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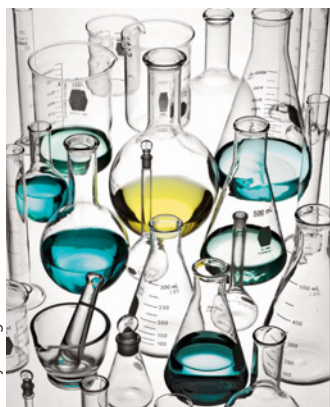
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ODS column with C18+anion+cation ligands

ODS + Anion Ligand

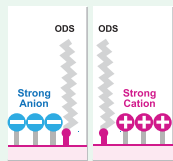


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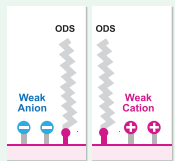


Reversed Phase
+
Anion Exchange
+
Cation Exchange
+
Normal Phase

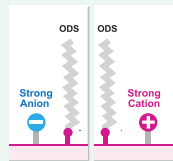
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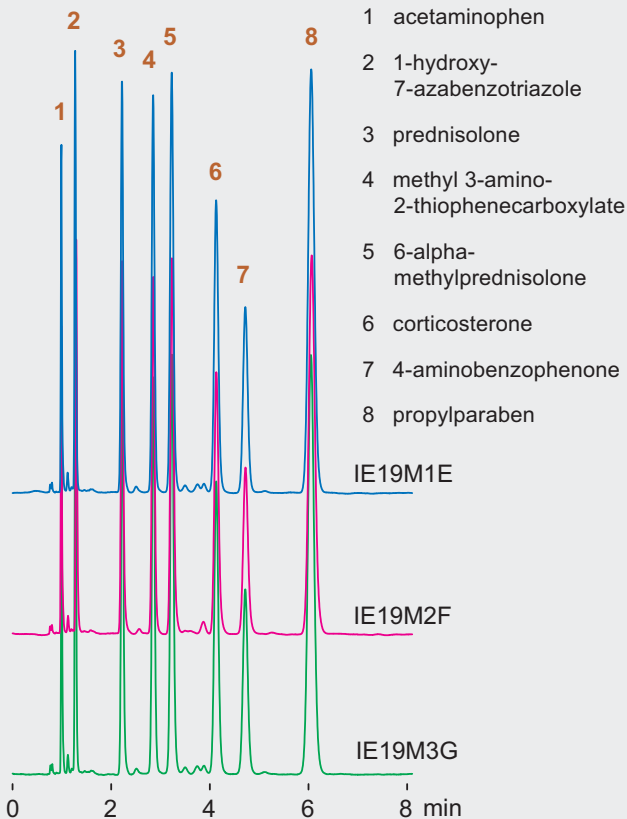
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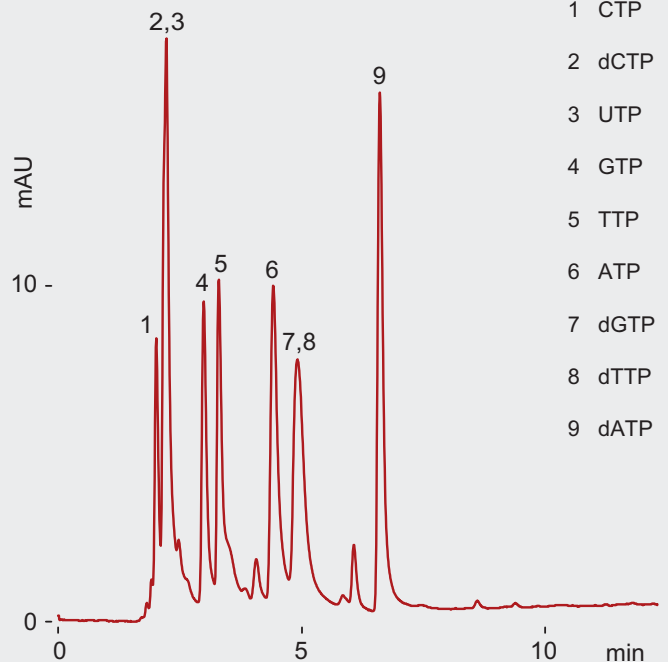
- LC-MS compatible without using ion-pair reagents
- Scherzo's unique blend of anion, cation, and reverse phase ligands allows for the recognition of compounds with slight differences in structure, such as UTP and TTP, which differ only in a methyl group
- Scherzo SM-C18 shows excellent batch to batch reproducibility between different lots

