

This month's "Sample Prep Perspectives" examines results from a survey of chromatography users conducted by LCGC and observes trends in sample preparation for chromatography. Ron Majors compares these results with those from two other surveys.

Ronald E. Majors
Sample Prep Perspectives Editor

Trends in Sample Preparation

Although many of the chromatographic instrumental techniques have matured and automation is commonplace, sample preparation still is considered to be slow, labor-intensive, and even a bottleneck in laboratory processes. Some high-throughput laboratories, particularly in the pharmaceutical industry, take advantage of the latest automation equipment to process hundreds and sometimes thousands of samples a day, but many laboratories use techniques based on age-old methodologies with some degree of miniaturization or low levels of automation.

LCGC occasionally surveys readers about topics ranging from instrumentation preferences to high performance liquid (HPLC) and gas chromatography (GC) column information. To obtain a current profile of sample preparation practices, LCGC sent a survey at the end of 2001 (1) to a statistically representative group of 1000 readers. This survey generated a total of 152 responses for a 15.2% response rate, somewhat lower than our previous sample preparation surveys in 1992 (2) and 1996 (3), but still sufficient to cite some general observations and make some broad-based conclusions about current sample preparation trends compared with earlier surveys. Some results from a more directed survey of 320 solid-phase extraction (SPE) users in the pharmaceutical industry helps to solidify the conclusions for the direction of this popular sample preparation technique (4). This "Sample Prep Perspectives" installment summarizes the survey results and observes trends in sample preparation for chromatography.

Survey Audience and Sample Types

Figure 1 provides a breakdown of the fields of work of survey respondents, and Figure 2 shows the typical sample types that they encounter. Most of these samples encountered require some pretreatment before injection into a chromatograph. In agreement with the 1996 survey (3), pharmaceu-

ticals represent the largest single sample category, followed closely by organic chemicals. The sample preparation of animal and biological tissues and physiological fluids in which sample types fall into the current research work in genomics and proteomics has increased since the last survey. If I had added the total percentage of respondents analyzing every form of environmental sample (wastewater, soil, drinking water, sediment, and air), then this category would have been the leading sample type.

When such a broad audience is surveyed, the physical state of samples varies widely. When asked about the state of matter of the samples encountered, 78% of the respondents reported that they deal with liquids, 67% with solids, 21% with gases, and 20% with gels or semisolids, which indicates that many of them deal with more than one type of sample.

Sample Preparation Techniques

Figure 3 depicts the 39 most popular sample preparation techniques used in order of their popularity. Some of the techniques — such as dialysis, cell disruption, ultrafiltration, and lyophilization — are market dependent and most often used for preparing biological samples. Other techniques, such as large-volume trace enrichment, are applied mainly in environmental laboratories for trace analysis of organic compounds in water. Usually several sample pretreatment steps are necessary between sampling and the placement of the prepared sample into the chromatograph. Most of the techniques depicted in Figure 3 were identified in earlier reviews, with only minor adjustments in their relative usage (2,3).

Sonication was the only technique that showed a large increase in use, which may be indicative of the large environmental presence in this survey and the fact that it was a write-in vote in the last survey. Ultrasonic extraction is a popular technique for preparing environmental solids (soil, sludge, and sediment), and it is recom-

mended in U.S. Environmental Protection Agency (EPA) Method SW-846 (5).

Relative to the 1996 survey, some newer extraction technologies have appeared. Pressurized-fluid extraction (6), sometimes called accelerated-solvent extraction, was used by 5.8% of the respondents, and microwave-assisted extraction (7) was used by approximately one-half of that number. Matrix solid-phase dispersion, covered in an

earlier "Sample Prep Perspectives" column (8), has attracted some attention. All of these techniques are useful for the preparation of solid samples. Microwave and pressurized-fluid extractions are particularly fast relative to the classic solid extraction approaches, with extraction times frequently less than 30 min. These two techniques have made inroads on the use of supercritical fluid extraction (SFE), which

has dropped quite dramatically since the last survey.

When respondents were asked which techniques of all 39 from Figure 3 they planned to use, SPE, microwave-assisted extraction, pressurized-fluid extraction, and, surprisingly, SFE came out on top, which indicates that these techniques could have bright futures.

