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LAB TECHNOLOGY BUYER'S REPORT

INSIGHTS ON LIQUID CHROMATOGRAPHY SYSTEMS

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TECHNOLOGY BUYER'S REPORT

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All articles by **Angelo DePalma**

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THE MORE IT MATURES, THE BETTER IT GETS

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Unlike many other mature analytical technologies, HPLC seems to have entered a period of intense innovation and competition. During the last decade we have witnessed the debut and evolution of ultra high-performance LC (UHPLC), widespread adoption of mass detectors, and greater appreciation for rapid methods based on novel or revived column technologies.

Dr. Stefan Schuette, Sr. Marketing Director for Liquid Phase Separations at Agilent Technologies (Waldbronn, Germany), identifies the major trends in HPLC as:

- UHPLC via both totally porous sub-2µ particle columns and superficially porous columns
- Multi-method/walk-up or "open access" systems that allow method-switching
- Bio-inert, metal-free UHPLC systems for sensitive biopharmaceuticals
- 2D LC
- Automated, online sample and standards preparation
- The revival of supercritical fluid chromatography (SFC) for both chiral and achiral applications
- "Green" LC achieved through solvent-sparing small-diameter columns' supercritical mobile phases
- Mobile HPLC by which instruments are brought to the sample

Yet the features most users look for in HPLC have not substantially changed, according to Schuette. "Users continue to seek performance, productivity in terms of speed and cost per analysis, data quality, and backward/forward compatibility. In other words, faster, cheaper, better."

Analytical labs were at one time interested primarily in reducing the cost per sample. No longer, says Simon Robinson, HPLC Product Manager at Shimadzu (Columbia, MD), who sums up the overriding trend in HPLC instrumentation as: "Speed, speed, speed." Companies are most concerned, he says, with getting through large numbers of samples, generating data quickly, and effectively managing time and human and physical resources.

For years the major technological trends in HPLC were instrument-related, says April DeAtley, Product Planning Manager for LC at PerkinElmer (Waltham, MA) and to a significant degree they still are. "Everyone was con-

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cerned with who had the highest pressure systems, or the latest bells and whistles." Today, at least from PerkinElmer's perspective, usability has moved to the top, or close to the top, of manufacturers' priorities.

"Current users are less experienced in chromatography, but more comfortable around 'technology,' than were previous generations," Ms. DeAtley says. "That is why HPLC, and analytical chemistry itself, are trending toward touch technology where users interact less with the instrument itself and more with the computer." She predicts that in the future methods will be "dialed up" rather than developed and tweaked by the user, similar to the way users operate consumer electronics. We have not quite reached the point of iPod-like control, "but within a few years we will definitely see instruments that are 'applicated,' where users select a method and go."

> "The overriding trend in HPLC instrumentation [is]: 'Speed, speed, speed.'"

LC Injector | 1290 Infinity
Agilent | www.agilent.com

Frank Steiner, Ph.D., Manager for Small Molecule Solutions at Thermo Fisher Scientific (Munich, Germany), concurs that system developers need to design UHPLC for accessibility and ease of use. "Customers don't want to have to undergo a lot of training to exploit these instruments fully," he says.

One could ask if the apparent decline in analytical skill may be in part caused by the growing reliance on advanced interfaces, or is it the other way around—users simply don't need to know as much about the inner workings of their instruments?

"It's probably a bit of both," Ms. DeAtley says. "In school we learn manual calculations in class and never carry them out again. To some extent the experience factor has declined because users just don't need to know or do some of these things anymore."

UPPING THE ANTE

Compared with HPLC, UHPLC provides improved resolution, sensitivity, and throughput through the use of sub-2µ particles, typically packed in 2.1mm or 1.0mm ID (internal diameter) columns. UHPLC is characterized by very high back-pressures resulting from the mobile phase passing through ultra-small particle beds packed tightly in long, thin columns. These factors result in a reduction in resolved peak elution volume—provided the instrument is optimized to reduce unnecessary volumes along the sample's flow path.

UHPLC speeds separations, which generates more data per unit time than conventional HPLC. Acquiring, managing, and reporting that data demands a faster data acquisition rate and chromatography data systems with scalable capabilities.

The success of sub-2µ UHPLC has been a vindication for Waters' (Milford, MA) strategy to introduce fast, low-volume, very high pressure LC. Waters shipped the first such instrument, trademarked UPLC© (Ultra Performance Liquid Chromatography), in 2004, and all major vendors have followed suit. Generic sub-2µ particle LC is referred to as UHPLC or u-HPLC.

UHPLC shows the highest uptake in QC labs, where the majority of installed LCs are located. Contributing to this ongoing momentum will be changes to USP Chromatography <621> and equivalent chapters in other pharmacopoeias, which allow greater flexibility for changing column dimensions and/or particle size.

Method development becomes less time-consuming with the improved workflows that faster LC provides, and this has led to enhanced software for rapid method screening, statistical analysis, and the rapid, iterative generation of robust methods. "This process was not feasible before UHPLC," observes Elizabeth Hodgdon, Senior Product Manager at Waters (Milford, MA).

