quality and safety** — Allergens; industrial contaminants; metals and metalloids; mycotoxins; marine and plant toxins; packaging and processing contaminants; pesticide residues; and veterinary drug residues.
- **General food analysis issues** — Authentication and fraud; bioactivity measurements; flavour and sensory analysis; foodomics; food forensics; nanoparticles; novel food and supplements; organic crops and foodstuffs; QA/QC; micro- and nano-food sensors; chemometrics; and data interpretation.

The conference programme will also be accompanied by several satellite events including:

- **Workshops on novel analytical strategies:** The 3rd European workshop on "Ambient mass spectrometry in food and natural products"; workshop

on "Infrared and Raman spectroscopy, and chemometrics for monitoring of food and feed products, bringing the lab-to-the-sample"; 1st European workshop on the "Analysis of nanoparticles in food, cosmetics, and consumer products"; workshop on "The application of micro/nano systems in food safety control"; and a workshop on "Smart data sets processing in food analysis".

- **Interactive seminar:** This interactive seminar will be on the topic of "Sample-prep, separation techniques, and mass spectrometric detection in food quality and safety: step-by-step strategies for fast development of smart analytical methods".
- **Food Authorities' summit, EU and beyond:** An FAO/IAEA workshop: Food safety — challenges for developing countries; an United States Department of Agriculture (USDA) seminar on "Food safety issues beyond the EU"; and an European Food Safety Authority (EFSA) information platform on emerging food quality safety issues of high concern.
- **Reference laboratories colloquium:** A workshop on "Experiences, achievements, and challenges of EU Reference Laboratories".
- **EU Framework programme seminar:** Tutorial for newcomers in HORIZON 2020, the EU framework Programme for Research and Innovation: a discussion platform mediating networking and joint planning of projects within the Societal challenge "Food security, sustainable agriculture, and forestry, marine, and maritime and inland water research and the bioeconomy" (chaired by an EC representative and supported by the Czech National Contact Point).

The keynote speakers have been announced and will include: Elke Anklam (European Commission, DG Joint Research Centre Director, Institute for Reference Materials and Measurements, Geel, Belgium): Quality Assurance of Methods Used for Food Safety and Quality Control; Paul Brereton (Fera Science Ltd., York, United Kingdom): Food Fraud — Old Problems New Solution; Christopher Elliott (Queen's University Belfast, Belfast, UK): Elliott Review into the Integrity and Assurance of Food Supply Networks — Final Report, A National Food Crime Prevention Framework; Carsten Fauhl-Hassek (Federal Institute for Risk Assessment, Berlin, Germany): Food Authentication: Challenges in Official Control; Jana Hajslova (University of Chemistry and Technology, Prague, Czech Republic): Pleasures Offered by Ion-Mobility MS to Food Chemists; Thomas Hofmann (Technische Universität München, München, Germany): Taste from Mother Nature and Culinary Art — Analytical Decoding by Means of the SENSOMICS Approach; Rudolf Krska (University of Natural Resources and Life Sciences, Vienna, IFA-Tulln, Austria): How Does Climate Change Impact on the Occurrence and the Determination of Natural Toxins; Erich Leitner (Graz University of Technology, Graz, Austria): Food Packaging Material and the Interaction with the Packed Good and the Analytical Challenges; Michel Nielen (RIKILT Wageningen UR, Wageningen, Netherlands): Ambient Mass Spectrometry Imaging of Food Contaminants; Bert Popping (Mérieux NutriSciences Corporation, Tassin la Demi-Lune, France): Out with the Old, In with the New: Novel Approaches in Allergen Detection Using MALDI-ToF-ToF and Mass Spectrometry; Michael Rychlik (Technische Universität München, München, Germany): Complementary Approaches in Food omics Towards New Horizons in Food Analysis; Michele Suman (Barilla Food Research Labs, Parma, Italy): Summary & Discussion Platform: Industry Perspectives.

An exhibition of recently introduced instrumentation in food analysis and other valuable equipment will be available during the symposium. Vendor seminars will also be organized to introduce recent developments and scientific strategies for advanced food quality and safety control.

Young scientists are encouraged to present their scientific work, with a number of RAFA 2015 Student Travel Grants available. The prestigious RAFA Poster Award will also be given for the best poster presentation by a young scientist, along with other sponsored poster awards. The deadline for registration at a reduced fee and submission of an abstract for oral presentation is 31 July 2015; deadline for submission of an abstract for poster presentation is 31 August 2015.

