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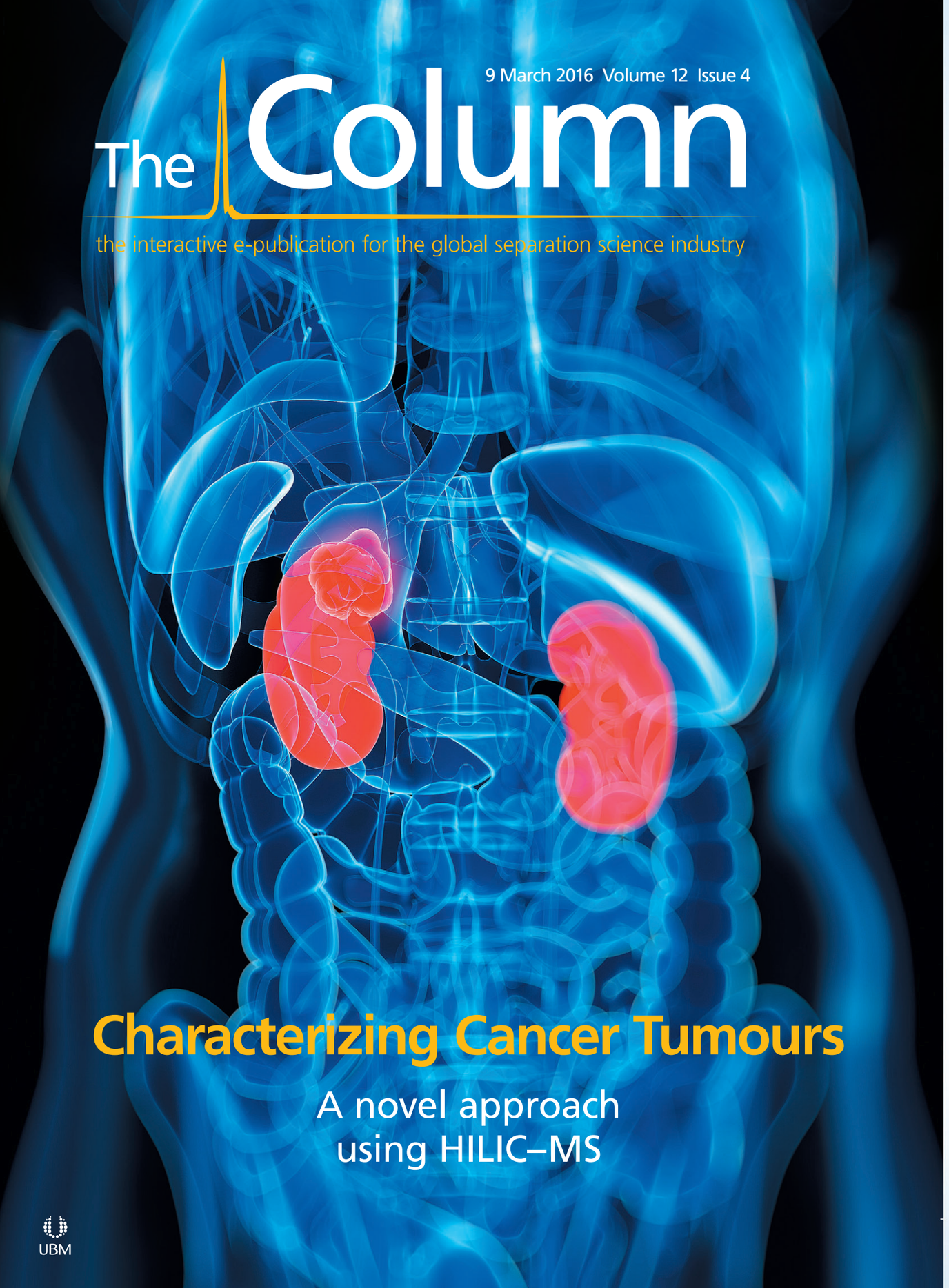
# The Column

the interactive e-publication for the global separation science industry



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UBM

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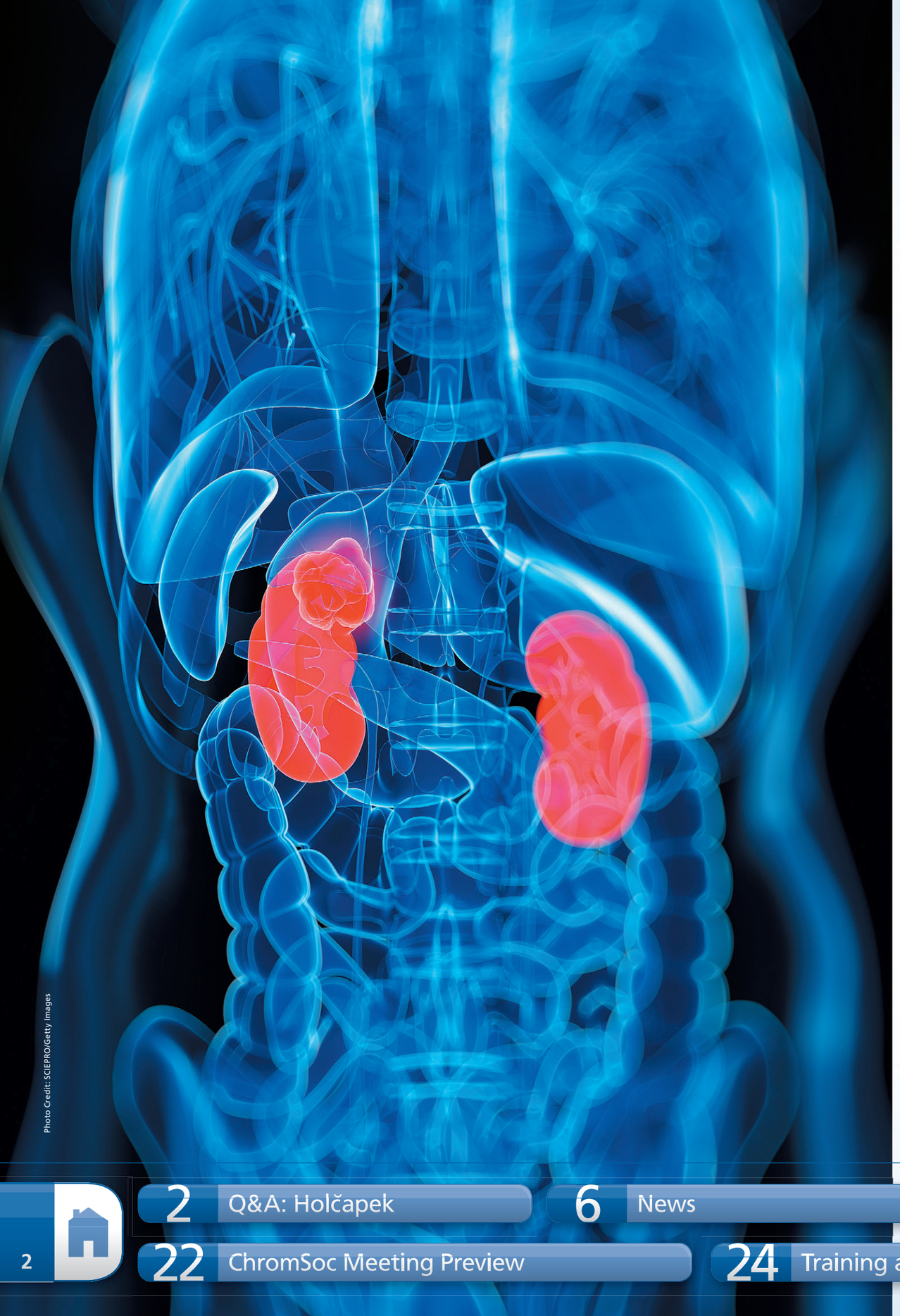

Photo Credit: SCIEPRO/Getty Images

# Characterizing Cancer Tumours Using HILIC–MS

**Early screening procedures are key in the fight against cancer. Michal Holčapek from the University of Pardubice, in Pardubice, the Czech Republic, spoke to Kate Mosford of *The Column* about his work using hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC–MS) in characterizing kidney cancer tumours.**

**Q. In 2015 your group published a study characterizing the differences between polar lipid classes in cancerous kidney tumours and in the surrounding normal tissue using hydrophilic interaction liquid chromatography (HILIC) and electrospray ionization mass spectrometry (ESI–MS).[1] What led your group to begin this study?**

**A:** We previously developed various cutting-edge separations of lipid isomers, but without strong biological applications. Later, I decided that I wanted to start up something with clear and easily explainable "benefits for humanity", so we started with the bioanalysis of human body fluids. We are now working on the large-scale ERC CZ project with the goal of finding lipid cancer biomarkers. In the framework of this project, we are analyzing a large number of clinical samples of tumour and healthy tissues obtained from oncological surgery, plasma, and urine of both cancer patients and healthy volunteers. The goal of this project is to find statistically relevant differences in the lipidomic composition of healthy individuals and those suffering from selected types of cancer, and then using this data to develop early diagnosis screening procedures.

**Q. What were your key findings?**

**A:** It is a well-known fact that tumour cells and normal cells are not identical. We already know some of the general differences between their lipidomes, such as the downregulation of phospholipids containing arachidonic acid with the expected upregulation of eicosanoids formed from arachidonic acid, which is currently being researched. Another general difference is the upregulation of some phospholipids containing combinations of saturated and


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monounsaturated fatty acyls, because those fatty acids can be synthesized *de novo* during the growth of tumour cells. Significant changes are also observed in the area of sphingolipids and glycosphingolipids. We are optimizing ultrahigh-performance liquid chromatography–mass spectrometry (UHPLC–MS) and ultrahigh-performance supercritical fluid chromatography coupled to MS (UHPSFC–MS) methods for their accurate quantitation.

