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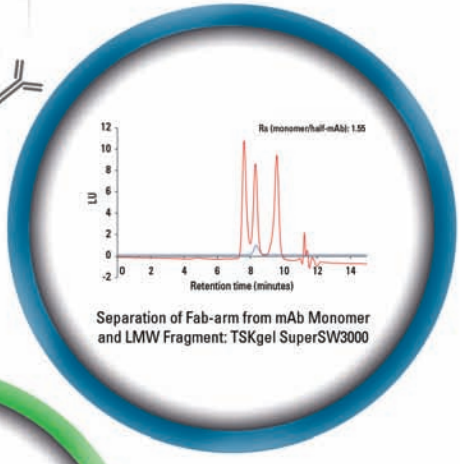
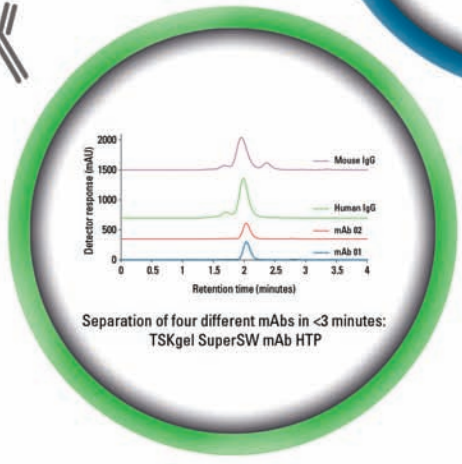
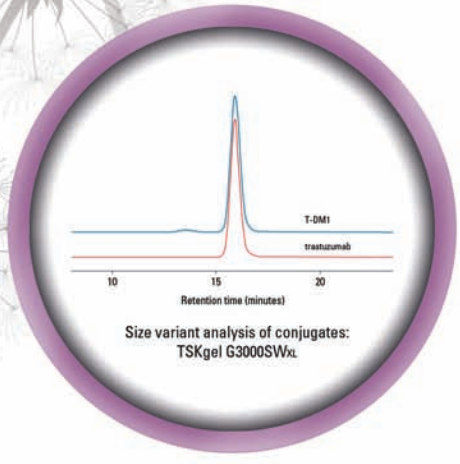


Separation of Fab-arm from mAb Monomer and LMW Fragment: TSKgel SuperSW3000



Separation of four different mAbs in <3 minutes: TSKgel SuperSW mAb HTP



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PEAKS of Interest

Jaap Venema Named Chief Science Officer of USP

The United States Pharmacopeial Convention (USP) has named Jaap Venema as the Chief Science Officer, beginning on April 15, and Chair of USP's standards-setting body, the Council of Experts, at the beginning of USP's next five-year cycle on July 1, 2015.

Venema recently was the Therapeutic Area Lead for Biotherapeutics, in Global Medical Affairs and Biologics Strategy Development at Abbvie (formerly Abbott Laboratories), where he provided scientific and medical leadership on key aspects of biotherapeutics across all therapeutic areas, including biosimilars and immunogenicity. In this role, Venema had global oversight for Asia, Europe, Latin America, and the United States. His 15-year tenure at Abbott and Solvay (acquired by Abbott in 2010) included leadership roles in immunology medical affairs, international medical development, vaccines development, and exploratory target biology.

Venema earned his PhD at the University of Leiden in the Netherlands. He also served on the faculty of the Vrije Universiteit Amsterdam (Free University Amsterdam) in the Netherlands.

In his new role at the USP, Venema will report to the CEO and serve as a member of USP's executive team. He will provide overall leadership for USP's scientific and standards-setting activities and will have management responsibility for a staff of more than 150 worldwide. Venema will work closely with scientific staff at USP's global laboratory operations in Rockville, Maryland; Hyderabad, India; Shanghai, China; and São Paulo, Brazil; and USP locations in Ghana, Switzerland, Ethiopia, and Indonesia.

Retiring CSO V. Srini Srinivasan, PhD, served USP in many capacities over a distinguished career of more than 30 years. "Dr. Srini," as he is affectionately known to USP staff worldwide, led USP's scientific efforts during an important time in the organization's growth, particularly in the establishment of USP's sites in India, China, and Brazil, as well as the development of USP's global activities in drugs, food ingredients, and dietary supplements. He served in many roles during his time at USP, culminating in becoming the Chief Science Officer in 2012. Dr. Srini's last day at USP will be May 1, 2015.

GC–MS Detects Chemical Cue for Mosquitoes in Soil

Cedrol, a sequisterpene alcohol found in the essential oil of conifers, could be a potent chemical cue for pregnant mosquitoes seeking the ideal location to lay their eggs. The compound was identified in a study looking at how mosquitoes find the ideal water body to lay their eggs. According to the study published in the *Malaria Journal,* cedrol could be used in the development of "attract and kill" traps targeting pregnant mosquitoes and reducing the spread of malaria (1).

Reference

(1) J.M. Lindh et al., *Malar. J.* **14**(119) DOI: 10.1186/s12936-015-0636-0 (2015). ■

LCGC*tv* New videos from *LCGC*

KEVIN SCHUG ON TRACE ANALYSIS OF ESTROGEN USING 2D LC–MS-MS
Schug, of the University of Texas at Arlington, talks about the advantages of using restricted access media for the on-line sample preparation of biofluids in a "trap-and-elute" LC–MS arrangement, and the possible extension of this approach to other application areas.

Other recent LCGC *TV interviews include:*

- Luigi Mondello on the fundamental principles of 2D GC and the advantages it has over 1D GC
- Giorgia Greco on the different options available for combining HILIC to reversed-phase LC, and how HILIC can be hyphenated with atmospheric pressure chemical ionization MS

Visit http://www.learnpharmascience.com/lcgc/index.php to see these videos and more.

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HIGHLIGHTS OF APPS FOR SEPARATION SCIENCE

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APP NAME: LC/GC Checker

OFFERED BY: David Baumgartner

PLATFORMS: Android (requires 2.3.3 and up); iPhone, iPad, and iPod touch (requires iOS 6.0 or later; optimized for iPhone 5)

WHAT IT DOES: The LC/GC Checker app is designed to provide four approaches to check an LC or GC instrument's performance. According to the company, it calculates the LC pump flow rate by stop watching the time of the eluent level in a volumetric flask, calculates the GC carrier gas flow based on the column dimension and retention time of a non-retained compound, and assists in measuring flow and temperature accuracy and precision. The app's IP Checker function calculates the injection precision of retention times, peak heights, or areas for repeated analyses.

COST: $6.04 (Android); $5.99 (iTunes)

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SAMPLE PREP PERSPECTIVES

New Sample Preparation Products and Accessories at Pittcon 2015

This yearly report on new products introduced at Pittcon (or in the preceding year) covers sample preparation instruments.

Douglas E. Raynie
Sample Prep Perspectives Editor

As expected, the new products introduced in the past year in the area of chromatographic sample preparation, while somewhat limited, mirror the current development in the field. That is, a few systems were developed to automate or streamline the sample preparation process; new sorptive phases and formats, including QuEChERS (quick, easy, cheap, effective, rugged, and safe), were developed; and accessories and other stepwise advances in the field were noted. In late 2014, the *LCGC* editorial staff submitted a survey to vendors of sample preparation products. Responses to this survey are compiled in this review. Additionally, a keyword search using the terms "sample preparation" and "extraction" was conducted for exhibitors at Pittcon 2015; then each of these vendors was visited. While attempts were made to be as inclusive as possible, we apologize for any oversight.

Hollow-Fiber Microextraction

Perhaps the highlight among new sample preparation products is an unheralded introduction by one of the smallest vendors. Biomics, Inc., brought forth devices for hollow-fiber microextraction (HFME) at Pittcon 2015. HFME has been developed for quite some time (more than a decade) and is performed in a variety of configurations; for example, it was reviewed in a 2010 "Sample Prep Perspectives" column (1). Biomics claims that their hollow-fiber product, available in single-vial and 96-well formats, is the only commercially available HFME product. Regardless of the validity of this claim, such products are certainly scarce and this development by Biomics should drive the acceptance of the technique. Figure 1 shows an example of the format of a 96-well plate HFME device. The HFME approach should work for the isolation of environmental, pharmaceutical, food, and nutraceutical samples.

Systems

Several sample preparation systems were introduced in the past year, typically with multisample capabilities and generally in the bioanalytical realm.

Similar to the HFME product introduction above, Phenomenex expanded its offerings with the Novum Simplified Liquid Extraction (SLE) product line. Available in both cartridge and 96-well plate formats, these liquid extraction products are designed to replace conventional liquid–liquid extraction (LLE) in bioanalytical, food safety, and environmental testing. In the suggested protocol with the Novum product, a sample is diluted with buffer solution and added to the SLE medium, and after a brief soaking period, elution with ethyl acetate or dichloromethane follows —the process is completed within about 15 min. The Extrahera system by Biotage supports both supported liquid extraction and solid-phase extraction (SPE), in either column (1, 3, and 6 mL) or plate formats. The Extrahera system also can be used in protein-crashing applications and uses positive pressure for more reproducible flow. Added automation to the Fotector Plus automated SPE system (Reeko Instrument

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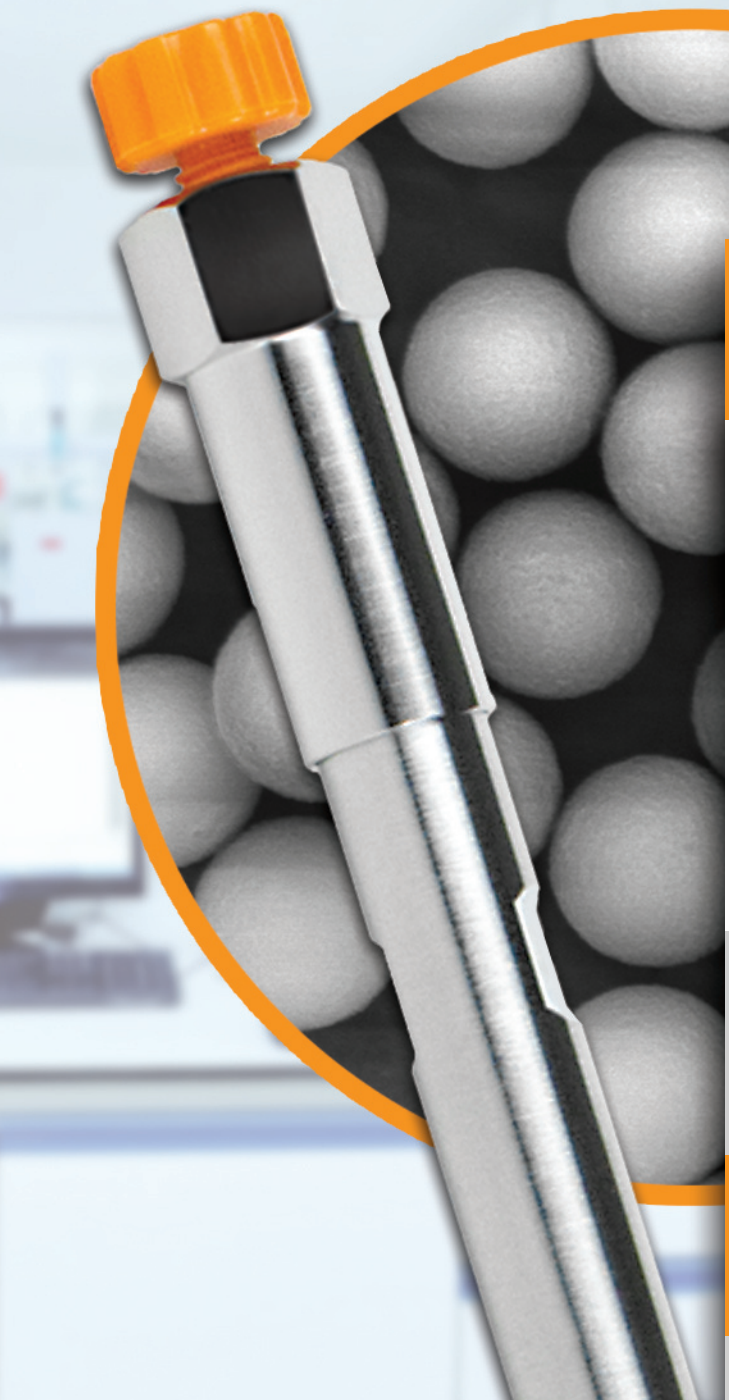
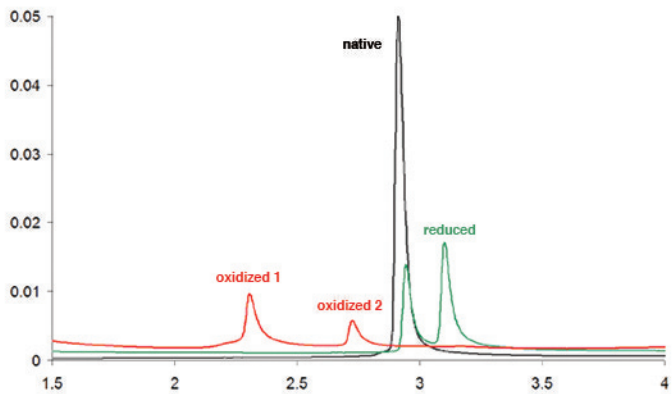


1st Carbon Core Shell Particle

Filgrastim (18.8 kDa): active ingredient in 1st biosimilar approved by US FDA



Column:	FLARE® C18 Wide Pore, 250 Å
Column Dimensions:	100 x 2.1 mm (ID# 16636. 1-2)
Mobile Phase:	A: 0.5% TFA, B: 0.5% TFA in ACN
Flow Rate:	0.2 mL/min
Gradient:	37-52% B in 4 min
Detection:	UV (280 nm, 20 Hz sampling rate)
Temp:	60 °C

Compared to USP method, separation had a reduction of:

- **Analysis time by a factor of 6**
- **Solvent consumption by a factor of 25**

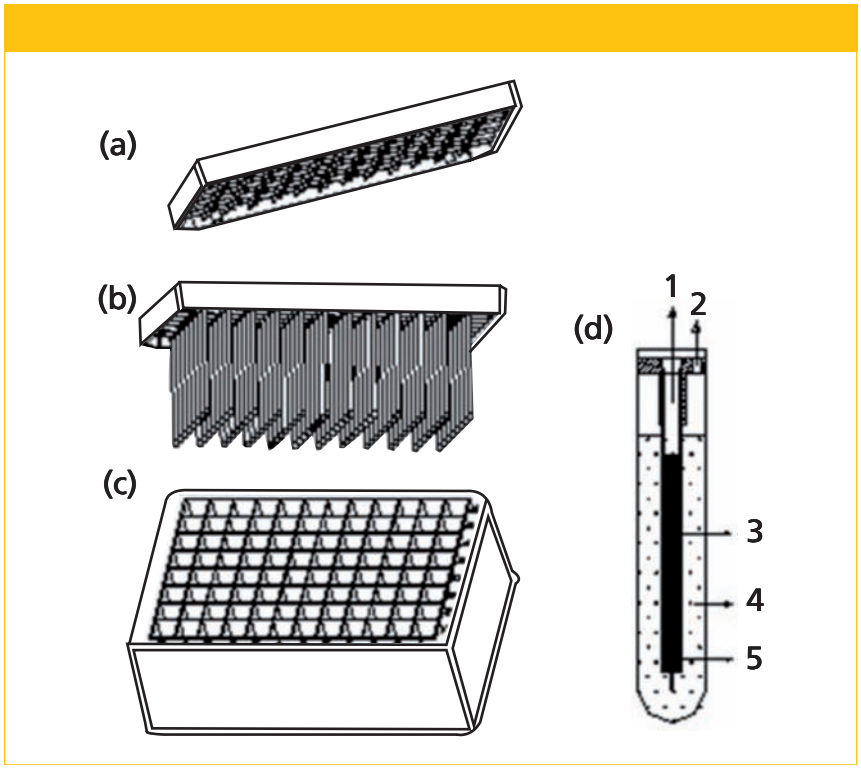


Figure 1: Hollow-fiber microextraction in a 96-well format: (a) the plastic base, (b) the attachment of hollow fiber to the plastic base, (c) the 96-well plate, and (d) an expanded view of the hollow-fiber device. 1 = hollow-fiber attachment tip, 2 = donor phase collection tip, 3 = acceptor phase, 4 = donor phase, 5 = hollow fiber. Adapted with permission from reference 2.

USA) provides capacity to run 48 samples continuously with positive pressure sampling and elution modes.

Keeping with developments in the bioanalytical area, the ECO_2Chrom flash chromatograph from Applied Separations uses liquid carbon dioxide to reduce organic solvent use and lower the analyte concentration time. The high diffusivity of the mobile phase allows smaller particle sizes to be used, allowing for greater efficiency or faster analysis times for the same efficiency as with liquid organic solvents. This flash chromatography system accommodates multiple sample introduction formats with time- or peak-triggered fraction collection. Meanwhile, wet or dry homogenization of biological samples can be performed with the Biotage Bead Ruptor 24. The bead mill uses 24 2-mL tubes, 12 7-mL tubes, or six 30-mL tubes simultaneously. The SiliCycle MiniBlock is a general purpose system that allows flow-through parallel processing of chemical reactions, including derivatizations, peptide synthesis, and screening, with resin agitation and washing. The system operates over a temperature range from -20 °C to 120 °C with capacities ranging from six 40-mL vials to 48 4-mL tubes.

Other significant introductions in the area of sample preparation systems were updates or product extensions, especially in systems for environmental analysis. The Pickering Laboratories DEXTech system uses columns with different formats for sample cleanup in the analysis of dioxins and polychlorinated biphenyls. Meanwhile, Horizon Technologies added plungers for greater flexibility to the SmartPrep Extractor automated SPE system and Environmental Express added chemistries to its SimpleDist system for the distillation of phenols. The Omni-Sampler Plus sample handling system from Entech Instruments updated cryogenic preconcentration for volatile organic compounds onto glass beads, with mild temperatures (60–100 °C) for the transfer of C_2–C_{24} compounds. The Omni-Sampler Plus sample handling system has

Table I: New sorbent products

Company	Product	Format	Notes
SiliCycle	SiliaQuick QuEChERS	Salt packets with centrifuge tubes for performing QuEChERS method	Available as $MgSO_4$ with primary secondary amine (PSA), carbon black, or C18.
UCT, Inc.	Styre Screen HL DVB	SPE cartridges	Cross-linked divinylbenzene for extraction of acidic, basic, polar, and nonpolar compounds with greater loading capacity than silica-based phases.
	Enviro-Clean QuEChERS	Salt packets with centrifuge tubes for performing QuEChERS method	Salt ratios optimized for biological samples with limited volumes. Protein precipitation not required for blood samples.
Separation Methods Technologies	SMT MEB	Bulk packings	Methyl (1% carbon load), ethyl (2% carbon load), and butyl (4% carbon load) silica-based phases, 35–50 µm particles, 60- or 150-Å pores. Selective for polar and nonpolar pharmaceuticals, natural products, and very hydrophobic proteins and biomolecules.
Bonna-Agela Technologies	Cleanert PEP-2	96-well, modular microplates	Five phases available: • PVB: functionalized vinyl pyrrolidone and urea to retain most acidic, basic, and neutral polar compounds without adjusting pH. Design for small sample amounts, resulting in one-third less evaporation time and reconstitution solvent. • PWCX: combines weak cation-exchange and reversed phases using carboxylate radical functional group for improved retention of basic analytes. • PWAX: combines weak anion-exchange and reversed phases on polymer support with amino functional group. • PAX: quaternary ammonium base functional group with reversed-phase and strong anion-exchange modes. Stable from 0–14 pH range. • PCX: sulfo-functional group with reversed-phase and strong cation-exchange modes. Stable throughout the pH 0–14 range.
Thermo Scientific	ASE Prep Sorbent cartridges	6-mL cartridges with 500-mg resin	Four resins available, designed for cleanup of extracts following accelerated solvent extraction: • Florisil for adsorption of polar compounds. • Alumina acid for anion exchange and adsorption of polar compounds. • Alumina base for cation exchange and adsorption of polar compounds. • Alumina neutral for adsorption of polar compounds, capable of anion or cation exchange with pH adjustment.

multiple modes for the determination of volatile analytes, including headspace sampling, thermal desorption, porous cartridge microextraction (a high-capacity version of solid-phase microextraction [SPME]), and on-column trapping. For water analysis, the 4100 Water/Soil Sample Handler from OI Analytical automates sample handling and processing in collaboration with the company's Eclipse 4660 purge-and-trap concentrator.

Sorbents

Various sorbents, in cartridges or as bulk phases, have been introduced in the past year. These phases are designed for SPE, including dispersive SPE (dSPE) approaches such as the QuEChERS method, high sample capability via polymer supports, and selectivity in sample cleanup. These sorbent products are summarized in Table I.

Accessories and Other Products

Several other sample preparation products were recently introduced to the market. Most notably, Supelco continues to develop its SPME product line in the area of biocompatible SPME. This product extension is more compatible with biological analyses such as the direct sampling of small animals like mice, as well as dried blood spot analysis, 96-well plates, and other microsampling situations. Since gas chromatography (GC) and derivatization reactions for GC are often considered mature technologies, it is somewhat surprising to see a new derivatization regent from Regis Technologies. *N*-Methyl-*N*-(trimethylsilyl)

trifluoroacetamide (MSTFA) with 1% trimethylsilyl chloride is also marketed by other vendors for the silylation of hindered hydroxyl groups that do not ordinarily react with MSTFA, along with secondary amines, amides, carboxyls, and steroids. Thermo Scientific addresses an expanding number of application areas for accelerated solvent extraction (ASE), particularly polymers, with the offering of ASE extraction thimbles for samples that melt at the operating temperatures used in ASE. The goal is to prevent the plugging of filters and tubing by fine particles by using cellulose or glass fiber filters. The GlycoWorks RapiFluor-MS N-Glycan kit from Waters is a 96-well plate product based on hydrophilic interaction chromatography. The GlycoWorks kit is used for the sample preparation of N-linked glycans released following rapid deglycosylation and labeling to provide enhanced sensitivity for both fluorescence and mass spectrometric determination. Sample analysis of glycoproteins can be completed in less than 1 h. Finally, J.G. Finneran Associates marketed a vial loader for 96-well plates with insert vial sizes ranging from 350-µL glass vials to volumes of 2 mL.

Conclusions

With this review of new product offerings in the field of chromatographic sample preparation, the natural question is: "What's next?" Based on this year's offerings and advancements in the field, it is anticipated that commercial developments in the current year will address several issues. Sorbent-based sample preparation will continue to see significant commercialization in several areas. QuEChERS will remain a growing area and the end of patent protection for SPME will bring new competitors to the field and new areas such as biocompatible SPME and SPME designed for liquid chromatography applications. Other advancements will accommodate serial or parallel sample processing for increased throughput. Bioanalytical and food safety applications will drive these developments.

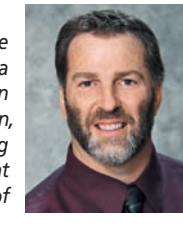
References

(1) L. Zhao, H.K. Lee, and R.E. Majors, *LCGC North Am.* **28**(8), 580–591 (2010).

(2) G. Borijijan, Y. Li, J. Gao, and J.J. Bao, *J. Sep. Sci.* **37,** 1155–1161 (2014).

Douglas E. Raynie

"Sample Prep Perspectives" editor Douglas E. Raynie is an Associate Research Professor at South Dakota State University. His research interests include green chemistry, alternative solvents, sample preparation, high resolution chromatography, and bioprocessing in supercritical fluids. He earned his PhD in 1990 at Brigham Young University under the direction of Milton L. Lee.



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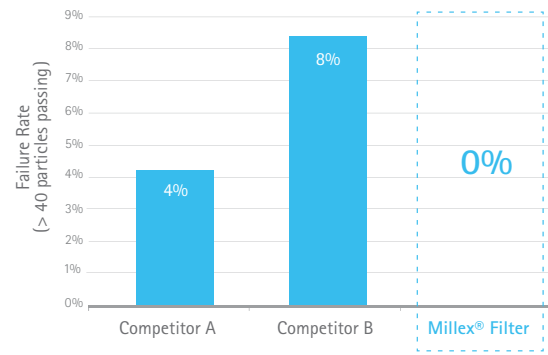

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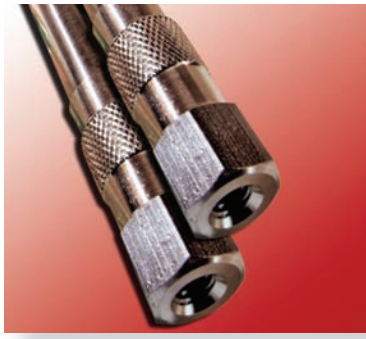

LC TROUBLESHOOTING

Calibration Problems — A Case Study

Unexpected results from calibration standards create confusion in a clinical liquid chromatography (LC) method.

John W. Dolan
LC Troubleshooting Editor

Recently, I received an inquiry from a reader regarding a problem he encountered with a routine liquid chromatography (LC) method in his clinical laboratory. He had prepared a fresh calibration standard (check sample) for the analyte of interest (I'll call it "X" to keep the reader's laboratory anonymous), yet when he assayed a blank sample spiked with 160 ppm of X, he found an indicated 400 ppm. This was puzzling and not a problem normally encountered, so he sent the sample to another laboratory that was analyzing the same compound by gas chromatography (GC), and their results showed that the spiked sample indeed contained 160 ppm of X. At this point he contacted me to help figure out what was happening. As we look at possible causes for and solutions to this problem, we can use this as a specific example to which we can apply general troubleshooting principles.

Background

Before we get further, let's take a look at the method, which is designed for the analysis of X in serum. Samples are prepared by taking an aliquot of serum, adding an aliquot of internal standard (IS), and a small amount of hydrochloric acid to acidify it. The solution is vortexed to mix, then an aliquot of dichloromethane is added, the solution is vortexed again, and then centrifuged to separate the two phases. The dichloromethane phase is removed, evaporated to dryness, and reconstituted in the injection solvent. The separation conditions comprise a reversed-phase column (size, stationary phase, and flow rate were not mentioned) with an isocratic mobile phase of acetonitrile, water, and trifluoroacetic acid. Ultraviolet (UV) detection is used. The chromatographic conditions give typical retention times of 9 min (IS) and 12 min (X), and the chromatogram is normally free of any other peaks. Calibration standards are prepared by spiking a stock solution of X into serum at 40, 120, and 160 ppm; these spiked calibrators are then extracted in the same manner as samples. A three-point calibration curve is run and if the regression is acceptable, this calibration curve is used for three months. With each batch of samples, a single injection of blank serum spiked to 160 ppm is made as a system suitability test; if this check sample assays at 160 ppm, the system is deemed stable and samples are run.