Outstanding batch-to-batch reproducibility



Scherzo SM-C18, 75 x 4.6 mm
10 mM ammonium acetate /acetonitrile = 65 /35
1 mL/min, 37 deg.C, 254 nm

Separation of nucleotide triphosphates



Scherzo SM-C18, 150 x 3 mm
A: 50mM HCOONH₄ : 50mM NH₄OH = 9 : 1, pH 8.6
B: (100mM HCOONH₄ : 100mM NH₄OH = 9 : 1, pH 8.6) /acetonitrile = 80 /20
0-50 %B (0-10min)
0.4 mL/min (8MPa), 37 deg.C, 260 nm, 1 uL (0.1ug, water)

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HPLC/UHPLC & LC-MS

For those who use HPLC, UHPLC, and LC-MS, keeping up with the latest trends and techniques is essential.

What trends have emerged in liquid chromatography-mass spectrometry (LC-MS) recently?

Classon: One of the newest trends is a move toward making LC-MS more compatible with ultrahigh-pressure liquid chromatography (UHPLC). As chromatography has become faster and chromatographic peaks have narrowed considerably, there is a greater need to perform quantitation on peaks that are less than 1-s wide, and perform quantitative and qualitative analysis on fast peaks in a single 1-min run. For high throughput labs such as clinical labs and contract research organizations (CROs), the lab that runs the most samples per day makes the most profit. It will be commonplace to see drug screens or pesticide screens with hundreds of target analytes performed in 1–2 min. Even as pharmaceutical companies are producing fewer new drug applications (NDAs), they still need to get better drugs into clinical trials faster. So, just about anyone with more than a few samples is trying to get faster results. LC-MS should not be the limiting step in getting a drug to mar-

ket. So, we should expect to see faster instruments that can acquire and process more data in a shorter period of time. As everything gets faster, there will be more of a need to handle overlapping peaks, perhaps hundreds at a time, so instead of LC-MS operating at a few thousand data points per second, we will see instruments producing hundreds of thousands of data points per second or more.

Taylor: The trend toward more complete solutions and kits has continued, particularly for many of the applications in food, environmental, and forensics markets. These kits provide users with a time saving approach to implementing analytical methods by providing databases and libraries of compounds of target analytes. These kits also include custom LC-MS methods optimized by application experts at customer sites.

Volatile buffers are just that, volatile. What techniques would you recommend to ensure mobile-phase integrity during chromatographic run sequences when using UHPLC?

Chakrabarti: Mobile-phase integrity during chromatographic run sequences when using UHPLC depends on the composition of the buffer, quality, and percentage of salt and organic in the buffer, how the user is storing and maintaining the buffer, the method of degassing it, and so on, particularly in the case of routine analysis where a bulk amount is made and used continuously. Nowadays most labs are

buying ready-made buffers as a 10× stock and then diluting it afresh when needed. Using a closed container to prevent evaporation, an antibacterial agent, filtering the sample, and checking the pH of the buffer from time to time to monitor aerial oxidation in case of pH-dependent analysis all help in maintaining the integrity of the mobile phase. If UHPLC is used for hyphenated techniques such as LC-MS, then compatibility of mobile phase for LC-MS analysis also should be considered.