**Q. What were the main challenges you encountered and how did you overcome them?**

**A:** By far the most serious problem in this type of research is biological variability. Proving statistically significant differences in cell composition are the result of the disease and not the result of biological variability is a major prerequisite in biomarker discovery. It is impossible to exclude or diminish this issue, because biological variability can be caused by many unavoidable variances, such as different living style, nutrition habits, physical activity, body-mass-index, age, sex, etc. This issue is the main reason why many previous biomarker studies failed in part or completely. We can still guarantee the quality of our analytical workflow using well optimized and validated quantitative methods, removing any additional uncertainty caused by natural sample-to-sample variations in the concentrations of individual metabolites.

**Q. What other analytical methods have been used in the analysis of lipids in tumours and how does your group's method compare?**

**A:** In recent years, MS-based approaches are the method of choice for a comprehensive quantitative analysis of lipids based on the following requirements: Internal standards for each lipid class to compensate for different extraction and ionization efficiencies, internal standards and analytes are ionized at the same time to avoid matrix effects and ion suppression, and finally the full validation of the whole analytical method. The combination of these factors sets the highest standards for lipidomic quantitation.

**Q. Kidney cancer is one of most prevalent cancers in both men and women. Could this analytical method be used to analyze polar lipids in other cancerous tumours?**

**A:** Yes, our method could be applied

to study various cancer types and other diseases with altered lipid metabolism. As well as kidney cancer, we can also analyze samples of breast, pancreas, prostate, and lung cancers. We focus mainly on cancer types with a high mortality, morbidity, with available treatment options, but a lack of reliable screening procedures.

**Q. What is your group working on next?**

**A:** We are still proceeding with this research because it was started less than three years ago. At the moment, we are measuring large sample sets, processing the data, and statistically evaluating them. When we find dysregulated lipids, we try to search for the biological consequences of these changes using metabolic pathways. Unfortunately, we are often unable to fully complete this task within our analytical laboratory, so we co-operate with skillful individuals with a strong biological background in lipidomics. The next step will be integrating our research into the context of metabolomics, proteomics focused on enzymes responsible for the lipid biosynthesis, and cancer biology. This type of research is certainly multidisciplinary.

**Q. You are chairing the upcoming HPLC 2017 symposium in Prague, have you started planning yet? Is there anything that the conference will focus on differently to previous years?**

**A:** We are already hard at work organizing and planning because we want to attract a large number of scientists from all over the world. The main goal of the symposium is to enable the active participation of many students and young researchers and ensure that they are given adequate space in the conference programme. We are preparing a range of interesting offers for younger scientists, such as low conference fees, cheaper short courses, travel grants, scientific awards, and a programme track devoted solely to short oral presentations by young researchers combined with tutorials given by leading experts during the whole conference. Our organizational efforts and plans for young scientists can be followed on the conference website (http://www.hplc2017-prague.org/) and Facebook (https://www.facebook.com/HPLC2017Prague/).

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1. Michal Holčapek *et al.*, *Journal of Chromatography B* **1000**, 14–21 (2015).

**Michal Holčapek** is a professor of analytical chemistry and head of the mass spectrometry group at the Department of Analytical Chemistry at the University of Pardubice, Czech Republic, where he obtained his Ph.D. in analytical chemistry in 1999. His research started with the structural analysis of organic and organometallic compounds using mass spectrometry (MS). This was then coupled with liquid chromatography (LC) and focused mainly on the metabolism of various pharmaceutical drugs and natural compounds. His present specialization is in the lipidomic analysis of biological samples using liquid chromatography (LC) or supercritical fluid chromatography (SFC) coupled to MS, shotgun electrospray ionization coupled to MS (ESI-MS), matrix-assisted laser desorption–ionization coupled to MS (MALDI-MS), and MS imaging. The main emphasis is on clinical research with an aim to find biomarkers for cancers and other serious human diseases. He has co-authored over 110 papers in peer-reviewed journals with a h-index of 32 and given over 300 conference presentations including more than 20 plenary or keynote lectures. He is a member of editorial boards on *Analytical Chemistry; Analytical and Bioanalytical Chemistry; Lipids;* a guest editor of special volumes on mass spectrometry — innovation and application in *Journal of Chromatography A* (2010, 2012 and 2016), editor of special issues on lipidomics in *Analytical Chemistry* (2014) and *Analytical and Bioanalytical Chemistry* (2015), editor of the book *Extreme Chromatography: Faster, Hotter, Smaller* (American Oil Chemical Society, 2011), past head of the Czech Mass Spectrometry Section and national representative in International Mass Spectrometry Foundation (2005–2013), and chairman of several international and national conferences.

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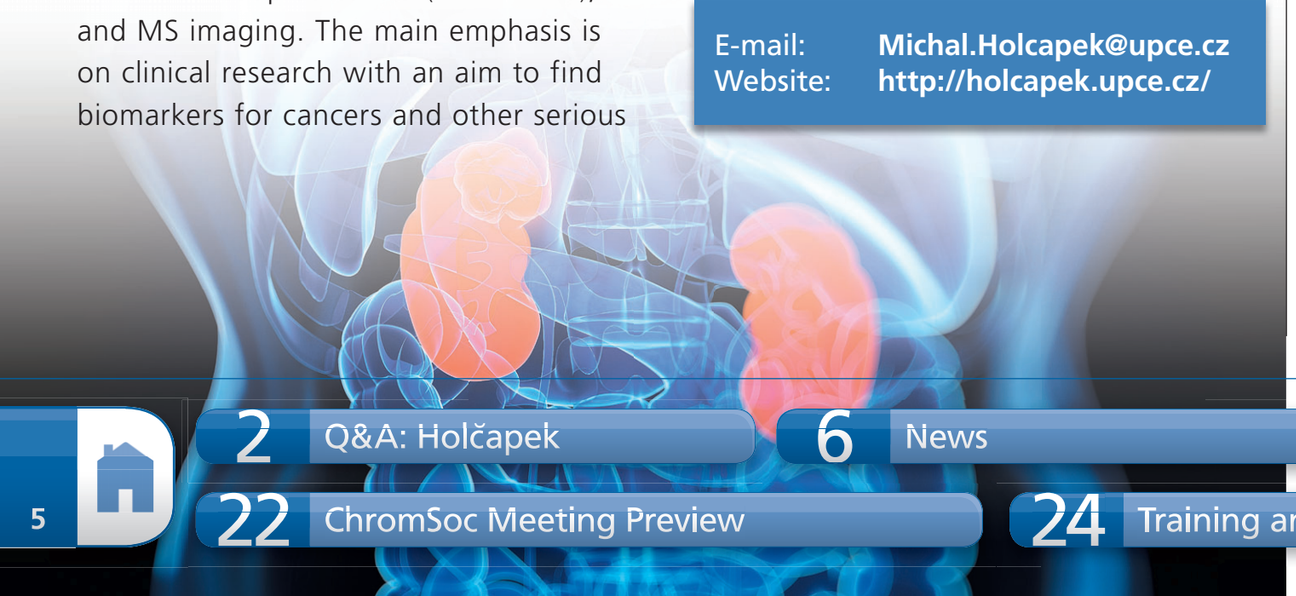



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# News

## Waters and BioCity Celebrate New Facility

Waters Corporation (Hertfordshire, UK) and BioCity (Cheshire, UK) have celebrated the opening of a new open access analytical laboratory located in the BioHub at Alderley Park, Cheshire, UK. The institute contains Waters' liquid chromatography (LC), mass spectrometry (MS), and informatics technologies, within a suite of analytical modules designed for use in the pharmaceutical and life sciences industries.

"At Alderley Park, and indeed across the BioCity group, we are working with a growth formula that provides emerging and growing companies with access to everything they need to succeed – be it people, facilities, finance, or technology," said Dr. Glenn Crocker (MBE) CEO, BioCity Group. He continued, "We are proud to be playing a leading role at the forefront of both the local and national economy."

"The companies located at BioHub are engaged in breakthrough research and development. Close engagement with these companies provides Waters with a deeper insight into their needs and challenges," said Rohit Khanna, Senior Vice President, Applied Technology Group, Waters Corporation.

Chancellor of the Exchequer, George Osborne, also praised the new laboratories, "This is more great news for Alderley Park and for Life Sciences in Cheshire. We are home to so many innovative and growing businesses in the sector and I was delighted to hear that BioCity Group and larger employers like Waters are helping to nurture new talent."

The laboratory will be resourced with many of Waters' equipment as well as a nuclear magnetic resonance (NMR) system provided by Manchester Science Partnerships, the owners of Alderley Park.

For more information please visit **http://www.biohubatalderley.co.uk/**

# Quantifying Methylergonovine Using LC–MS–MS

A team of researchers from China and the USA has developed a liquid chromatography coupled to tandem mass spectrometry (LC–MS–MS) method for quantifying methylergonovine (ME), a semi-synthetic ergot alkaloid used for the treatment and prevention of postpartum haemorrhage (PPH), in human plasma.[1] PPH results in 44,000 to 86,000 deaths each year around the world, making it the leading cause of death during pregnancy.

ME has been used more recently in the control of refractory headaches and can be used as a chemosensitizer for cancer. However, this alkaloid sometimes causes elevated blood pressure, and so quantification in biological matrices is necessary.