The method had been running acceptably until he ran out of the 1000 ppm stock of X used for spiking the check sample. When the new stock was prepared, the problem of a 400 ppm assay for the 160 ppm sample appeared.

Consider the Possibilities

In a case like this, I like to divide the case up into several possible problem areas, then see how many of these possibilities I can eliminate with the data at hand. This helps to focus my attention on the source of the problem so that it can be investigated further, if necessary, and corrected. We can broadly, and somewhat arbitrarily, divide the possible problem areas into chemistry, hardware, sample-related, and calibration. Let's look at each of these in more detail.

Chemistry

By chemistry, here I mean the chromatographically related chemical

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Solvent extraction is a sample preparation technique used to remove analytes from solid samples such as soil and tissue. This technique uses elevated temperature to accelerate the partitioning of analytes from the matrix and collects solvent extracts that can be analyzed by chromatographic techniques. Analytical laboratories often use solvent extraction prior to analysis by GC/GC-MS or LC/LC-MS. This technique has become an integral part of the complete laboratory workflow solution.

Solvent extraction is often used in the following industries:

- Environmental Testing (Dioxins, Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), Pesticides, Brominated Flame Retardants)
- Food and Beverage (Lipid Content, Oil Content, Vitamins, Pesticides)
- Chemical & Petrochemical (Consumer Products, Plastics & Electronics, Biofuels)
- Life Science (Herbal Supplements, Natural Products, Pharmaceuticals)

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Table 1: Comparison of solvent extraction techniques (*per sample basis)

Technique	Solvent Usage*	Extraction Time*
Soxhlet	200 - 500 mL	4 - 48 hours
Automated Soxhlet	50 - 100 mL	1 - 4 hours
Sonication	150 - 200 mL	0.5 - 1 hour
Supercritical Fluid Extraction	5 - 50 mL	0.5 - 2 hours
Microwave	25 - 50 mL	0.5 - 1 hour
Accelerated Solvent Extraction	15 - 50 mL	0.2 - 0.3 hour

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influences. These are the nature of the sample, the column, the mobile phase, and the column temperature. We can quickly eliminate these as the likely sources of the problem. If the column chemistry, mobile-phase chemistry, or column temperature had changed, we would expect a shift in retention for X and the IS, but this was not observed. The sample chemistry, or identity, is unlikely to have changed, because the check sample had no apparent retention problems in either the LC or GC assay.

Hardware

LC system hardware could malfunction in terms of flow rate, injection problems, or detection. The flow rate must be correct or the retention times would shift for both X and the IS. It is possible that the autosampler is not working properly, but this is unlikely to cause the noted problem, because any volume error in the autosampler would be compensated by the use of the IS. The purpose of the IS is to add it early in the sample preparation process so that any loss of sample volume or injection error would not matter, because it is the ratio of X/IS that is used in the calibration process, not the absolute response of either compound.

Problems related to the detector are a possible source of error, and should be checked. Two obvious possibilities are that the wrong wavelength was selected or that there is something wrong with the detector lamp. The response of X and the IS would be expected to change if the detection wavelength was changed, and a change in the relative response of X and the IS would be likely. This would generate a different X/IS ratio for a given concentration, which in turn would change the assay value for X in the check sample. A change in lamp energy as the detector lamp aged could also cause a change in response, and although I would expect that such intensity would affect X and the IS similarly, that is not a certainty. The proper wavelength should be verified and the lamp energy should be compared to normal values to determine if either of these items could be the problem source.

Sample-Related Problems

We know that the identity of the sample is correct and that the standard was made at the proper concentration because the sample assayed at 160 ppm by GC. The reader did not state if a new batch of IS stock was made at the same time, but if we consider the method, either the new batch of IS was made correctly or the old one was still good and was used. The check sample is made by spiking serum, and serum would never be injected directly, so it follows that the check sample was spiked with IS and extracted in the normal manner. One of the reasons for adding IS is to account for the inevitable changes in sample volume that take place during sample preparation.

Let's review the sample cleanup procedure: 300 µL of serum is combined with 50 µL of IS and 200 µL of dilute hydrochloric acid (550 µL total), centrifuged and extracted with 600 µL of dichloromethane. All of X and the IS should transfer into the dichloromethane, so the concentration of X and IS is 550/600 of its concentration in the original diluted serum. Next, 400 µL of the dichloromethane is removed, evaporated to dryness, and reconstituted in 50 µL of methanol. This concentrates the dichloromethane extract by 400/50 or eightfold. With the extraction, evaporation, and reconstitution steps, there will be inevitable volumetric errors introduced, which is why the IS is added — the same losses of X and IS should occur, so the X/IS ratio should stay constant. All this leads me to conclude that the GC method would be very unlikely to give an assay value of 160 ppm of X by an external standard method, even if the results were adjusted for the theoretical changes in concentration. Instead, I conclude that the IS method was used for GC, as well, and because the assay was as expected, it tells me that the check sample was made correctly, even though it doesn't assay properly by the LC method. The bottom line here is that it is unlikely that the current problem lies with the sample or sample preparation.

Calibration

At this point we've eliminated chemistry problems, hardware problems (assuming the detector wavelength is set correctly and the detector lamp is in acceptable condition), and sample-related problems. This leaves calibration problems as the most likely problem source (assuming that we haven't overlooked something else obvious, which is always a possibility).

My initial interaction with the reader simply indicated that the check sample did not assay correctly by LC, but gave the expected answer by GC. When I requested more information about the method, I learned of the practice of calibrating every three months and using the system suitability check sample to verify that the method was working properly. Although the rules are a bit different in the clinical laboratory industry, this goes strongly against the analysis of the same drugs in serum or plasma to support drug development in the pharmaceutical industry. The latter techniques fall under guidelines from the United States Food and Drug Administration (FDA). The FDA's "Guidance for Industry: Bioanalytical Method Validation" (1) discusses validation of methods for the analysis of small molecular weight drugs in plasma and other tissues (generally called "bioanalytical" methods, as opposed to methods for the analysis of biological compounds). In this document in the section titled "Application Of Validated Method To Routine Drug Analysis" (pp. 13–14), it is stated:

> A calibration curve should be generated for each analyte to assay samples in each analytical run and should be used to calculate the concentration of the analyte in the unknown samples in the run . . . . The calibration (standard) curve should cover the expected unknown sample concentration range in addition to a calibrator sample at LLOQ [lower limit of quantification].

It goes on to say:

> Once the analytical method has been validated for routine use, its accuracy and precision should be monitored regularly to ensure that the method continues to perform satisfactorily. To achieve this objective, a number of QC [quality control] samples prepared separately should be analyzed with processed test samples at intervals based on the total number of samples. . . . The QC samples in duplicate at three concentrations . . .

Additionally, it is noted:

> A matrix-based standard curve should consist of a minimum of six standard points, excluding blanks (either single or replicate), covering the entire range.

This says that the calibration curve should be run with each batch of samples, not once every three months. The calibration curve should cover the expected sample concentration range, and include the LLOQ. Furthermore, QC samples should be run at three concentrations that fall within the range of sample concentrations. These guidelines also make good sense from an analytical chemistry standpoint.

There are just too many potential problems that can occur that might cause the calibration curve to be different on different days. I have been involved with research and development (R&D) studies where the reference standards were so rare and valuable that it was not possible to run them every day, but a surrogate standard was found to verify that the original calibration was still adequate. That may seem to align with the current problem, but in fact the drug X and its IS are very common compounds that can be purchased in reference standard grade for reasonable prices, so it is hard to justify trimonthly calibration on economic grounds.

The fact that the check sample was formulated at 160 ppm and verified by GC underlines the probability that the source of the problem lies with the calibration curve. My best guess is that something in the LC system has drifted over time, most likely the detector response (or an improper wavelength setting), and has caused the current response to the X/IS ratio to be much larger than it was when the calibration curve was run originally.

What Now?

I recommend that the proper wavelength setting and detector lamp performance be verified before proceeding. After these are found to be satisfactory, I would generate a new calibration curve using freshly prepared standards of X spiked into blank serum and extracted normally. I believe that the check sample will now assay correctly, closing the loop on identifying the problem source.

Technically, the check sample has done exactly what it was intended for — it has alerted the operator to a problem with the assay before valuable patient samples were run. However, I would modify the method to comply more with the industry standard of the FDA guidelines (1). This would require running a calibration curve, containing samples with at least six concentrations, each day with each batch of samples run. In addition, a set of check samples, or QCs, should be prepared and included in each sample batch to show that during the analysis, the method gives the expected results for samples of known concentration. There will be some documentation required to make these changes, but the method reliability will be much improved and should

justify this extra work. The quality of the results produced should improve, as well. Finally, should the laboratory be audited by a regulatory agency, there will be much less likelihood of negative findings by the auditors.

In terms of day-to-day added work, there should be only a small impact on the total batch run time for a potentially large improvement in data quality. The calibration and check samples can be quickly spiked with known amounts of X and extracted with QC samples and samples to be analyzed. A total of six calibrators and six QC samples (duplicates at three concentrations) would add 12 samples to the day's run. At a 12-min retention time for X, this would increase the run time for the batch by about 2.5 h. It may be very easy to compensate for this increase in run time by increasing the flow rate; with an isocratic run, the separation should not be affected by the flow rate. The pressure would rise in proportion to the increase in flow rate, but it is fairly rare with conventional LC runs that pressure is a limiting condition, so the added pressure is unlikely to be an issue.

Conclusions

We have used a specific example of a method problem to illustrate how to break down the problem into several potential problem sources. Most of these sources could be eliminated by careful consideration of the method and how the results deviated from the expected ones. This left us with two likely problem sources. First, a problem with the detector wavelength setting or detector lamp energy. These could be quickly checked by examining the instrument. The second potential problem source was that the instrument response to X or the IS had drifted between the time the original calibration curve was run and the problem was noted.

The recommended solution was to first check for detector problems, and second rerun the calibration curve. A more permanent fix to the problem would be to change the method to comply better with current FDA guidelines and general analytical chemistry practices of running calibrators contemporaneously with samples.

References

(1) United States Food and Drug Administration, *Guidance for Industry: Bioanalytical Method Validation* (FDA, Rockville, Maryland, 2001).

John W. Dolan

"LC Troubleshooting" Editor John Dolan has been writing "LC Troubleshooting" for LCGC *for more than 30 years. One of the industry's most respected professionals, John is currently the Vice President of and a principal instructor for LC Resources in Lafayette, California. He is also a member of* LCGC*'s editorial advisory board. Direct correspondence about this column via e-mail to John.Dolan@LCResources.com*

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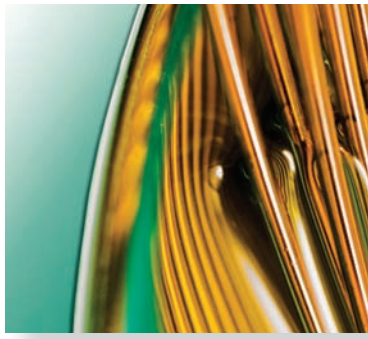

GC CONNECTIONS

New Gas Chromatography Products, 2014–2015

In this installment, John Hinshaw reviews gas chromatography (GC) instruments, columns, and accessories that were newly on display at the Pittsburgh Conference in New Orleans, Louisiana, during March 2015, or were introduced to the marketplace in the preceding year.

John V. Hinshaw
GC Connections Editor

From March 8–12, 2015, the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon) returned to the Morial Convention Center in New Orleans, Louisiana, for its 66th annual meeting. This was the first time since 2008 that the conference had taken place in New Orleans, and the city welcomed the 14,272 registered attendees with open arms, brass bands, and beignets. There were 919 exhibitors in 1690 booths, and this year 90 countries were represented. Although I did not attend many of the technical sessions, they were of as high quality and as well attended as in previous years. Of particular interest to the readers of *LCGC* was the half-day session devoted to the presentation of the 2015 *LCGC* Lifetime Achievement in Chromatography Award to Jack Kirkland (Advanced Materials Technology) and the *LCGC* Emerging Leader in Chromatography Award to Caroline West (Université d'Orléans, France). Please see the February 2015 issue of *LCGC North America* (1) for more information about this year's awards.

Pittcon will head to Atlanta in 2016, where conferees will enjoy a second consecutive year of Southern hospitality. In 2017, the conference returns to Chicago.

This annual installment reviews gas chromatography (GC) instrumentation, columns, and accessories shown at this year's Pittcon or introduced during the previous year. For a review of new products in other areas of chromatography, columns, and related accessories, please see the additional coverage in the April issue as well as this issue of *LCGC North America* (2–4), which are also available on-line at *LCGC*'s website.

The information presented here is based on manufacturers' replies to questionnaires, as well as additional information from manufacturers' press releases, websites, and product literature about the past year's products, and not on actual use or experience of the author. During Pittcon, I took time to stroll around the convention aisles and see some of the new products firsthand as well as discover a number of items that weren't covered by the questionnaires. Every effort has been made to collect accurate information, but because of the preliminary nature of some of the material, *LCGC North America* cannot be responsible for errors or omissions. This column installment cannot be considered to be a complete record of all new GC products introduced this year at Pittcon or elsewhere because not all manufacturers chose to respond to the questionnaire or attend the conference, nor is all of the submitted information

Table I: Companies introducing new GC products

Company Name
Agilent Technologies
AFP
Baseline Mocon
DANI
Defiant Technologies
Gow-Mac
Ionicon
JEOL
LECO
Phenomenex
Qmicro
Restek
SGE
Shimadzu
Thermo Scientific
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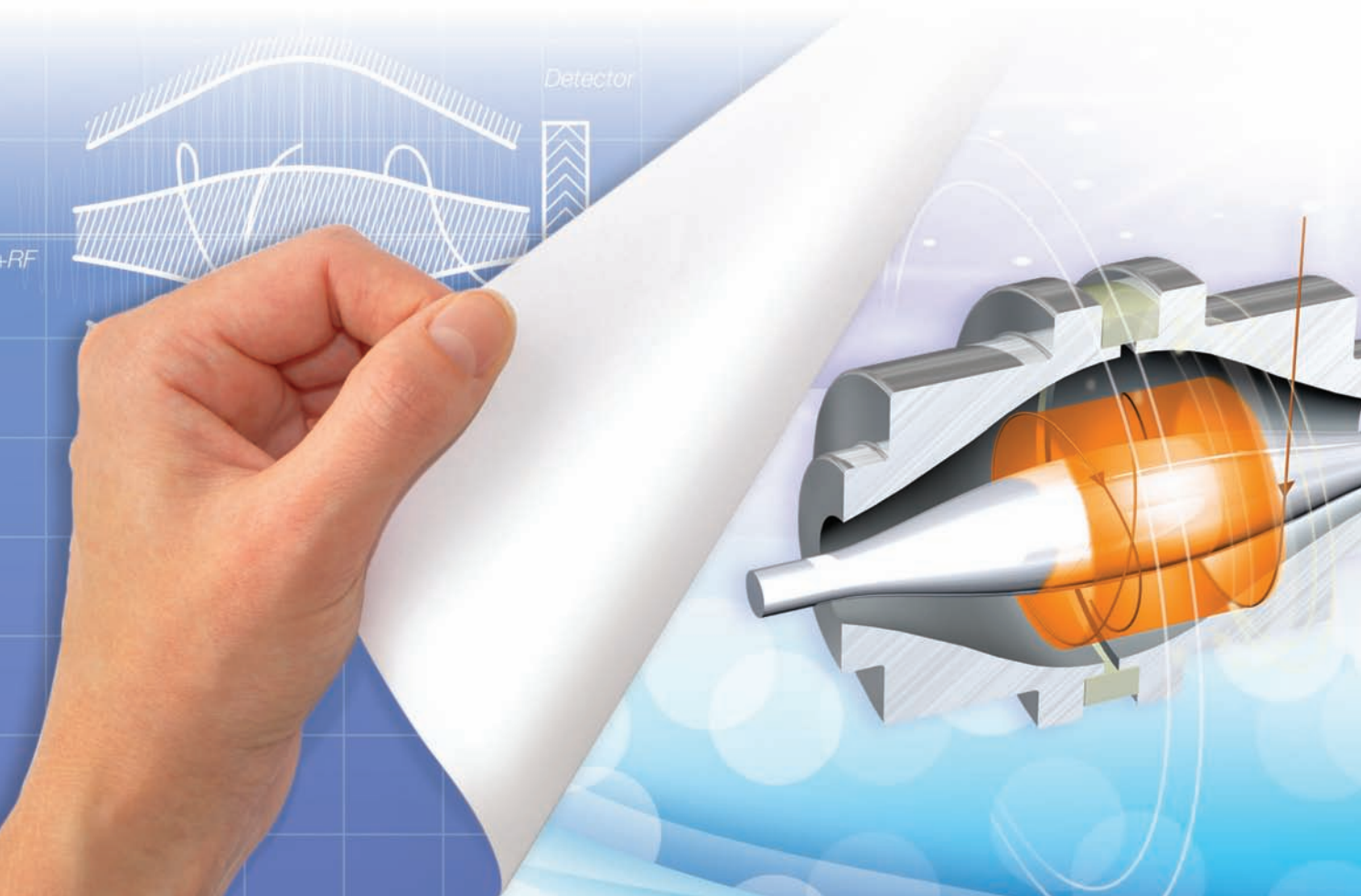

Table II: New GC instruments

Product	Company	Description
7010 Triple-Quad GC–MS system with improved EI source	Agilent Technologies	Agilent's 7010 triple-quadrupole GC–MS system features a redesigned high-efficiency electron ionization (EI) source that enables attogram detection limits. The system's new EI source creates more than 20 times as many ions as the current generation of EI sources, according to Agilent, to deliver an instrument detection limit (IDL) of 0.5 fg OFN. The new EI source technology is also available to current and future 7000C owners in the form of an upgrade package. Specifications: mode of operation: EI standard, CI optional; ion source temperature: 150–350 °C; dual filaments for EI; electron energy: 10–300 eV; mass filters: proprietary monolithic hyperbolic gold-coated quadrupole; mass axis stability: less than ± 0.10 u over 24 h (10–40 °C); quadrupole temperature: 106–200 °C; mass range: *m/z* 10–1050; resolution: selectable, 0.4–4.0 Da, custom tune; scan rate: up to 6250 u/s; detector: Triple-Axis HED-EM with extended-life EM and dynamically ramped-iris; MRM speed: 800 transitions/s; minimum MRM dwell: 0.5 ms; collision cell: linear hexapole; collision cell gas: nitrogen with helium quench gas; collision energy: selectable up to 60 eV.
7200B Q-TOF GC–MS system	Agilent Technologies	The Agilent 7200B Series Q-TOF GC–MS system with Agilent MassHunter software replaces the company's 7200A offering and provides an improved mass accuracy specification of less than 3 ppm over an extended mass range, with acquisition rates up to 50 Hz. The system must be combined with the high performance Agilent 7890B gas chromatograph. 7200B specifications: EI (high sensitivity extraction source), PCI, and NCI ionization mode as standard; ion source temperatures: 106–350 °C; electron energy: 10–200 eV; removable ion source without breaking vacuum through an isolation valve; dual filaments for EI source, single filament for CI source; quad isolation mass range (*m/z*) 20–1050; resolution (full width at half height) settable from 0.4 to 4.0 Da; dynamic range (electronic) greater than 10^5; quadrupole mass axis stability less than ±0.10 Da over 24 h (10–40 °C); quadrupole temperature: 100–200 °C; collision cell: linear hexapole, nitrogen collision cell gas; collision energy: selectable up to 60 eV; ion extraction and mirror: two-stage second-order corrected; TOF flight pathlength: 2 m; microchannel plate/scintillator/PMT detector; TOF mass range (*m/z*): 25–1700, extended 15–3000; TOF detector sampling rate ADC: 32 Gbits/s; autotune or manual tuning; spectra acquisition rate: 1–50 spectra/s; EI instrument detection limit of 240 fg or less of OFN.
AccuTOF-GCx time-of-flight GC–MS system	JEOL	The fourth-generation AccuTOF-GCx features high sensitivity (S/N >300 at OFN 1 pg/µL) and offers improved resolution, accuracy, and sensitivity, while retaining the power and flexibility of the company's previous models. The GCx offers both powerful chromatographic separation and high-resolution mass spectra in combination with comprehensive 2D GC (GC×GC) using the Zoex thermal modulator. Specifications: mass resolution: 10,000 (FWHM); mass range: 4–6000 (*m/z*); data acquisition speed: up to 4 GS/s; spectrum acquisition speed: up to 16,000 spectra/s; spectrum recording speed: up to 50 spectra/s; sensitivity: 1 pg octafluoronaphthalene (OFN) S/N ≥ 300.
AQMAlert Ozone Precursor system	Baseline Mocon	The company's AQMAlert multiple-GC field system combines two Series 9100 GC systems with a Series 9300 Preconcentrator into a photochemical assessment monitoring station (PAMS). The first GC system uses flame ionization detection (FID) for light hydrocarbon detection; the second GC system incorporates photoionization detection (PID) for the remaining components. The optional preconcentrator is a dual-tube desorption system that allows lower detection limits.
Gas chromatography cartridge	Qmicro	The new Qmicro gas chromatography cartridge is based on an innovative micro gas analysis platform with integrated injector and thermal conductivity detection systems plus columns, backflush to detector, and temperature programming, all packaged in a small palm-size oven. Backflush enables protection of sensitive columns by minimizing exposure to harmful gas components — such as carbon dioxide and water on a molecular sieve 5A column — thus increasing lifetime. Backflush to detector functionality allows quantification of the total backflushed sample peak. This enables fast analysis of total C_{6+} or C_{9+} content of a natural gas. The cartridge is based on silicon chips made by MEMS microtechnology and micro assembly technologies, for virtual zero dead volumes and microscopic small flow channels. The cartridge is intended for OEM partners and system integrators to integrate micro GC technology for fast, small, and reliable analyses into instruments and systems.
GCMS-TQ8040	Shimadzu	Shimadzu's GCMS-TQ8040 triple-quadrupole GC–MS system includes the following features: Smart MRM (multiple reaction monitoring), which can combine over 400 compounds into a single MRM method without losses in sensitivity or selectivity; MRM analysis at up to 800 transitions/s; high-speed scanning control at 20,000 u/s; an MRM optimization tool that automatically determines optimum transitions and collision energies for all compounds in a single sequence; an off-axis design that eliminates neutral noise; UFsweeper technology that accelerates ions out of the collision cell to eliminate crosstalk; an automatic adjustment of retention times (AART) function that updates retention times in both the acquisition and data processing methods after column maintenance, without changing chromatographic conditions or requiring multiple injections of standards. The system also has Shimadzu's Smart Database Series software to create MRM and scan-MRM methods automatically, and a scan-MRM mode that simultaneously acquires accurate library-searchable mass spectra and low-level MRM quantitation in a single analysis.
Pegasus GC-HRT 4D	LECO	LECO's newest GC×GC–MS system combines the company's Pegasus GC×GC system with high resolution TOF MS and ChromaTOF-HRT software, which uses high-resolution deconvolution (HRD) for component detection, National Institute of Standards and Technology (NIST), and Accurate Mass Library searches, pseudomolecular ions (via chemical ionization), retention time matching, isotope patterns, and mass accuracy of deconvoluted fragments within a complete package for data acquisition, processing, and reporting. According to LECO, the system can produce resolution up to 50,000 FWHM, mass accuracies less than 1 ppm, and acquisition rates up to 200 spectra/s.