Analysts seldom are lucky enough to be able to inject samples with no pretreatment. Table I shows that more than 50% of the respondents use two or more techniques per sample. In fact, 5% of the respondents used seven or more individual techniques, which could indicate the complexity of their samples. My estimation of the weighted average indicated that the average respondent used approximately three techniques per sample analyzed. For example, the use of liquid-liquid extraction and evaporation to dryness of the collected analyte solution followed by reconstitution in a suitable solvent for injection into the chromatograph could be a typical sequence for a liquid sample. In other cases, a single step such as the dilute-and-shoot mode of a pharmaceutical liquid formulation could be all that is necessary. The fewer sample preparation techniques before injection, the better. A clear and optimized sample preparation strategy is necessary to minimize the number of steps because each step represents additional time and a potential source of error (9).

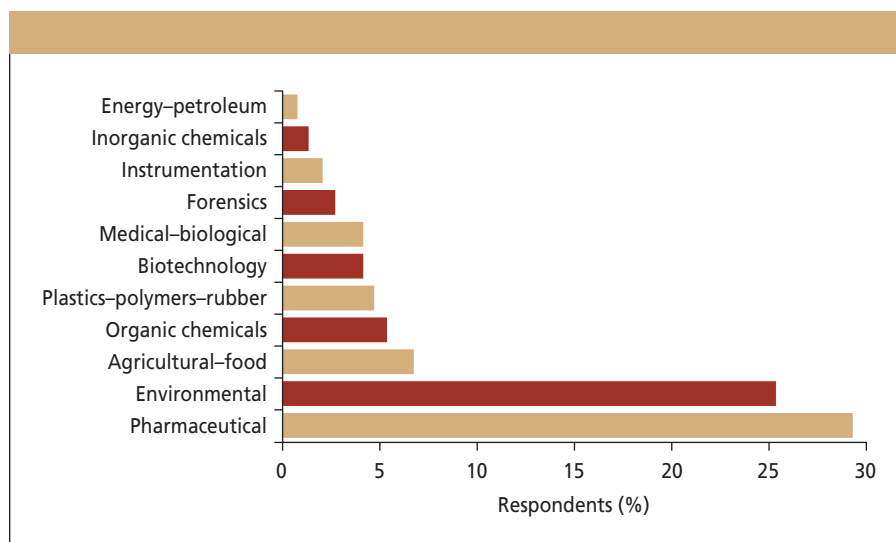


Figure 1: Fields of work of survey respondents (normalized).

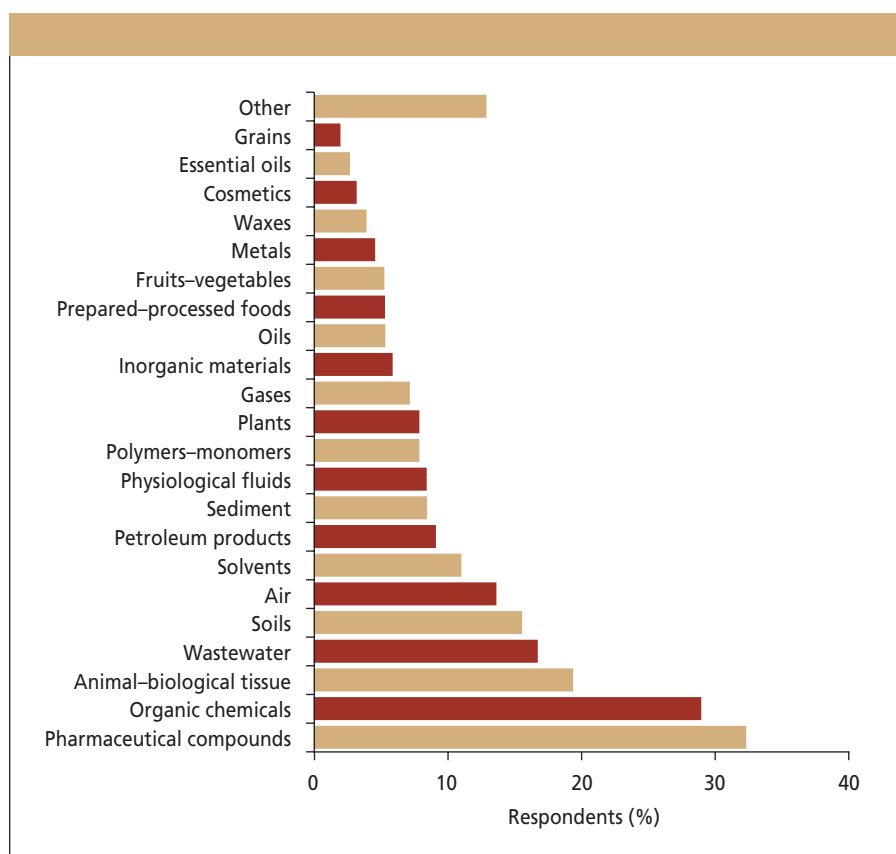


Figure 2: Sample matrices analyzed by survey respondents.