The team extracted ME from 500-µL plasma samples using liquid–liquid extraction under alkaline conditions and detected using positive multi-reaction-monitoring mode (+MRM) mass spectrometry (MS). The method was validated according to US FDA guidelines and covered a working range from 0.025 to 10 ng/mL with a lower limit of quantification (LLOQ) of 0.025 ng/mL.

The team concluded that they have developed a rapid, sensitive, selective, and accurate LC–MS–MS method, which was successfully applied to a clinical pharmacokinetics study in female volunteers. They determined that it is suitable for both preclinical and clinical studies on ME. — K.M

Photo Credit: SCIEPRO/Getty Images

## Reference


1. Hongxiang Lou *et al.*, *Journal of Chromatography B* **1011**, 62–68 (2016).

# Quantifying Nicotine Levels in Necrophagous Insects Using GC–MS

Researchers studying necrophargous blowflies for forensic investigations have developed a novel gas chromatography–mass spectrometry (GC–MS) method for the detection of nicotine.[1] Nicotine has been attributed to a number of accidental or sudden, suicidal, and homicidal deaths.[2,3,4,5,6]

Entomotoxicology is the combination of entomology, the study of insects, and toxicology, the study of the adverse effects of chemicals on living organisms. These analytical procedures and methods are useful for forensic investigations into suspicious deaths.

"In the forensic context of a suspicious death, the identification of drugs or other toxicological substances may help determine the cause and/or the circumstances of death," explained Paola Magni from Murdoch University, in Perth, Australia. She continued, "Unfortunately the common toxicological analyses conducted on decomposed tissues are generally less sensitive and yielded almost erroneous results. Several studies have demonstrated that the toxicological analyses of the insects feeding on a body are able to provide a more reliable result even long after the death event."

Nicotine is a commonly known chemical derived from the leaves and stems of the common tobacco plant. Its acute toxicity is often ignored, despite being considered one of the most deadly poisons known to man. "The fatal dose of nicotine is estimated to be 30–60 mg in adults and 10 mg in children," Magni told *The Column*. "Contextually, cigarettes typically contain 10–20 mg of nicotine, but approximately only 1–1.5 mg is absorbed systemically during smoking."

Nicotine can be readily absorbed across numerous membranes. A wide range of potential poisoning methods exist including poisoning from insecticides. "Non-fatal nicotine poisoning is sometimes the result of accidental intoxication, caused by unorthodox treatments against worms, eczema, and constipation, or suicide attempts using insecticides, transdermal nicotine patches, and e-cigarette refills. Recipes are readily available on the internet on how to extract pure nicotine from smoking tobacco. Also, the content of e-cigarette refills is potentially lethal for adults and children if taken other than directed, and children could be attracted to ingest such solutions because of their pleasant flavours," added Magni.

Researchers reared blowfly larvae (*Calliphora vomitoria*) on different concentrations of nicotine-spiked liver before sample preparation. Analytical determinations for the detection of nicotine and its metabolites were performed using GC–MS. The nicotine detection method was validated according to ISO/IEC 17025 requirements and ICH guidelines.

Further data regarding the effects of nicotine on *C. vomitoria* larvae development was gained. "The results of this research show that *C. vomitoria* immatures accumulate both nicotine and its metabolite cotinine, and that the length and survival of *C. vomitoria* feeding on nicotine-containing liver can be significantly affected by the presence of the drug. Interestingly, although the effect on survival is dose dependent, the dose does not have effects on the total duration of their life cycle, from egg to adult. Furthermore, the presence of nicotine in the food substrate has effects on the body structure of *C. vomitoria*, as both larvae and pupae result shorter than those of the control," explained Magni.

The ability to detect the presence of potentially lethal doses of nicotine in blowfly larvae means that the method could prove useful in future forensic investigations where nicotine is suspected to be involved in some form. This is especially true in cases where high levels of decomposition are an issue. Nicotine products are easily available and highly toxic to living beings and therefore the possibility of nicotine overdose, accidental or intentional, should not be ignored.

The research teams from Murdoch University (Australia), University of Turin (Italy), and Boston University (USA) who are responsible for this study are currently conducting a similar study on ketamine in blowfly. The dissociative anaesthetic has seen a surge in popularity for recreational use and is increasingly used in cases of rape. Furthermore, the effects of ketamine on the blowfly's survival and growth rate will be investigated. — L.B.

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2. W.D. McNally. *J. Lab. Clin. Med.* **8**, 83–85 (1922).
3. J.M. Corkery *et al.*, *Forensic Sci. Int.* **199**, e9–e13 (2010).
4. N. Dillion, California Nuclear Power Plant Engineer Convicted of Killing Wife with Nicotine (NY Daily News, 2014).
5. A.K. Chaturvedi *et al.*, *Forensic Sci. Int.* **23**, 265–275 (1983).
6. C. Lardi *et al.*, *Forensic Sci. Int.* **236**, e14–e18 (2014).


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# *LCGC* TV Highlights

***LCGC* TV: Applying Linear Solvation Energy Relationships to Chiral Separations**
Chiral separations are highly qualitative. Apryll Stalcup from the Irish Separation Science Cluster, and an organizer of ISC 2016 in Cork this year, discusses why she wants to look at these separations more closely and explains the role of linear solvation energy relationships (LSERs) in this research.
**Watch Here>>**

***LCGC* TV: The Potential for Ionic Liquid-Based Coatings in SPME**
More and more analytes are now being found in complicated matrices. Jared Anderson from the Iowa State University recently designed new SPME coatings based on polymeric ionic liquids suitable for these matrices. He discusses the potential for these coatings to improve on SPME.
**Watch Here>>**

# Peaks of the Week

**The *LCGC* Blog: Hidden Problems in Your LC–MS Data?** — If you don't examine your LC–MS data closely, you could be missing some of these effects, and those problems with your quantitative data will never be fixed. **Read Here>>**

Photo Credit: malerapaso/Getty Images

**A Collaborative Approach to Water Analysis** — Contaminants in surface water and drinking water supplies arising from pharmaceutical and personal care product use as well as other compound sources pose a difficult challenge for analytical chemists. Thomas Letzel from the Technical University of Munich in Germany, spoke to *The Column* to discuss collaborative research taking place in Europe to address contaminants of emerging concern in water analysis. **Read Here>>**

Photo Credit: Witchaphon Saengaram/Getty Images

**The Rise of Hydrophilic Interaction Chromatography in Untargeted Clinical Metabolomics** — Hydrophilic interaction chromatography (HILIC) was introduced more than two decades ago and is now considered an attractive alternative to reversed-phase liquid chromatography (LC) for many applications. This review presents an overview of the most recent technical improvements and applications of HILIC analysis in untargeted clinical metabolomics and discusses important practical considerations. **Read Here>>**

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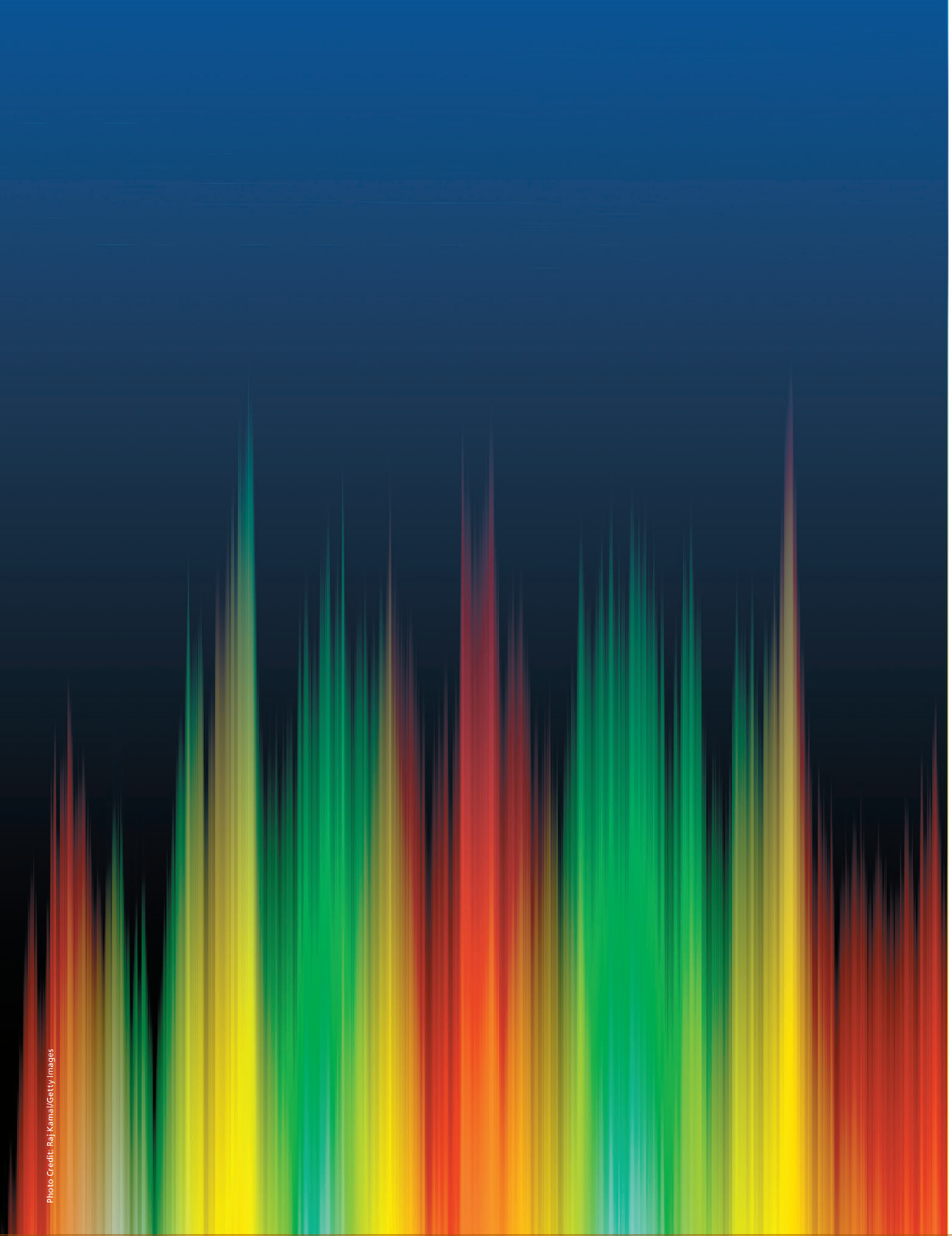
# News In Brief