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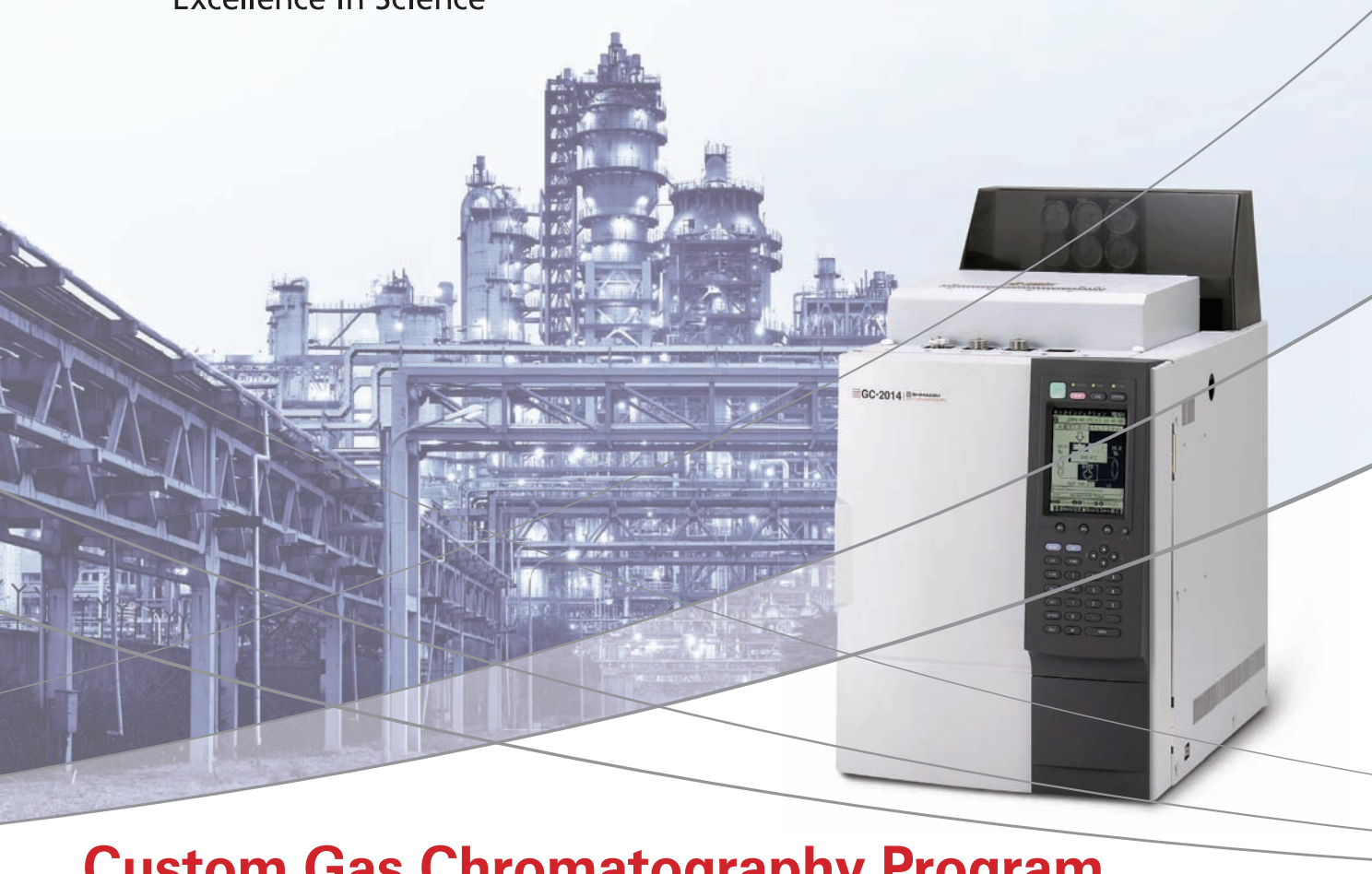

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Table II: New GC instruments (continued)

Product	Company	Description
Series 8100 Programmable GC system	Gow-Mac	Gow-Mac's new Series 8100 GC system is a custom, application-specific system configurable for research, industrial, laboratory, academic, and quality assurance (QA) and quality control (QC) environments. The system accommodates up to two independently controlled detectors that can be operated either individually, in series, or in parallel depending on the ordered configuration. Detection currently available includes TCD and FID. Features of the instrument include an ambient plus 5 °C to 450 °C operating temperature; independently programmed and controlled temperatures at injection ports, detectors, and column oven; the column oven accommodates up to five packed, wide-bore capillary, or capillary columns; a column oven temperature programming rate of 0.1 °C to 40 °C /min in 1 °C increments; an oven cooling rate of 350 °C to 75 °C in 5 min; method storage of three internal methods and an infinite number external methods; manual differential flow controllers or pressure regulators (detector dependent); and direct on-column (direct packed–capillary or split–splitless) or gas sample valve injection methods; and a full array of optional output capabilities that include analog outputs of 0–1 V, 0–1 mV, and 0–10 V VDC, or digital outputs to RS-232, USB, and ethernet utilizing MODBUS and PROFINET (read only) communication protocols.
TOCAM	Defiant Technologies	The TOCAM miniature GC-based toxic organic chemical monitor for airborne volatile organic compounds (VOC) includes a microconcentrator, micro-GC column, and two miniature photoionization detectors for rapid screening as well as detailed analysis of trapped and desorbed compounds of interest. The first detector responds directly to desorbed compounds. High levels can trigger a detailed GC analysis onto the second detector. The portable or mountable instrument features a 10.6-eV detector lamp, a 2.5-m or 4.6-m GC column, and operates from a 9–2 VDC AC wall adapter.
TSQ Duo triple-quadrupole GC–MS-MS system	Thermo Scientific	The Thermo Scientific TSQ Duo triple-quadrupole GC–MS-MS bridges from single-quadrupole full scan and SIM methods to the high selectivity and sensitivity of SRM (selected ion monitoring) methods. The system operates in both single and triple quadrupole modes with automatic selected reaction monitoring (AutoSRM) software for method development and optimization. A selected ion monitoring bridge method migration tool easily and accurately migrates existing methods to either SIM or SRM methods. The system uses Thermo Scientific Dionex Chromeleon chromatography data system software. Other system specifications include a mass analyzer with quadrupole scanning up to 20,000 u/s; heated, off-axis ion guide for noise reduction and solid, homogeneous, noncoated, maintenance-free quadrupole rods; automatic tuning down to 0.4 u; selectable SRM resolution settings in method at autotune value, 0.7, 1.5, and 2.5 u; Thermo Scientific DynaMax XR detection system, with off-axis 10 kV dynode, discrete dynode electron multiplier and electrometer, linear range of greater than 10^7 (0–68 µA); a collision energy range of 0–60 eV; a mass range of 1.2–1100 u; and scanning capabilities of up to 20,000 u/s with the ability to acquire more than 97 scans/s in FS when scanning over a range of 125 µ; 1.0 ms minimum SRM dwell times, and up to 300 SRM transitions/s.

necessarily included here because of the limited available space and the editors' judgment about its suitability.

Gas Chromatography in 2014–2015

Gas chromatography again displayed renewed vigor in the past year, which certainly was evident at the 2015 Pittcon conference. Comprehensive GC×GC continues to yield significant advances, in particular when combined with mass spectrometry (MS) detection. MS detectors for GC alone experienced no fewer than six new or enhanced product introductions. Advances in fast mini- and micro-sized GC systems were evident, too, as well as a nice assortment of valves, fittings, syringes, gas accessories, and columns.

In the instrument system area, Agilent Technologies introduced the 7200B quadrupole time-of-flight (QTOF) GC–MS system plus the 7010 Triple-Quad GC–MS system with an improved electron ionization (EI) source. Both of these offerings work with the company's gas chromatographs. Also in the GC–MS area, the AccuTOF-GCx TOF GC–MS system from JEOL works with comprehensive two dimensional (2D) GC to make a powerful GC×GC–MS analyzer. LECO displayed its newest GC×GC–MS system, the Pegasus GC-HRT 4D. Also coming in with a new product in this area, Shimadzu introduced the GCMS-TQ8040 triple-quadrupole GC–MS system. Finally, Thermo Scientific had its TSQ Duo triple-quadrupole GC–MS-MS system on-hand. Please see Table II for the details and specifications supplied by the manufacturers for these high-end hyphenated GC instruments.

The AQMAlert Ozone Precursor system from Baseline Mocon combines two of the company's model 9100 GC systems and a preconcentrator into a photochemical assessment monitoring station. From Gow-Mac, the Series 8100 programmable GC system represents a new customizable application-specific laboratory system with multiple inlets, detectors, and other options. In miniature and micro GC systems, Defiant Technologies showed the TOCAM toxic organic chemical monitor, based on a microconcentrator and micro-GC column for rapid screening and more detailed analyses. A new entry to micro-GC, Qmicro brought examples of its GC cartridge with integrated sampling valve, detector, and backflushing options, to be made available to original equipment manufacturers (OEMs).

Table III lists new GC accessories such as autosamplers, detectors, and more. From DANI, the Peakblade 77 GC×GC modulator is a liquid-nitrogen-free device with rapid and programmable thermal modulation. Two fast GC accessories were shown at Pittcon: the fast GC conversion kit from VICI that integrates the company's resistively heated columns and controller with Agilent GC systems, and a fast GC add-on for Ionicon's PTR-TOF gas analyzer. Analytical Flow Products (AFP) introduced a modular multipurpose valve oven, a miniature version of the company's multiport valve, and a new design for zero-dead-volume fittings. Shimadzu introduced two accessory prod-

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Table III: New GC accessories

Product	Company	Description
5-mL Diamond headspace syringe	SGE	SGE enhanced its line of Diamond headspace syringes with a 5-mL model. These syringes incorporate features for headspace sampling such as a unique "energized" plunger tip that eliminates dead volume at the end of the plunger stroke and is compatible with a wide range of solvents and a gas-tight seal that is not affected by temperature cycling up to at least 150 °C.
AOC-6000	Shimadzu	The Shimadzu AOC-6000 system is capable of exchanging syringe modules automatically between liquid injection, headspace injection, or solid-phase microextraction (SPME) injection. The autosampler features automated pretreatment with automatic syringe exchange (10 µL to 1000 µL) and mixing functions that automate the preparation of calibration curve samples, addition of internal standard substances, and sample dilution. The device performs sample pretreatment and analysis in parallel so that no time is lost in the continuous analysis of samples requiring headspace sampling or other time-consuming pretreatments.
ECD-2010 Exceed	Shimadzu	The Shimadzu ECD-2010 Exceed system uses contact-free technology in which the sample gas flow makes as little contact as possible with the collector electrode or the ^{63}Ni radiation source, which greatly improves the the ECD cell durability by reducing deposition of sample residue on the detector and radiation source. The detector has a limit of detection of 4.0 fg/s and a dynamic range of 10^5 for γ-BHC.
EZGC software suite	Restek	Restek has upgraded its EZGC software suite to include a method translator, flow calculator, and chromatogram modeler. The software application is available in an on-line version. For new methods, the chromatogram creator uses a database of thousands of compounds to accurately predict the best column and conditions for a wide range of applications. For optimization and troubleshooting of established methods, the method translator and flow calculator helps analysts easily change column formats and carrier gases for faster analyses or improved capacity, and also calculates splitless hold times for applications using splitless injections.
Fast GC conversion kit for HP6890 and Agilent 7890 systems	VICI	This conversion kit from VICI includes the parts and tools to convert HP6890 or Agilent 7890 instruments to fast GC systems. An adapter plate relocates the detector next to the injector to make room for the high-speed components. The kit enables use of VICI's resistively heated columns that are wrapped with nickel wire and heated by applying a low-voltage current. Column temperature is controlled by regulating the amount of current; a small fan provides rapid cooling of the low-mass column to near-ambient temperatures. The company's fast temperature programmer (a separate item) provides precise temperature programming with rapid heating and cooling in an eight-state profile at up to 1200 °C/min with a 5-m long or 500 °C/min with a 15-m-long nickel-wire or nickel-clad resistively heated column.
fastGC add-on for PTR-TOFMS series	Ionicon	Ionicon PTR-TOF systems are capable of measuring trace gas samples in real-time with a high mass resolving power. The new fastGC module adds an optional chemical preseparation step before the analysis. The module consists of a short GC column with an advanced heating concept for ultrafast heating and equally fast cooling rates that makes the preseparation step nearly real time. The fastGC module is integrated with the PTR-TOF and the normal sample gas inlet is used, which allows researchers to perform real-time measurements and add fastGC runs at time points of interest for enhanced separation and identification. Winner of the *Gases & Instrumentation International Magazine*'s 2015 Golden Gas Award in the gas chromatography category.
IMOv Intelligent Modular oven	AFP	The IMOv Intelligent Modular oven is a modular GC oven system offering flexibility in the number of column ovens and amount of space for valves and other accessories. The valve oven is independent from the column, with the choice of one to three column ovens. The valve oven has a preperforated bottom plate to accommodate AFP's valves and comes with custom tube brackets for easy routing. All electronics such as the company's IVD intelligent valve driver, electronic relays, electronic pressure control (EPC), flow meters, and communications ports are accessible from the back of the controlled temperature zone. The system also features a built-in leak detection system and has software drivers for various third-party instruments.
IPAPS: Intelligent Plasma Assisted Purifier system	AFP	The Intelligent Plasma Assisted Purifier system from AFP is a gas purifier that uses a plasma to increase the lifetime for the heated gettering alloy. The purifier features an end-of-life monitoring system, impurities overload detection, and a capacity of 1 L/min. The device is suitable for gas purification, zero gas generation for calibration of on-line analyzers, mass spectrometers, and GC detectors such as plasma emission, helium ionization, discharge ionization, flame ionization, pulsed-discharge ionization, and electron capture. The purifier can deliver a total outlet impurity level of less than 1 ppb, with 99.9999% grade inlet gas. It supports AFP's IMOv and other accessories through a serial communications link.
LipSeal fitting	AFP	AFP introduced a new patented fitting concept that provides two levels of sealing, requires a much lower torque, and permits an increased number of remakes. Ultrafine-pitch threads transfer the sealing force to the ferrule and an antifriction and antigalling coating is applied to the front and the threaded portions of the nut, which reduces the friction at least by a factor of 10 and eliminates rotation of the ferrule when the nut is tightened. Gold-plated ferrules optimize sealing performance, and a fine lip is added on the bottom of the detail that creates a metal-to-metal seal between the tube end and fitting body with no dead volume. The front portion of the ferrule is longer to occupy more of the empty volume.
MRV: Mini rotary valve	AFP	AFP introduced a smaller form factor rotary valve that includes features from the company's larger valve series such as a double stopper to prevent side loading of the rotor, which results in a longer lifetime; two dowel pins to lock the stator body in place to prevent rotation; and a treated rotor to enable the use of the high temperature version at ambient temperature without damaging the valve. The MRV works with the company's Mini Pneumatic Actuator.

Table III: New GC accessories (continued)

Product	Company	Description
PeakBlade 77 GC×GC modulation system	DANI	The DANI PeakBlade 77 GC×GC modulator is able to reach down to 77 K without using liquid nitrogen, which opens up analysis of the most demanding molecules and allows fast chromatography with sharp peaks. The modulator cuts chromatographic peaks to unfold complex sample composition. Driven by a cryocooler system, a cool tip connects to the cryofocusing area of the GC×GC system by heat conduction to allow an effective focusing process into the GC oven. The system features an innovative heater design, that allows ultrafast vaporization of cryofocused molecules resulting in an ultranarrow peak bandwidth. The modulator can achieve as much as 450 °C difference between temperature of the cold tip and the GC oven when the oven is at 350–400 °C. The system is capable of the analysis of compounds down to C_3. The modulator can use different vaporization temperatures to adapt to different compounds, programmable during the run. It is possible to change modulation frequency for target peaks or in timed intervals to better select sensitivity versus peak-cutting. The modulator is available as part of the DANI Master GC×GC/TOF-MS system or can be sold to support other GC and GC–MS systems on the market.

Table IV: New GC columns

Product	Company	Description
Rt-Silica BOND	Restek	The Rt-Silica BOND column is aimed at analysis of permanent gases, chlorofluorocarbons (CFC), light hydrocarbons, and sulfur analyses. The column has the retention and capacity of a traditional PLOT column, but the company's manufacturing process for this column nearly eliminates particle release, which minimizes detector spikes, maintenance, and downtime. The silica phase easily stands up to water, eliminating the need for long bakeouts after injections of water-contaminated samples. Each column is individually tested with C_4 hydrocarbons, including methyl acetylene and 1,3-butadiene, both of which are extremely sensitive to changes in inertness and selectivity.
Rxi-1301Sil MS	Restek	The Rxi-1301Sil MS column targets solvent analyses, glycols by MS, and EPA Method 551. It features an arylene-stabilized cyano phase, combining retention and selectivity for volatiles with the highest maximum temperature and lowest bleed of any cyano column. This allows for reliable MS analyses for volatiles and polar compounds, fast elution of less-volatile analytes for faster cycle times, as well as improved robustness.
ZB-CLPesticides-1 and ZB-CLPesticides-2 column set	Phenomenex	Phenomenex's new columns are intended for application-specific analysis of chlorinated pesticides, herbicides, and polychlorinated biphenyls (PCB) by GC–ECD. The columns provide baseline separation of all analytes of interest in under 10 min and are available as a two-column kit. The column set supports seven EPA methods (8081, 8082, 8151, 504, 505, 508, and 552) without changing columns, which reduces down-time by eliminating column installations otherwise needed when changing methods. The columns have temperature limits of 320 °C isothermal and 340 °C programmed temperature. The ZB-CLPesticides-1 column is available with film thicknesses of 0.25, 0.32, and 0.50 µm, and the ZB-CLPesticides-2 column is available with 0.20-, 0.25-, 0.42-, and 0.50-µm films.

ucts: the AOC-6000 autosampler, which automates calibration sample preparation, and the ECD-2010 Exceed electron-capture detector. SGE introduced a new version of its Diamond headspace syringe line. The EZGC Software Suite Online from Restek has been expanded with some additional calculation and translation capabilities.

Only two companies submitted information about new GC columns, as shown in Table IV. Restek has two new columns, the Rt-Silica BOND porous-layer open-tubular (PLOT) column for permanent gas separations and the Rxi-1301Sil MS column, which targets solvent analyses with MS detection. Phenomenex introduced a two-column set that consists of the company's ZB-CLPesticides-1 and ZB-CLPesticides-2 columns and is targeted for multiple polychlorinated biphenyl (PCB) US Environmental Protection Agency (EPA) methods that use electron-capture detection.

Acknowledgments

I would like to thank the manufacturers and distributors that kindly furnished the requested information, which allowed a timely report on new product introductions over the past year. For those manufacturers who did not receive a "New Products" questionnaire this year and would like to receive one and be considered for early inclusion into the 2015–2016 new GC and related product introductions review, please send the name of the primary company contact, the mailing address, fax number, and e-mail address to Laura Bush, Editorial Director, *LCGC North America*, lbush@advanstar.com, with the subject line "2016 New GC Products." The questionnaire will be sent out in December 2015.

References

(1) M. L'Heureux, *LCGC North Am.* **33**(2), 108–121 (2015).
(2) M. Swartz, *LCGC North Am.* **33**(4), 232–247 (2015).
(3) M.W. Dong, *LCGC North Am.* **33**(4), 232–247 (2015).
(4) D.E. Raynie, *LCGC North Am.* **33**(5), 306–310 (2015).

John V. Hinshaw

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For more information on this topic, please visit www.chromatographyonline.com/column-gc-connections

THE HISTORY OF CHROMATOGRAPHY

Georges Guiochon: Separation Science Innovator

When Professor Georges Guiochon passed away last October, at the age of 83, the separation science community mourned the loss of one of the great pioneers of the field. In this interview with Fabrice Gritti, conducted about a year earlier, Guiochon talked about his life and work.

Fabrice Gritti

Gritti: How did you enter the field of analytical chemistry and come to specialize in gas chromatography (GC)?

Guiochon: After graduating from École Polytechnique in Paris, in 1953 with a Masters degree in engineering, I accepted a graduate student position to research why ammonium nitrate was unstable under certain conditions.

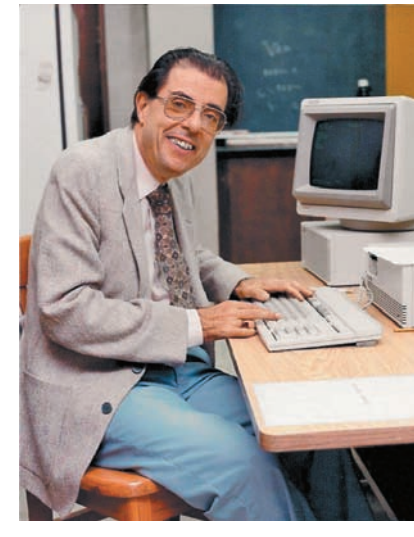
In 1947 ammonium nitrate had been the source of two devastating explosions on cargo ships, one in Texas, and another in Brest, France, that resulted in numerous casualties and widespread devastation.

On April 16, 1947, in Texas, a cargo ship, The Grandcamp, was being loaded with bags of ammonium nitrate fertilizer when a fire was detected in the hold. However, at this point 2600 tons of ammonium nitrate were aboard. The captain responded by tightly closing the hold and pumping in pressurized steam. One hour later, the ship exploded, killing several hundred people and setting fire to another vessel, The High Flyer, which was moored 250 meters away and contained 1050 tons of sulfur and 960 tons of ammonium nitrate. The Grandcamp explosion also created a powerful earthshock that broke windows as far as 40 miles away and knocked two small planes flying at 1500 feet (460 m) out of the sky.

The High Flyer exploded the next day, during the night, after having burned for 16 hours. A store of 500 tons of ammonium nitrate in the nearby warehouse also burned, but without exploding, probably because it was less tightly packed. All but one member of the Texas City fire department died. An estimated 567 people died in Texas City and 5000 people were injured; it was the worst industrial disaster in US history.

On July 28, 1947, in Brest, another cargo ship, The Ocean Liberty, was loaded with 3300 tons of ammonium nitrate and various inflammable products when it caught fire. The captain also ordered the hold to be sealed while pressurized steam was pumped in. As this did not stop the fire, the vessel was towed out of the harbor at 14:00, and exploded at 17:00. The explosion caused 29 deaths on the quay side and serious damage to the harbor of Brest.

The objective of my research was to determine regulations required



for the safe manufacturing, packaging, storage, and transportation of ammonium nitrate, which was growing in demand, particularly for agricultural applications. At around this time my mentor, Professor Leon Jacque, professor at École

Polytechnique, received a visitor who wanted research performed into a hypothesized phenomenon. He believed that strong ultrasonic vibrations from the ship hull generated by hoists uploading bags of ammonium nitrate aboard the ship could cause the transmutation of nitrogen into carbon monoxide (which has roughly the same molecular weight: 28).

The mixture of carbon monoxide and air would then result in an explosion. This hypothesis was far-fetched because nitrogen transmutation would require the input of a very large amount of energy. The mass of CO is less than that of N_2. It would have been possible for a spontaneous transmutation to occur in the opposite direction.

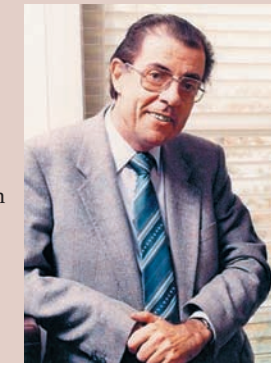
On realizing that the visitor was the son-in-law of the French Prime Minister at the time, rather than rejecting the idea, my mentor answered that a powerful, highly sensitive analytical instrument would be needed to detect traces of carbon monoxide in air. This exchange took place shortly after the publication of the paper by James and Martin on GC, when gas chromatographs began to be manufactured.

My mentor requested that the Office of the Prime Minister provide the funds to buy a GC instrument to research the hypothesized phenomenon. The decision was positive, fast, and we rapidly obtained this instrument, even though the purchase of American goods was tightly controlled at that time. Obviously, I did not detect any CO in the decomposition gases of ammonium nitrate, even under high-energy ultrasonic irradiation. This was not important because the French Prime Minister was replaced in the mean time. I soon put the instrument to good use by investigating other fields of interest of my mentor such as the compositions of natural essential oils, gasoline, and other petroleum distillation fractions. From then on I was hooked on GC, from which I was later attracted to high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC).

Gritti: You focused on GC between 1955 and 1984. What do you regard as your main contributions to both the fundamental and practical aspects of GC?

Guiochon: Some interesting work involved the investigation of nonlinear GC for the development of methods for the preparative production of butadiene, 2-methylbutadiene, and related olefins for a French company that was producing high-molecular-weight elastomers. The molecular weight obtained by polycondensation of these olefins is limited by the presence of certain impurities (for example, isoprene) that interrupt the polycondensation when they become bonded at the end of a monomeric chain. It was useful for the company to measure the properties of elastomers made with known concentrations of these impurities and to derive the specifications for the reagents needed for the production of different brands of elastomers.

Georges Guiochon (1931–2014) obtained a Master's in engineering at École Polytechnique (Paris, France) and a PhD in chemistry at the University of Paris (France). In the late 1950s, Guiochon developed a research group on the fundamentals and applications of GC, later becoming involved in the development of HPLC. He was a professor of chemistry at École Polytechnique (1958–1985) and at the University of Paris (1968–1984). He joined Georgetown University in Washington, D.C., in 1984–1987 and later the University of Tennessee in Knoxville, where he was a Distinguished Scientist since 1987. At the University of Tennessee Guiochon developed the theory of nonlinear chromatography and its applications in gas, liquid, and supercritical chromatography. He published five books and more than 1100 scientific papers in peer-reviewed journals and organized many scientific meetings. Among his many honors are the Tsvett Medal from Advances in Chromatography (1976); the Stephen Dal Nogare Award (Chromatography Forum of the Delaware Valley, 1977); the Silver Medal of the Centre National de la Recherche Scientifique (Paris, France, 1978); the Tswett Memorial Medal (Academy of Sciences of URSS, 1978); the A.J.P. Martin Award (Chromatographic Society, UK, 1980); the ACS Awards in Separation Sciences (1991) and in Chromatography (1998); the Alexander von Humboldt Award to Senior American Scientists (1994); the Istvan Halasz Award of the Hungarian Society of Separation Sciences (Siofok, 2000); and a JSPS Fellowship for Research in Japan (2002). He also held honorary PhD degrees from the Technical University in Budapest (Hungary, 1982); the University of Pardubice (Czech Republic, 1999); the University Ramon Llull (Barcelona, Spain, 2002); and the University of Ferrara (Italy, 2003). He is survived by three children and seven grandchildren.



We built several GC preparative instruments using 4-cm, 10-cm, and 30-cm diameter packed columns (one was several meters long) and produced ultra-pure C_4, C_5, and C_6 olefins by the kilogram. This gave me a good empirical understanding of nonlinear and preparative GC that was helpful later in doing similar studies in HPLC. These early attempts at producing high-purity compounds by gas chromatography were essentially experimental work as there was little interest in fundamental work at that time in France. It became clear that there were not many valuable compounds with a vapor pressure high enough to permit significant production rates using GC.

Gritti: After a first successful academic career in France, you decided to go to the US. There, you started a vast research program in preparative liquid chromatography (prep LC). What drove you there and why did you want to focus on prep LC?

Guiochon: An important event in France at this time was the election of Francois Mitterand as President of the Republic in 1981 because this major political shift had unexpected consequences. Before this election, French universities and administrations that supported academic research were supervised by administrators appointed by the government and confirmed by a conservative congress that oversaw nonpolitical distribution of grants.

When Mitterand was elected, and formed the first socialist government, generous spending began on many

popular social projects. Credits for academic research were also markedly increased. After a couple of years, the government realized that there was much less money available than they had dreamt and austerity became enforced. Life became much worse in France than in the US universities. As a result, research grants were thinned out, university organization was changed, work became less pleasant, and politics pervaded the academic system. Since I had many friendly colleagues in the US, I decided to move there.

Gritti: What has the theory of prep LC, which you developed over 25 years, brought to the practical challenges met by the industry?

Guiochon: Preparative chromatography is important for a number of reasons, including the role it plays in the production of pure chemicals. Books on preparative chromatography are always in demand, as are other publications that clarify and make it more practical. An annual meeting on preparative scale chromatography (PREP-1985 to PREP-2015) attracts hundreds of participants from industry, government laboratories, and academy. My involvement in preparative-scale chromatography is a source of satisfaction because I observed the field evolve and saw the realization of what was once a promising idea. It is especially satisfying to discuss matters with someone who applies preparative chromatography, to reply to questions, and to enjoy

recognition in this important area of separation science.

Gritti: How important is the role of prep LC in the chemical and pharmaceutical industries at present? How do you think it will evolve in the future?
Guiochon: To meet specifications, many pharmaceuticals and fine chemicals are made from intermediates — chemicals that are extracted or purified by preparative chromatography. Similarly, more and more biopharmaceuticals must be extracted from cells or other organisms involved in their production and separated from other compounds and viruses that might have adverse effects on patient health.

Various forms of chromatography used for extraction and purification will remain important. There is considerable opportunity for separation scientists to address issues in the biotechnology area, specifically in the separation of biochemicals.

Gritti: SFC analysis and prep SFC have grown up over the last few years. You have recently engaged yourself in bridging the gap between prep GC and prep HPLC. Could you comment on the future benefits of SFC and prep SFC?
Guiochon: Although it is more complex, preparative SFC is an attractive alternative to preparative HPLC. The main reason for industry interest is because of the fast rate of mass transfers in SFC columns and the low viscosity of the eluents used for SFC separations. Columns used in SFC have as high an efficiency as those used in HPLC, but should be operated at a flow rate that is several times higher. As a result, SFC analysis is much faster, more productive, with higher analytical throughput. Because of its complexity, developing SFC separations requires the attention of engineers and technicians who are well-educated in the fundamentals of chromatography. One specific area where SFC overtakes GC and HPLC is the purification of large amounts of pure enantiomers.