Stefan Schuette defines UHPLC in terms of column technology rather than system, pressure, or detector speed. "We refer to all types of LC employing stationary phase particle sizes of less than 2µ as UHPLC, for example

columns packed with 1.7µ particles, with a 3mm or 4mm internal diameter and a length of 15mm, which can achieve very rapid runs at high resolution," but at pressures normally associated with conventional HPLC.

Next to the ability to withstand very high pressures, the single most critical design feature for UHPLC systems, according to experts, is minimizing dispersion or band spreading. Dispersion arises from volumetric factors within the instrument that cause peaks to elute in larger volumes, thus eroding the high resolving power of small-particle columns. For this reason, vendors trim extraneous volumes when designing instruments.

➤ *I-Class System | ACQUITY UPLC® Waters | www.waters.com*

system to the requirements of the new column will result in no gain, or even worse performance than with conventional HPLC columns," notes Bill Letter, Sr. Scientist and Consultant at Chiralizer Services (Newtown, PA).

The need for speed, as exemplified by UHPLC systems, brings other benefits that are now taken for granted, such as reduced usage of mobile phase and smaller sample injections. But these present their own challenges, for example the precision of injection volumes, carryover, system maintenance, column selection, and temperature stability. These issues caused a significant backlash against UHPLC during the mid-2000s and persist to this day.

"Usability has moved to the top, or close to the top, of manufacturers' priorities."

➤ *UHPLC System | ExpressHT™-Ultra System Eksigent | www.eksigent.com*

Users need to consider and control factors affecting band spreading as well. Dispersion is tolerable in relatively large-volume HPLC systems, but in UHPLC, tubing IDs and lengths should be minimized, and care should be taken in making connections.

For example, 5µ particle columns have void volumes of about 3mL, which provides acceptable resolution for HPLC. But void volumes for most sub-2µ columns are just 10 percent as large. "Failure to optimize the

▲ *UHPLC RRHD 300SB-C18 1.8 µm columns | ZORBAX Agilent | www.agilent.com*

WHAT IT TAKES TO RUN HPLC/UHPLC

Although by no means the only operational issue involved in HPLC, cost of ownership is something everyone considers and ultimately comes to grips with. Cost of ownership for an HPLC system is complicated by several factors, for example operational robustness, minimal repairs and downtime, and rapid diagnostics for maintenance and repair.

It makes sense that cost of ownership increases with instrument complexity and usage. To manage operating costs, vendors vie to deliver systems that require less frequent maintenance and are easier to service. "If we look at throughput and quality of data, the cost of ownership has dropped," says Shimadzu's Simon Robinson. The key is ensuring that whether a set of runs is conducted in 20 minutes or 50 hours, cost per sample is roughly equivalent.

HPLC operators, regardless of technical level, require some degree of training in instrument use and maintenance, and in keeping systems and columns in top condition. For example, sample prep and mobile phase quality became serious issues for 5µ particle systems, and they are absolutely critical below 2µ. Vendors do their part to ensure that users are properly trained, but this becomes increasingly difficult after the unit ships.

> "Cost of ownership for an HPLC system is complicated by several factors."

Users of both UHPLC and HPLC can perform routine checks on valves, seals, pistons, and general wear, with the higher-pressure instruments being somewhat more difficult and prone to problems. Manufacturers have designed instruments for accessibility to critical components, but most large companies, particularly in regulated industries, service their instruments through their vendor or a third-party maintenance organization.

Because higher pressures stress instrument components, UHPLC systems operating above about 600 bar (about 9000 psi) require more frequent maintenance, usually at higher cost, than do standard HPLC systems. Additionally, the smaller column particles and narrower internal diameter lines require that users filter all samples and mobile phase components to prevent clogging. "In my experience, most users often skip these extra steps, resulting in more service calls," notes Chiralizer Services' Bill Letter. Additionally, the sub-2µ particles used in UHPLC columns contain more fines that can clog column frits and system lines.

Despite higher operating costs for UHPLC vs. HPLC, the former may return its investment rather quickly through improved throughput and reduced solvent usage, depending on workflows. "But users must run genuine UHPLC methods to attain these benefits," cautions Elizabeth Hodgdon of Waters.

▲ *Core-Shell HPLC/UHPLC Columns | Kinetex®*
Phenomenex | www.phenomenex.com

Scientific's Accucore brand, and PerkinElmer's Brownlee columns. Waters discovered the SPP phenomenon during the 1960s but abandoned the idea "in favor of fully porous particles and the benefits they bring," according to a Waters web page.

SPPs consist of a solid silica core surrounded by a porous shell. Conventional particles are porous throughout. The basis for SPPs' enhanced performance is more rapid mass transfer through the particle bed, which occurs at the expense of binding capacity.