Using HPLC researchers have investigated the antioxidant and α-glucosidase inhibitory activity of 19 edible seaweed species. The results showed *Ascophyllum nodosum* and *Fucus vesiculosus* to be rich in antioxidants, equaling a Trolox equivalent antioxidant capacity of 135 and 108 mM Trolox $mg^{-1}$ extract, respectively.
**doi:10.1016/j.foodchem.2016.02.001**

Researchers from the University of Maribor, Slovenia, have used highly porous functionalized poly(glycidyl methacrylate) materials prepared by the polymerization of high internal phase emulsions (polyHIPE) to selectively remove silver, lead, and cadmium ions from contaminated water samples.
**doi:10.1016/j.chroma.2016.02.012**

A recent publication from the Singapore Institute of Manufacturing Technology, Singapore, has detailed the use of a disposable integrated microfluidic device for the rapid sample stacking, separation, and extraction of multiple DNA fragments from a relatively large amount of sample. DNA fragments of 200 bp, 500 bp, and 1 kbp were successfully separated and collected in the extraction chamber within 25 min.
**doi:10.1016/j.chroma.2016.01.076**



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# Split Injection GC: Inlet Liner Choice for Shoot and Dilute GC

**Jack Cochran,** Restek Corporation, Bellefonte, Pennsylvania, USA.

**Jack Cochran's new column "Practical GC" provides readers with practical advice and new experimental evidence for how to get the best results from their gas chromatography (GC) systems. This instalment looks at GC inlet liner choice for "shoot and dilute" GC.**

In my previous article, I not only defined when split injection "has" to be done (for example, with highly concentrated samples so a gas chromatography (GC) column does not become overloaded), but I also demonstrated that split injection has practical use in trace-level GC when used with a modern, ultra-sensitive detector.[1] I also listed the numerous features and benefits of split injection, with a hope that users who are having problems with splitless injection might give split injection, or "shoot and dilute" GC a try. I want to list the merits of split injection again in case you missed the last piece:

1. Split injection introduces a very narrow band of sample to maintain GC column separation efficiency, which is particularly important for narrower bore GC columns (0.25 mm i.d. and less).
2. Split injection offers highly efficient and consistent transfer of a wide volatility range of compounds to the GC column.
3. Split injection allows a higher GC oven start temperature, which helps increase throughput.
4. Split injection eliminates peak splitting that occurs when using polar solvents with non-polar GC stationary phases (or non-polar solvents with polar GC phases).
5. Split injection increases GC column lifetime by decreasing the amount of non-volatile material that goes on the column with every dirty sample injection.
6. Split injection minimizes sensitive compound degradation by decreasing compound residence time in the hot GC inlet.
7. Split injection improves quantitative accuracy by reducing adsorption of active compounds to GC inlet liner surfaces.
8. Split injection reduces lower volatility compound-of-interest loss to inlet liner surfaces for samples that contain non-volatile material.

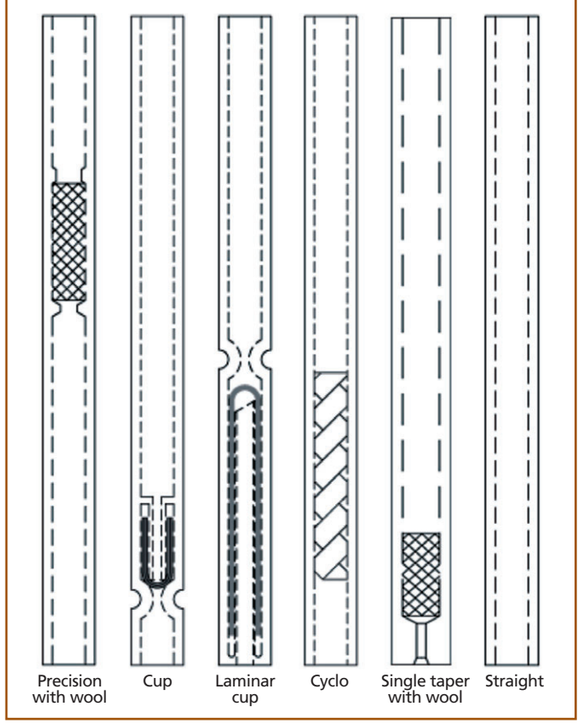



**Figure 1:** The first four GC inlet liners from left to right are for split injection. They either have wool or an obstruction in order to encourage vaporization and homogenization of injected sample prior to the sample reaching the GC column and split point at the bottom of the inlet. The last two inlet liners on the right are for splitless injection, but were included in split injection experiments here to show the impact of incorrect split GC inlet liner choice.

If increasing throughput, eliminating peak splitting, and avoiding compound degradation are of interest to you, I invite you to review the split injection examples I presented in my first "Practical GC" article.[1]

In the past, the development of liners for split injection revolved around the need for good sample homogenization before reaching the column entrance and split point at the bottom of the GC inlet. While inexpensive borosilicate glass wool provided an excellent homogenization/vaporization area for split injection, its adsorptive and catalytic activity compromised certain analyses. These concerns led to a slew of split liner offerings designed around providing a tortuous mixing path between points of injection and split, while avoiding the use of glass wool or other packing materials. Included (but not limited to) are the cup-type and cyclo- (or helix-) type split liners shown in Figure 1. While these split liners were designed with good principles in mind, based on the lack of current data demonstrating their advantages (if any), it could be argued that some have outlived their usefulness. This is especially true given the introduction of highly deactivated quartz wool packing for split liners. Unfortunately, once a liner makes it into a company's catalogue, it is rarely discontinued, which is why new laboratory experiments are needed to help differentiate one split liner from another.

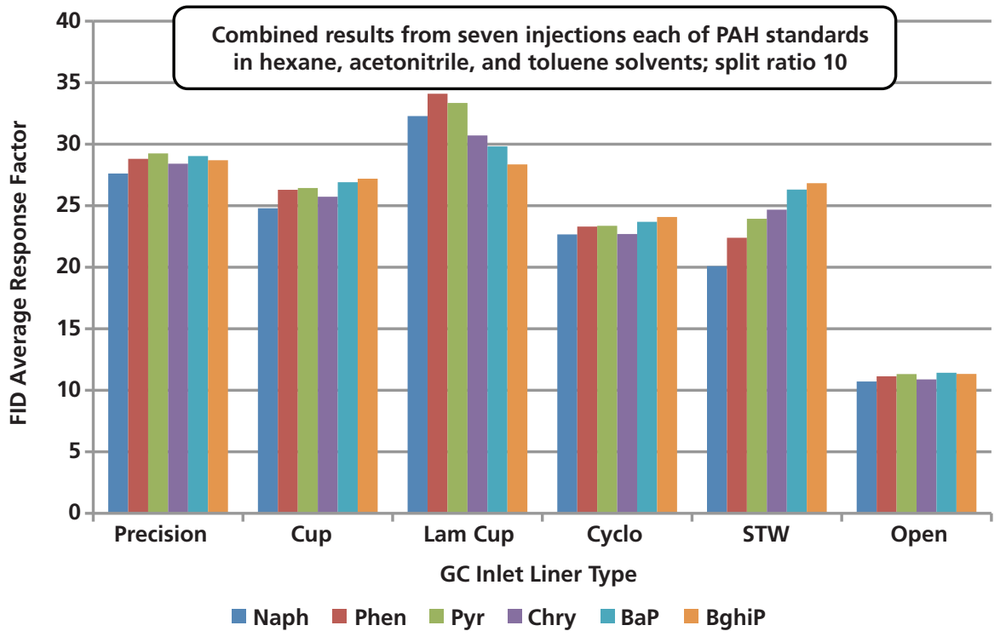
## Experimental

A 30 m × 0.25 mm, 0.25-µm 5% diphenyl - 95% dimethyl polysiloxane GC column (Restek) with helium carrier gas at a constant flow of 1.4 mL/min was used for all experiments. The GC oven programme was as follows: 100 °C (0.1 min), 7 °C/min to 330 °C. The flame ionization detector (FID) (Agilent) was at 350 °C. Nitrogen makeup + column flow was at 50 mL/min. Fast 1 µL autosampler injections of standards containing 18 polycyclic aromatic hydrocarbons (PAHs) at 2 ng/µL each PAH were made into various liners used for either split or splitless GC (Figure 1). The split ratio was always 10. Six PAHs from volatile to involatile were used to define liner efficacy for split injection with a variety of different sample solvents spanning a range of volatility and polarity. Seven replicate injections of each PAH solvent standard were performed into the differing liners.