Gritti: During your whole career you have been very active at the interface between the academic and the industrial world. What do you think will be the next challenges for the future generation of analytical chemists?
Guiochon: It was fun to be in this position because it was where the action was, at the border between several important domains. My education background — a Master's degree in chemical engineering and a PhD in physical chemistry — combined with intense research in analytical GC helped me in finding critical problems, formulating them, and developing solutions.

Chromatography involves a wide range of physical chemistry areas: Adsorption and solvation equilibria, mass transfer kinetics, hydrodynamics, the consolidation of beds of particles, spectrophotometric, and other detection methods. Success requires an advanced knowledge in multiple fields. Future chromatog-

raphers will have to build on past achievements and develop powerful, useful methods. As in all areas of science and technology, separation science may suffer slowdowns with progress limited by a lack of funding. At this point it remains lively and demanding.

Gritti: Are there any specific separation scientists that have inspired you? If so, how?

Guiochon: Geza Schay and Istvan Halasz taught me chromatography. Keulemans introduced me to A.J.P. Martin, to Janak, and to many Eastern European colleagues. Attending many meetings across the world, I met Ernst Bayer, Ervin Kovats, Cartony Phillips, Dennis Desty, Marcel Golay, John Calvin Giddings, Csaba Horvath, Josef Huber, Roy Keller, John Knox, A. Zlatkis, J.J. van Deemter, J.H. Purnell, and so many others who came to be famous in the 1950s to 1980s. Whether these individuals remain famous or not is unimportant. The contributions of these scientists and others who were involved in chromatography since Tswett will remain important in the future because we all build on previous knowledge even if we don't know the source.

I was lucky to find a scientific domain that involved so many different branches of science and technology at a period where its understanding and expansion was critical for a wide range of practical applications and where many important new problems had to be identified and solved. I was happy to meet many fine people who helped me to contribute to this task and who supported me.

Gritti: You were involved in the early days of computing in chromatography. Could you elaborate on your early work, how the field has evolved and where it is going?

Guiochon: I moved to Knoxville, Tennessee, at the time when minicomputers, desktop computers (and later laptop computers) were becoming available and affordable. At the same time, useful programs were available that allowed the storage and interpretation of data using appropriate models. There were excellent theories of nonlinear chromatography that had not been used because of insufficient computational power. Suddenly, this power appeared, within a few years I was ready and began to make intensive use of computers in preparative and nonlinear chromatography.

Gritti: What do you regard as the unexplored avenues of chromatography?

Guiochon: I feel that the most important areas of pure chromatography that have not been sufficiently explored are multidimensional chromatography combined with various detection techniques, and the preparation of efficient chromatographic beds.

Over the last 40 years the efficiency of columns has markedly improved. Column efficiency can be conveniently expressed as the HETP, $H = h\,d_p$, where d_p is the average size of the particles used to pack the columns. Forty years ago, d_p was about 20 µm; now it is 1.6 µm to 2.6 µm depending on the column length that we can afford. In the mid-1970s, h used to be 4 to 5 before it decreased to 2.0–2.3 in the late 1990s. Now it has dropped to 1.5–1.8. On theoretical grounds, we anticipate a possible minimum around 0.9. This could provide great progress, but it is still uncertain how to do it and this will require considerable improvements in the instruments used to operate the columns.

The applications of chromatography to the extraction and purification of biochemicals raise important difficulties. Many biochemicals found in nature are mixtures of conformers that are difficult to separate and identify. The systematic use of high performance liquid chromatography coupled with nuclear magnetic resonance spectroscopy (HPLC–NMR) could potentially provide a useful method of approach. This tool is not yet widespread in academic laboratories, but promises great rewards. Another interesting area is miniaturization that may lead to advances in medical and forensic testing. All these promising areas add to the vibrancy of the separation science field.

Fabrice G. Gritti received a B.S. degree in chemistry and physics from the University Joseph Fourier of Grenoble (France) in 1995, a graduate Engineering school degree in chemistry and physics from the University of Bordeaux I (France) in 1997, and a PhD in chemistry and physics from the University of Bordeaux I (France) in 2001. He arrived in the US in 2002 for a post-doctoral study at the University of Tennessee with Professor Georges Guiochon. He is now a research scientist in the Department of Chemistry at the same university.

Gritti's research interests involve liquid/solid adsorption thermodynamics and mass transfer in heterogeneous media for characterization and design of new HPLC column technology. He developed experimental protocols that helped refine the models of adsorption isotherms used in preparative chromatography for the prediction of the band profiles of neutral and ionizable compounds; and the theory of band broadening along analytical columns in linear chromatography. He has published more than 190 papers.

This interview has been edited for length and clarity. It was originally published in LCGC Europe *in February 2014.*

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Gas Chromatography with Reduction Gas Detection for the Characterization of Parts-Per-Billion Levels of Ethylene in Various Matrices

A highly sensitive and selective mercuric oxide–based reduction gas detector was successfully used for parts-per-billion level gas chromatography (GC) determination of ethylene in various matrices such as in the monitoring of fruit ripening, ambient air, and industrial solvents. Separation of ethylene from the sample matrix was achieved by isothermal analysis using a silica-based packed column. Backflushing was incorporated to ensure overall system cleanliness and to improve sample throughput, keeping analysis time under 4 min.

Monica Lin, Ronda Gras, Clayton Bleile, Kaelyn Gras, Jim Luong, and Robert A. Shellie

Reduction gas detection (RGD) was originally conceived as an ultratrace gas chromatography (GC) detection method for the measurement of carbon monoxide and hydrogen for the electronics industry. Compounds such as acetone, aldehyde, and olefins reduce mercuric oxide and elicit a respectable RGD response (1–8). Amongst the olefins, ethylene is industrially significant because it is one of the major building blocks for downstream products such as ethylene oxide, ethylene glycol derivatives, linear low density polyethylene, and high density polyethylene. Ethylene is found in the atmosphere at the parts-per-billion level from natural and anthropogenic sources. Ethylene is generated in nature by the metabolic activities of vegetation and microbes. Ethylene is also a by-product of combustion from sources such as motor vehicles, fires, and volcanic activities (9,10). From an emission perspective, ethylene can be generated through catalytic cracking of natural gas; thus, atmospheric emissions are monitored by regulatory agencies (11,12). Ethylene plays a role in agriculture such as impacting the quantity and quality of crop production, the shipping of fruits and vegetables, and fruit preservation to maximize the availability of food across seasons.

Headspace gas chromatography (GC) with flame ionization detection (FID) is one of the most popular approaches for trace ethylene measurement (13,14). However, headspace GC is limited by the amount of sample that can be reliably introduced into the analytical system. Dynamic enrichment, or trapping, is an alternative to headspace GC that has the advantage of concentrating the analytes to detectable levels, thereby reducing detection limits. Solutions of mercuric acetate or mercuric perchlorate have been used as nonspecific traps for olefins based on complexation. The addition of chloride salts to these solutions degrades these complexes and releases ethylene for headspace analysis (15). These methods suffer from low trapping efficiency, considerable sample preparation time, and the necessity of using toxic reagents. Thermal desorption technology combined with adsorbent trapping for light hydrocarbon analysis is another alternative, but substantially higher cost of ownership and high frequency of maintenance from trap contamination are key limitations (16).

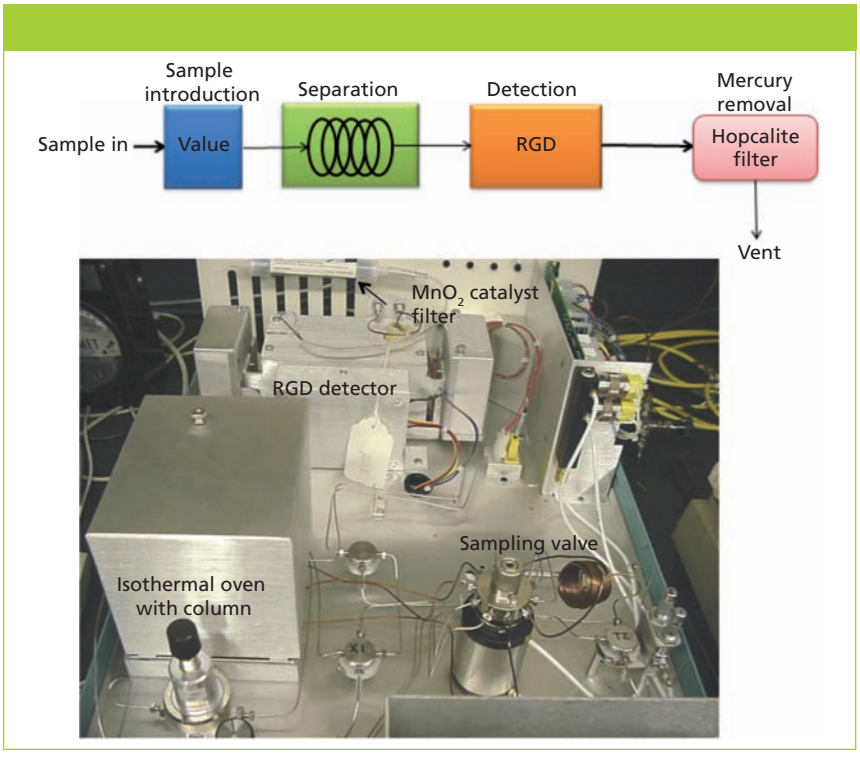

Figure 1: A block diagram of the analytical system.

In this article, a simple and reliable GC procedure with RGD was developed for the determination of ultratrace levels of ethylene in the fruit ripening process, in industrial solvents, and in ambient air monitoring.

Experimental

A Peak Performer 1 GC analyzer (Peak Laboratories) was used for all of the experiments. Figure 1 shows a block diagram of the GC system, which includes a rotary valve, a packed column, an RGD system, and an MnO_2 catalyst (hopcalite) filter. Sampling and backflushing were conducted with A C10WP 10-port sample injection valve (Valco Instruments Co.) fitted with a 1-mL sampling loop.

Figure 2: A picture of the sampling vessels.

Separation of ethylene was conducted with a 31 in. × 1/8 in. (79 cm × 0.32 cm) stainless steel column packed with Unibeads 3S, 60/80 mesh silica (Alltech Assoc.) at 130 °C using nitrogen carrier gas at 30 mL/min. A second column with dimensions of 16.5 in. × 1/8 in. (41.9 cm × 0.32 cm) with the same packing was used as a guard column. Backflushing was conducted 70 s after injection to isolate the detector from heavier hydrocarbon components and water and maintain a lower overall analysis time. These short columns were packed in-house.

The detector consists of a heated mercury oxide reaction bed, where Hg(II) is reduced to Hg(0) vapor by the analyte of interest. The reaction bed can be constructed from red or yellow mercury oxide, either granulated or powder sieved to a uniform particle size of approximately 40 µm, roughly spherical in shape. The reaction bed of this construction typically has a diameter of 0.063 in. and is 0.063 in. deep and contains approximately 20 mg of mercury oxide. The reaction bed requires minimal maintenance. A single charge lasts in excess of two years under normal operation conditions. Symptoms of a worn-out bed include a high background detector noise and erratic response. The temperature of the reaction bed was 265 °C based on manufacturer's recommendation. This value is consistent with published articles in literature to be optimum for use with olefins such as ethylene and isoprene (17). The mercury liberated in the reduction reaction passes directly into a quartz optical cell, where it is measured by its atomic absorption of light from a mercury lamp source at 254 nm.

Standards were obtained from Sigma-Aldrich. A 10 ppm (v/v) stan-

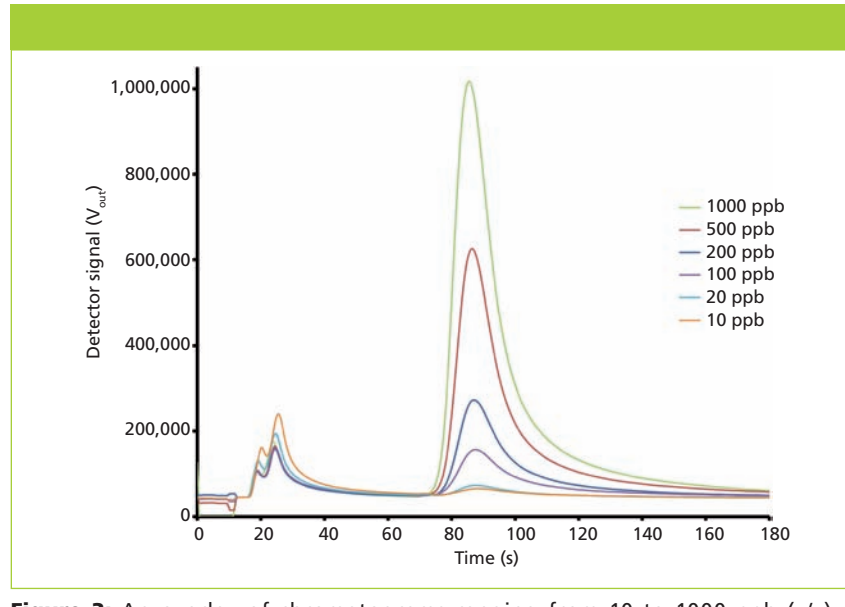

Figure 3: An overlay of chromatograms ranging from 10 to 1000 ppb (v/v) of ethylene in air.

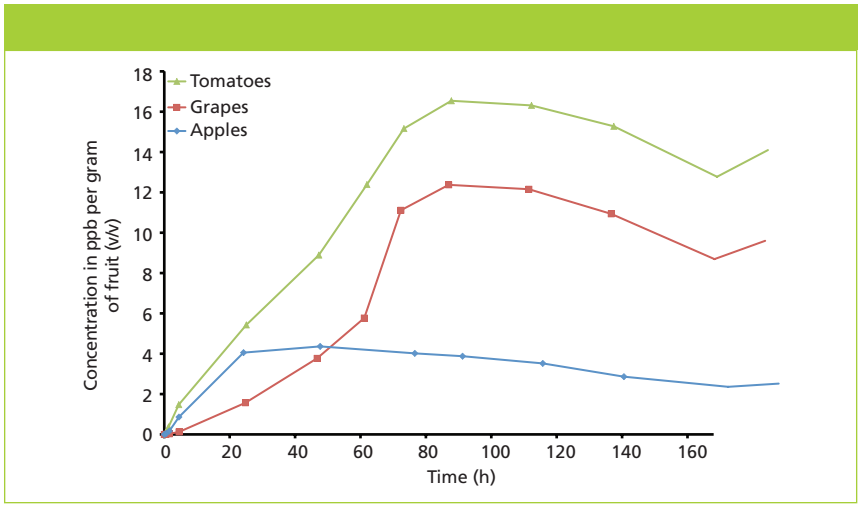

Figure 4: A plot of the amount of ethylene generated by various matrices over the course of 168 h (seven days).

dard of ethylene in air was obtained from Praxair Canada Inc. The standard was serially diluted with an in-house dynamic diluter to obtain standards over a range of 10–1000 ppb (v/v) in air. Tomatoes and grapes were purchased from a local grocery store, and apples were organically grown in the residence of one of the authors. The vines and stems were not removed for the experiment and no bruised or damaged fruit was used.

A 1-L (nominal volume) polypropylene bottle (Nalgene, Thermo Fisher Scientific) was used as a sampling vessel (Figure 2) to characterize the ripening of fruit. The cap of the bottle was drilled out with a 4-mm titanium drill bit and a 1/16-in. bulkhead Swagelok union (Edmonton Valve and Fitting) was installed. The bulkhead union was sealed to the surface of the polypropylene cap by compression force using two 3-mm-thick PTFE-lined silicone septa. The silicone sides of the septa were pressed against the cap to provide a leak-free seal. The threads of the bottle were wrapped with PTFE tape with a minimum of five layers to prevent the potential diffusion of ethylene around the cap. The vessel was pressure tested and determined to be leak-free in up to 6 psig of air. To verify the vessel was leak-free, it was submerged in a water bath. The end of the 1/16-in. bulkhead union was fitted with a 1/16-in. to 1/4-in. adapter and terminated with a 1/4-in. nut that holds a 9-mm Supelco LB-2 septum. For direct needle insertion into the vessel, an in-house 1/16-in. union was made from a 16-gauge disposable needle tip (Thermo Fisher Scientific). This thin-walled larger internal diameter tip was required for the sampling needle to enter the vessel. A 50-mL B&D Yale hermetically sealed syringe

(Thermo Fisher Scientific) was used with a 6-in., 20-gauge, stainless steel needle from Sigma-Aldrich to access the vessel at a consistent depth. The sealed vessels were pressurized with an additional 500 mL of ambient air (approximately 6 psig). Six sampling vessels were built for the study. Air samples of 10–20 mL were obtained from the headspace above the fruit. The contents of the syringe were used to purge the sampling loop, and a 1-mL aliquot was injected into the analytical system.

For the characterization of entrenched ethylene in industrial solvents, the industrial solvents were obtained on site. The samples were prepared by total evaporative technique using 20 µL of solvent in a 250-mL serum vial and heating them to 80 °C.

Results and Discussion

The performance of the analytical system was optimized for best separation with the shortest time possible to enhance sample throughput. We determined 130 °C as the optimal temperature for the GC oven and used this temperature for all subsequent experiments. Under these conditions, ethylene is eluted at 84 s and total analysis time is 4 min. Figure 3 shows an overlay of chromatograms ranging from 10 to 1000 ppb (v/v) of ethylene in air under the conditions described. Six moles of mercury vapor are generated for each mole of ethylene, leading to the high sensitivity and selectivity of the detector.

Determination of Ethylene in the Fruit-Ripening Process

A study was conducted with climacteric fruits such as apples and tomatoes, which continue to ripen after being harvested. Grapes, a nonclimacteric fruit that only ripens while still attached to the plant, were also used in this experiment (18). The emission of ethylene from these matrices can be monitored using the simple sampling device. The frequency of sampling and the amount of sampling have a direct impact on the duration of the study. In general, if the vessel is pressurized to 6 psig, with the removal of approximately 10–20 mL/day, there is a sufficient volume to satisfy a sampling period of seven days.

Figure 4 shows a plot of the amount of ethylene generated per gram of fruit by various matrices over the course of 168 h. Over this seven day period, tomatoes generated the most ethylene per day, increasing to a maximum concentration of approximately 16.5 ppb (v/v) per gram. Apples reached a maximum level of ethylene production at 8 ppb (v/v) per gram of fruit at about 48 h and then leveled off for the remainder of the study. Grapes had a steady increase in ethylene for the duration of the study. The rate of ethylene generation of the tomatoes and grapes increased significantly after approximately 50 h, with a substantial amount of mold visible on the tomatoes and grapes. The rate of increase of ethylene might have been caused by a mold such as *Passalora fulva* that can accelerate the decomposition process on tomatoes or other uncontrolled factors like bacteria growth such as *Xanthomonas ampelina,* which is common in grapes. No forensic work was conducted to ascertain the type of mold observed. In contrast, mold was not visually observed

on the apples. Regardless, our results show that the analytical procedure developed with the novel sampling vessel described can be an effective means of monitoring the fruit ripening process by measuring the net emission of ethylene; that is, the difference in the rate of emission and the rate of absorption of the matrix in a sealed container.

Determination of Ethylene in Air

Ethylene in ambient air can be studied very accurately with the procedure described. This finding is consistent with results that have been reported in the literature (19,20). As a demonstration of the capability of the analytical system, two sources of air (Shanghai, China, and Folsom, California) were obtained from inflatable cushioning packages that are commonly used to protect fragile items during shipment, and ambient air in Edmonton, Alberta, Canada, was collected. It was found that the concentration of ethylene in these locations was 63, 10, and <10 ppb (v/v), respectively. Figure 5 shows an overlay of the results obtained. It should be noted that the level of ethylene in ambient air varies from day to day depending on industrial activities, wind velocity, and temperature. Additionally, ethylene has a half-life of 2.2 days.

Determination of Ethylene in Industrial Solvent

Ethylene is soluble in a variety of polar and nonpolar process solvents at atmospheric temperatures and pressures. The presence of ethylene in industrial solvents can have a negative impact on the flammability of chemical processes, particularly in chemical and petrochemical operations (21–23). Also, the recovery of ethylene from a chemical process can improve economic profitability since ethylene is a feedstock in many chemical processes. Figure 6 shows a plot of entrenched ethylene in commercially available industrial solvents. The concentration of ethylene detected ranged from 13 to 330 ppb (v/v), which demonstrates the high degree of selectivity and sensitivity of the analytical technique.

Conclusions

We successfully developed and implemented a GC approach using short packed columns and RGD for the characterization of ethylene in a variety of matrices, notably in tracking the presence of ethylene in the fruit-ripening process, in ambient air, and in industrial solvents. Ultratrace detection of ethylene in various matrices was conducted without sample preconcentration. The approach has a high degree of reliability and is easy to maintain and operate when compared to other methods for trace ethylene analysis. This technique has the potential for use in other volatile gas applications where the analytes of interest can be reduced with mercuric oxide.

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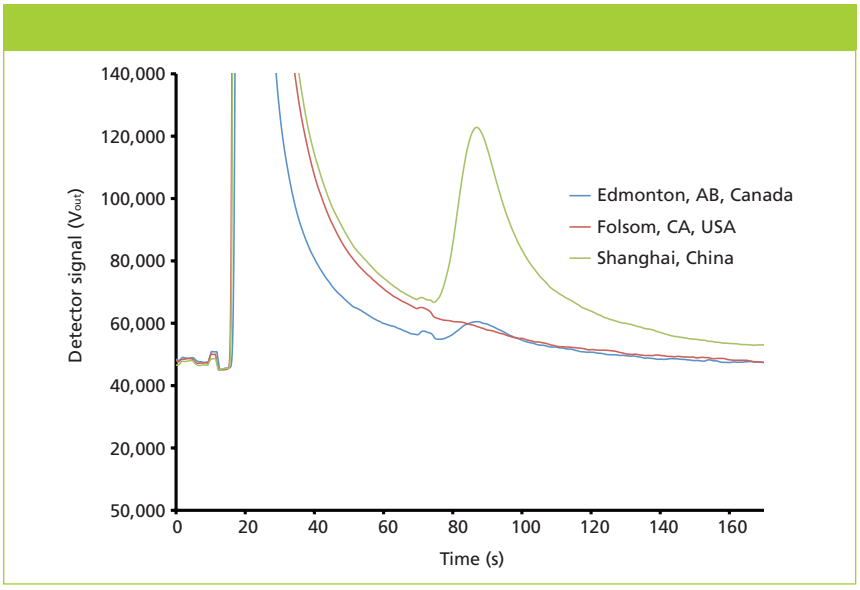

Figure 5: Overlay of chromatograms of ethylene in air from three different locations.

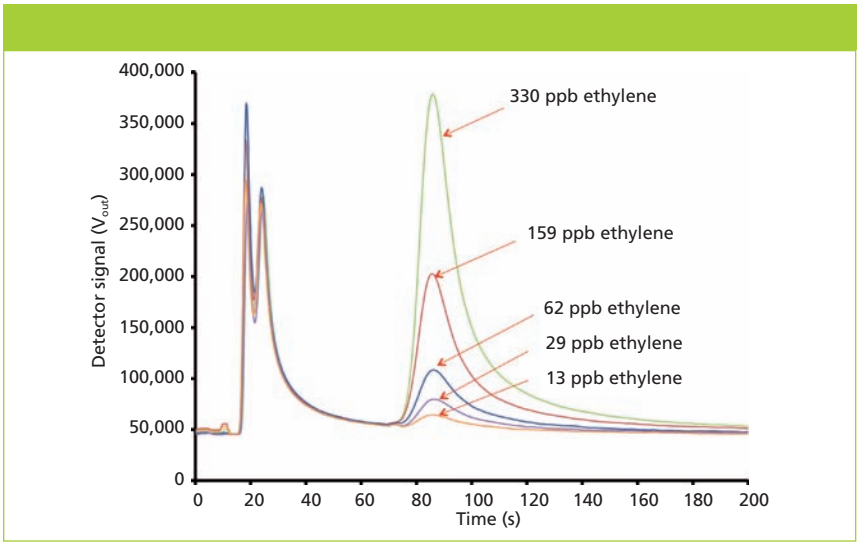

Figure 6: Overlay of chromatograms of entrenched ethylene in commercially available industrial solvents.

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References

(1) X.L. Cao, C. Hewitt, and K. Waterhouse, *J. Chromatogr. A* **679,** 115 (1994).

(2) X.L. Cao, C. Hewitt, and K. Waterhouse, *Anal. Chim. Acta* **300,** 193 (1995).

(3) M. Succi and J. Moncur, *Process Anal. Chem.* **57,** 2287 (1991).

(4) C. Ostrander, US patent 4,411,867, October 25, 1983.

(5) C. Ostrander, D. Oharra, C. McDowell, and S. Hartmann, US patent 6,368,560, April 9, 2002.

(6) M. Doizaki and M. Levitt, *J. Chromatogr. A* **285,** 210 (1984).

(7) T. Kawano, N. Tsuboi, H. Tsujii, T. Sugiyama, Y. Asakura, and T. Uda, *J. Chromatogr. A* **1023,** 123 (2004).

(8) T. Kawano, N. Tsuboi, H. Tsujii, and T. Uda, *J. Chromatogr. A* **1157,** 408 (2007).

(9) B. R. Glick, *FEMS Microbiol. Lett.* **251,** 1 (2005).

(10) Alberta Environment, "Assessment Report on Ethylene for Developing Ambient Air Quality Objectives" (2002).

(11) D. O'Hara and H. Singh, *Atmosph. Environ.* **22,** 2613 (1985).

(12) X.L. Cao and C. Hewitt, *J. Chromatogr. A* **648,** 191 (1993).

(13) F. Abeles, *Ethylene in Plant Biology* (Academic Press, New York, 1973).

(14) M. Saltveit and S. Yang, *Principles and Practice of Plant Hormone Analysis, Vol. 2* (Academic Press, New York, 1987), pp. 367–401.

(15) R. Young, H. Pratt, and J. Biale, *Anal. Chem.* **24,** 551 (1952).

(16) E. Butrym and T. Hartman, "Determination of Ethylene by Adsorbent Trapping and Thermal Desorption — Gas Chromatography," Application note 65, Scientific Instrument Service (1999).

(17) B.E. Taylor, G.M. Silver, and R. Fall, *Atmos. Environ.* **27A,** 2689 (1993).

(18) C. Chervin, A. El-Keramy, J.P. Roustan, A. Latche, J. Lamon, and M. Bouzayen, *Plant Sci.* **167,** 1301 (2004).

(19) F.B. Abeles and H.E. Heggestad, *J. Air Waste Manage. Assoc.* **23,** 517 (1973).

(20) M.S. Aziz and A.J. Orr-Ewing, *J Environ. Monit.* **12,** 3094 (2012).