Most SPP sizes are in the 2.6µ range. Phenomenex also manufactures a sub-2µ SPP-based column that Philip J. Koerner, Ph.D., Senior Technical Manager, says provides the best of both technologies. "Superficially porous particles provide the efficiency of sub-2µ without the immediate need to purchase a new UHPLC system." SPPs enhance the capabilities of HPLC, while stressing the instrument less due to lower-than-UHPLC pressure operation.

"Most large companies, particularly in regulated industries, service their instruments through their vendor or a third-party maintenance organization."

Planned maintenance is particularly important for long-term UHPLC performance. The quality and cleanliness of parts, design, manufacturing, and packaging all play into maximizing performance in instruments operating at up to 1000 bar (15,000 psi). At these pressures, fluid path components can fail or malfunction if part tolerances are not rigorously controlled.

Due to UHPLC's higher sensitivity, service must follow exacting protocols that limit the introduction of contaminants such as chemicals, oils, or particulates. "What may be an acceptable level of particulate matter in HPLC is not acceptable for UHPLC," Ms. Hodgdon explains.

COLUMNS: KEY VALUE DRIVERS

Column technology has been a rich area for HPLC R&D. As reasonably expensive consumables, columns are a significant factor in LC operating costs and performance—in other words, key value drivers.

Superficially porous particles (SPPs) represent a true breakthrough in column technology. SPPs go by different names depending on the vendor. The Kinetex® line of what Phenomenex (Torrance, CA) calls "core shell" particle columns competes with Agilent's Poroshell, Advanced Materials Technology's (Wilmington, DE) Fused-Core™ products, Thermo

As Waters points out, SPPs will not provide significant performance enhancements unless one addresses the system contributions to band broadening. Dr. Koerner agrees, but notes that "this is done relatively easily."

▲ *HPLC Columns | Accucore™*
Thermo Fisher Scientific | www.thermo.com

Manufacturers provide kits for minimizing band broadening, or dispersion, through modifications to connecting tubing or UV detector cells.

UHPLC columns, with their minimized volumes and high theoretical plate numbers, are demanding with respect to system effects on peak broadening. "Your separation can be destroyed between the column and de-

tector, or within the detector itself," notes Frank Steiner of Thermo Fisher. "This requires adapting detector flow cell volume to a minimum of one-tenth the expected peak volume. Otherwise you cannot exploit the column's theoretical resolution." Other trouble spots include tubing, pre-heaters, column thermostat, and autosampler.

Almost any standard HPLC system with a pressure maximum of less than 600 bar (8500 to 10,000 psi) can utilize columns packed with 2.1µ-diameter to 5µ-diameter particles. These provide rapid resolution analysis, often with no further system modification, according to Bill Letter. "Columns containing 2.1µ to sub-5µ particles in smaller formats can often provide many of the same benefits of UHPLC systems, at lower pressures, and still reduce throughput times and increase solvent savings."

▲ *HPLC Columns | XSelect Waters | www.waters.com*

These columns, he says, are "more reliable and rugged" than sub-2µ columns since the latter are more difficult to pack uniformly and reproducibly. Furthermore, standard HPLC systems provide adequate means to adjust the internal delay volume sufficiently to accommodate narrow columns with smaller internal void volumes. "These savings have been available for decades to anyone who wishes to utilize them."

Despite the value of SPP columns, and the fact that even UHPLC manufacturers are eager to sell them, their eventual effect on the course of HPLC/UHPLC is subject to debate. Tom Jupille, President of LC Resources (Walnut Creek, CA), who is not unfriendly to older HPLC technology and likes SPPs, says the extent to which they extend the life of conventional HPLC systems will not be dramatic. "I'm not certain they will be a game changer."

PerkinElmer's April DeAtley disagrees. "As column technologies catch up, I believe more people will choose lower-pressure systems simply due to their convenience and lower maintenance. Most users will probably own a UHPLC pump, and they will prefer to obtain results using column technology in conjunction with their instrument, while avoiding issues encountered at ultra-high pressures."

BEYOND THE COMMON COLUMN

Specialty columns are an oft-overlooked avenue to higher performance. How many readers have heard of Jordi Labs, Sepax Technologies, Nacalai Tesque, Dikma, Chromenta, or Sepax Technologies? In addition to manufacturing conventional columns (often at significant discounts compared with better-known manufacturers), these small column companies specialize in stationary phases that can only be described as exotic. For example Jordi's (Bellingham, MA) stationary phases are based on

> "Columns are a significant factor in LC operating costs and performance."

polymer particles, not silica, and are claimed to be stable from pH 0 to 14 and 100 percent aqueous to 100 percent organic mobile phases. Nacalai Tesque's (Tokyo, Japan) columns include cholesteryl chemistry, pyrenylethyl (which separates based on pi-pi electron interactions), and pyrenylpropyl (for fullerenes).

"Not too many people know these chemistries even exist," says Columnex President Ken Tseng, Ph.D. Columnex sells columns from 13 boutique manufacturers.

Also in the niche category are mixed-mode columns that combine ion exchange with reverse phase or HILIC. Large manufacturers like Agilent, Dionex, and GE Healthcare offer mixed-mode columns, as do several firms in the Columnex stable, particularly Sielc (Prospect Heights, IL) and Intakt (Tokyo, Japan).