## Results and Discussion

Generally speaking, split liner performance should be independent of compound volatility and solvent type, which in this case means the PAHs should show approximately the same FID average response factors and low RSD% values for replicate injections. Figure 2 is a summary of all the data collected for hexane, acetonitrile, and toluene solvents, and indicates the excellent average response factor performance for the precision-type liner. Laminar cup liner results are biased high and the single-taper-with-wool liner results

**Figure 2:** A precision-type GC liner for split injection allows accurate transfer of volatile to involatile compounds to the GC column with minimal discrimination, independent of sample solvent type. Other liners give less accurate results, especially the open liner where there is no wool or obstruction to help with sample homogenization prior to the sample reaching the split point at the bottom of the inlet.




Precision = precision-type liner with wool; Cup = cup inlet liner; Lam Cup = laminar cup inlet liner; Cyclo = cyclo inlet liner; STW = single taper liner with wool (recommended for splitless injection); Open = straight inlet liner.
Naph = naphthalene; Phen = phenanthrene; Pyr = pyrene; Chry = chrysene; BaP = benzo[a]pyrene; BghiP = benzo[ghi]perylene.

indicate compound-volatility discrimination. The performance differences in favour of the precision-type liner become more obvious when viewing the repeatability results in Figure 3. Remember that the data represents a compilation of three solvent-types chosen for the work, which makes the very low RSD% values for the precision- (or focus-) type liner more impressive.

Readers may be wondering why a liner without mixing capability (straight/open liner) was included in this work. Uh, I am too... While an open liner can be used for split work where the sample is already a gas or vapour, it does not work well for liquid samples. The fast autosampler injection throws the sample liquid droplet past the column entrance to the split point, where sample exits prior to vaporization,



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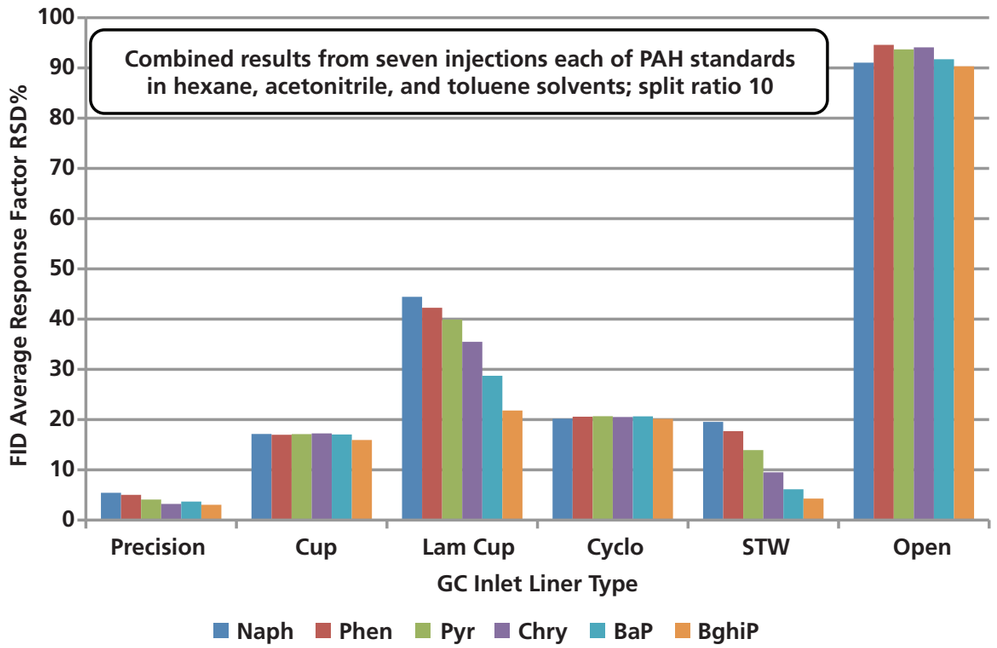
**Figure 3:** A precision-type GC liner for split injection shows the best repeatability across the whole volatility range for PAHs, and is excellent for non-polar, polar, and aromatic solvent types. The other liners show less repeatability overall, and in some cases, show worse repeatability based on compound volatility and sample solvent type. The open liner does not allow for adequate mixing of sample prior to splitting, which leads to huge variability.



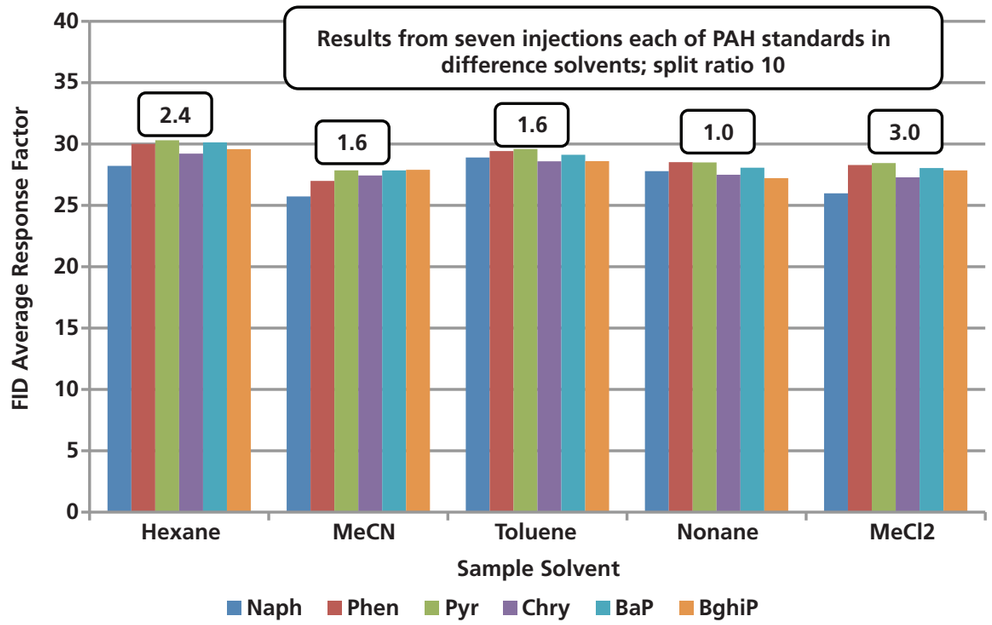

Precision = precision-type liner with wool; Cup = cup inlet liner; Lam Cup = laminar cup inlet liner; Cyclo = cyclo inlet liner; STW = single taper liner with wool (recommended for splitless injection); Open = straight inlet liner.
Naph = naphthalene; Phen = phenanthrene; Pyr = pyrene; Chry = chrysene; BaP = benzo[a]pyrene; BghiP = benzo[ghi]perylene.

leading to severely low response factors for all PAHs (Figure 2). Repeatability is also unacceptably poor for the straight liner when used for split injection with liquid samples (Figure 3).

After deciding that the precision-type liner gives the best comprehensive split injection performance, a closer look should now be taken at average response factors and repeatability for specific solvent types (Figure 4). Both average response factor data and repeatability data indicate excellent consistency for the precision-type liner across compound- and solvent-types.

I am often asked by gas chromatographers, "Is there one liner I can use for all split and splitless GC work?" The single-taper-with-wool liner is sometimes touted as the universal

**Figure 4:** The precision-type GC liner delivers similar FID response factors for a wide range of PAH volatilities in most solvents, including non-polar, polar, volatile, less volatile, halogenated, and aromatic solvents for split injection. The means for repeatability for all PAHs evaluated are listed above each group of data.

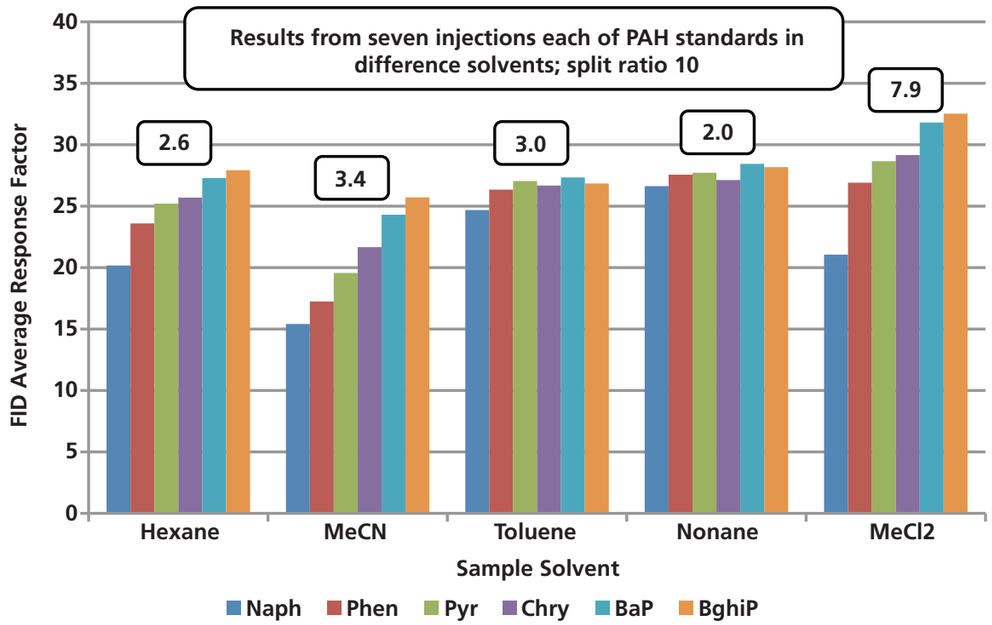



MeCN = acetonitrile; MeCl2 = methylene chloride.
Naph = naphthalene; Phen = phenanthrene; Pyr = pyrene; Chry = chrysene; BaP = benzo[a]pyrene; BghiP = benzo[ghi]perylene.

split and splitless GC inlet liner because it has wool for good sample vaporization and homogenization, and the taper protects the sample from being lost to the metal bottom seal. However, the split injection data shown in Figure 5 for the single-taper-with-wool liner reveals discrimination effects for PAHs depending on the solvent type, and repeatability that is not as good as seen for the precision-type liner. Is it possible then that the precision-type liner is the universal liner for split and splitless GC? My answer is "no" for splitless injection, but data to back up that assertion will have to wait until later.