Layne: Use mobile-phase reservoirs with screw caps with minimal openings to allow for evaporation — openings in caps will accommodate mobile-phase tubing with a sinker, but only need the one line — block off all other openings to prevent evaporative loss of volatile buffers. Make fresh mobile phase daily and filter; this serves two purposes: no worries about evaporative loss of volatile buffer, and aqueous buffers are excellent media for microbial growth, which can wreak havoc on UHPLC systems and columns.

Parmar: Use high-quality solvents and modifiers. Extra care needs to be taken to eliminate particulates including staying away from solubility limits and filtering.

This technology forum was edited for length and clarity. For the full set of questions and answers please visit:

chromatographyonline.com/BuyersGuideLC2012. ■

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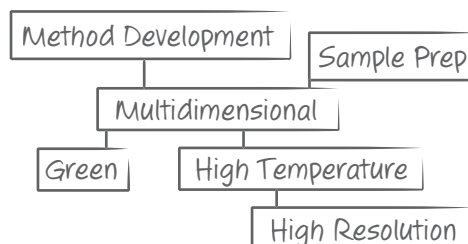
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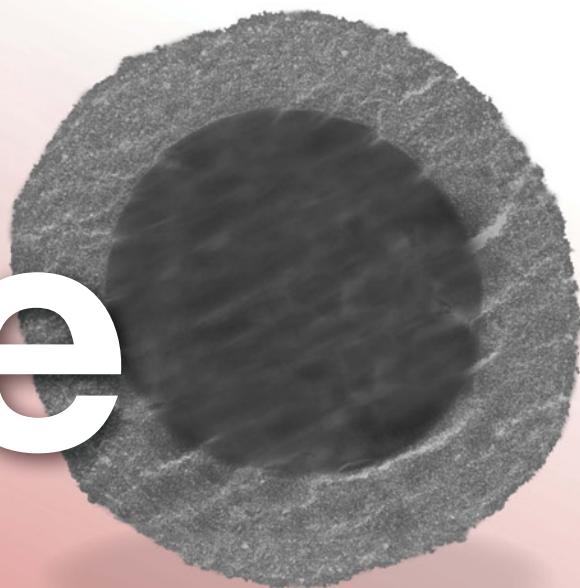
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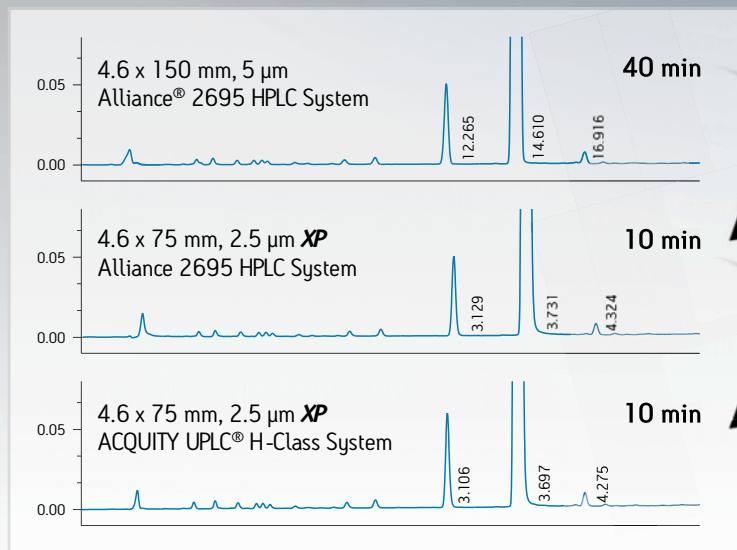
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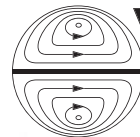
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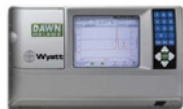


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Gas Chromatography

Gas chromatography (GC) continues to be a workhorse technique, but users now face choices of upgrading their systems to newer time-saving technologies or possibly switching to hydrogen carrier gas.