(21) H. Lin and B.D. Freeman, *J. Membr. Sci.* **239,** 105 (2004).

(22) A. Sahgal, H.M. La, and W. Hayduk, *Can. J. Chem. Eng.* **56,** 354 (1978).

(23) Y. Sato, N. Hosaka, H. Inomata, and K. Kanaka, *Fluid Phase Equilib.* **344,** 112 (2013).

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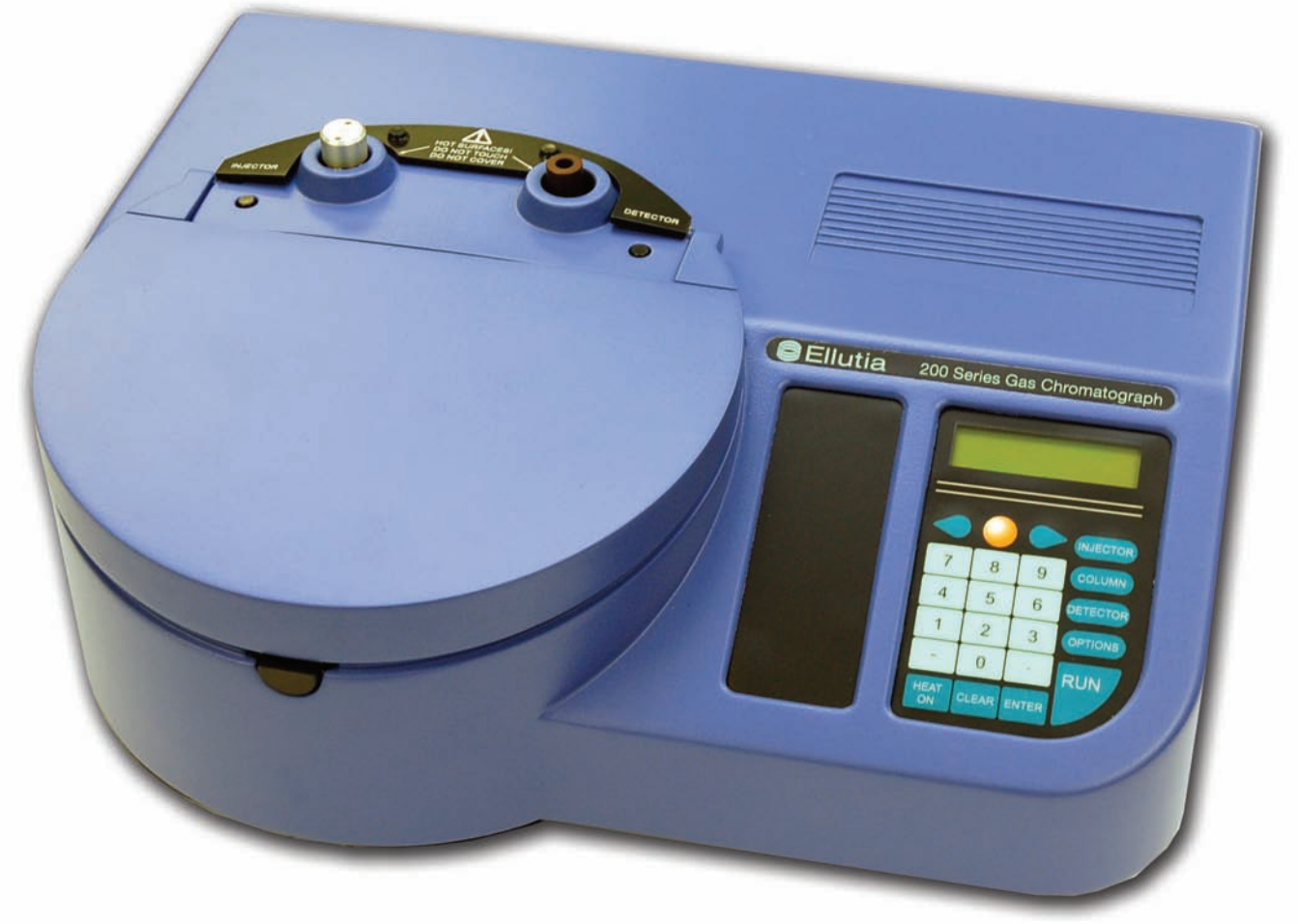

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Joseph Jack Kirkland: *LCGC*'s 2015 Lifetime Achievement Award Winner

Joseph Jack Kirkland, considered by many to be one of fathers of high performance liquid chromatography (HPLC), is the winner of *LCGC*'s 2015 Lifetime Achievement Award. Kirkland's vast contributions to the field include the development of the first spherical packing designed specifically for modern HPLC, the development of siloxane bonded phases, and establishing processes for manufacturing spherical, small-particle (5-µm) totally porous packings that introduced a major leap forward in the performance of HPLC columns. Kirkland recently spoke to *LCGC* about his career and work.

Were you always interested in a scientific career?

Kirkland: Having been gifted a Gilbert chemistry set at the age of 10, I quickly developed a strong interest in science and chemistry in particular. My subsequent A.B., M.S., and PhD degrees focused on chemistry in general and analytical chemistry in particular. I was always interested in the quantitative aspects of chemistry and the techniques of solving problems.

How did you get started in chromatography?

Kirkland: My dissertation studies required that I conduct some simple classical chromatographic separations, but my real interest in chromatography occurred in 1955 at DuPont. I had a problem in analyzing mixtures of methylamines, but after several weeks of using classical methods and infrared spectroscopy my work was largely unsuccessful. Then I learned that there was a chemist in another DuPont department, Dr. Steven Dal Nogare, who was conducting research on a new separations method called gas chromatography (GC). When I contacted him, it was suggested that I give him some samples of interest, which I did. The next day he called and said that my problem was solved. That result obviously got my attention, so I immediately arranged to get a duplicate simple, DuPont-constructed GC instrument, through Steve's help. My career with GC then began.

Who was the biggest influence on your career when you were just getting started?

Kirkland: My analytical chemistry professor and subsequent close friend at Emory University, R.A. Day, was strongly influential in my approaching a career in analytical chemistry. To this day, I remember his first lecture, which was focused on measurement precision and accuracy, with an emphasis on significant figures. At DuPont I was fortunate to be hired by the department manager, Dr. Ralph K. Iler, an internationally renowned silica and colloid chemist. It was even more fortunate that our laboratories were adjacent, as Ralph was still conducting research in addition to managing. As a result, some of his incredible knowledge rubbed off on me strictly by association. Dr. Iler was largely the catalyst for my interest in silica chemistry and its utility in gas and liquid chromatography.

Why did you decide on a career as an industrial scientist rather than an academic?

Kirkland: I have always thought of myself as a problem solver, which is more suited for industry needs. I really never seriously even considered teaching until Dr. Lloyd R. Snyder and I agreed to work on an educational program in HPLC for the Continuing Education Department of the American Chemical Society (ACS) in 1971. That teaching project lasted for 21 years and drew in more than 5000 attendees who were interested in learning about HPLC.

Despite being an industrial scientist, you still contributed to the education of a lot of scientists through your many books, scientific publications, lectures, and short courses. How did you get involved in those projects, and why did you think it was important to participate?

Kirkland: The ACS courses that Dr. Snyder and I taught were extremely important in the development of our skills in HPLC. Attendees not only asked questions for which we had to find answers, but we also learned what was important to those using HPLC to solve real problems. The input that came from these courses really gave us the background for books on HPLC that we coauthored over many years. My scientific publications partially resulted in my interest in "spreading the word" about HPLC, but some of the publications contained technical information about new products or techniques that were designed to increase the knowledge and utility of the HPLC method. I suspect that all of this indicates that the science relating to chromatography was not only important for my career, but that it is also a strong and rewarding hobby.

You are hailed as a pioneer of modern liquid chromatography. What was the first major breakthrough you had with LC? How did that impact the rest of your career?

Kirkland: My first major breakthrough in HPLC was the development of the 30-µm silica superficially porous particle (SPP) in about 1967 (which DuPont commercialized and called Zipax). Before commercialization,

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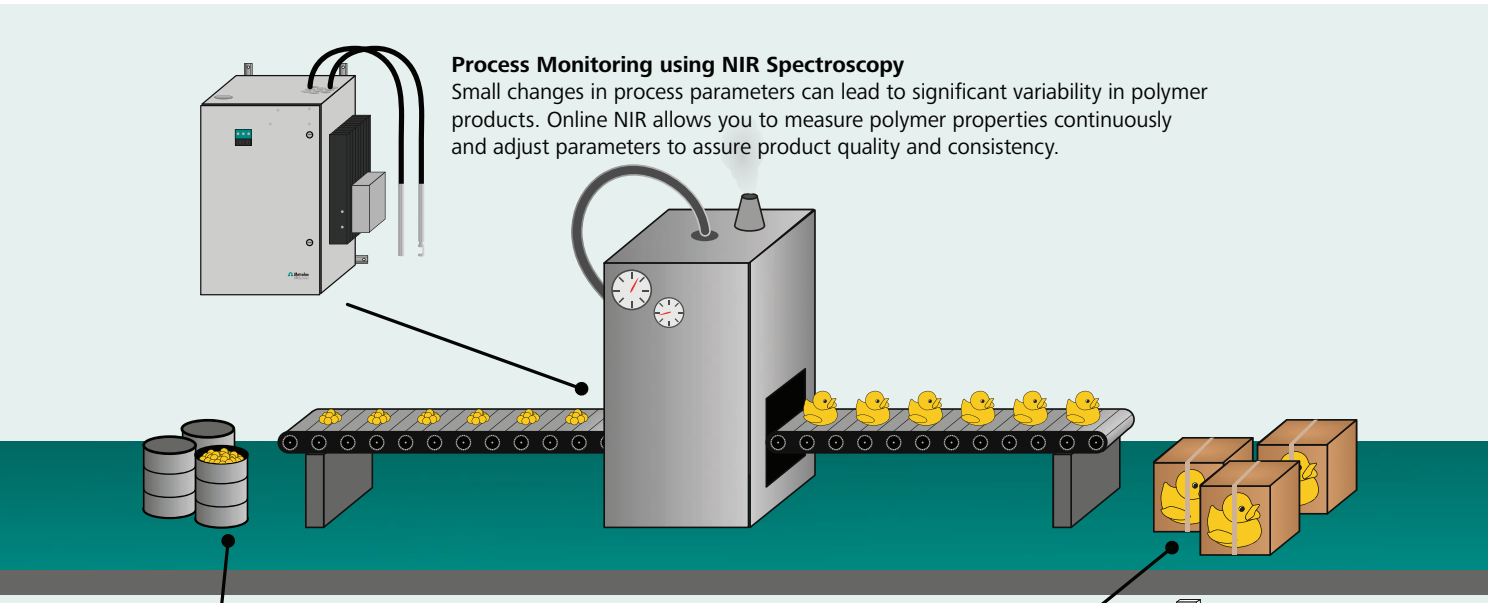

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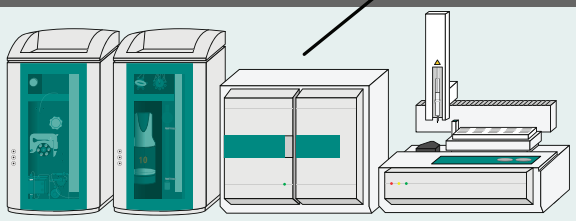

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we were effectively using this material in the Agricultural Chemicals Department for analyzing pesticides by HPLC. The commercial and technical success of Zipax enhanced my interest in SPP, and I continued to research SPP technology throughout my career. My development of small (6-µm) totally porous silica microspheres occurred in about 1972 (DuPont called the commercial particles Zorbax totally porous microspheres). The availability of these small particles ended the commercial aspects of SPP like Zipax. Subsequently, I developed high-purity porous silica microspheres, called Zorbax Rx, that reinforced the utility and value of this approach. It was more than 20 years before SPP technology reemerged commercially when I developed the 5-µm Poroshell particles for peptide and protein separations. However, the real boost in SPP came in 2006. While I was directing research at Advanced Materials Technology (AMT), we introduced small (2.7-µm) SPPs for the very fast separation of small molecules. This technology was strongly embraced by HPLC users. Other manufacturers quickly cloned and extended this technology so that it now is widely used globally. Since that time, AMT has developed and commercialized very small wide-pore SPPs optimized for separating peptides and proteins.

What did you first think about HPLC when it was introduced in the 1960s? What do you think of HPLC and ultra-high-pressure liquid chromatography (UHPLC) today?

Kirkland: I first learned about what we now call HPLC when visiting Eindhoven Technical University in 1964. There I found Dr. J.F.K. Huber doing some of the first experiments in what we now call HPLC. This demonstration galvanized me to return home and gain the support of my manager to conduct research on this new technique. The reason was that my department had many difficult analysis problems with nonvolatile compounds that could not be solved by GC, so liquid chromatography seemed to be the answer. This certainly proved to be the case for us, and HPLC and the higher pressure UHPLC now are widely used as major analysis tools throughout the scientific community.

Can you tell us about the development of the first spherical packing designed specifically for HPLC (Zipax)?

Kirkland: When I began research on HPLC in 1965, I recognized that two problems that existed at that time needed some help to make the method sufficiently powerful and practical: a sensitive and reliable detector and better column packing materials. The former problem was relatively easy to resolve, as I adapted a recently developed, highly sensitive, and stable DuPont UV process monitor with a low-volume flow cell to produce a very useful UV detector. The second problem was more difficult. At that time, 100–125 µm irregular silica particles normally used for GC were used in HPLC columns. Papers by Professor Calvin Giddings and others had predicted that smaller particles would be required to produce better separations, so I focused on smaller particles. I had previously used Ralph Iler's particle multilayering method to prepare some GC packing materials, so it occurred to me that this method might be used to develop spherical particles for HPLC. I decided to use sized silica glass beads as a core starting material. Silica has a negative charge, so a positively charged polymer was selected as the layering agent to be held by ionic forces. After layering this polymer, the coated beads were then positively charged, so that a single layer of 200-nm negatively charged silica sol particles could then be added to the beads held by ionic forces. The polymer-silica sol treatment was continued four more times, so that a 1-µm-thick layer was produced. The organic polymer was removed by heating and the resultant beads sintered at high temperature to produce the required strength. Considerable effort was needed for the sintering step to gain the desired strength without collapsing the porous shell. The final particles were about 30 µm overall with a 1-µm-thick porous shell composed of 1000-Å pores. Development of these particles required about three months (part-time, as I was also working on other analytical problems). These SPPs were used for liquid–liquid chromatography by filling the pores with stationary phase liquid. DuPont commercialized these patented particles as Zipax column packing. Zipax later was bonded with a nonsoluble silicone, which eliminated some of the disadvantages of the liquid–liquid method, and allowed reversed-phase technology to be developed with gradient separations. DuPont called this Zipax-modified commercial material Permaphase column packing.

Can you tell us about the development of the spherical small-particle totally porous packings (Zorbax)?

Kirkland: The development of the previously mentioned totally porous silica microsphere particles for HPLC, Zorbax, again occurred as an extension of one of Ralph Iler's projects. Dr. Iler found that by coacervating various inorganic sols with a urea–formaldehyde polymer, small spherical particles resulted. The polymer was removed by heating, leaving porous microspheres. I used this technology as a basis and found that by varying the size of silica sols, porous silica microspheres with definable pores and narrow pore size distributions could be made. By altering synthesis conditions, silica particles of a particular size also could be made, so I concentrated on 5–6 µm particles, which I believed would be useful for performing fast HPLC separations. A main problem in this project was how to load these small particles into efficient columns for separations. It was found that the then-used dry method of packing larger Zipax particles produced poor results. A wet particle slurry method previously reported for preparing columns of large polymeric ion-exchange particles was studied and adapted to produce efficient columns of the porous silica microspheres. In my 1972 ACS Chromatography Award address, I described the utility of these particles, which at that time were used for liquid–liquid and liquid–solid (adsorption) chromatography. The use of these particles for reversed-phase HPLC was quickly developed largely by chemists in DuPont's HPLC Application Laboratory. Later, it was found that silanes could be bonded to the surface of these silica particles to create appropriate stable and useful stationary phases. Studies continued so that porous silica microspheres with different pore sizes were synthesized for columns to perform high-performance size-exclusion chromatographic separations for synthetic polymers of DuPont's interest. These studies on synthetic polymers were conducted with my coworker and friend, Dr. Wallace W. Yau. The research on the porous microspheres came over a two-year period that was interrupted by my transfer from the Agricultural Chemicals Department to DuPont's Central Research Department in 1972.

Of the 32 U.S. patents you hold, which ones do you think have had the greatest impact in your field?

Kirkland: Patent-wise, my work on Zorbax and Zorbax Rx particles probably had the most total impact on HPLC

utility, as these materials continue to be widely used globally.

What led you to cofound Rockland Technologies? What did you learn from that experience and what advice would you offer a scientist trying to start a new company?

Kirkland: I was still working in Central Research in the late 1980s when DuPont decided to abandon the HPLC column business. DuPont contacted my friend and ex-coworker, Dr. Joseph DeStefano, (now in Central Research), who previously managed this technology to see if there was interest in a leveraged takeover. There was strong interest by DuPont in continuing to supply customers that were using DuPont HPLC columns. Dr. DeStefano contacted me and three other ex-DuPonters with HPLC technical and business experience. As a result, an agreement was made with DuPont to take over the column business. Dr. DeStefano then retired from DuPont, but I continued my DuPont position. However, DuPont allowed me to conduct research activities for the new company, Rockland Technologies, on my own time as a consultant. I retired from DuPont in 1992 and then participated full-time as research director for Rockland Technologies. My involvement with Rockland exposed me to the business community, of which I had limited previous experience. Decisions for research were then often based on commercial possibilities, and activities were more focused on user needs and desires. Starting a new company like Rockland Technologies requires a strong technical background in product focus, a decent business and marketing experience, a willingness to take a chance for an opportunity, and hard work.

Your have an impressive body of publications. How important is it to you to share your work with the scientific community? Has anything you published led to unexpected collaborations?

Kirkland: Sharing my work with the scientific community has been a two-way street. This effort has led to many important discussions and sharing of ideas, and in the process has resulted in my getting acquainted with some top-flight scientists and some very nice people. An unexpected collaboration resulted in interactions with DuPont biochemists in a project on arthritis (one of my personal problems). We studied the progressive ageing-deterioration of proteoglycans in bovine joints using sedimentation field flow fractionation equipment that I had developed for characterizing microparticles and very high molecular weight components. Another unexpected highly successful collaboration was developed with Dr. Henk Claessens at Eindhoven Technical University in developing technology and insights regarding the use of silica-based column packing for high-pH HPLC separations. High-pH operation with silica-based columns was not popular at that time because of the dissolution of silica support during use. We were able to develop a stationary phase and operating conditions that allowed useful and practical separations at high pH.

What chromatography problem would you most like to see solved in the next 5–10 years? Do you have any plans to solve it yourself?

Kirkland: The next 5–10 years for HPLC technology will be focused on high-performance separations in the biosciences. Rapid, efficient, and effective separations will be required for a wide variety of biomaterials for which there is now a lack of experience. I am currently working in this area, but my years are limited as the calendar moves on. It is likely that field flow fractionation methods will be required to solve some of the difficult problems involving very large biomolecular components and particulates, as HPLC is much less effective for separations of these materials.

What advice would you offer a scientist just starting out?

Kirkland: Effective research needs a good formal educational background to get started. However, to continue to perform effective research through the years, real interest in science must be continuously stimulated. To maintain skills, literature must be assimilated, technical meetings attended, and networks with other scientists developed. In fact, science must also be sort of a hobby, where new technology is always promoted.

This interview has been edited for length and clarity. For more interviews like this please visit chromatographyonline.com ■

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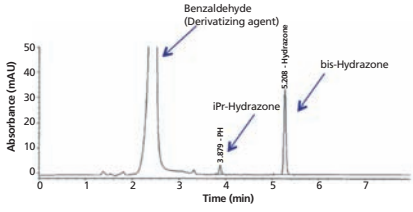
Analytical Technologies for Genotoxic Impurities in Pharmaceutical Compounds



Genotoxic impurities (GTIs) have gained considerable attention from health authorities as well as from the pharmaceutical industry in recent years. Analysis and control of these impurities in pharmaceutical compounds pose a significant challenge, often requiring selective, sensitive, and robust trace-level methods for analysis. This article focuses on the method development strategy and associated technologies for the analysis of GTIs. GTIs typically have a wide range of physicochemical properties such as volatility, stability, presence of UV chromophores, ionizable groups, and derivatizable functional groups. Various separation and detection technologies are available and can be used to identify and analyze. Choosing the appropriate analytical technique depends on the GTI physicochemical properties, required sensitivity, and the consideration of the matrix interference.

Archana Kumar, Kelly Zhang, and Larry Wigman

Genotoxic impurities (GTIs) belong to a class of compounds that interact with DNA and induce genetic mutations. These compounds add significant risk without adding any benefit to patients and are also known as mutagenic impurities. Therefore, exposure to even low levels of such impurities may be of significant toxicological concern.

Genotoxic assessment is required throughout synthetic route development and stability duration for the presence of any GTI alerts. Demonstrating that GTIs are controlled to safe levels is of the utmost importance for patient safety (1). In recent years GTIs in pharmaceuticals have become a topic of increasing concern, consequently several review papers have been published focusing on the regulatory and toxicology assessment (2–7), control strategy (7–9), and risk assessment (10). Effective analytical technologies for selective, sensitive, and robust trace-level detection methods are required for low-level quantitation. Physicochemical properties of the GTIs that need to be considered include volatility, stability, presence of UV chromophore, ionizable groups, and derivatizable functional groups.

Regulatory Overview

GTIs are required to be controlled at lower limits (11,12) compared to less toxic impurities controlled in new drug substances (13,14) and drug products (15). The concept of staged threshold of toxicology concern (TTC) was developed to allow higher limits for compounds in development based on the duration of exposure rather than lifetime exposure (16).

The International Conference on Harmonization's (ICH) M7 document "Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to limit Potential Carcinogenic Risk" provides recommendations for toxicology assessment, identification, categorization, and control of actual and potential mutagenic impurities that are likely to arise during the manufacturing and long-term storage of a new drug substance and drug product (17).

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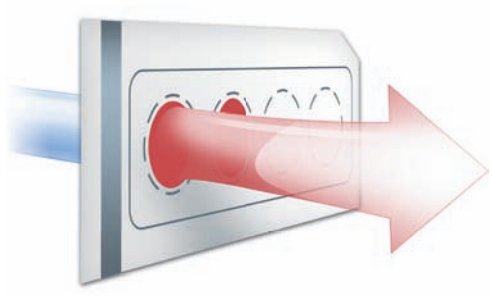

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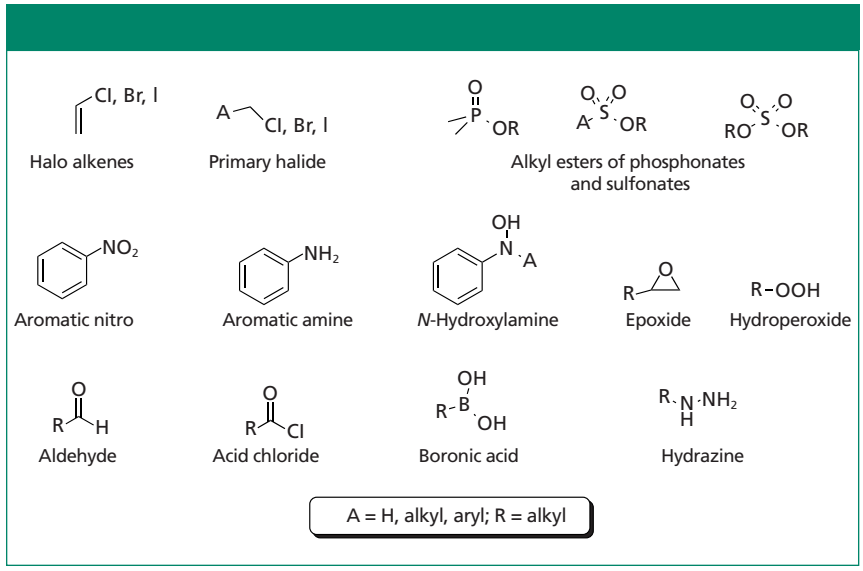

Figure 1: Structures of commonly observed GTIs.

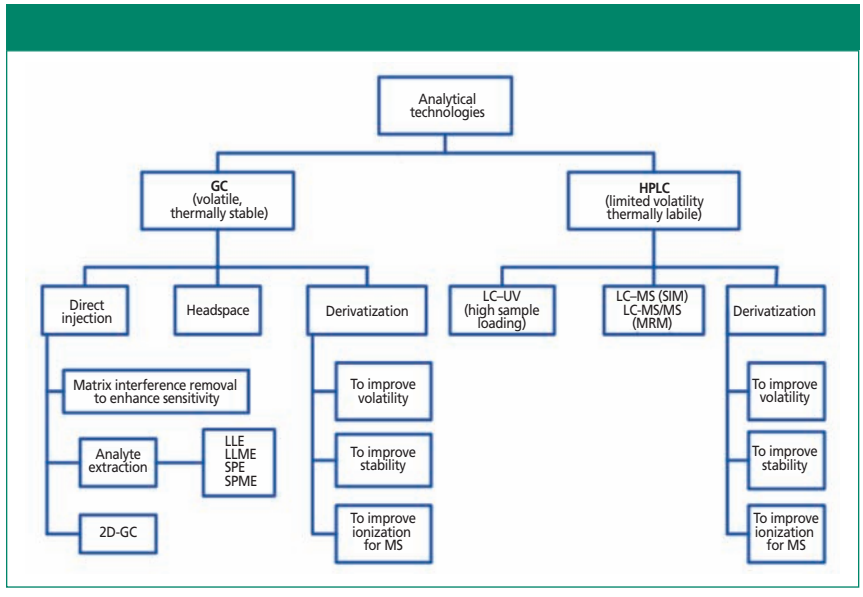

Figure 2: Analytical method development strategy.

Compound-specific calculations for acceptable intakes may be applied where sufficient carcinogenicity data are available or if the impurity is similar to a known carcinogen compound class. Performing spiking and purge studies during the development stage and justifying the presence at higher limits for the impurities may not only mitigate routine analysis at trace levels, but also may mitigate the need to specify control levels in drug substance specifications.

ICH S9 document guidance supports the concept that higher limits may be appropriate for a drug candidate or commercial product targeted for advanced cancer with life-threatening malignancies (18).