Given my suggestion in the previous "shoot and dilute GC" article to try the precision-type liner, fortunately for me and any readers who grabbed that liner and went to the laboratory, the data presented here validate its choice as an excellent split injection liner.

**Figure 5:** The single taper inlet liner with wool, which is recommended for splitless injection, shows some discrimination for split injection of certain PAHs depending on the sample solvent. The means for repeatability for all PAHs evaluated listed above each group of data may be acceptable, but are still about twice those seen for the precision-type liner for most solvents.




MeCN = acetonitrile; MeCl2 = methylene chloride.
Naph = naphthalene; Phen = phenanthrene; Pyr = pyrene; Chry = chrysene; BaP = benzo[a]pyrene; BghiP = benzo[ghi]perylene.

## Reference

1. J. Cochran, *The Column* **11**(21), 14–20 (2015).

**Jack Cochran** is a Director of New Business and Technology at Restek Corporation. He is a recognized expert in GC and GC×GC for the analysis of pesticides and priority pollutants. He serves on the Board of Directors for FLAG Works (sponsor of NACRW) and the Centre of Oil and Gas Research and Development at the University of Manitoba. Jack is also an Adjunct Professor in the Forensic Science Program at The Pennsylvania State University.

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# The *LCGC* Blog: System and Column Volumes in HPLC: We Still Haven't Gotten the Message

**Tony Taylor,** Crawford Scientific, Strathaven, Scotland.

**What is required to produce a highly efficient modern high performance liquid chromatography (HPLC) system/column combination? This instalment of the *LCGC* Blog explains more.**

Some modern high performance liquid chromatography (HPLC) systems resemble spacecraft in terms of their technology, designed as they are to operate to the highest efficiencies, compared to traditional systems.

However, I still see countless examples where high efficiency columns are used on systems that are not matched and cannot support the highest efficiencies offered by the column.

There are many factors to consider when studying the optimum efficiency obtainable from a system/column combination. I've outlined some of the variables that can be easily controlled within the laboratory and those data acquisition variables which need to be considered.

For reference, I'm not going to consider capillary or nano columns here because to take advantage of these technologies you will have already considered all of the information presented here.

You will need to know the extra column volume associated with your HPLC column, which, as a first approximation, can be calculated using the information in Table 1.

The fact of the matter here is that shorter, narrower columns and those packed with highly efficient packing materials (< 2 mm or superficially porous particles) need to be used with HPLC systems whose extra column volumes are low, to avoid the system being the dominant factor in determining the maximum achievable efficiency.

The extra column volume (ECV) within a system is additive alongside the dispersion that occurs within the column in determining the peak volume (peak width); there is a very nice rule of thumb which states that the ECV of the system should be less than half of the total peak width to achieve more than 90% of the column resolving power.

Figure 1 is what we should be aiming for. The chromatographic peak volume can be estimated by drawing tangents to estimate 4σ peak width — in this case the peak width is 0.5495–0.5240 min = 0.0255 min and the flow rate was 600 µL per minute giving an estimated peak volume of 15.3 µL. This would dictate that the total system extra column volume (ECV) should be less than 8 µL (approximately) to achieve the optimum resolving power of the column/system combination.

Some typical maximum allowable ECV contributions for columns of various dimensions are shown in Table 2.

Photo Credit: Dennis O'Clair/Getty Images



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**Figure 1:** Measuring peak volume to assess system dispersion requirements.

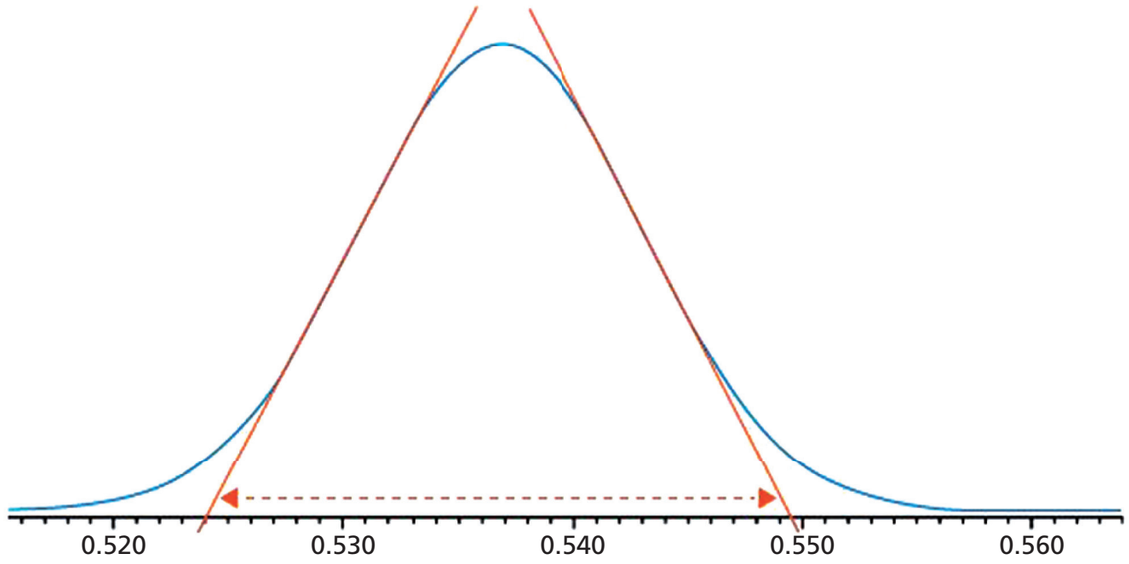


**Table 1:** Calculating the extra column volume.

| |
|---|
| 2.1 mm i.d. — column length (mm) × 2 = column void volume ($V_m$) (µL) |
| *50 mm long 2.1 mm i.d. column has void volume of approximately 100 µL* |
| 3.0 mm i.d. — column length (mm) × 5 = column void volume ($V_m$) (µL) |
| *100 mm long 3.0 mm i.d. column has void volume of approximately 500 µL* |
| 4.6 mm i.d. — column length (mm) × 10 = column void volume ($V_m$) (µL) |
| *150 mm long 4.6 mm i.d. column has void volume of approximately 1500 µL* |

So, what do we need to take care of in order to optimize our experiments from a practical perspective?

Well, I've compiled a brief list below of the items that we review on each system after measuring the ECV to reconcile the measured value against what is calculated:

1. Volume of sample injected
2. Tubing from autosampler to column heat exchanger
3. Column heat exchanger
4. Tubing from heat exchanger to column

**Table 2:** Maximum allowable extra column volume for columns of various dimensions.

| Column Dimensions (L × i.d., mm) | Maximum Extra Column Volume (µL) |
|---|---|
| 100 × 4.6 | 35 |
| 50 × 4.6 | 25 |
| 100 × 3.0 | 15 |
| 50 × 3.0 | 10 |
| 100 × 2.1 | 7 |
| 50 × 2.1 | 5 |
| 30 × 2.1 | 4 |

**Table 3:** Volume contribution from tubing of various internal diameters.

| Tubing Internal Diameter (mm) | ECV Contribution (µL/cm) |
|---|---|
| 0.127 | 0.13 |
| 0.178 | 0.25 |
| 0.229 | 0.41 |
| 0.254 | 0.51 |

and column to detector
5. Detector internal tubing volume (often negligible but sometimes not!) and flow cell volume
6. Fittings and in-line filters/unions etc.

So — how can you estimate the ECV value?
1. Known
2. Use Table 2 to calculate
3. Should be written on the heat exchanger or in your manufacturer's literature
4. See 2 above
5. From manufacturer; cell volume will be written on the cell or via part number
6. Zero contribution from fittings if zero dead volume (ZDV) type used

To calculate the ACTUAL extra column volume of the system, replace the column with a zero dead volume union or capillary



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**Figure 2:** Effect of data sampling rate on peak shape and efficiency. Right — use of time constant to adjust noise, which is especially important in high frequency measurements.