Dr. Tseng explains that mixed-mode HPLC has always been around but not recognized as such. "People tried to isolate one mode or the other." For example, depending on the pH, an amino column works through either the –NH2 functional group (cation exchanger) or the –NH3+ (anion exchanger). "Now that we have better control over stationary phases, we can put those modes back in and make them work for us."

Evaluating alternatives to the hard upgrade from HPLC to UHPLC has become an interesting and instructive exercise, but not all options are considered equal, and not every solution is for everyone.

SURVEY SAYS: ARE YOU IN THE MARKET FOR AN HPLC COLUMN?

High-performance liquid chromatography (HPLC) columns are considered the "heart" of the instrument used to transport the analyte and the mobile phase and provide the environment in which separation is achieved.

HPLC columns are stainless steel tubes generally 30 to 300 mm in length with internal diameters of 2 to 5 mm, internally coated with a stationary phase. Plastic or glass may also be used, but steel supplies the highest mechanical strength. Conventional columns are filled with porous particles coated with a polymeric material that interacts with the injected sample. In contrast to gas chromatography columns, HPLC has a true stationary phase: column "chemistries" are bonded tightly to the base material and do not bleed off.

Reverse-phase and normal-phase chromatography separation methods are based on polarity. Reverse-phase columns separate analytes based on their hydrophobicity, with the more hydrophobic compounds being retained longer on the column. Separations based on charge utilize ion-exchange chromatography.

More recently, many companies have introduced hydrophilic interaction chromatography (HILIC) columns for analysis of polar analytes. Reverse-phase is the most popular method among survey respondents.

Reverse phase	**26%**
Normal phase	**17%**
Ion exchange	**13%**
Ion chromatography	**9%**
Hydrophilic interaction (HILIC)	**7%**
Chiral	**7%**
Gel permeation (GPC)	**7%**
Gel filtration (GFC)	**5%**
Affinity	**5%**
Ion exclusion	**3%**

As particles decrease in size from conventional 10-, 7-, 5-, and 3-micron diameters, back pressure build-up increases exponentially. Thus, a 3-micron column is about twice as efficient as a 5-micron column, but attendant pressures are three times as high. While additional separation efficiencies are possible by further reducing particle size (to below 2 μ), more expensive hardware is required to handle extremely high pressures. Such systems are referred to as UHPLC, a significant trend in LC column technology.

Analytical scale	**54%**
Narrow-bore (1 to 2 mm diameter)	**20%**
Large ID (>10 mm diameter)	**11%**
Capillary (<0.3 mm diameter)	**11%**
Chip-level (microfluidic)	**2%**
Other	**2%**

Of the three common chain lengths, C4 is generally used for proteins and C18 is used to capture peptides or small molecules. Peptides are smaller and need longer chain lengths to be captured, so C8 and C18 are appropriate.

In reverse-phase HPLC, the stationary phase is often a silica-based packing covalently bonded with hydrophobic alkyl chains of C8 (octyl group) or C18 (octadecyl group), though there are many variations on this theme.

C18	**20%**
Silica	**19%**
C8	**12%**
C18 (polar end-capped)	**10%**
Anion exchange	**9%**
Phenyl	**8%**
Cation exchange	**8%**
Cyano	**6%**
Amino	**6%**
Biphenyl	**3%**
C4	**3%**
PFP	**3%**
Other	**1%**

It's a challenge is to pick the right column to analyze the right sample correctly. Several factors, including particle and pore sizes, can affect separation efficiency, inertness, resolution, solvent usage and more.

Lab professionals need to know that the column will elute the analyte peaks at the same time, every time. Along with elution times, getting good peak shapes—sharp, narrow, symmetrical peaks—is important for various applications.

	Important
Technical performance of HPLC columns	**98%**
Shorter run times/Increased throughput	**94%**
Lot-to-lot reproducibility of HPLC columns	**93%**
Ruggedness/durability of HPLC columns	**92%**
Lower operating costs (reduce solvent use and waste)	**87%**
Reputation of column manufacturer	**75%**
Purchase price of column	**72%**
Breadth of HPLC column offering (selectivity)	**70%**
Applications support	**62%**
Method validation/compliance support	**57%**
Covers/lids	**73%**
Safety and health features	**70%**

In an effort to be economical, many users are moving to smaller columns, packed with smaller particles (sub-2 μ) because they use less solvent. However, slower, longer columns that offer better resolution are sometimes preferred to separate sample components in extremely complex samples.

Since high-pressure instruments work with both conventional and UHPLC columns, users might prefer an instrument with greater capability even if they don't yet need its higher-end performance. Some vendors have discontinued older HPLC systems in favor of those that can handle both conventional columns and ones that generate very high back pressures.