Structures of Commonly Encountered GTIs

Structures of some of the impurities commonly observed in pharmaceutical synthetic routes that get structural alerts are shown in Figure 1. These functional groups are generally linked to genotoxicity and are identified based on the chemistry and structure–activity relationship (16). Boronic acids were recently recognized as mutagens, but do not have enough carcinogenicity data. Galloway and colleagues (19) compared the data for 361 various chemicals used in the synthetic route that had structural alerts and were tested for Ames. They reported that 80% of aromatic nitro and boronic acid derivatives had positive Ames results, which was the highest among the selected samples, and 50–60% of alkylating agents, acid chloride derivatives and hydrazines, were positive.

Analytical Method Development Strategy for GTI Analysis

GTIs were controlled at a TTC of 1.5 µg/day at commercial stage. For a 1-g daily dose of the marketed product, a GTI would require control at a level of 1.5 ppm ([1.5 µg/day]/[1 g/day] = 1.5 ppm), which is several hundred times lower than the 0.05% (500 ppm) control level per the ICH Q3 guidance for traditional pharmaceutical impurities analysis. Further, in case of the presence of three or more mutagenic impurities in the drug substance specification, total mutagenic impurities should be limited to 5 µg/day for clinical development and marketed products. Considering such a low level, robust and sensitive analytical methods are a critical element of the control and analysis of GTIs. Before developing a method, it is important to understand a few factors:

- the purpose of testing — whether the method will be implemented for API release testing or used during development (spiking and purge studies);
- the need for a limit test or quantitative method — the requirements for method validation vary;
- the physicochemical properties of the analyte;
- and the end user of the method — it can be a development laboratory, a commercial user, or a manufacturing laboratory.

With frequent changes in dose and duration during the clinical trial stage, it is often necessary to collect quantitative data at the development stage. Quantitative data collection requires a method with lower detection limits than the analytical specifications limit. After the process understanding is gained and lower levels are attained for each impurity compared to the control level, a limit test can be used rather than a quantitative test. Based on physicochemical properties of the analyte, gas chromatography (GC) or high performance liquid chromatography (HPLC), two of the most commonly used analytical techniques, may be used (Figure 2).

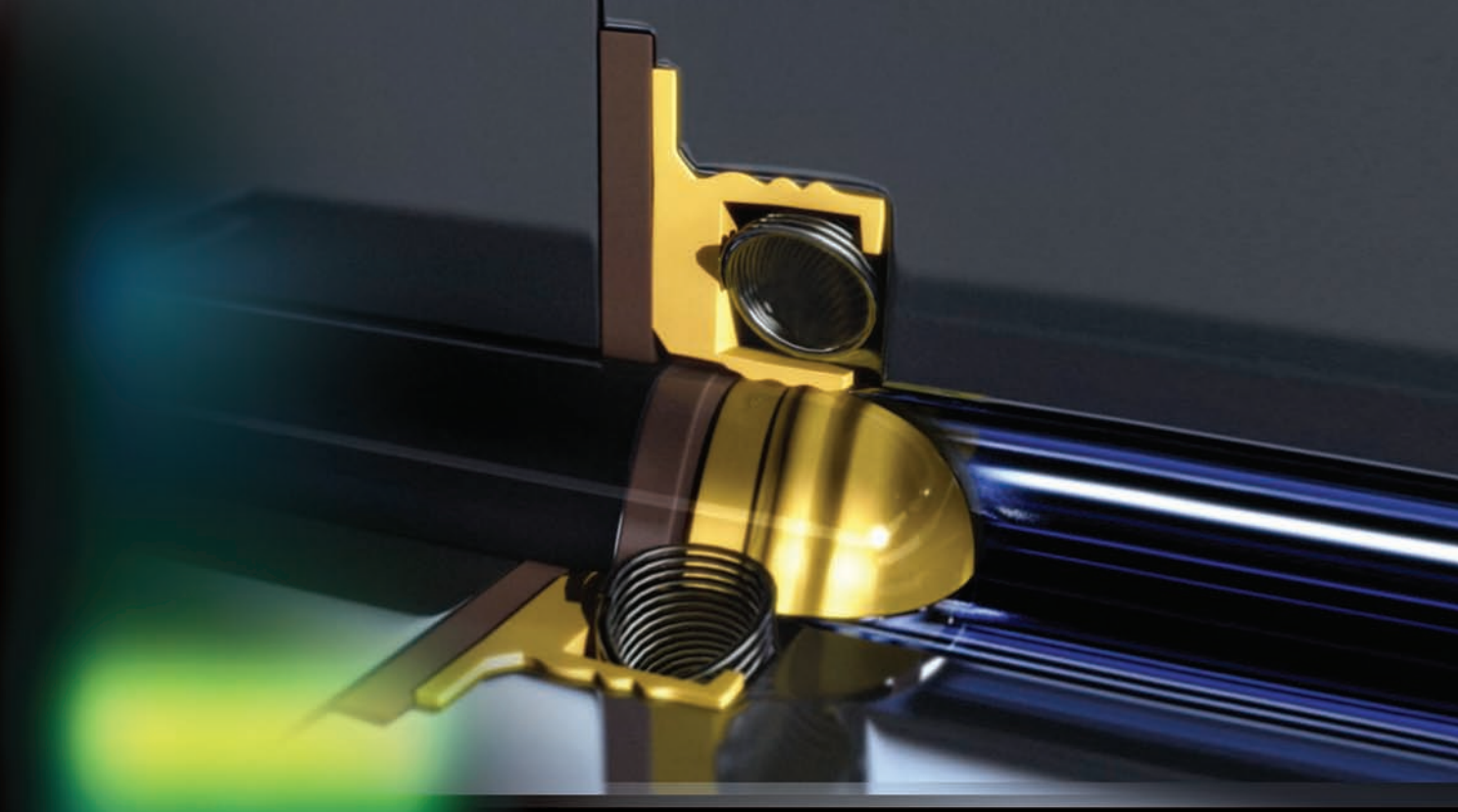

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Table I: Literature references for sensitivity enhancement by removal of matrix interference

Sample Preparation Technique	Detection	Analyte	DL (QL)	Reference	Year
Headspace GC	FID	Alkyl chlorides	0.8 (13.5) ppm	Kaleemullah (23)	2011
Matrix deactivation	MS	Various	<1 ppm	Sun (24)	2010
Solid-phase microextraction	MS (SIM)	Sulfonates	5 ppm	Colon (25)	2005
LLE direct injection (deuterated internal standard)	MS (SIM)	DMS	0.3 (1) ppm	Zheng (26)	2009
LLE direct injection	MS	Mesylates, besylates	<0.1 ppm	Wollein (27)	2012
SAX-SPE direct injection	MS (SIM)	2-Chloroethanol	1.7 ppm	Garcia (18)	2012
2D-GC	MS	Multiple	<1 ppm	Frank (28)	2010

Table II: Literature references for application of derivatization followed by GC for GTI analysis

Derivatizing Agent	Detector	Analyte	DL (QL)	Reference	Year
Methanol	MS	Benzoyl chloride	0.2 ppm	Raman (32)	2014
Ethanol	MS	Formaldehyde	3 ppm	Raman (32)	2014
BSTFA	MS	4-Chloro-1-butanol	0.05 (0.08) ppm	Harigaya (33)	2014
Sodium thiocyanate	FID MS (EI)	Mesylates, DMS	5–10 ppm ≤0.05 ppm	Lee (34)	2003
PFTP	MS (SIM)	Sulfonate esters	<1 ppm	Alzaga (35)	2007
PFTP	MS	Ethyl mesylate	<0.5 (1) µg/mL	Jacq (36)	2008
Benzaldehyde	NPD	Hydrazine	<1 ppm	Gyllenhaal (37)	1990
Acetone	MS	Hydrazine	0.1 ppm	Sun (38)	2009
Benzaldehyde	ECD	Hydrazine	<1 ppm	Carlin (39)	1998
Acetylation	NPD	Aziridine	0.2 ppm	de Haan (40)	1989
Acetylation	ECD	2-Chloroethylamine	0.5 ppm	de Haan (40)	1989
Acetylation	ECD	Chlorophenols	6–122 ng/L	Morais (41)	2011

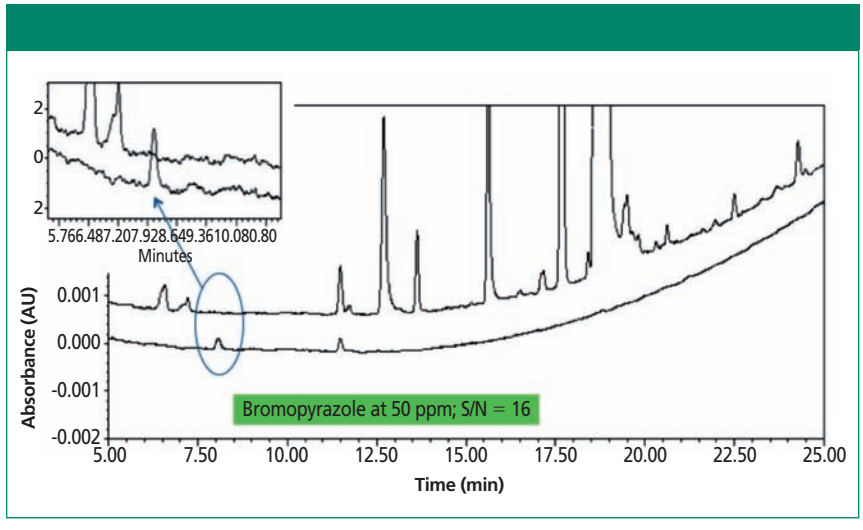


Figure 3: Overlay HPLC–UV chromatogram of bromopyrazole spiked in a drug substance. Column: 150 mm × 4.6 mm, 3.5-µm Zorbax Eclipse XDB-C18 Rapid Resolution.

Analysis of Genotoxic Impurities by GC

GC is a preferred separation technique for the analysis of volatile analytes. Direct liquid injection and headspace are two commonly used injection modes for GC analysis. The specification limit calculated based on the daily dose is a key factor in determining the desired sensitivity of the method and subsequently the selection of the detector.

GTI Analysis Using Direct Injection

GC analysis using direct liquid injection is the primary choice for analysis of volatile and thermally stable analytes such as alkyl mesylates that lack the sufficient vapor pressure required for the headspace technique (20). The dissolve-and-inject approach significantly simplifies the sample preparation before analysis. Flame-ionization detection (FID) is preferred because of its easy availability and versatility. FID may be used for higher control levels that are justified by low dosage drugs; however, sensitivity is somewhat limited (21). The high sample loading technique may be applied to obtain the desired sensitivity (17).

A hyphenated GC–mass spectrometry (MS) technique in selected ion monitoring (SIM) mode provides better sensitivity and selectivity for impurities analysis at sub-part-per-million levels. The most prominent fragment ion is selected to obtain the desired sensitivity and resolution. The GC–MS method using SIM and repetitive scanning has been reported for the analysis of mesylates (20) and various alkyl halides (22). However, this method has a restriction of 1 µL for injection volume because of the accumulation of active pharmaceutical ingredients (APIs) and resulting matrix interferences, which additionally

require frequent changing of the inlet. Inlet contamination and resulting matrix interference are the primary limitations of the direct injection analysis method. Most drug substances are nonvolatile, and solutions might need to be prepared at higher concentrations to achieve sensitivity. This contamination may result in irreproducibility and low recovery problems during method validation and testing.

Enhancing Sensitivity by Removing Matrix Interference

The presence of excessive matrix interference may have a significantly adverse impact on method sensitivity. Using headspace GC for the analytes with sufficient vapor pressure may be a quick solution because most APIs are not volatile. However, for complex samples, the introduction of nonvolatile and reactive materials in the GC inlet may be mitigated by matrix deactivation, analyte extraction, or two dimensional (2D)-GC. These techniques may also be used to increase analyte stability, for selective extraction, and for enrichment of analytes. Table I summarizes the various technologies used for the removal of matrix interference and hence increasing the sensitivity.

Analyte Extraction

Extraction methods such as liquid–liquid extraction (LLE), liquid-phase microextraction (LPME), solid-phase extraction (SPE), and solid-phase microextraction (SPME) help enhance the analyte concentration and sensitivity, thereby reducing matrix interference, especially where the GTI and matrix have diverse chemical properties. Small neutral molecules, such as alkylating agents, have high solubility in organic solvents and may be extracted out from ionizable API matrix, which will have better solubility in water in an ionized form (14). The solvent *n*-hexane is the best solvent for liquid extraction since only sparse amounts of API or potential impurities are extracted from the matrix. *n*-Hexane does not impact the sensitivity or selectivity by ion depression being a nonpolar solvent (27). However, this procedure is laborious and may be prone to interferences because of tough-to-break emulsions, the use of organic solvents, and concentration steps. Additionally, an internal standard needs to be used to compensate for any loss of analyte during the extraction procedure. LPME offers the advantage of much improved concentration power as only microvolumes are used for extraction. SPME is a solvent-free extraction technique that involves the use of a coated fiber to extract various analytes that can be in liquid (direct injection) or gas (headspace) phase (25). The working pH range for the currently available fibers is pH 4–9. That being said, the adjustment of pH is a critical factor for the ionization and extraction of potential interferences. Polymeric ionic liquids (PILs) with variable chemical properties have also been evaluated as selective SPME sorbent coatings for the analysis of alkyl halides and aromatics. This allows quantitation of GTIs in ultratrace level (29). The ability to modify their chemical structures

to achieve different analyte solvation capabilities is an advantage. However, this approach is still in an early stage and further studies are underway to understand the effect of various PILs.

2D-GC

Compounds such as epoxides and Michael reaction acceptors do not have sufficient volatility for headspace. 2D-GC with a Dean switching valve may be used to divert the matrix to waste and the fraction containing desired GTIs to the detector. Using this approach, the API, solvent, and derivatization agents are not introduced on the second column and MS detector. Enhanced sensitivity is observed mainly because of the removal of background interference. Moreover, this technique also results in the complete separation of two analytes present in the same sample (28).

GTI Analysis Using Headspace GC

Headspace analysis minimizes the potential contamination of the injector, column, and detector. In headspace analysis, nonvolatile APIs do not partition into the headspace and thus do not get injected and enter the GC system (23). High boiling solvents such as dimethyl sulfoxide, *N*,*N*-dimethylacetamide, or *N*,*N*-dimethylformamide are commonly used for such analysis. Recently, the use of ionic liquids has also been reported for the analysis of high boiling analytes (30,31). Ionic liquids offer an advantage because they provide high thermal stability and low volatility without causing significant background noise at elevated headspace oven temperatures. This technique may be used for analytes that have sufficient vapor pressure to be injected using headspace GC and that have demonstrated stability at high temperatures.

Table III: Literature references for use of LC–MS for GTI analysis

Detector	Analyte	DL (QL)	Reference	Year
MS (SIM)	Besylates, tosylates	<0.1 ppm	Taylor (21)	2006
MS (SIM)	Nitro, chloro, and amino	0.2–0.6 ppm	Liu (44)	2009
MS/neg. mode (SIM)	Arylsulfonamine	(0.2) ppm	Liu (44)	2009
LC–MS (ESI)/IC	Alkyl halides	<1 ppm	Lee (45)	2000
MS-MS (MRM)	Methyl and ethyl mesylate	0.002 (0.01) µg/mL	Kakadiya (46)	2011
MS-MS (SIM)	2-Chloromethyl-3,4-dimethoxy pyridine	0.1 (0.3) ppm	Venugopal (47)	2012
LC–MS-MS-ESI (MRM)	4-chloro-1-hydroxy butane sulfonic acid	0.17 ppm	Narayana (12)	2012
MS-MS (MRM)	4-DMAP	0.03(0.1) ppm	Szekely (48)	2012
MS-MS (MRM)	Substituted hydrazines	0.02 (0.06) ppm	Reddy (49)	2014
MS (ESI), HILIC	Quaternary hydrazine derivatives (polar imp)	3 ppm	Hmelnickis (50)	2008

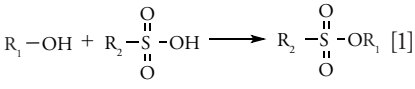
Table IV: Literature references for use of derivatization before analysis by LC for GTI analysis

Technique or Derivatization Agent	Detection	Analyte	DL (QL)	Reference	Year
2,4-DNPH	UV diode array	Formaldehyde	0.5 (1.5) ppm	Nageswari (53)	2012
2,4-DNPH	UV diode array	Formaldehyde	0.1 (0.33) ppm	Soman (54)	2008
Derivatization	GC, ion, and three different	Formaldehyde		Manius (55)	1993
2,4-DNP	MS	Aldehyde	0.1 ppm	Raman (32)	2014
Benzaldehyde	MS	Methyl hydrazine-carboxylate	1.2 ppm	Raman (32)	2014
Aniline	MS	Ethyl chloroformate	10 ppm	Raman (32)	2014
Triethylamine	MS (SIM)	DMS	(0.5) ppm	Liu (44)	2009
2-Mercaptopyridine	MS (SIM)	Chloroethyl chloroformate	5 ng/mL	Liu (8)	2010
DMA	ESI-MS	Epoxide	1 ng/mL	Liu (8)	2010
DMAP-SPE (weak cation exchange)	MS-MS (MRM)	4-Fluorobenzyl chloride	0.5 ng/mL	Yang (56)	2005
DMAP-HILIC	MS-MS	Alkyl halides	1 mg/Kg	Van Wijk (57)	2011
DMA	MS	Alkyl halides epoxide	1 ng/mL	Bai (58)	2010
TMA-TEA-HILIC	MS (SIM)	Alkyl sulfonates; dialkyl sulfates	1–2 ppm	An (59)	2008
Acetonitrile-gas phase Meerwein reaction	MS (SIM; MRM)	Epoxides	1 ppm	Wu (60,61)	2010, 2011
Salicylaldehyde, benzaldehyde-LLE	TLC	Hydrazine	1–20 ppm	Kean (11)	2006
2-OH-1-Naphthaldehyde		Hydrazine	20 ppb	Mañes (62)	1988

GTI Analysis Using Derivatization Followed by GC

Sample derivatization is an efficient way to alter the physicochemical properties of the analyte and increase the sample stability, volatility, and ionization efficiency needed for MS. Table II summarizes the application of the derivatization technique for various analytes, hence increasing the stability and sensitivity.

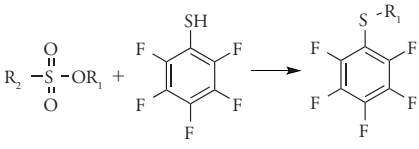
Sulfonate esters are known DNA reactive genotoxins and can be formed from the reaction of residual alcoholic solvents used in the manufacturing process and sulfonic acids (equation 1).



[1]

Sulfonate esters do not have sufficient vapor pressure to be analyzed using headspace GC. Analysis using direct injection is prone to artifacts because of ester hydrolysis, decomposition of API salts, and matrix interference.

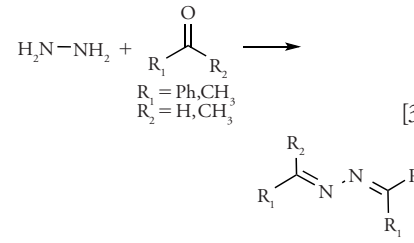
Derivatization of sulfonate esters before analysis not only stabilized the analyte, but also increased volatility because of the low polarity of derivatized esters (34–36). For instance, pentafluorothiophenol is a commonly used derivatization reagent for sulfonate esters. As shown in equation 2, sulfonate esters react with pentafluorothiophenol to form volatile sulfide:



[2]

However, this method does not distinguish between different alkyl groups (R_2 in equation 2) and provides common derivatization products for the analysis of various alkyl sulfonates and sulfates, which limits the selectivity. This approach was demonstrated by Jacq and colleagues (36) to monitor the formation of ethyl mesylate from ethanol and methanesulfonic acid in the reaction mixture.

Hydrazine, a GTI, is a common building block in the synthesis of many drug substances and also is a known degradation product of the antituberculosis drug isoniazid. The drug is highly reactive with a high polarity, low molecular weight, limited volatility, and lack of UV chromophore, which makes it challenging for the analysis of residual hydrazine. Derivatization using benzaldehyde to provide resulting benzalazine has been a favorite approach for the analysis of hydrazines followed by analysis using various detectors (equation 3) (37–39). The resulting benzalazine may be analyzed using GC or HPLC analysis.



[3]

Derivatization reaction time is reported to be critical as an increase in benzalazine has been observed if the aqueous reaction sample is allowed to stand for a longer time before analy-

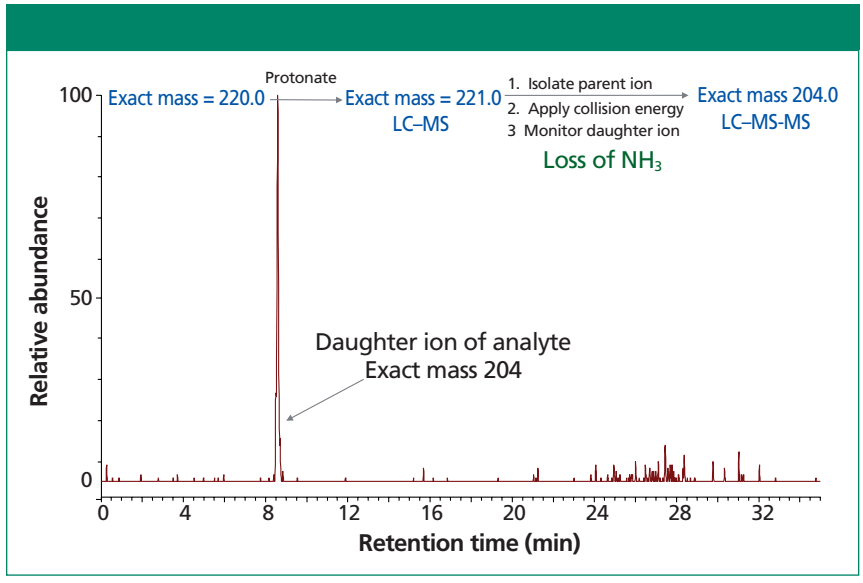
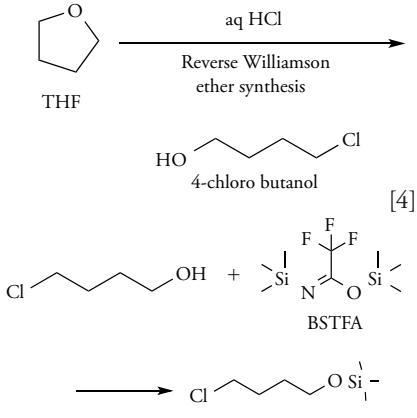


Figure 4: Representative chromatogram of hydroxylamine impurity MRM at 1 ppm level.

sis. In situ derivatization of hydrazine using acetone or acetone-d_6 followed by GC–MS analysis of the resulting acetone azine has an advantage because acetone may be used as a derivatizing agent as well as a diluent (38).

Alkyl chlorides, which are alkylating agents, may be formed because of the use of various alcohols and aqueous hydrochloric acid during the API synthesis. A genotoxic impurity such as 4-chloro-1-butanol may also be generated if tetrahydrofuran and hydrochloric acid are used during the manufacturing process of an API (equation 4).



Because of low sensitivity and a tendency to react back to tetrahydrofuran, 4-chloro-1-butanol needs to be derivatized before analysis. This may be done easily by silylation using *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, a common reagent for hydroxyl group derivatization); the resulting trimethylsilyl ether is analyzed by headspace GC. To ensure accuracy and precision, 3-chloro-1-butanol may be used as an internal standard (33).

We also used derivatization with the BSTFA technique followed by GC–MS analysis in SIM mode to track the residual amount of 4-chloro-1-butanol in the final API as a limit test, with limits of detection (LOD) of <1 ppm and sample concentration of 1 mg/mL.

Determination of low levels of small alkyl aldehydes, such as formaldehyde, poses an analytical challenge because of their volatility and stability issues. Formaldehyde is eluted very fast and no specific ions are present for selective and sensitive detection by GC–MS. Derivatization is often applied to enhance stability and sensitivity with the change in its physicochemical properties (32). Formaldehyde is derivatized to diethoxymethane by addition of ethanol in the presence of *p*-toluenesulfonic acid as a catalyst. A similar approach has been reported for the analysis of unstable acid chlorides, aziridines (40), and chlorophenols (41). A combined approach of extraction followed by derivatization has been developed for the determination of small, water-soluble amines such as aziridine and 2-chloroethylamine in drug substances at trace levels before quantitation by GC (40).

Analysis of GTIs by LC

LC is the primary separation technique for nonvolatile and thermally labile analytes. LC techniques include reversed-phase chromatography, normal-phase chromatography, and hydrophilic-interaction chromatography (HILIC). Reversed-phase HPLC is typically the first choice used for the analysis of nonvolatile GTIs. HILIC may be used to retain and analyze very polar analytes, which are difficult to retain and are eluted at solvent front with reversed-phase LC. Detection by UV is the first choice for the GTI analysis. However, MS is becoming a fairly common and universal detection method these days because of its enhanced sensitivity and selectivity. Other detection methods reported in the literature are evaporative light-scattering detection (ELSD) and charged aerosol detection (CAD) for the analysis of GTIs that lack a UV chromophore. Derivatization may be used to introduce a chromophore for UV analysis, to increase ionization for MS, or enhance stability of the analyte.

Direct Analysis (No Derivatization)

GTI Analysis Using High Sample Loading, LC–UV

Similar to GC, the control level of analytes drives the required sensitivity of the method and detector to be used with LC. UV detection is typically less selective and less sensitive compared to MS. However, at the level of 100 ppm, for compounds with strong UV chromophores and no matrix interference, UV still remains the most preferable detector for routine analysis because of its robustness, ease of use, and easy transfer (13–15,42,43).

The HPLC–UV impurity method for drug substances may be used as an initial platform followed by increasing the sample load to increase sensitivity. Similar technology has been used in our laboratory for the determination of a GTI, bromopyrazole, in a drug substance. In this case, the GTI control level was determined to be 160 ppm based on the TTC approach. First, the drug substance impurity method was assessed for the matrix interference and then the sample concentration was increased from 0.4 mg/mL to 1 mg/mL and the injection vol-

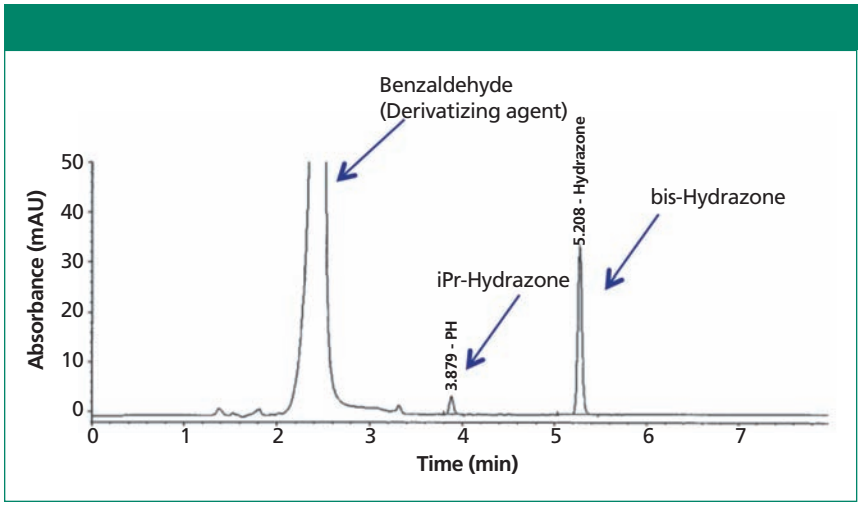


Figure 5: A representative chromatogram for analysis of hydrazine and isopropyl hydrazine. Column: 150 mm × 4.6 mm, 3.5-µm Waters Xbridge C18; mobile-phase A: water; mobile-phase B: acetonitrile; temperature: 50 °C; injection volume: 5 µL; flow rate: 1 mL/min.

ume was increased from 10 µL to 20 µL to enhance the sensitivity. Figure 3 shows overlay chromatograms of API unspiked and spiked with bromopyrazole at the 50 ppm level. This method demonstrated excellent precision, spiked recovery, and linearity assessed up to 180 ppm (R^2 = 1.0).