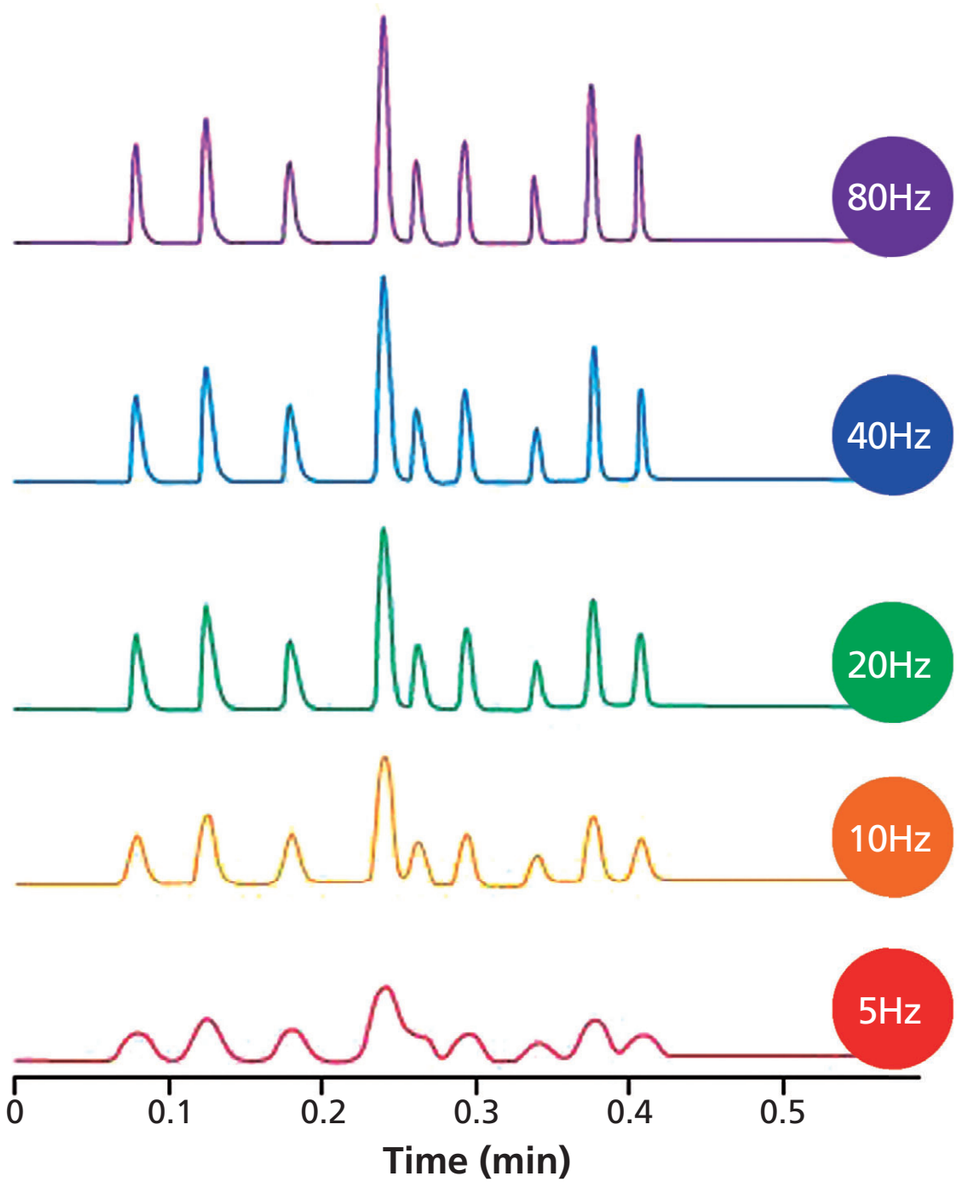
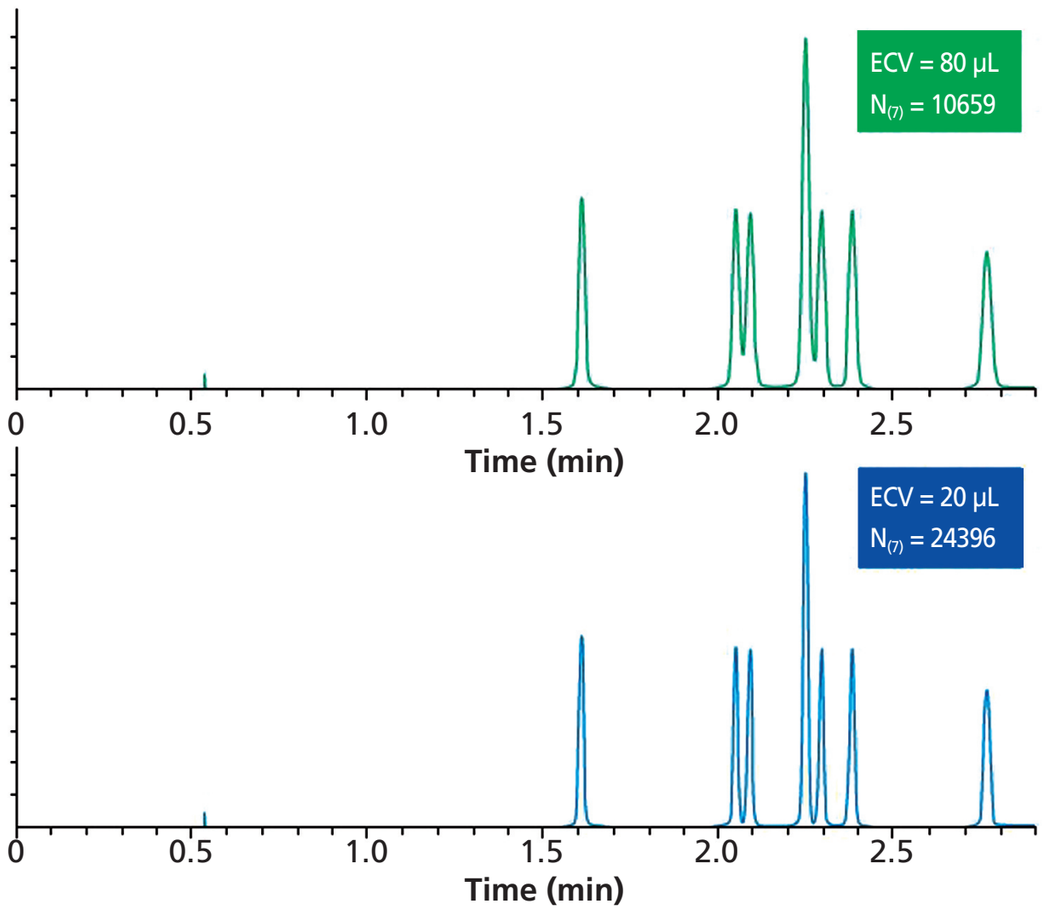


**Figure 3:** Effects on chromatographic performance of systems with varying extra column volume.




restrictor and inject an aliquot of 0.1% *v/v* acetone in water and monitor at 270 nm with your UV detector. Measure the 4 s peak width and calculate the peak volume as per Figure 1. This value is the ECV value for the system you are using.

One should carefully consider, and reduce where possible, the tubing length and internal diameter being used, especially between the column and detector, the number of unions, and any in-line filters or couplings which may not be necessary. Our earlier example with

**Table 4:** Effect of flow cell volume on chromatographic performance.

| Cell Volume (µL) | Path Length (mm) | Signal-to-Noise Ratio | Efficiency* |
|---|---|---|---|
| 13 | 10 | ++++ | + |
| 5 | 6 | +++ | ++ |
| 2 | 3 | ++ | +++ |
| 0.08** | 6 | +++ | ++++ |

* — ultimate efficiency depends upon the total system extra column volume and column dimensions
** — based on light pipe design

**Table 5:** Recommended data acquisition rates for columns of different internal diameter.

| Column Dimensions (L × i.d., mm) | Minmum Data Rate (Hz) | Maximum Time Constant (S) |
|---|---|---|
| 50 × 4.6 | 10 | 0.16 |
| 50 × 3.0 | 20 | 0.16 |
| 50 × 2.1 | 20 | <0.1 |

the 8 mL maximum ECV constraint would start to suffer problems if any more than 32 cm of 0.178 mm i.d. tubing is present in the whole system!

You should note the comment made earlier regarding zero contribution from ZDV unions. Well, this is only true if the connection is properly made! Ensure that the tubing is fully butted up inside the fitting and that it remains that way during the tightening operation — this is basic good practice that is often not followed. Also remember that PEEK fittings deform only so many times and old PEEK column nuts will eventually lose their ability to deform to the internal volume of the column end fitting.

Given the constraint of keeping below 8 mL ECV, one may also need to consider for our system, the required injection volume. Clearly injection volumes need to be scaled when using lower volume columns and a nice estimate of maximum injection volume is around 15% of the peak volume. So restricting injection volume to no more than 2.25 mL would be sensible for our example here. If scaling down a method to a smaller column dimension, and assuming a constant particle porosity, then a simple scaling relationship is:

$$V_{inj\,(2)} = V_{inj\,(1)} \times (i.d._{(1)} \times L_{(1)} / i.d._{(2)} \times L_{(2)}) \quad [1]$$

where $V_{inj\,(1)}$ is original injection volume, $i.d._{(1)}$ the internal diameter of the original column, and so forth…

Another very useful rule of thumb in reducing ECV and minimizing peak dispersion to realize maximum efficiency is to restrict the flow cell of the detector to less than 10% of the calculated peak volume. In our previous example the peak volume was approximately 15 µL, which indicates a maximum flow cell volume of around 1.5 µL. This indicates the need for a flow cell based on light pipe principles, which have very low internal volume whilst still maintaining good sensitivity.

Table 4 outlines the relative merits of flow cells of various dimensions.

The most surprising contributor to peak dispersion is the data acquisition parameters that we apply to a method. The data sampling rate can have a direct effect on peak width (efficiency) and as such these are important parameters that must be matched to the widths of peaks generated by various column dimensions.

However, data sampling rate is not the only important factor in determining peak width and variables such as bunch rate or time constant, that is, the digital bunching and filtering applied by the detector, are also of importance and some guideline as to these variables are shown in Table 5.

To conclude, Figure 3 shows the effect of extra column volume on a chromatographic separation using a column of 100 × 2.1 mm with an ECV of 20 µL (bottom) and 80 µL (top).

It should be clear from Figure 3 that the upper separation would not find favour in regular use and that the efficiency of peak 7 has fallen by more than half due to a 4× increase in system extra column volume.

It is very important when using reduced dimension HPLC columns and high efficiency particle morphologies that the system volume and data acquisition parameters are optimized to realize the full efficiency benefits.