What can we do to reduce analysis time and increase resolution?	**21%**
How do I determine which column(s) makes the most sense for my lab?	**19%**
What should I consider when selecting a column(s) for faster throughput and higher resolution?	**18%**
What are the considerations for selecting a column(s) to achieve longer column life/retention?	**18%**
Are newer models of LC columns significantly better for developing faster LC methods?	**15%**
What type(s) of column(s) could be used to reduce solvent use and waste?	**12%**
Other	**1%**

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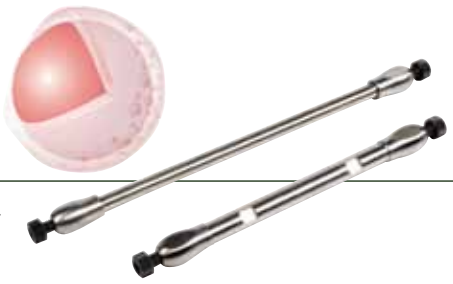

➤ *Core-Shell HPLC/UHPLC Columns | Aeris Phenomenex | www.phenomenex.com*

TO UPGRADE OR NOT TO UPGRADE?

Nowhere is the controversy over instrument upgrades more animated than in HPLC, or more correctly, in the debate over switching from HPLC to UHPLC. Before delving into that controversy, it is useful to note that more users than ever view upgradeability almost as a legal right. It ensures that ordinary operators will be able to swap out a column, replace a 10 Hz detector with an 80 Hz model, or substitute a higher-pressure pump as needed, without calling the service organization.

One aspect of upgradeability is backward/forward compatibility, which Agilent's Stefan Schuette refers to as "investment protection that provides a stepwise upgrade path." Instruments with these capabilities are attractive for obvious reasons, provided the price tag for added functionality is reasonable.

On the HPLC/UHPLC question, it is safe to say that opinions are quite diverse.

LC Resources' Tom Jupille has long believed that plenty of life remains in older LC technology. He nevertheless appreciates UHPLC for what it is, the benefits it provides, and its inevitability.

"It's hard to make a business case for rushing to upgrade from HPLC, but when you do replace your equipment, that instrument you replace it with will probably have UHPLC capability."

"You must consider how the changes will benefit you from a cost and time basis, including time for revalidating methods."

UHPLC's future is perhaps best explained demographically. According to surveys, the prevalence of 10µ columns is asymptotically approaching zero, and 5µ technology peaked around five years ago; 3µ technology is still increasing but will begin to wane over the next few years, while sub-2µ technology is increasing inexorably. "The shifts are not revolutionary, but are evolutionary toward smaller particles," Mr. Jupille says.

Adoption of UHPLC, moreover, follows historical trends in that end-user complaints about sub-2µ technology sound eerily similar to grumbles regarding 3µ particle columns. "We're hearing the same issues," Jupille tells *Lab Manager Magazine*, "that the new systems are more difficult to use, the columns clog, and solvents must be filtered."

Another skeptic, Bill Letter of Chiralizer, follows a similar script: "In my opinion, many chromatographers do not need to change or upgrade to a new UHPLC system. The high operating pressures are not for everyone."

However, he admits that if analysis speed is critical and sub-2µ columns are available with the appropriate reproducibility and ruggedness, then users should consider UHPLC systems. "As with everything in life, you must consider how the changes will benefit you from a cost and time basis, including time for revalidating methods."

▲ *Liquid Chromatograph | EASY-nLC 1000*
Thermo Fisher Scientific | www.thermo.com

Vendors of course want to sell new instruments, but to their eternal credit LC companies take great pride in their established instrument base and their ability to service decades-old installations. As PerkinElmer's April DeAtley observes, "65 percent of the market is still running and purchasing HPLC. We still have to support this group."

For Shimadzu's Simon Robinson, the most compelling business case for upgrading to UHPLC is compressed method development times. "And the benefits flow downstream from there, to data quality and throughput. But if it works, don't change it! We have installed instruments that are 20 years old and the customer is still very happy with them."

Method transfer is perhaps co-equal with acquisition cost and high-pressure operation as a justification for *not* upgrading to UHPLC. "Chromatographers want the latest and greatest, and they also want to execute legacy methods on these systems and obtain the same result," observes Stefan Schuette. Yet strange things happen when an uncorrected HPLC method is attempted on a UHPLC. "Peaks elute at different times, resolution may change, peaks appear and disappear or switch positions."

"Strange things happen when an uncorrected HPLC method is attempted on a UHPLC."

The easiest fix is to use an HPLC column on the UHPLC system, but here the smaller system volumes will skew results. This has led some to suggest adding volume to the UHPLC system to approximate that of the older technique. Dr. Schuette likens this "old column on a new instrument" approach to "pulling a caravan with a Ferrari. It works, but it's not smart."

Several manufacturers offer strategies that compensate for differences in column size and system volumes by adjusting injection volumes, flow rate, and time program. These approaches, based on sub-2µ particles at modest (less than 600 bar or about 9000 psi) pressures, comprise what one vendor refers to as "UHPLC-style" analysis. Agilent appears to be the only vendor that offers an internal method, Intelligent System Emulation, that automatically reproduces HPLC methods on a modern UHPLC platform.