Sample Loads

In the last survey, a majority of respondents (51.8%) indicated that their sample loads would remain the same in the next two years (3). The results depicted in Table II, which compares the 2001 data with the 1996 data, indicate a different trend. In the entire sampled population, the number of samples handled per instrument per week increased by approximately 7%. At the lower end of sample loads (analysts who processed fewer than 100 samples/week), a slight decrease (2%) occurred. Analysts at the upper end (who process more than 100 samples/week) saw their workloads increase by 17%. Although the absolute numbers of users on the upper end (18%) was smaller than the lower end (83%), their higher sample throughput per instrument skewed the results. In this latest survey, a majority (57%) felt that their sample loads would remain the same in the next two years and only 4.2% foresaw a decrease. The rest (38.9%) felt that their sample loads would increase. Increased sample loads should

favor laboratory automation (see the "Automation" section below).

In the pharmaceutical survey, users were queried about the total number of samples that they analyzed per instrument per week (4). Interestingly, when I compared the *LCGC* survey results with the pharmaceutical survey results, the number of samples per analytical instrument per week was nearly identical for similar ranges studied. For example, both the general audience and the pharmaceutical audience that analyzed 0–20 samples per week were approximately 33% of the total, and those who analyzed more than 50 samples per week were 40.6% in the pharmaceutical survey

and 39% in the *LCGC* survey. For the 21–50 samples per week range, the pharmaceutical survey listed 26.3% of the respondents and the *LCGC* survey tallied 27.5%.

Sample Characteristics

Volume: Before injection into chromatographs, samples usually are in a liquid form. For liquid samples, the initial volume of sample can vary greatly. For example, trace organic contaminants that occur in environmental water can be in a large sample volume greater than 1 L, but the organic compounds must be trapped, concentrated, or enriched before analysis.

Blood samples collected from neonates or tiny mammals can have volumes less than 0.5 mL. Overall, the current survey revealed that initial sample volumes for liquid samples are decreasing. For example, 5.5% of the respondents in 1996 had total sample volumes less than 1 mL, but 18% of the respondents reported this lower initial sample volume in 2001. On the larger end of the volume spectrum, the percentage of respondents handling sample volumes greater than 100 mL stayed about the same — 22% in 1996 and 21% in 2001. The use of smaller sample volumes could indicate the increased sensitivity of analytical instruments that necessitates a smaller amount of sample to obtain an adequate analytical measurement.

Solid samples must first be put into a liquid form, or the soluble components of insoluble solids must be extracted. In the survey, we asked about the initial sample mass in increments from 50 mg to 50 g of total sample. Within experimental error, I observed no particular trend in the mass available; the amount of available sample was divided evenly among respondents.

A benefit of most sample preparation techniques is their ability to concentrate samples before injection. Respondents reported a range of typical sample volumes after concentration but before injection — less than 1 mL (25%), 1–2 mL (27%), 3–10 mL (29%), and greater than 10 mL (19%). These results were similar to those