GTI Analysis Using LC–MS and LC–MS-MS

MS has become the detection method of choice and is widely used for GTI analysis because of its high selectivity and sensitivity. Table III summarizes the applications of MS for the analysis of various analytes in pharmaceutical compounds. However, matrix interference is a higher risk than GC–MS and may cause analyte ion suppression or enhancement.

The recent advancement of MS instrumentation has established another level and possibility to achieve much higher sensitivity for GTIs (12,46,47,49–51). Switching from selected ion monitoring (SIM) to multiple reaction monitoring (MRM) mode for the analysis of 4-dimethylaminopyridine resulted in increased sensitivity and lower limits of quantitation (LOQ) (48). A highly sensitive and precise analytical method using LC–MS-MS was reported for the quantitation of 2-chloromethyl-3,4-dimethoxypyridine hydrochloride in APIs and the final tablet form (47). Trace levels of ammonium acetate were added to the mobile phase to enhance ionization and detection. In our laboratory, LC–MS-MS was also successfully applied to quantify a hydroxylamine impurity present at a low level in a drug substance (52). LC–MS in SIM mode did not provide the required sensitivity of 10 ppm. To increase the sensitivity, the parent ion obtained from LC–MS (SIM) was

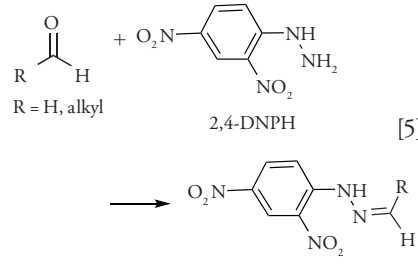
isolated and a collision energy of 17 eV (optimized) was applied using a triple-quadrupole mass spectrometer. The resulting daughter ion was monitored, providing an LOD of 1 ppm, excellent linearity, and percent recovery of 91% at the 2 ppm level. Figure 4 shows the representative chromatogram for the daughter ion.

HILIC using MS-compatible mobile phases in conjunction with MS has also been used as an alternative to reversed-phase and ion pair chromatography for the analysis of polar analytes (50).

GTI Analysis Using Derivatization Followed by LC

As is the case for GTI analysis by GC, direct accurate analysis of some of the genotoxic impurities by LC is challenging because they react rapidly, decompose, lack a UV chromophore or ionizable functional groups, or need to be derivatized before analysis. Derivatizing agents are selected based on the functional groups of the GTIs and are focused mainly toward the improvement of sensitivity, the generation of chromophores, and analyte stability (32). Table IV summarizes the application of derivatization for various analytes enhancing stability, introducing UV chromophore, and ionizable functional groups before LC analysis.

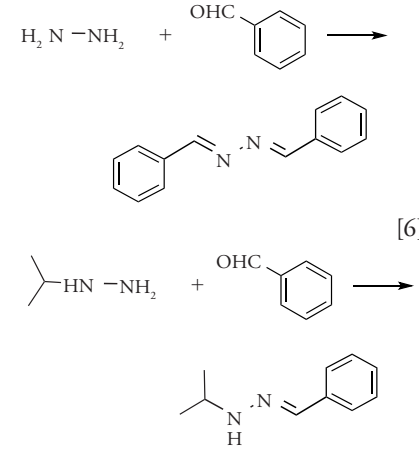
Aldehydes are prone to undergo partial aerial oxidation leading to inaccurate determinations. A favorite derivatizing agent for the analysis of aldehydes has been 2,4-dinitrophenylhydrazine (2,4-DNPH), which provides respective hydrazone derivatives that have absorbing maxima at 360 nm (equation 5):



[5]

The resulting hydrazones are analyzed either by HPLC–UV (53,54) or LC–MS (32).

Derivatization has been one of the main analytical techniques for the analysis of hydrazines as well because they are highly reactive, polar, and lack UV chromophores. Aryl aldehydes are most commonly used to yield respective benzalazine derivatives, which have UV chromophores and are stable for analysis (11,62). Determination of residual hydrazine and isopropyl hydrazine in our laboratory was performed by derivatization followed by LC–UV (equation 6).



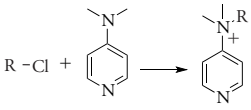
[6]

A solution of 5% benzaldehyde in methanol was added to the sample solution in water in an HPLC vial, and derivatization was carried out at 50 °C for 30 min. The derivatization time was optimized by comparing the peak areas of the derivatized product at different intervals. The detection wavelength was selected as 300 nm, which was the absorption maximum of the derivatized product, and LC analysis was carried out using a Waters XBridge C18 column. Figure 5 shows a chromatogram of the starting material spiked with hydrazine and isopropyl hydrazine. Linearity was demonstrated over the 1–40 ppm range for hydrazine and the 5–100 ppm range for isopropyl hydrazine (based on the sample concentration of 10 mg/mL), with a correlation coefficient of >0.999 and excellent precision.

The method was further optimized to determine the residual hydrazine in a drug substance since the drug substance was not aqueous soluble; the diluent was changed to methanol and the concentration of the derivatizing agent was changed from 5% to 10% in methanol to keep the same ratio in the derivatizing mixture. The method demonstrated excellent spiked recoveries (98.9%).

Various alkyl and aryl halides, which are not sufficiently volatile and are thermally labile, have been derivatized using

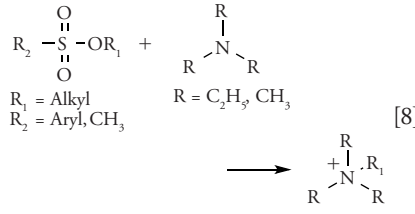
4-dimethylamino pyridine (4-DMAP) or dimethylamine (DMA) forming a stable quaternary amine salt derivative before LC analysis (equation 7) (56–58).



[7]

HILIC–MS-MS has been used to retain polar derivatized products and quantify the amount of derivatives. HILIC using a mass compatible buffer provides a great advantage compared to reversed-phase HPLC in which MS non-compatible ion-exchange or ion-pairing buffers are generally used. Another key advantage is that reagent-related fragments produced for derivatives allow for low-level screening of alkyl halides. A three-step weak cation-exchange SPE procedure was used to remove excess DMAP and the resulting solution was analyzed by HPLC–MS-MS in MRM mode. Chloroformates tend to be much more moisture sensitive and hydrolytically unstable, so derivatization is used to stabilize the analyte. It was reported that strong basic derivatization reagents caused decomposition of the desired derivatives. Therefore, residual chloroethylchloroformate (CECF) in API was derivatized using 2-mercaptopyridine, a less basic nucleophile (8). The authors suggested that both glassware and solvents needed to be suitably dried to prevent interference from residual moisture and achieve the desired sensitivity and reproducibility.

Techniques similar to alkyl halides can also be applied for the analysis of sulfonate esters (equation 8) (44,59).



[8]

A method comprising generic derivatization and LC–MS (SIM) in combination with a HILIC stationary phase has been used for the analysis of a group of commonly encountered alkyl esters of sulfonates or sulfates present in drug substances at low parts-per-million levels. Trimethyl and triethylamine have been used as derivatizing agents and a HILIC stationary phase was used to retain highly polar quaternary ammonium derivatization products and separate them from the API peak.

Chemical derivatization and coordination ion spray ionization MS have been used to enhance the detection sensitivity of neutral analytes such as epoxides (58). The chemical derivatization converts analytes into highly ionizable, permanently charged derivatives, which become readily detectable by MS, whereas the coordination ion spray MS approach improves ionization by forming neutral ion adducts with metal ions such as Na^+, K^+, or NH_4^+, which are introduced into the electrospray ionization (ESI) source.

Innovative Analytical Techniques for the Analysis of GTIs

Because of the wide variety of GTIs present and their physicochemical properties, many innovative analytical

Table V: Literature references for use of innovative techniques for GTI analysis

Analytical Technique	Analyte	DL (QL)	Reference	Year
Matrix deactivation	Various alkyl halides	0.4–4 ppm	Sun (24)	2010
Molecular imprinting-scavenger resins	Acetamide arylsulfonates		Székely (51)	2012
2D-GC–MS	Multiple	<1 ppm	Frank (28)	2010
Metal ion coordination ion spray MS	Epoxide	7.5 ng/mL	Bai (58)	2010
Coordination ion spray MS	Various		Bayer, Harvilla, and Gao (63–65)	1999, 2000, 2005
Meerwein reaction-MS	Epoxides	1 ppm	Wu (60,61)	2010, 2011
Capillary electrophoresis	DMS, chloroacetyl chloride	3 (10) ppm	Khan (66)	2012
Capillary electrophoresis	Alkyl halides	(0.05%)	Hansen (67)	2005
Derivatization (dansyl hydrazine)–CE	Formaldehyde	200 ppb	Feige (68)	1996
2D HPLC, on-line reduction, fluorescent detector	3-Nitrobenzanthrone	0.002 (0.006) ng	Hasei (69)	2012
LC–ELSD, LC–CAD			Yuabova (15)	2008
HILIC–CLND	Hydrazines	0.02%	Liu (70)	2009
Extractive ESI	On-line reaction monitoring	2.5 ppm	McCullough (71)	2011
Atmospheric-pressure thermal desorption-extractive ESI-MS	*p*-Toluenesulfonate	0.1 ppm	Devenport (72)	2013
LC–MS (SIM) SFC–MS (SRM)	Alkyl halides	0.03 (0.1) ppm	Huybrechts (75)	2007
Derivatization (3-iodobenzoyl chloride), LC–ICP-MS	4-Chloro-1-butanol	<1 ppm	Harigaya (73)	2014
NMR	Trifluoronitrobenzene	9 ppm	Parmar (74)	2013

techniques have been developed and reported in the literature to reduce matrix interference and enhance sensitivity. Table V summarizes these techniques for various analytes. Matrix deactivation provided an innovative approach to stabilize reactive or unstable analytes (24). This deactivation has been achieved chemically either by protonating and masking the basic functionalities or scavenging the hypothetical reactive species in the sample matrix. Molecularly imprinted polymers have high affinity binding sites for target analytes, whereas APIs have a different size and structure and work as scavenger resins. The efficiency of these polymers has been demonstrated for the quantitative removal of acetamide and aryl sulfonates in the presence of APIs (51).

The analysis of neutral GTIs provides a challenge because of their poor ionization efficiency. Chemical derivatization followed by coordination ion spray MS improved sensitivity for some neutral epoxides by forming neutral ion-adducts with metal ions such as Na^+, K^+, or NH_4^+, which are then introduced into the ESI source and analyzed by MS (58,63–65). Formations of adduct ions in positive or negative detection mode is applicable. The gas-phase derivatization via Meerwein reaction provided another alternative for LC–MS analysis of trace-level epoxides (60,61). Ethylnitrilium ions generated by atmospheric pressure ionization (during MS when acetonitrile is used as mobile phase for HPLC) react with epoxides, and then Meerwein reaction products are analyzed

by either SIM or MRM modes. This technique is a great alternative in cases when an analyte is difficult to analyze directly because it has poor ionization and is unstable in solution-phase derivatization.

Genotoxins are mostly determined using HPLC–UV–MS and GC–MS techniques for their robustness. However, the use of capillary electrophoresis (CE) can complement the existing techniques for analysis and open new horizons. CE has been reported for the analysis of dimethyl sulfate, chloroacetyl chloride (66), formaldehyde (68), and alkyl halides (67). It also appears to be more robust to matrix effects and provides highly efficient separations and consumes very low amounts of reagents. However, it has the drawbacks of poor sensitivity and precision needed for trace analysis.

The potential applicability of supercritical fluid chromatography (SFC)–MS in selective reaction monitoring (SRM) mode has been reported as a complementary and orthogonal approach to HPLC for the analysis of a GTI at sub-part-per-million levels (sample concentration 10 mg/mL) in an unknown API (29). SFC may prove to be an advantageous technique when attaining a high sample concentration is a significant challenge because of limited solubility. A novel LC–inductively coupled plasma (ICP)-MS method has been used for the analysis of 4-chloro-1-butanol (73). To enhance the selectivity, a 2D LC system is further connected with ICP-MS. Then, 4-chloro-1-butanol is derivatized with 3-iodobenzoyl chloride in acetonitrile and the resulting derivative is analyzed for iodine (*m/z* 127). Methanol is selected as the organic solvent for the mobile phase of the second LC system, which is more suitable for ICP-MS because of its lower carbon content compared to acetonitrile.

Quantitative nuclear magnetic resonance (NMR) spectroscopy (74) is also feasible with recent developments of high strength systems (such as 400–600 MHz) combined with cryoprobe technology. This technique has advantages of being quantitative in nature, easy sample preparation, and the ability to provide quick answers. A comparison experiment may be performed with and without spikes at an appropriate level to provide a quick answer if it is feasible to be used for a particular analysis. ^{19}F-NMR is widely used for the quantitation of fluorinated impurities. Because of the high sample concentration (1 g in 300 µL DMSO-d_6) and hence high viscosity, analysis is done at 343 K to provide a sharp signal, and $^{19}F_1$ at -156 ppm is selected for quantitation because it provides the simplest multiplet structure. However, this approach does have some notable disadvantages, such as being less sensitive (requiring high sample concentration) and more expensive.

Conclusion

Controlling GTIs in pharmaceutical compounds is of the utmost importance and is a requirement throughout the clinical development and commercial stage. A good process understanding and sensitive, specific analytical methods are needed to control these impurities below the TTC level.

Many factors, including physicochemical properties of the analytes, need to be considered as an effective analytical method development strategy. Direct analysis without extensive sample preparation is preferred. However, sometimes sample preparation becomes an integral part of the analytical method if there is matrix interference. Hyphenated techniques are gaining popularity, and with advancements in MS instrumentation, MS is becoming the detection of choice because it provides the required sensitivity and specificity in SIM and MRM modes. Chemical derivatization offers an alternative to achieve the needed sensitivity and sample stability. Following method development, the method is validated to demonstrate linearity over the desired range, spiked recovery, and sensitivity.

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References

(1) S. Görög, *J. Pharm. Biomed. Anal.* **48,** 247 (2008).

(2) T. McGovern and D. Jacobson-Kram, *TrAC, Trends Anal. Chem.* **25,** 790 (2006).

(3) D.A. Pierson, B.A. Olsen, D.K. Robbins, K.M. DeVries, and D.L. Varie, *Org. Process Res. Dev.* **13,** 285 (2009).
(4) D.I. Robinson, *Org. Process Res. Dev.* **14,** 946 (2010).
(5) N.V.V.S.S. Raman, A.V.S.S. Prasad, and K. Ratnakar Reddy, *J. Pharm. Biomed. Anal.* **55,** 662 (2011).
(6) A. Giordani, W. Kobel, and H.U. Gally, *Eur. J. Pharm. Sci.* **43,** 1 (2011).
(7) U. Yadav, P. Dhiman, N. Malik, A. Khatkar, N. Redhu, and D.P. Singh, *J. Biomed. Pharm. Res.* **2,** 39 (2013).
(8) D.Q. Liu, M. Sun, and A.S. Kord, *J. Pharm. Biomed. Anal.* **51,** 999 (2010).
(9) L.K. Dow, M.M. Hansen, B.W. Pack, T,J. Page, and S.W. Baertschi, *J. Pharm. Sci.* **102,** 1404 (2013).
(10) N.A. Charoo and A.A. Ali, *Drug Dev. Ind. Pharm.* **39,** 947 (2013).
(11) EMEA-CHMP, EMEA/CHMP/QWP/251344/2006 London (June 28, 2006).
(12) Draft Guidance for Industry: Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches. FDA, CDER, December 2008.
(13) International Conference on Harmonization, *ICH Q3A (R2): Impurities in New Drug Substances* (ICH, Geneva, Switzerland, October 2006).
(14) International Conference on Harmonization, *ICH Q3C (R4): Impurities: Residual Solvents* (ICH, Geneva, Switzerland, February 2009).
(15) International Conference on Harmonization, *ICH Q3B (R2): Impurities in New Drug products* (ICH, Geneva, Switzerland, June 2006).
(16) L. Müller, R.J. Mauthe, C.M. Riley, M.M. Andino, D.D. Antonis, C. Beels, J. DeGeorge, A.G.M. De Knaep, D. Ellison, J.A. Fagerland, R. Frank, B. Fritschel, S. Galloway, E. Harpur, C.D.N. Humfrey, A.S. Jacks, N. Jagota, J. Mackinnon, G. Mohan, D.K. Ness, M.R. O'Donovan, M.D. Smith, G. Vudathala, and L. Yotti, *Regul. Toxicol. Pharmacol.* **44,** 198 (2006).
(17) International Conference on Harmonization, *ICH M7 (Step. 4): Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk* (ICH, Geneva, Switzerland, June 2014).
(18) International Conference on Harmonization, *ICH S9: Nonclinical Evaluation for Anticancer Pharmaceuticals* (ICH, Geneva, Switzerland, March 2009).
(19) S.M. Galloway, M.V. Reddy, K. McGettigan, R. Gealy, and J. Bercu, *Regul. Toxicol. Pharmacol.* **66,** 326 (2013).
(20) H.G. Ramjit, M.M. Singh, A.B. Coddington, *J. Mass Spectrom.* **31,** 867 (1996).
(21) G.E. Taylor, M. Gosling, and A. Pearce, *J. Chromatogr. A* **1119,** 231 (2006).
(22) Y. Mamilla, K. Chetyala, R. Venna, R. Gajjela, K. Katragadda, S. Govindasamy, S. Mulukutla, and D. Datta, *Chromatographia* **71,** 733 (2010).
(23) T. Kaleemullah, M. Ahmed, H.K. Sharma, K.V.S.N. Raju, and M.N. Kumar, *Der Pharma Chemica* **3,** 390 (2011).
(24) M.J. Sun, L. Bai, G.J. Terfloth, D.Q. Liu, and A.S. Kord, *J. Pharm. Biomed. Anal.* **52,** 30 (2010).
(25) I. Colón and S.M. Richoll, *J. Pharm. Biomed. Anal.* **39,** 477 (2005).
(26) J. Zheng, W.A. Pritts, S. Zhang, and S. Wittenberger, *J. Pharm. Biomed. Anal.* **50,** 1054 (2009).
(27) U. Wollein and N. Schramek, *Eur. J. Pharm. Sci.* **45,** 201 (2012).
(28) F. David, K. Jacq, P. Sandra, A. Baker, and M. Klee, *Anal. Bioanal. Chem.* **396,** 1291 (2010).
(29) T.D. Ho, M.D. Joshi, M.A. Silver, and J.L. Anderson, *J. Chromatogr. A* **1240,** 29 (2012).
(30) M. Andre, J. Loidl, G. Laus, H. Schottenberger, G. Bentivoglio, K. Wurst, and K.H. Ongania, *Anal. Chem.* **77,** 702 (2004).
(31) T.D. Ho, P.M. Yehl, N.P. Chetwyn, J. Wang, J.L. Anderson, and Q. Zhong, *J. Chromatogr. A* **1361,** 217 (2014).
(32) N.V.V.S.S. Raman, A.V.S.S. Prasad, K.R. Reddy, *J. Pharm. Biomed. Anal.* **89,** 276 (2014).
(33) K. Harigaya, H. Yamada, K. Yaku, H. Nishi, and J. Haginaka, *Chem. Pharm. Bull.* **62,** 395 (2014).
(34) C.R. Lee, F. Guivarch, C. Nguyen Van Dau, D. Tessier, and A.M. Krstulovic, *Analyst* **128,** 857 (2003).
(35) R. Alzaga, R.W. Ryan, K. Taylor-Worth, A.M. Lipczynski, R. Szucs, P. Sandra, *J. Pharm. Biomed. Anal.* **45,** 472 (2007).
(36) K. Jacq, E. Delaney, A. Teasdale, S. Eyley, K. Taylor-Worth, A. Lipczynski, V.D. Reif, D.P. Elder, K.L. Facchine, S. Golec, R.S. Oestrich, P. Sandra, and F. David, *J. Pharm. Biomed. Anal.* **48,** 1339 (2008).
(37) O. Gyllenhaal, L. Grönberg, and J. Vessman, *J. Chromatogr. A* **511,** 303 (1990).
(38) M. Sun, L. Bai, and D.Q. Liu, *J. Pharm. Biomed. Anal.* **49,** 529 (2009).
(39) A. Carlin, N. Gregory, and J. Simmons, *J. Pharm. Biomed. Anal.* **17,** 885 (1998).
(40) P. E. de Haan, D. de Jong, J.H.M. van den Berg, and C.G. Kruse, *J. High Res.Chromatogr.* **12,** 604 (1989).
(41) P. de Morais, T. Stoichev, M.C. Basto, P. Carvalho, and M.T.D. Vasconcelos, *Anal. Bioanal. Chem.* **399,** 2531 (2011).
(42) V. Madireddy, K.S. Babu, and P. Narayanreddy, *Global J. Anal. Chem.* **2,** 198 (2011).
(43) L. Valvo, R. Alimenti, S. Alimonti, S. Raimondi, F. Foglietta, and F. Campana, *J. Pharm. Biomed. Anal.* **15,** 989 (1997).

(44) D.Q. Liu, T.K. Chen, M.A. McGuire, and A.S. Kord, *J. Pharm. Biomed. Anal.* **50,** 144 (2009).
(45) C.R. Lee, M. Hubert, C.N. Van Dau, D. Peter, and A.M. Krstulovic, *Analyst* **125,** 1255 (2000).
(46) P.R. Kakadiya, B.P. Reddy, V. Singh, S. Ganguly, T.G. Chandrashekhar, and D.K. Singh, *J. Pharm. Biomed. Anal.* **55,** 379 (2011).
(47) N. Venugopal, A.V.B. Reddy, K.G. Reddy, V. Madhavi, and G. Madhavi, *J. Pharm. Biomed. Anal.* **70,** 592 (2012).
(48) G. Szekely, B. Henriques, M. Gil, A. Ramos, and C. Alvarez, *J. Pharm. Biomed. Anal.* **70,** 251 (2012).
(49) A.V.B. Reddy, N. Venugopal, and G.Madhavi *J. Anal. Sci. Technol.* **5,** 18 (2014).
(50) J. Hmelnickis, O. Pugovičs, H. Kažoka, A. Viksna, I. Susinskis, and K. Kokums, *J. Pharm. Biomed. Anal.* **48,** 649 (2008).
(51) G. Székely, E. Fritz, J. Bandarra, W. Heggie, and B. Sellergren, *J. Chromatogr. A* **1240,** 52 (2012).
(52) K.C. Gu, H.J.W. Comstock, L. Wigman, C.J. Venkatramani, and A. Deese, "Quantitative Analysis of Potential Genotoxic Impurities by High Resolution Mass Spectrometer," presented at 60th ASMS Conference on Mass Spectrometry and Allied Topics Vancouver, BC, Canada (2012).
(53) A. Nageswari, K.V.S.R. Krishna Reddy, and K. Mukkanti, *Chromatographia* **75,** 275 (2012).
(54) A.Q. Soman, Y. Chan, and Q. Li, *J. Chromatogr. Sci.* **46,** 461 (2008).
(55) G. Manius, L.-F. Wen, and D. Palling, *Pharm. Res.* **10,** 449 (1993).
(56) E. Yang, S. Wang, C. Bowen, J. Kratz, M.J. Cyronak, and J.R. Dunbar, *Rapid Commun. Mass Spectrom.* **19,** 759 (2005).
(57) A.M. van Wijk, B. Beerman, H.A.G. Niederländer, A.H.G. Siebum, and G.J. de Jong, *Anal. Bioanal. Chem.* **400,** 1375 (2011).
(58) L. Bai, M. Sun, J. An, D.Q. Liu, T.K. Chen, and A.S. Kord, *J. Chromatogr. A* **1217,** 302 (2010).
(59) J. An, M. Sun, L. Bai, T. Chen, D.Q. Liu, and A. Kord, *J. Pharm. Biomed. Anal.* **48,** 1006 (2008).
(60) L. Wu, D.Q. Liu, and A.S. Kord, *J. Am. Soc. Mass Spectrom.* **21,** 1802 (2010).
(61) L. Wu, D.Q. Liu, F.G. Vogt, and A.S. Kord, *J. Pharm. Biomed. Anal.* **56,** 1106 (2011).
(62) J. Manes, M.J. Gimeno, J.C. Molto, and G. Font, *J. Pharm. Biomed. Anal.* **6,** 1023 (1988).
(63) E. Bayer, P. Gfrörer, and C. Rentel, *Angew. Chem., Int. Ed.* **38,** 992 (1999).
(64) S. Gao, Z.-P. Zhang, and H.T. Karnes, *J. Chromatogr. B* **825,** 98 (2005).
(65) C.M. Havrilla, D.L. Hachey, and N.A. Porter, *J. Am. Chem. Soc.* **122,** 8042 (2000).
(66) M. Khan, K. Jayasree, K.V.S.R.K. Reddy, and P.K. Dubey, *J. Pharm. Biomed. Anal.* **58,** 27 (2012).
(67) S.H. Hansen and Z.A. Sheribah, *J. Pharm. Biomed. Anal.* **39,** 322 (2005).
(68) K. Feige, T. Ried, and K. Bächmann, *J. Chromatogr. A* **730,** 333 (1996).
(69) T. Hasei, H. Nakanishi, Y. Toda, and T. Watanabe, *J. Chromatogr. A* **1253,** 52 (2012).
(70) M. Liu, J. Ostovic, E.X. Chen, and N. Cauchon, *J. Chromatogr. A* **1216,** 2362 (2009).
(71) B.J. McCullough, T. Bristow, G. O'Connor, and C. Hopley, *Rapid Commun. Mass Spectrom.* **25,** 1445 (2011).
(72) L.C.S.N.A. Devenport, F. H. Alruways, D.J. Weston, J.C. Reynolds, and C.S. Creaser, *Anal. Chem.* **85,** 6224 (2013).
(73) K. Harigaya, H. Yamada, K. Yaku, H. Nishi, and J. Haginaka, *Anal. Sci.* **30,** 377 (2014).
(74) A.K.M.K. Parmar, R. Patel, and S. Prajapati, *Int. J. ChemTech Res.* **5,** 312 (2013).
(75) T. Huybrechts, "Successfully developing and validating methods for the quantification of genotoxic impurities in APIs," presented at Informa Genotoxic Impurities Meeting, Prague, Czech Republic, 2007.