▲ *HPLC Liquid Flowmeter | FlowCal 5000*
Tovatech | www.tovatech.com

The upgrade imperative is ultimately a business decision, according to Tom Jupille. "If you can reduce run times from 15 minutes to 3 minutes, that's a genuine productivity boost." Yet run times are only part of the picture. Users must still log samples in, prepare samples, and process the data. In some workflows the actual chromatographic run is negligible, in terms of time, compared with re-equilibration, sample and solvent preparation, and data processing.

One gets the strong impression, from talking to industry experts, that LC manufacturers truly want to provide no more than what customers need. But the story doesn't always end there. "You can tell a customer, based on their methods and workflows, that they really don't need a UHPLC system," says April DeAtley, "but the customer wants what the customer wants, and of course that is what you sell them."

▲ *Triple Quad LC/MS | 6490*
Agilent Technologies | www.agilent.com

THE RIGHT CHOICE FOR

UHPLC SYSTEMS

by Rachel Muenz

UHPLC – ultra high performance liquid chromatography – systems have been around since 2003 and continue to grow. These systems, while having better performance than traditional HPLC, have more limited surface chemistries than HPLC. Surface chemistries give columns their selectivity. Recently, UHPLC has been catching up in this area, however, and many more options are available today than in the past. When deciding if UHPLC rather than HPLC is right for your lab, it's important to note that these systems can mean savings over the long term through increased productivity and throughput. The slower flow rates of UHPLC systems also mean they consume much lower amounts of costly solvents as well as providing higher quality data to users. As for methods, transferring HPLC methods to UHPLC or developing UHPLC methods is much easier nowadays through the kits most vendors offer, which are made up of a conventional column and a sub-2-micron column of the same chemistry. To find out the latest offerings in UHPLC systems, and to find out which is right for you, contacting your vendor is usually a great first step.

APPLICATIONS

- Quality control in pharmaceuticals
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THERMO FISHER EASY-NLC 1000

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Thermo Scientific's EASY-nLC 1000 liquid chromatograph provides effortless split-free, nano-flow UHPLC performance up to 1000 bar with minimal investment. The instrument's dual in-line flow sensors before mixing give uncompromising gradient precision. This machine can also allow users to identify more peptides and increase productivity with higher pressure without compromising reliability and robustness.

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- Easy to use
- Can integrate with Thermo's complete range of MS systems
- Significantly shortens analytical cycles

A Q&A WITH SELECT HPLC EXPERT END-USERS

OUR EXPERTS:

Mary Snider, *Chemist,* Catalent Pharma Solutions, Somerset, NJ

Prof. Richmond Sarpong, Ph.D., *Department of Chemistry,* University of California, Berkeley

David Norris, *President,* David Norris Analytical, Kent, UK

Liang Zhao, Ph.D., *Research Analyst,* Johns Hopkins University, Baltimore, MD

Ravi Orugunty, Ph.D., *Senior Scientist,* Worldwide Clinical Trials, King of Prussia, PA

Barry E. Boyes, Ph.D., *Director of Bioscience R&D,* Advanced Materials Technologies, Wilmington, DE

PROVIDE SOME GENERAL IMPRESSIONS ABOUT YOUR LC INSTRUMENTATION

Mary Snider: I like Agilent's ease of changing lamps and the self diagnostics.

Richmond Sarpong: HPLC is indispensible for unearthing minor impurities that we cannot observe using other techniques such as NMR. We also employ it for conducting enantiomeric excess assays.

David Norris: HPLC is tough and reliable and it works for most of our samples. UHPLC is probably great for analysis of pesticides in drinking water, but for us it is lacking for difficult separations.

Ravi Orugunty: The Shimadzu Nexera pumps are robust; the Autosampler SIL 30 uses a unique method of sample delivery using two different valves – a low pressure valve that is part of the injection port, and a high pressure valve that contains the sample loop and the entire UHPLC setup. The system has extensive capability for washing the external surface and internal surface of the needle to remove carryover, as well as port washing. This system allows us to study carryover in an analytical method and take steps to eliminate it.

"There definitely is a learning curve with UPLC systems."

UHPLC provides faster sample run times, improved sensitivity, and lower solvent use. But there are tradeoffs in terms of the amount of material we can load onto the column, plus band broadening. Solving these issues requires deep knowledge of the system configuration, dead volumes, pump and mixing efficiencies, and the correct choice of column. In other words, there definitely is a learning curve with UPLC systems.

Barry E. Boyes: I like what works with high reliability and performance and low maintenance. Most modern HPLC systems show low extra-column band dispersion, which saves me the trouble of refitting them for our applications. This becomes critical for LC/MS work, as we are always fighting interface issues for post-column effects. A few microliters of bad design can turn a very useful separation into blobs!

I also like that current pumps, injectors and detectors come from the factory with appropriate fittings and tubing, and work pretty much without issues with the software and data handling capabilities.