Gas tanks for GC are rapidly becoming a thing of the past. How can GC users be encouraged to switch to hydrogen carrier gas?

Phillips: GC users will not need any encouragement to switch from helium to hydrogen as a carrier gas. The drivers for this change are a combination of scientific and economic factors, with some politics thrown in. Hydrogen can provide faster analysis times and more efficient separations on GC columns. If we use the van Deemter equations and graph those, hydrogen provides a wider and faster optimal flow rate range for chromatographic separations. If we add the expense of purchasing helium tanks, not to mention the production through distillation of natural gas and shipping, it is no wonder that laboratories are looking for other carrier gases. Of course, nitrogen could be used. It is plentiful. However, there is a problem because it is just not as efficient in GC. Separations are longer and peaks are wider, not a good combination. Hydrogen is also plentiful. The easiest way to get to it is with a hydrogen generator, which uses water. These will just use a relatively small amount of electricity to provide the hydrogen gas. So, the movement to hydrogen as a carrier gas for GC has been happening for some time. It is increasing more rapidly because of economic (and partially political) concerns.

There are some things to keep in mind when moving to hydrogen generators though. The gas must be dry, high purity, and of sufficient pressure to perform the analyses. Moisture is still not good for GC columns. The generator used must also produce enough pressure to cover the flows that are needed by the GC even at high temperatures or high injector split flows. The chromatographic results may be a little different than what you are used to with helium as a carrier gas. Some adjustments to the GC or GC–mass spectrometry (MS) method may be needed and should be expected. The GC–MS system should be built with hydrogen carrier gas in mind. This will prevent a lot of problems from occurring when tuning the system.

Taylor: We see two issues with switching to hydrogen as a GC carrier gas, the first being safety and the second being method redevelopment. As helium becomes more expensive and less available, the demand for alternatives will increase. Hydrogen will be the natural replacement as long as the issues mentioned above are addressed.

There are already some safety measures in place. Most hydrogen generators have a pressure sensor that will force the shutdown of hydrogen production in the case of a leak. Most modern GC systems have a similar feature in their electronic carrier gas delivery hardware. New GC models will have hydrogen sensors in the oven that will have the ability to sense the presence of small leaks and to stop carrier flow, thus adding an additional level of safety assurance.

In terms of method development, hydrogen has a higher optimum linear velocity than helium, and the minimum in the van Deemter curve is much broader. This means that it will provide

optimal separations at a broader range of linear velocities. It will also mean that users will be able to potentially speed up their analyses without compromising peak resolution.

Modern GC software will include helium-to-hydrogen method translation, allowing users to quickly make the switch. They can either maintain their current peak retention times by not adjusting the helium liner velocity from their previous method or further optimize the chromatography by speeding up the linear velocity and shorten their analyses.

van den Heuvel: Helium is considered one of the best carrier gases for GC, but it is only available from gas tanks and is running short. On the one hand, helium is nonrenewable once vented in the atmosphere and is a by-product from natural gas, which has a declining availability. On the other hand, the applicability of helium is increasing; MRI scanners are the most helium demanding instrument today. Both trends will lead to an increased market price for helium and, thus, an increased operation cost for the GC. Alternatively, nitrogen can be used, however its use as carrier gas requires much higher operating pressures compared to helium or hydrogen. The benefit of hydrogen is that it can be produced rather easily, ensuring high purity at a relative low price. The only disadvantage of hydrogen is its flammability. Risk minimization is achieved by local production of small amounts and relative low pressures, and the ability of GC systems to shut off the gas supply as soon as gas supply settings are not met.

This technology forum was edited for length and clarity. To see the full set of questions and answers please visit: chromatographyonline.com/BuyersGuideGC2012. ■

Participants

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Hans van den Heuvel
Bruker Chemical & Applied Markets



Together, we can eliminate helium, reduce costs and improve lab safety.