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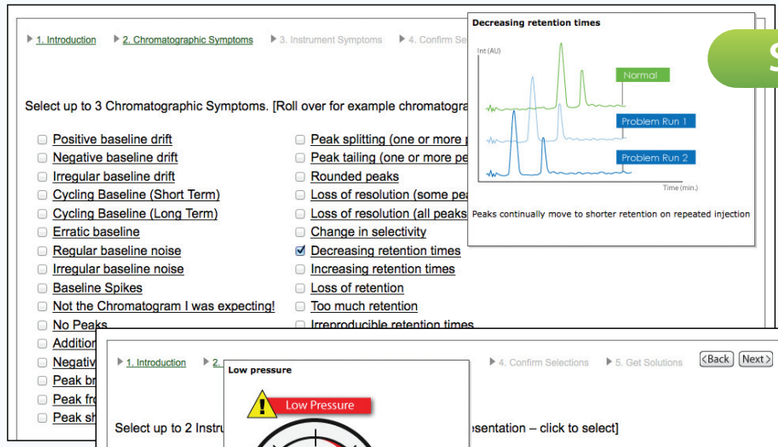
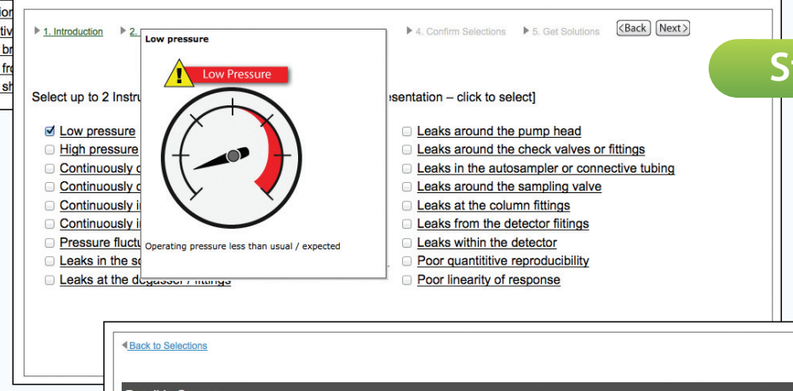
3. The troubleshooter returns a list of possible causes. Each cause has a concise summary of the problem and recommended solutions. These solutions are supported by over 1000 references, feature articles, and CHROMacademy content written by our experts.

HPLC Troubleshooter



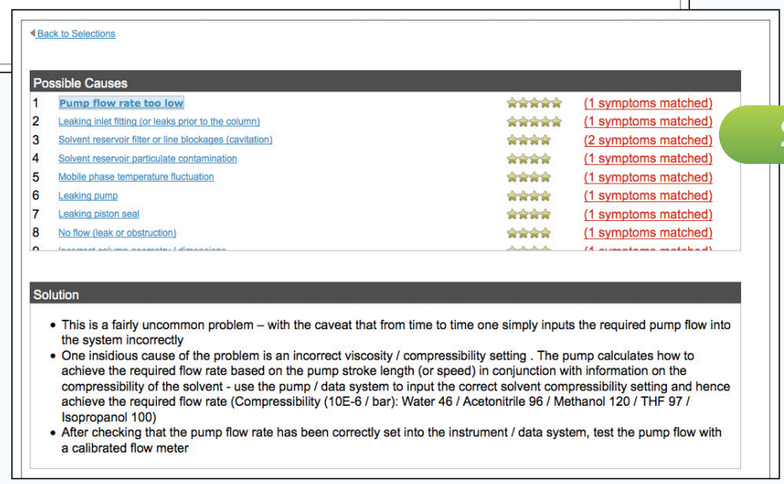
Step 1

Select your chromatographic symptoms.



Step 2

Select your instrument symptoms.



Step 3

See a list of probable causes and the recommended solutions.

Still got a problem? Ask our experts.

If you have a specific enquiry, or just need more information, one of our technical experts will contact you within 24 hours and will work with you until your problem has been resolved.

"Ask the Expert" is available only to Premier Members.