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PRODUCTS & RESOURCES

Laboratory management software

The NuGenesis laboratory management system from Waters is designed as an alternative to a traditional laboratory information management system. According to the company, the system combines data, workflow, and sample management capabilities to support the product lifecycle from discovery through manufacturing.

Waters Corporation,
Milford, MA.
www.waters.com

Pure gases

Alphagaz pure gases from Air Liquide are supplied in cylinders designed with the company's Smartop lever-activated valve. According to the company, a built-in residual pressure valve protects gas purity from back-flow contamination, and automatic flow restriction helps prevent injury in case of an accidental pressure breach.

America Specialty Gases LLC,
Plumsteadville, PA.
www.airliquide.com

Solid-phase extraction system

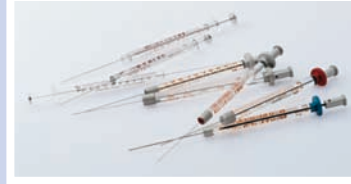
The EconoTrace parallel solid-phase extraction (SPE) system from FMS is designed with positive pressure pumping for delivery of the sample and conditioning, washing, and elution solvents. According to the company, up to eight extractions can run simultaneously and the system uses any size or type SPE cartridge.

FMS, Inc.,
Watertown, MA.
www.fms-inc.com

Autosampler syringes

Autosampler syringes for liquid and gas chromatography from Hamilton are designed specifically for CTC PAL liquid chromatography autosampler systems. According to the company, the S-Line syringes complement its existing C-Line and X-Type syringes, and are suitable for everyday use.

Hamilton Company,
Reno, NV.
www.hamilton.com



GC–MS system

The Pegasus GC-HRT 4D system from LECO combines a GC×GC system with a time-of-flight mass spectrometer. According to the company, advances in the instrument are paired with LECO's ChromaTOF-HRT software with a High Resolution Deconvolution feature, which is designed for the analysis of high-resolution data using NIST and accurate mass libraries.

LECO Corporation,
St. Joseph, MI. www.leco.com

HPLC columns

Machery-Nagel's spherical silica Nucleosil columns are designed for use in validated methods. According to the company, available phases include C_{18}, C_{18} HD, C_8, CN, NH_2, SiOH, and others, and a universal screw-on guard column holder system can be used for the protection of the main column.

Macherey-Nagel Inc.,
Bethlehem, PA.
www.mn-net.com

Chemistry analyzer

The Flow Solution 3700 continuous flow chemistry analyzer from OI Analytical is designed to improve laboratory workflow and productivity by automating chemical analysis of validated ASTM, ISO, and US EPA methods. According to the company, the analyzer has modular hardware that allows FIA, SFA, and SFIA methods to be run interchangeably on the same unit.

OI Analytical,
College Station, TX.
www.oico.com

LC system

PerkinElmer's Altus UPLC system is designed for use in food, environmental, and industrial laboratories for the detection of adulterants, contaminants, and pollutants. According to the company, the system is controlled through Waters's Empower 3 software and combines advanced fluidics with hybrid particle columns.

PerkinElmer, Inc.,
Waltham, MA.
www.perkinelmer.com

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powered by crawford scientific

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CHROMacademy is the world's largest eLearning website for analytical scientists

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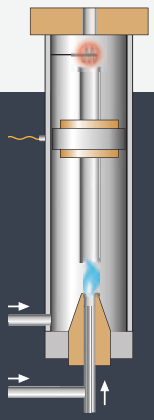

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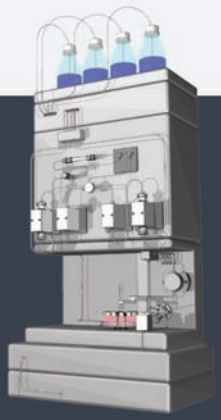


To find out more about CHROMacademy Premier membership contact:
Glen Murry on +1 732 - 346 - 3056 | e-mail: gmurry@advanstar.com

www.chromacademy.com

Hydrogen generators

Proton OnSite's S-Series hydrogen generators are designed to produce ultrahigh-purity hydrogen for use with multiple gas chromatography systems and for other laboratory applications. According to the company, with a production rate of 4.8, 9.6, or 18.8 slpm, the generator produces the equivalent of four cylinders of better-than-UHP-grade hydrogen per day.

Proton OnSite,
Wallingford, CT;
www.protononsite.com

GC capillary columns

Quadrex's 007-624 bonded GC capillary columns are based on a mid-polarity cyanopropylphenyl methylopolsiloxane phase (USP G43) designed for the separation of volatile organic compounds and residual solvents such as those found in USP 467 methods. According to the company, the columns are available in several standard configurations.

Quadrex Corporation,
Woodbridge, CT.
www.quadrexcorp.com

Autosampler

Shimadzu's AOC-6000 robotic autosampler is designed with a syringe tool exchanger that allows users to change injection techniques before starting the next injection. According to the company, users can select different volumes and types of syringes, and the system can perform sample pretreatment and analysis in parallel so no time is lost during continuous analysis.

Shimadzu Scientific Instruments, Columbia, MD.
www.ssi.shimadzu.com

Mobile app update

The mobile app "Innovative Analytical Products" from Supelco has been updated. According to the company, the number of ionic liquid capillary GC column chromatograms was increased from 5 to 57, and all 57 chromatograms are now organized in eight industry buckets to make it easier for end users to locate appropriate choices for their applications.

Sigma-Aldrich/Supelco,
Bellefonte, PA.
www.sigma-aldrich.com/mobileapps

HILIC columns

Tosoh's TSKgel Amide-80 hydrophilic interaction liquid chromatography (HILIC) columns are now available packed with 2-µm spherical silica particles. According to the company, the columns offer higher resolution and faster analysis than the versions of the columns that have 3-µm particles.

Tosoh Bioscience, LLC,
King of Prussia, PA.
www.tosohbioscience.com

Forensic analytical kits

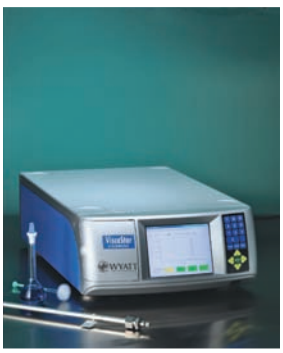
UCT's forensic analytical kits are designed to perform toxicology extractions and analyses. According to the company, the kits contain buffer reagents, SPE columns, HPLC columns, hydrolyzing reagents, and stock drug standards. Certificates of analysis for all standards and recommended extraction and analytical procedures are included.

UCT, LLC,
Bristol, PA.
www.unitedchem.com

Viscometer

The ViscoStar viscometer from Wyatt Technology is designed as an on-line chromatography detector for determining specific and intrinsic viscosities. According to the company, the viscometer has a transducer protection system that prevents accidental damage to transducer membranes.

Wyatt Technology Corp.,
Santa Barbara, CA.
www.wyatt.com



LC columns

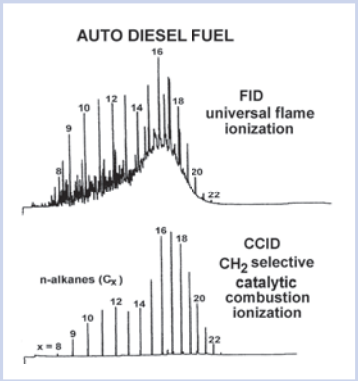
YMC America's Triart ExRS HPLC and UHPLC columns for hydrophobic substances and isomers are designed with an organic and inorganic hybrid silica particle that uses polymeric bonding. According to the company, the columns are chemically stable at pH extremes (pH 1–12) and are mechanically stable at elevated pressures and temperatures.

YMC America, Inc.,
Allentown, PA.
www.ymcamerica.com

Catalytic combustion ionization detector

DETector Engineering's catalytic combustion ionization GC detector is designed to selectively detect chains of methylene functional groups in alkane, FAME, and triglyceride constituents of petroleum, biofuel, and biological samples, with discrimination versus compounds containing carbon double bonds.

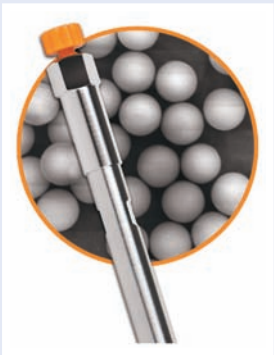
DETector Engineering & Technology,
Walnut Creek, CA.
www.det-gc.com



HPLC columns

Diamond Analytics' Flare columns for HPLC are designed to offer an expanded pH range (1–13), elevated temperature capabilities (up to 100 °C), increased longevity, and unique selectivity, without compromising efficiency. According to the company, the columns' core–shell particles are composed of a solid, spherical carbon core and a functionalized porous nano-diamond shell.

Diamond Analytics,
Orem, UT.
www.diamond-analytics.com



Water purification systems

EMD Millipore's AFS 40E, 80E, 120E, and 150E water purification systems are designed to provide clinical laboratories with purification for daily water volumes of up to 3000 L. According to the company, the systems provide users with continuous real-time monitoring and remote control.

EMD Millipore,
Billerica, MA.
www.emdmillipore.com

Combustion ion chromatograph

Metrohm's combustion ion chromatograph (CIC) is designed to automate the determination of halogens and sulfur. According to the company, the system's autosampler can run both solid and liquid samples, and flame sensor technology is used to measure the light intensity from the pyrolysis oven during combustion.

Metrohm USA,
Riverview, FL.
www.metrohmusa.com/CIC

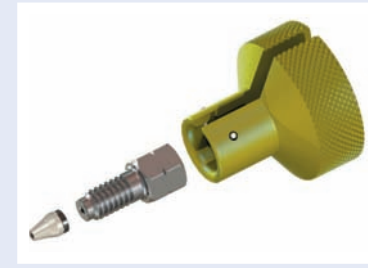
Column storage

MicroSolv's storage cabinet for HPLC columns is designed to hold most analytical and semipreparative columns in a customizable foam insert. According to the company, the five drawer stainless steel cabinet can hold up to 30 30-cm columns and has foam cutouts to accommodate 5-, 12.5-, 15-, or 30-cm columns.

MicroSolv,
Eatontown, NJ.
www.mtc-usa.com/columnstore.asp

UHPLC nano fittings

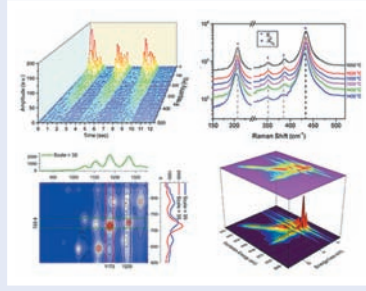
EXP2 UHPLC fittings from Optimize Technologies are designed with titanium hybrid Ti-LOK ferrules for PEEKsil tubing. Male fittings reportedly feature a hex head with a removable slotted knurled wrench for hand tightening to more than 15,000 psi, depending on the tubing internal diameter. According to the company, uses for the fittings include nano and UHPLC connections as well as sample loops.



Optimize Technologies, Inc., Oregon City, OR.
www.optimizetech.com

Data analysis and graphing software

Origin and OriginPro 2015 data analysis and graphing software from OriginLab add more than 100 new features and improvements. According to the company, enhancements include collapsible menus, project file search for string, thumbnail previews of graphs, and tooltips that display folder or window comments in Project Explorer.



OriginLab, Northampton, MA.
www.originlab.com

Syringe filters

Acrodisc syringe filters with GHP membranes from Pall are designed for compatibility with acids and bases, aggressive organic solvents, and halogenated solvents. According to the company, the membranes allow the syringe filters to be used across a range of sample preparation applications.

Pall Life Sciences,
Westborough, MA.
www.pall.com

Capillary tubing

Tight Tolerance Vision System flexible fused-silica capillary tubing from Polymicro Technologies, offered by Molex, is designed to provide precise internal diameter tolerances. According to the company, the tubing is drawn from high-purity synthetic fused-silica preforms and has industry-standard outer diameter dimensions.

Polymicro Technologies,
Phoenix, AZ.
www.molex.com/polymicro/capillarytubing.html

Terpenes standards

Multicomponent terpenes standards for medical cannabis analysis are available from Restek. According to the company, the certified reference materials meet strict ISO requirements and were developed specifically for cannabis analysis.

Restek Corporation,
Bellefonte, PA.
www.restek.com/medical-cannabis

Integrated HPLC systems

Shimadzu Scientific's Prominence-i and Nexera-i integrated HPLC systems are designed to provide a more efficient workflow for conventional to ultrahigh-speed analysis. According to the company, the data acquired by these systems via an interactive communication mode is sent to a laboratory's data center by the systems' LabSolutions network and managed uniformly by a server.

Shimadzu Scientific Instruments,
Columbia, MD.
www.ssi.shimadzu.com

Mass spectrometer

Thermo Fisher Scientific's Q Exactive Focus LC–MS-MS system is designed for laboratories performing food and environmental testing, clinical research, forensic toxicology, pharmaceutical and biopharmaceutical measurements, and other applied analyses. According to the company, the instrument delivers up to 70,000 resolution at *m/z* 200, and a scan speed of up to 12 Hz.

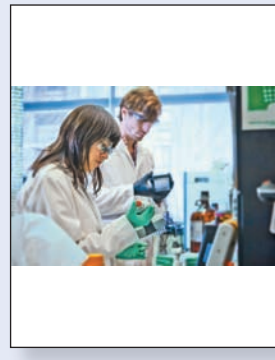
Thermo Fisher Scientific,
San Jose, CA.
www.thermofisher.com/qefocus

Literature

Application notebook

The newest edition of Waters' *Peptide and Protein Bioanalysis Applications Notebook* contains information on more than 20 applications that use the company's LC–MS-MS methods for peptide and protein bioanalysis. A free copy is available to download.

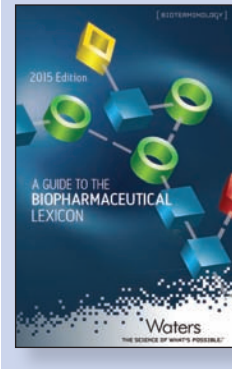
Waters Corporation,
Milford, MA.
www.waters.com/peptidebioa



Analytical methods repository

The AppsLab free on-line knowledge base from Thermo Scientific is designed to help laboratories reduce time spent on researching, developing, or implementing analytical methods. According to the company, the repository contains more than 800 validated methods for a range of common workflows.

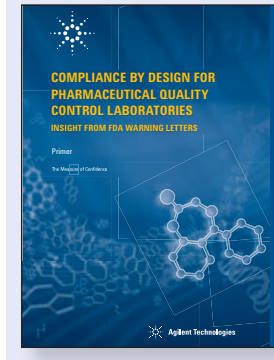
Thermo Fisher Scientific,
San Jose, CA.
www.thermoscientific.com/AppsLab



Biopharmaceutical lexicon guide

Waters' 2015 publication, *A Guide to the Biopharmaceutical Lexicon,* from *BioPharm International* is available for download or by mail. According to the company, the 70-page edition of the guide contains definitions for more than 1000 terms, and dozens of suggested references.

Waters Corporation,
Milford, MA.
www.waters.com/lexicon



Compliance primer

Agilent's primer for pharmaceutical quality control laboratories provides an overview of citations from FDA warning letters. According to the company, the primer, written by Dr. Ludwig Huber, contains advice on appropriate improvements with respect to observed deviations.

Agilent Technologies, Inc.,
Santa Clara, CA.
www.agilent.com/chem/compliance-primer

CALENDAR

16–21 May 2015
39th International Symposium on Capillary Chromatography and 12th GCxGC Symposium
Fort Worth, TX
www.isccgcxgc2015.com

20–21 May 2015
3rd Nordic Symposium on Convergence Chromatography
Gothenburg, Sweden
www.waters.com/waters/eventInstance.htm?locale=en_SE&eiid=134826144

31 May–4 June 2015
63rd ASMS Conference on Mass Spectrometry & Allied Topics
St. Louis, MO
www.asms.org/conferences/annual-conference/annual-conference-homepage

2–4 June 2015
Microbiology Week
Philadelphia, PA
www.cbinet.com/conference/PI15056#.VPSASUtNv1o

21–25 June 2015
42nd International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2015)
Geneva, Switzerland
www.hplc2015-geneva.org

22–24 June 2015
IFCC-EFLM European Congress of Clinical Chemistry and Laboratory Medicine
Paris, France
www.paris2015.org

28 June–1 July 2015
RDPA 2015: Recent Developments in Pharmaceutical Analysis
Perugia, Italy
rdpa2015.chimfarm.unipg.it

30 June–3 July 2015
21st International Symposium on Separation Sciences (ISSS 2015)
Ljubljana, Slovenia
www.isss2015.si

22–24 July 2015
SFC 2015 — 9th International Conference on Packed Column SFC
Philadelphia, PA
www.greenchemistrygroup.org/Registration.html

26–29 July 2015
PREP 2015: 28th International Symposium on Preparative and Process Chromatography
Philadelphia, PA
www.prepsymposium.org

17–19 August 2015
Conference on Small Molecule Science (CoSMos 2015)
San Diego, CA
www.cosmoscience.org/blog/2015-cosmos/

19–20 August 2015
International Conference on Environmental Forensics
Putrajaya, Malaysia
www.ienforce.upm.edu.my/index.php

23–28 August 2015
35th International Symposium on Halogenated Persistent Organic Pollutants (DIOXIN2015)
São Paulo, Brazil
www.dioxin2015.org

28–30 September 2015
4th International Conference on Forensic Research & Technology
Atlanta, GA
forensicresearch.conferenceseries.com

5–7 October 2015
International Symposium on Applied Chemistry (ISAC)
Bandung, Indonesia
situs.opi.lipi.go.id/isac2015/

16–20 October 2015
Native Mass Spectrometry-Based Structural Biology
Pacific Grove, CA
www.asms.org/conferences/asilomar-conference/asilomar-conference-homepage

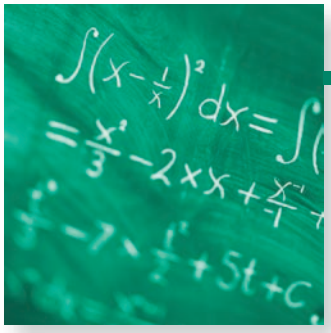
20–21 October 2015
Gulf Coast Conference 2015
Houston, TX
www.gulfcoastconference.com/index.cfm

3–6 November 2015
7th International Symposium on Recent Advances in Food Analysis
Prague, Czech Republic
www.rafa2015.eu/RAFA_2015_1st_flyer.pdf

12–15 November 2015
GPSS: Gazi Pharma Symposium Series
Antalya, Turkey
www.gpss2015.org

15–17 November 2015
12th International Symposium on Persistent and Toxic Substances (ISPTS)
Riverside, CA
pts2015.ucr.edu

18–19 November 2015
Petroleum, Refining, and Environmental Monitoring Technologies (PEFTEC 2015)
Antwerp, Belgium
www.peftec.com



SHORT COURSES

GC

19–21 May 2015
Modern Practice of Gas Chromatography
West Chester, PA
www.cfdv.org/course/modern-practice-gas-chromatography-0

10–13 June 2015
Forensic GC
Chicago, IL
www.forensicchromatography.com/forensic-chromatography/

8–11 September 2015
Gas Chromatography: Fundamentals, Troubleshooting, and Method Development
Chicago, IL
proed.acs.org/course-catalog/courses/gas-chromatography-fundamentals-troubleshooting-and-method-development

HPLC

1–4 September 2015
High Performance Liquid Chromatography: Fundamentals, Troubleshooting, and Method Development
Chicago, IL
proed.acs.org/course-catalog/courses/high-performance-liquid-chromatography-fundamentals-troubleshooting-and-method-development/

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

AD INDEX

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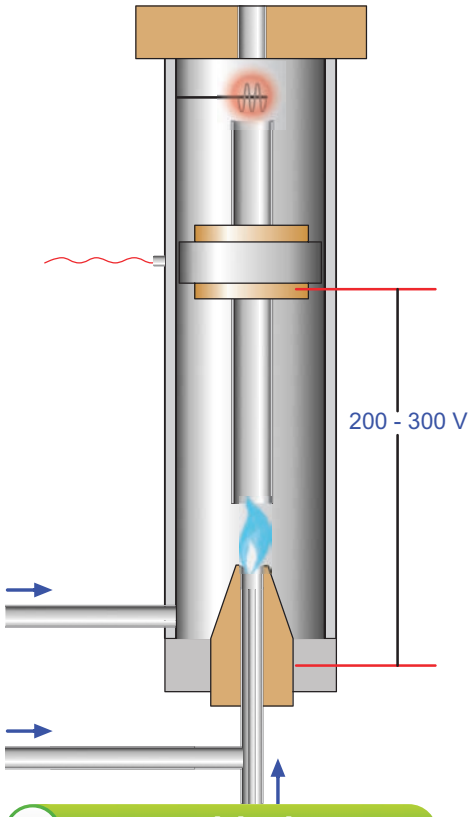

GC Troubleshooter

HPLC Troubleshooter

THE ESSENTIALS

Excerpts from LCGC's professional development platform, CHROMacademy.com

Choosing the Correct Sample Preparation Technique

There are many reasons for choosing to implement sample preparation, but most fall under the three general aims shown in Table I.

In many cases the type of sample preparation used will depend on the analyte type, the sample matrix, or the instrument technique that will be used for the analysis. In other words, various industries and applications are suited to certain types of sample preparation — some of these have been outlined in Figure 1, alongside the relative specificity of some common sample preparation techniques.

All sample preparation adds the potential for error to the analytical protocol, so choosing less-complicated techniques will help to reduce inherent error. The use of preconcentration with an inert gas stream for volatile samples or extracts can add significant error to the method.

Knowing the target analyte concentration will help to inform the correct choice of sample preparation technique. The attainable limit of detection (LOD) or limit of quantitation (LOQ) will be directly related to the specificity of the sample preparation technique. It is this parameter that needs to be optimized, as far as is possible, to achieve the cleanest extracts and the lowest limits of detection.

Specificity in sample preparation is optimized when any differences in the chemistry or physicochemical properties of the analyte molecules and the sample matrix are exploited. Strategic ways in which selectivity might be optimized include

- Adjusting aqueous sample pH to ensure ionizable analytes are ion-suppressed (nonionized) and will partition more efficiently into the organic phase in liquid–liquid extraction (LLE).
- Increasing the salt concentration of the aqueous phase to drive polar analytes into the organic phase during LLE.
- Using prebuffered supports in support assisted LLE to ensure ionizable analytes are preferentially retained either within the organic or aqueous solvent systems (ionized form in the aqueous sample initially applied or nonionized in the organic extraction solvent).
- Optimize the salt concentration and sorbent type to remove as much of the sample matrix as possible while leaving target analytes behind in dispersive solid-phase extraction (SPE).
- Use the most selective sorbent in SPE based on the chemistry of the analytes, and include an electrostatic element to the retention mechanism wherever possible to optimize the strength of retention and therefore specificity. Use the strongest wash solvent and weakest elution solvent strength (this can be in terms of the organic:aqueous solvent ratio, pH or buffer ion type, or concentration) to optimize the selectivity of the process.
- For stir-bar sorptive extraction (SBSE), solid-phase microextraction (SPME), and single drop microextraction (SDME), choose the surface chemistry carefully to be as closely matched to the analyte of interest as possible and optimize factors such as pH, temperature, and headspace versus immersed sampling to improve the specificity of the extraction.

For more information on applications and considerations such as extraction method capacity and practical implementation see the CHROMacademy tutorial.

More Online:

Get the full tutorial at
www.CHROMacademy.com/Essentials
(free until June 20)

Table I: Aims of sample preparation

Aim of Sample Preparation	Notes
Sample cleanup	Increase instrument maintenance intervals and improve column lifetime. Deconvolute chromatogram improving resolution.
Increase analyte concentration	Achieve sensitivity requirements of the analytical method.
Solvent or matrix switching	Swap solvents to achieve compatibility with the analytical technique (generally aqueous solvents for high performance liquid chromatography [HPLC] and organic solvents for gas chromatography [GC]).
Chemical derivatization of the analyte	Chemically alters the analyte to improve detectability and peak shape.

Technique	Relative Specificity	Method of Separation	Typical Applications
Sample preconcentration (blow down) Filtration Centrifugation Protein precipitation Soxhlet extraction Liquid–liquid extraction (LLE)	Least specific	Physical (volatility) Physical (size) Physical (mass) Chemical (denaturation) Physical (volatility) Chemical (hydrophobicity, polarity, extent of ionization)	Tablet assay Bioanalysis of whole blood Bioanalysis of plasma Dioxins from stack emissions Pesticides from river water
Support-assisted liquid–liquid extraction (SALL)			Metabolites from urine
Dispersive SPE (dSPE, QuEChERS) Single-drop microextraction (SDME) Stir-bar sorptive extraction (SBSE) Solid-phase microextraction (SPME) Solid-phase extraction (SPE) • Hydrophobic mechanism			Pesticides from whole foods Pesticide residue analysis Flavors from beverages Off odors in wine and beer
• Electrostatic mechanisms			Hydrophobic analytes from river water Ionizable drugs from wastewater
• Mixed-mode mechanisms	Most specific		Acid, basic, and neutral drugs from blood plasma

Figure 1: Description of the various commonly used sample preparation techniques. Note that the list of typical applications is for illustrative purposes only and is not meant to be exhaustive.

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GC–MS has long been applied to pesticide residue analysis and still remains a key technique for the more non-polar pesticides analyzed. Quadrupole based mass spectrometry, both single and triple quadrupole, dominates this analysis, with the latter becoming the system of choice due to the high selectivity and subsequent sensitivity. Even with selective systems, to work efficiently within the required quality criteria for target compounds for a wide range of sample types is challenging, especially in routine settings.

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Series Moderator

Richard Fussell, Ph.D.
Global Vertical Marketing Manager, Food and Beverage, Chromatography & Mass Spectrometry Division
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Presenter:

Dominic Roberts
Senior Applications Specialist GC-MS/MS, Chromatography and Mass Spectrometry
Thermo Fisher Scientific

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- The application of GC–MS technology for both screening and accurate quantification of pesticides in food matrices
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