The global shortage of helium has caused prices to increase significantly. Many GC users are considering switching to hydrogen as a carrier gas. Hydrogen provides many advantages over helium including, higher resolution, shorter run times, longer column life, and cost savings. Many instrument manufacturers now provide resources to make switching carrier gas easier.

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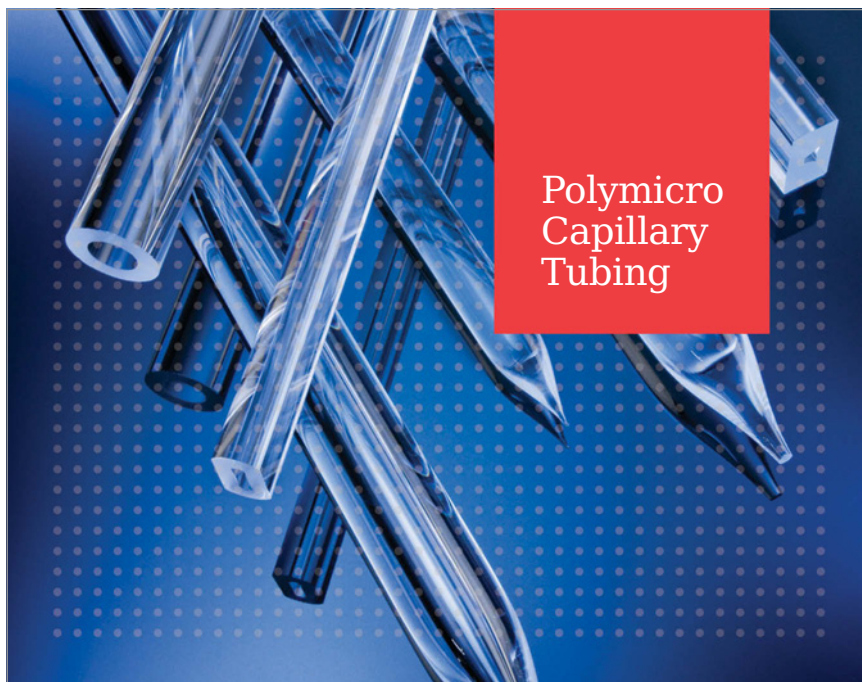
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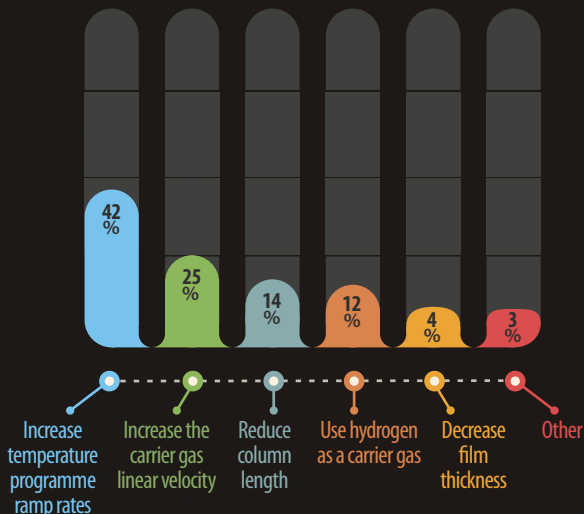
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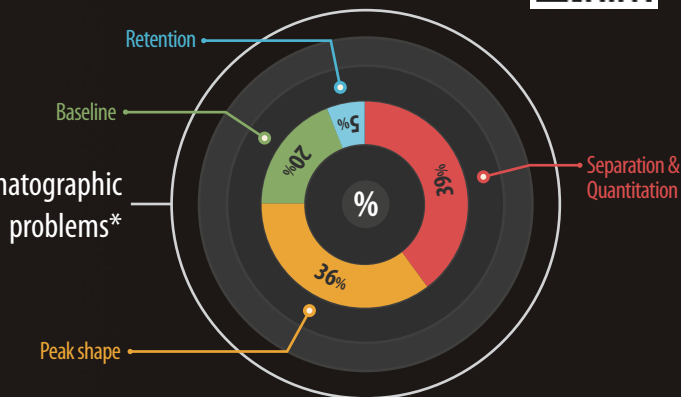
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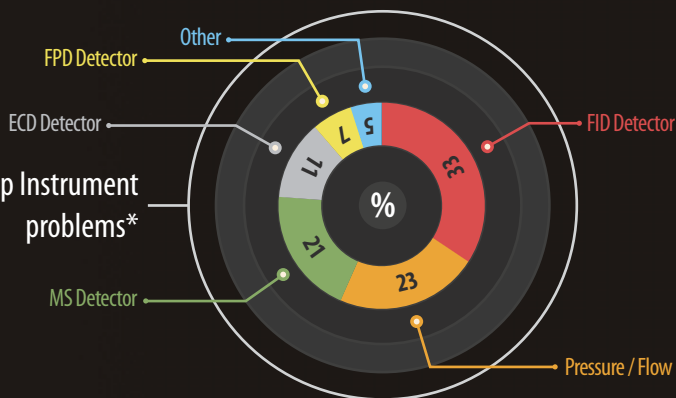
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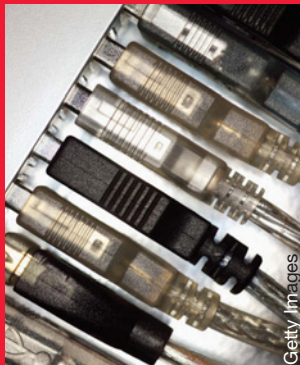
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Sample Preparation