For more tutorials on LC, GC, or MS, or to try a free LC or GC troubleshooting tool, please visit **www.chromacademy.com**

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Website: **www.chromacademy.com**

# The Chromatographic Society: Advances in Microcolumn and Related Separation Technologies

**A preview of The Chromatographic Society's first meeting in 2016, which will take place on Tuesday 22 March in London, UK.**

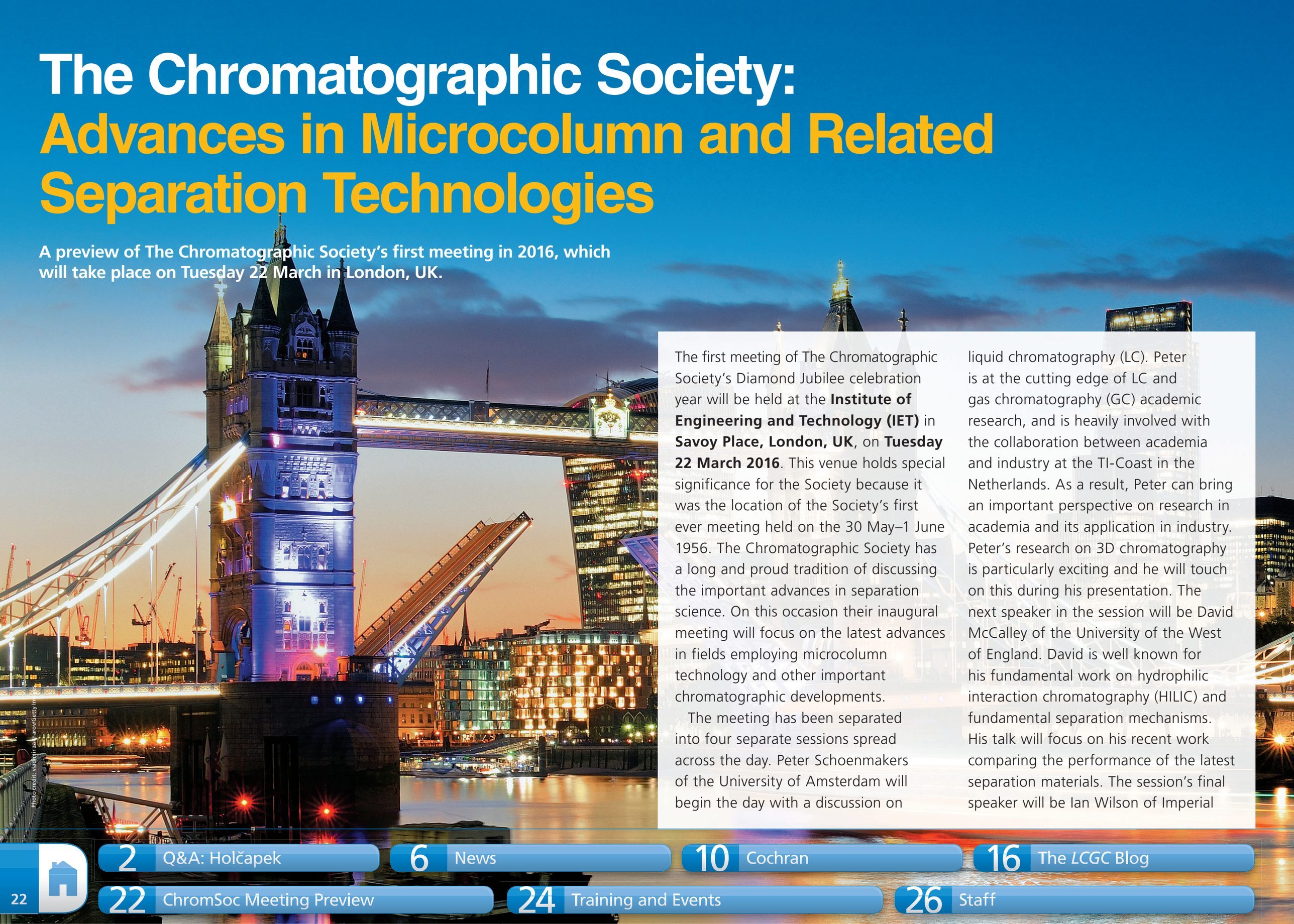

Photo credit: Vadmyr zakharov/Getty Images

The first meeting of The Chromatographic Society's Diamond Jubilee celebration year will be held at the **Institute of Engineering and Technology (IET)** in **Savoy Place, London, UK**, on **Tuesday 22 March 2016**. This venue holds special significance for the Society because it was the location of the Society's first ever meeting held on the 30 May–1 June 1956. The Chromatographic Society has a long and proud tradition of discussing the important advances in separation science. On this occasion their inaugural meeting will focus on the latest advances in fields employing microcolumn technology and other important chromatographic developments.

The meeting has been separated into four separate sessions spread across the day. Peter Schoenmakers of the University of Amsterdam will begin the day with a discussion on liquid chromatography (LC). Peter is at the cutting edge of LC and gas chromatography (GC) academic research, and is heavily involved with the collaboration between academia and industry at the TI-Coast in the Netherlands. As a result, Peter can bring an important perspective on research in academia and its application in industry. Peter's research on 3D chromatography is particularly exciting and he will touch on this during his presentation. The next speaker in the session will be David McCalley of the University of the West of England. David is well known for his fundamental work on hydrophilic interaction chromatography (HILIC) and fundamental separation mechanisms. His talk will focus on his recent work comparing the performance of the latest separation materials. The session's final speaker will be Ian Wilson of Imperial


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College London. A former President of the Society, Ian's work now focuses on a variety of complex separation types including metabolomics and he will share some of his state-of-the-art research with us at this meeting.

A range of sponsors will present the second session of the day. As well as the sponsor presentations, there will be a sponsor exhibition in the IET library highlighting the latest separation science products and related technologies.

The third session of the day will focus on GC and will commence with Pat Sandra from the University of Ghent, who has an extensive background in GC. His talk will discuss some of the most recent automation developments regarding sample preparation and injection for capillary GC. Tom Lynch of BP will follow and he will discuss GC column technology. Kevin Van Geem of the University of Ghent will close this session with a talk on multidimensional GC, an approach that is becoming more prevalent for the analysis of complex sample types.

The final session will focus on other separation approaches and Frederic Lynen from the University of Ghent will begin with a discussion of his group's recent work in the field of capillary chromatography. The next presentation will be from Jean-Luc Veuthey from the University of Geneva, who will discuss some of the latest developments in the field of supercritical fluid chromatography (SFC). The final speaker in the session will be Peter Myers from the University of Liverpool, recipient of the Society's 2016 Martin medal and a former Chromatographic Society committee member. He will bring the meeting to a close with a discussion on the future of microcolumn formats for separation science.

This speaker line up will provide chromatographers with an exciting glimpse into the cutting-edge of separation science and will also highlight an important Diamond Jubilee year in the Chromatographic Society's history.

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**Website: www.chromsoc.com**




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# Training Courses

## GC

### The Theory of GC
**Website:** http://www.chromacademy.com/gc-training.html

### Hands-On GC Theory and Methods
**11 April 2016**
The Open University, Milton Keynes, UK
**Website:** http://www.anthias.co.uk/training-courses/handson-GC-theory-methods

### Fundamental GC
**19 April 2016**
Caledonian University, Glasgow, UK
**Website:** http://www.crawfordscientific.com/training-online-calendar.asp

### GC–MS for the Chromatographer
**10 May 2016**
Caledonian University, Glasgow, UK
**Website:** http://www.crawfordscientific.com/training-online-calendar.asp

## HPLC/LC–MS

### 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

### Practical HPLC Method Development
**7–8 June 2016**
Caledonian University, Glasgow, UK
**Website:** http://www.crawfordscientific.com/training-online-calendar.asp

### HPLC Masterclass
**2–24 August 2016**
Laserchrom HPLC Laboratories Ltd, Rochester, Kent, UK
**Website:** http://www.hplccourses.com/index.htm

## SAMPLE PREPARATION

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

### Sample Prep, SPE & Data Integrity (3 Short Courses)
**12 April 2016**
Reading, UK
**Website:** www.hichrom.co.uk

### Hands-On Sample Preparation
**1–13 May 2016**
The Open University, Milton Keynes, UK
**Website:** http://www.anthias.co.uk/training-courses/hands-on-sample-preparation

## MISCELLANEOUS

### Basic Lab Skill Training
**Website:** http://www.chromacademy.com/basic-lab-skills-training.html

### Introduction to IR Spectroscopy
**Website:** http://www.chromacademy.com/infrared-training.html

### INstruments of Change Tour
**6–21 April 2016**
Various locations around the UK and Ireland
**Website:** www.perkinelmer.com/INTour2016

### Troubleshooting, Method Translations, and Exploiting Selectivity (3 Short Courses)
**21 April 2016**
Bristol, UK
**Website:** www.hichrom.co.uk

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

# Event News

### 3–7 April 2016
**32nd International Symposium on Microscale Separations and Bioanalysis (MSB 2016)**
Niagara-on-the-Lake, Canada
**Chairman:** Philip Britz-McKibbin
**E-mail:** info@msb2016.org
**Website:** www.msb2016.org

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### 20–21 April 2016
**XI International Conference ION CHROMATOGRAPHY 2016**
Zabrze, Poland
**Contact:** Rajmund Michalski
**E-mail:** rajmund.michalski@ipis.zabrze.pl
**Website:** www.ipis.zabrze.pl

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### 10–13 May 2016
**Analytica 2016**
Messe München GmbH, Munich, Germany
**Tel:** +49 89 949 20720
**E-mail:** info@messe-muenchen.de
**Website:** http://www.analytica.de

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### 29 May–3 June 2016
**40th International Symposium on Capillary Chromatography and 13th GC×GC Symposium**
Palazzo dei Congressi, Riva del Garda, Italy
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