To find out more about CHROMacademy Premier membership contact:

Glen Murry on +1 732 - 346 - 3056 | e-mail: gmurry@advanstar.com

To discuss CHROMacademy Group or Corporate memberships contact:

Allen Basis on +1 732 - 346 - 3013 | e-mail: abasis@advanstar.com

The CHROMacademy troubleshooters are an on-line interactive version of the much-loved troubleshooting poster you see on lab walls all over the world.

Our troubleshooters are available to Lite and Premier members and are used globally as a resource for analytical scientists who wish to more accurately diagnose issues with equipment and separations.

Training Courses

GC

GC–MS Interpretation
12 August 2015
Hilton Glasgow Grosvenor,
Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Hands-on GC–MS Theory and Methods
26 August 2015
The Open University,
Milton Keynes, UK
Website: http://anthias.co.uk/training-courses/handson-GC-MS-theory-methods

Gas Chromatography: Fundamentals, Troubleshooting, and Method Development
8–11 September 2015
Axion Analytical Laboratories,
Chicago, Illinois, USA
Website: http://proed.acs.org/course-catalog/courses/gas-chromatography-fundamentals-troubleshooting-and-method-development/

HPLC/LC–MS

HPLC Troubleshooting and Maintenance
2 September 2015
Caledonian University,
Glasgow, UK
Website: http://www.crawfordscientific.com/training-online-calendar.asp

Basic Concepts of LC, Instrument Basics & Troubleshooting & Column Care & Maintenance
24 September 2015
MediCity Nottingham, UK
Website: www.hichrom.co.uk

How to Develop Validated HPLC Methods: Rational Design with Practical Statistics and Troubleshooting
14–15 October 2015
MicroTek, Edison,
New Jersey, USA
Website: http://proed.acs.org/course-catalog/courses/how-to-develop-validated-hplc-methods-rational-design-with-practical-statistics-and-troubleshooting/

The Theory of HPLC
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/hplc-training.html

Fundamental LC–MS
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/mass-spec-training.html

HPLC Troubleshooter
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/hplc_troubleshooting.html

METHOD VALIDATION

Validation and Transfer of Methods for Pharmaceutical Analysis
30 September–2 October 2015
Hilton Garden Inn, London Heathrow Airport
(formerly Jurys Inn Heathrow),
London, UK
Website: http://www.mournetrainingservices.co.uk/course_list.html#vampa

SAMPLE PREPARATION

Solid-Phase Extraction
On-line training from
CHROMacademy
Website: http://www.chromacademy.com/sample-prep-training.html

Hands-On Sample Preparation
5–8 October 2015
The Open University,
Milton Keynes, UK
Website: http://anthias.co.uk/training-courses/hands-on-sample-preparation

MISCELLANEOUS

Introductory & Advanced ½ Day Courses: MALS, DLS, and More
2 November 2015
Wyatt Technology Headquarters, Santa Barbara, California, USA
Website: http://www.wyatt.com/events/ilsc/2015-short-courses.html

Please send your event and training course information to Kate Mosford kmosford@advanstar.com

Event News

23–28 August 2015

35th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2015)
Hotel Maksoud Plaza, Sao Paulo, Brazil
Tel: +55 11 3056 6000
E-mail: dioxin2015@acquacon.com.br
Website: www.dioxin2015.org

2–4 September 2015

10th Balaton Symposium on High-Performance Separation Methods
Hotel Azúr, Siófek, Hungary
Tel: +36 1 2250210
E-mail: diamond@diamond-congress.hu
Website: www.balaton.mett.hu

3–4 November 2015/5 November 2015

24th International Light Scattering Colloquium (ILSC)/2015 Focus Meeting
Four Seasons Resort The Biltmore, Santa Barbara, California, USA
E-mail: programs@wyatt.com
Website: www.wyatt.com/events/ilsc/2015-ilsc-program.html / http://www.wyatt.com/events/ilsc/2015-focus-meeting.html

27–29 January 2016

14th International Symposium on Hyphenated Techniques in Chromatography and Separation Technology (HTC-14)
Ghent, Belgium
Tel: +32 10 454777
E-mail: info@LODrganisation.com
Website: http://www.ldorganisation.com/v2/content.php?langue=english&cle_menus=1238916064

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Mission Statement
The Column (ISSN 2050-280X) is the analytical chemist's companion within the dynamic world of chromatography. Interactive and accessible, it provides a broad understanding of technical applications and products while engaging, stimulating and challenging the global community with thought-provoking commentary that connects its members to each other and the industries they serve.
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