The areas in which sample preparation is being used have expanded, and the techniques required for optimum results continue to evolve.

In which area is sample preparation growing the fastest?

Coyer: Obviously, dilute and shoot has seen the widest growth. Liquid chromatography–tandem mass spectrometry (LC–MS–MS) has changed the complexion of sample preparation. And 96-well plates are also becoming more popular with the autoextractors, ultrahigh-pressure liquid chromatography (UHPLC) systems, and the ease of configuring LC autosamplers with 96-well plates.

Richter: Based on our experience, we believe that food safety is one of the fastest growing areas for sample preparation in general. There are many countries that want to export more food. More regulations are being put in place because of recent issues with food safety and health concerns. Food samples can also represent very complex matrices that can be difficult to extract. We all want our food supply to be safe. All of these and other factors combine to put pressure on laboratories that prepare and analyze food samples, so they are constantly looking for better and faster ways to get the job done. However, bioanalysis and clinical work flows continue to be a major user of solid-phase extraction (SPE), and will continue to be so for the foreseeable future. These high-throughput environ-

ments will continue to come under pressure from FDA guidelines, which will concentrate on reproducibility of their assays. As a result, new levels of reproducibility will be demanded from their SPE protocols, and this is where new SPE technological breakthroughs will play a fundamental role.

Stevens and Robarge: We believe both the food testing and environmental analysis areas are where sample preparation is growing the fastest. While many contaminants have been known for decades, the formation and presence of certain chemicals and compounds in foods and the environment have been discovered recently. These are the so-called emerging contaminants, for example, acrylamides, 3-mono-chloro propane-1,3-diol (3MCPD), 4-hydroxynonenal (4-HNE), and pharmaceutical and personal care products (PPCP). Both food and environmental samples can be very complex, and sometimes require volume reduction and concentration. In most cases, to achieve the levels of detection determined by regulatory agencies, sample preparation must be implemented. Therefore, sample preparation is essential for the sensitive and reproducible analysis of contaminants. One really cannot ignore the value of sample preparation; eliminating sample preparation from the analysis process can lead to maintenance and sensitivity issues within your analysis system.

How difficult is it to find sample prep technology that is safe, fast, environmentally friendly, and cost effective?