Almost all newer HPLCs and UHPLC systems have the ability to monitor themselves for maintenance and repair. This is important, particularly with instruments that track by module identity, since components get moved around between instruments.

Q: How can LC workflows be improved?

A: **Mary Snider:** We have a lot of problems running Chemstation with Chemstore data management software. For example, Chemstation does not link the column to the method unless you are using the ID tags, and requires us to calculate signal to noise manually. I would also like Agilent to improve the autosamplers on its 1200 and 1260 models and improve reliability. Our well plate autosampler has required repair twice in the last year.

Richmond Sarpong: The improvements I envision are not necessarily with the instruments. I would like to see columns that are more robust and do not fail easily after a short period of use. Furthermore, instruments that are easier to clean and dismantle the parts easily (i.e., modular) to allow routine trouble-shooting would be great. I would also welcome more user-friendly manual processing.

David Norris: I would like to see software that makes manual data processing easy again. Auto-integration, which all the software packages seem to assume, is not for every user and method.

Liang Zhao: If any aspect needs improving it would be columns. I would like to see new column types with better selectivity for specific analytes, for example glycopeptides and other post-translational modifications of peptides. I would also like Internet connectivity so I can monitor instrument performance remotely.

"Almost all newer HPLCs and UHPLC systems have the ability to monitor themselves for maintenance and repair."

Ravi Orugunty: In a CRO business model the ultimate driver is the number of samples that we analyze per unit time. I personally would like to see multiplexing UHPLC systems that are easily deployed for use in a GLP environment. In addition, almost all HPLC systems that I have seen in the market have very limited ability to store the pressure and other operational data that is provided by the various sensors on the HPLC systems. This data is critical as it provides valuable insight into column durability and catastrophic failure events. It also helps in trouble-shooting.

Barry E. Boyes: The sticker price of LC systems has gotten out of hand. LCs should not be $100,000 instruments! I recently helped out some students on "optimizing" their instruments, and the tubing, fittings and minor accessories costs were thousands of dollars. A small piece of tubing with fittings should not cost $150, nor should the cost of accessories, tubing, and fittings be punitive.

Reconfiguring all of the various late model LC instruments to move between applications is more complex than it needs to be. Changing injection sample volume ranges should not require pulling the manual every time, then following a 23-step process.

The software systems for automated data collection and analysis are still too convoluted and specialized for many normal humans. Across platforms, I have limited problems learning new menu structures, but teaching it to a novice is unreasonably painful. Most people are PC conversant, and I wonder how isolated from reality some of the software engineers have become.

By the way, I have had two very bad high pressure LC systems in my lab that were launched before their time. There is another very popular system that I won't have in my lab as the plumbing and internal design is so unrealistically complex. My rule of thumb is that if I can't fix 90 percent of the likely problems, then I don't want it.

"HPLC makes more sense for large-volume injections, whereas for low-concentration samples (for example proteomics) one should probably select UHPLC."

Q: Would you consider switching to or expanding your capabilities through acquiring UHPLC instrumentation, or switching more of your methods to UHPLC?

A: **Mary Snider:** Because we're a contract lab and employ validated methods, we cannot use UHPLC. If we were given a validated method to transfer that required a UHPLC, we would purchase one.

Richmond Sarpong: We have not had any exposure to UHPLC, so it is an unknown quantity for us. We have been happy with the capabilities of HPLC for our purposes.

David Norris: We must use both UHPLC and HPLC as many of our methods cannot convert from HPLC to UHPLC. We got our first 1200 Series UHPLC three years ago. It does an acceptable job for easy assays, but for impurity assays we often must overload the column to see minor

impurities, and peak shapes suffer badly for the on-scale impurities. In our hands, UHPLC lacks reproducibility. We have found storage stability studies to be quite difficult, as the instrument does not hold retention time over long periods if other assays are conducted in the interim. In other words, storage stability studies conducted at 3-6-9-12 and 24 months require dedicated columns, which is not the case with conventional reverse-phase columns. UHPLC columns have such low capacity that we are unlikely to see impurities we look for without using highly sensitive detectors such as MS-MS-MS.

Liang Zhao: If shorter run times and cost made sense for our lab, we would consider switching to UHPLC. HPLC makes more sense for large-volume injections, whereas for low-concentration samples (for example proteomics) one should probably select UHPLC.

Ravi Orugunty: This is a tricky question. The answer depends on the analyte, how it separates from the matrix, and the presence of interferences. If the analyte(s) show good behavior under classic reverse phase conditions, then it is reasonable to expect that the method would translate to a UHPLC system. Analytes that are chromatographed using ion exchange or HILIC I tend not to try on UHPLC, though in the future we may have UHPLC columns for these modes as well.

Q: For which samples/workflows do you use HPLC and UHPLC?

A: **David Norris:** We originally used UHPLC for storage stability and HPLC for chemical profiling, but we are beginning to convert our standard methods back to HPLC

Ravi Orugunty: Workflows that involve very clean extracts (such as liquid liquid extraction or solid phase extraction of analytes from a biological matrix that provide a reasonably clean sample can usually be incorporated into UHPLC methods. If the extracts for the analysis are very dirty, I would usually go with HPLC conditions. This usually avoids catastrophic column failures, unusually large retention time shifts due to secondary chromatography of matrix components, such as phospholipids, on the column.

Barry E. Boyes: UHLPC instruments are employed for higher flow rate separations, and when coupling several columns in series to take advantage of 100,000 plate-plus separations for very complex samples. I personally find the popularization of UHPLC as an acronym kind of irritating. I suppose that this is better than saying "low band dispersion contemporary HPLC instruments with extended pressure tolerance." Oh well, that's marketing in action! Since the instruments have gotten so pricey, I tend to reconfigure the instruments for various workflows (small molecules, large, complex, simple), rather than dedicate an instrument for a particular need.

SURVEY SAYS: ARE YOU IN THE MARKET FOR AN HPLC SYSTEM?

The origins of high-performance liquid chromatography (HPLC) date back to the invention of chromatography in the early 20th century, then to partition and paper chromatography in the 1940s, and finally to the introduction of liquid chromatography in the early 1960s. Shortly thereafter, the need for better resolution and high-speed analyses of non-volatile samples led to the development of HPLC.

In HPLC, a pump pushes the mobile phase with the sample through the column. This is similar to classical LC, in which the mobile phase and analyte are driven through the column by gravity alone. HPLC separation media are typically very dense, which creates a high back pressure, usually measured in the hundreds to thousands of psi. This allows for higher resolution and faster separation on columns of shorter length when compared to open column chromatography.

HPLC is, for many scientists, an essential piece of apparatus for the separation, identification, purification and quantification of various compounds. Users of HPLC work in a variety of fields including biomedical research, and the cosmetics, energy, and food industries. UHPLC is gaining rapid acceptance for its performance, speed of analysis and low consumption of eluent.

Types of HPLC systems respondents are using.

Analytical HPLC	53%
UHPLC	14%
Ion chromatograph	12%
Preparative HPLC	11%
GPC	5%
FPLC/Bio	3%
Don't know	1%
Other	1%

Many LC systems incorporate a detector that analyzes different fractions. As a researcher, your detector needs to be matched to identify your compounds of interest. UV/Vis, a common detector, comes in a few different types, including diode array. Other popular detectors include ultraviolet, fluorescence, mass spectroscopy (MS) and refractive index (RI).

UV/Vis	22%
Ultraviolet (UV)	18%
Fluorescent	14%
Mass spectroscopy (MS)	12%
Refractive index (RI)	11%
Conductivity	7%
Light scattering (LS)	6%
Electrochemical	4%
Other	6%

Separations based on polarity utilize reverse-phase chromatography (most popular) and normal phase. The reverse-phase columns separate analytes based on their hydrophobicity, with the more hydrophobic compounds being retained longer on the column. Separations based on charge utilize ion exchange chromatography. Recently, many companies have introduced hydrophilic interaction chromatography (HILIC) columns for analysis of polar analytes.

Reverse phase	26%
Normal phase	19%
Ion exchange	9%
Ion chromatography	8%
Ultra high-performance (UHPLC)	8%
Hydrophilic interaction (HILIC)	7%
Size exclusion (SEC)	7%
Chiral	6%
Gel permeation (GPC)	4%
Affinity	3%
Gel filtration (GFC)	2%
Ion exclusion	2%

Manufacturers typically offer HPLC components as a system; however, there can be a mixing of the system components from various vendors. Today, 95 percent or more of HPLC systems from major manufacturers ship with autosamplers, a testament to the improved reliability and reproducibility of autosampler hardware and controls. No wonder it's the most commonly used HPLC component among survey respondents.

Autosampler	24%
Column heater	20%
Data system	19%
Degasser	19%
Automated valve	9%
Fraction collector	7%
Solvent recycler	2%

Average annual budget for supplies, accessories, maintenance, repairs, etc.

$0 to $5,000	20%
$5,000 to $10,000	22%
$10,000 to $15,000	17%
$15,000+	32%
Don't know	9%

The need for speed and quality of data among researchers has led to faster, more efficient HPLC separations. Some purchasers prefer an integrated chromatography system in which all components are supplied in a single unit. Other purchasers prefer a modular system, in which individual components are purchased separately.

More researchers are identifying operating and acquisition cost, service, support and training as key factors in their decision-making process. Vendors recommend, when looking for a new HPLC instrument, to look a little into the future (about six to 12 months ahead) to figure out exactly what you need. For a smaller lab with lower throughput, cheaper machines working at low pressure may suffice, depending on the application. All vendors offer a range of different machines, and there are instruments that fall somewhere between HPLC and UHPLC that are sometimes 20 percent cheaper.

Top ten factors/features	
Accuracy	100%
Low maintenance	100%
Quality of data	100%
Sensitivity	100%
Service, support, training	100%
Availability of supplies/accessories	99%
Price	99%
Precision and accurate flow rates	99%
Resolution	99%
Ease of use	96%

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