Coyer: Environmentally friendly is lower solvent use. Single-step SPE, dilute and shoot, and smaller sample volumes all lend to that. As far as safety goes, smaller sample volumes minimize

that as an issue. Resins that do not need conditioning help the situation as well.

Richter: The answer to this can be dependent on the application area being considered. If one is considering environmental or food samples it is relatively easy to find safe, fast, environmentally friendly, and cost-efficient technology for sample preparation. However, there are other application areas or sample matrices for which sample preparation technology that satisfies these criteria might not be readily available.

Stevens and Robarge: It is not difficult at all. There are many sample preparation techniques that fit these requirements. When determining the environmental footprint associated with sample preparation, take into account not only the solvents used, but also the disposables, refuse, and process used to clean reusable containers and glassware. QuEChERS (quick, easy, cheap, effective, rugged, and safe) and supported liquid extraction (SLE) are two sample preparation techniques that minimize the amount of organic solvent used relative to liquid–liquid extraction (LLE) or the modified Luke method. QuEChERS and SLE can drastically reduce solvent waste and are therefore environmentally friendly. In addition, both of these sample prep methods are very quick, safe, and cost-effective. Other sample preparation techniques like solid-phase microextraction (SPME) and on-line SPE reuse the extracting phase, drastically reducing disposal, time, and cost. SPME, which is a solventless sample preparation technique, is very environmentally friendly. Therefore, there are numerous sample preparation techniques that can still be implemented in one's sample workflow that are safe, fast, environmentally friendly and cost effective . . . there's really no excuse. ■

Participants

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UCT

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Thermo Fisher Scientific

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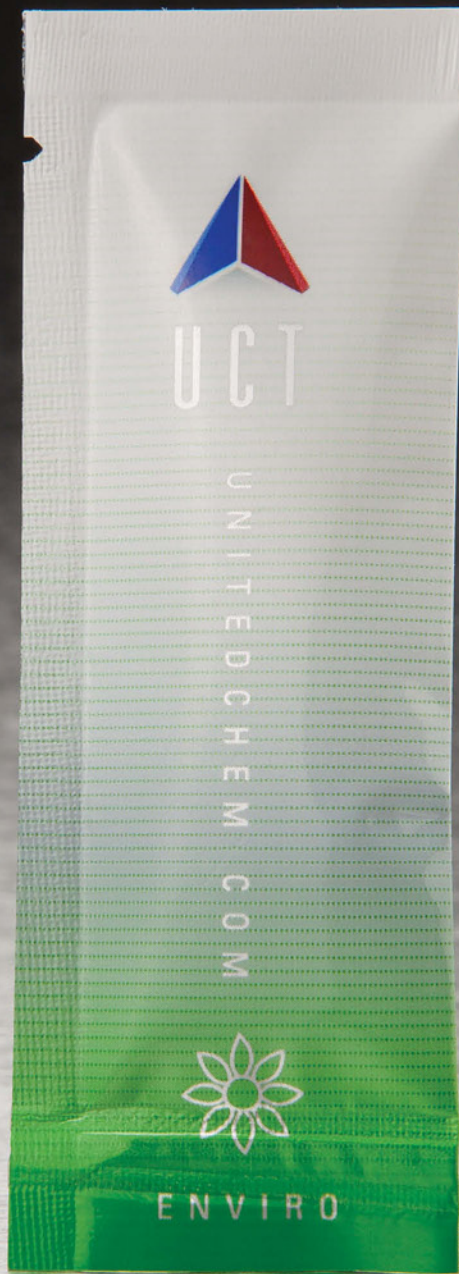
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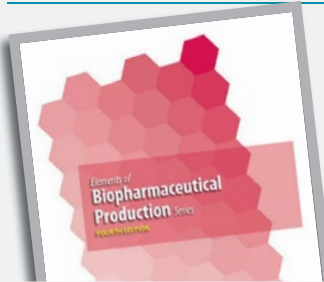
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