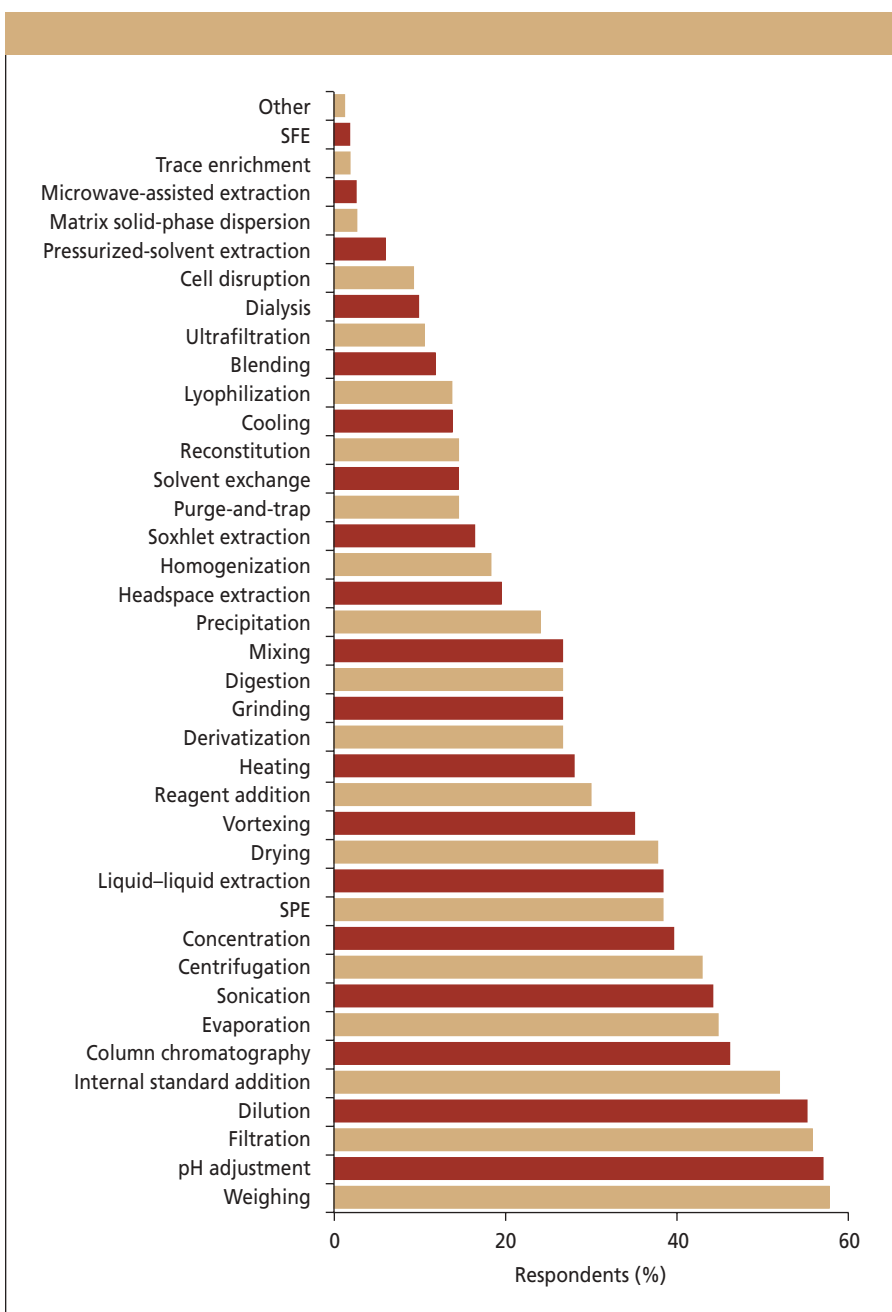


Figure 3: Sample preparation procedures currently in use.

Table I: Number of sample preparation techniques required per sample

Number of Techniques	Respondents (%)
1	19.3
2	28.6
3	20.7
4	15.0
5	8.6
6	2.1
7	0.7
More than 7	5.0

Table II: Number of samples analyzed per instrument per week

Number of Samples	Respondents in 2001 (%)	Respondents in 1996 (%)
Less than 20	33.2	29.7
21–50	27.5	36.7
51–100	21.8	18.4
101–150	7.0	5.8
151–200	2.8	4.4
More than 200	7.7	4.9

of the 1996 survey. Sample volumes of less than 2 mL are suited to the standard 2-mL vials used in GC; HPLC instruments also use these vials, but vials as large as 5 mL are available. Microvials also are available for sample volumes less than 100 μ L, but the survey did not ask about this volume range. Of course, the actual volume injected into a chromatograph depends upon the analyte concentration in the prepared samples. With modern injection techniques, liquid volumes greater than 50 μ L often can be accommodated by both GC and HPLC.

Concentration: According to Table III, 47% of the samples encountered have initial analyte concentrations of less than 1 ppm, more than the 33% reported in the 1996 survey. In this survey, I observed a marked increase in initial sample concentrations of less than 1 ppb (10% in 2001 compared with 3.7% in 1996). Thus, it appears that more samples are falling into the trace analysis category. Optimized sample preparation techniques become more critical for these dilute concentrations. For these samples, the challenge of sample preparation is to ensure that sample loss does not occur by adsorption on the walls of the container, through evaporation, oxidation, or the like.

High enrichment factors are needed for the sample preparation methods. Techniques such as SPE or micro-extraction techniques can provide high enrichment factors (10). Sensitive detectors such as the electron-capture detector for GC and the mass spectrometry (MS) detectors in HPLC can be used to measure smaller amounts of analyte. For compounds that lack strong chromophores or fluorophores in their molecular structure, derivatization will provide higher sensitivity. As indicated in Figure 2, 26.6% of the respondents use derivatization to improve detectability or separation characteristics.

Table III: Typical concentration range of original sample

Concentration Range	Respondents in 2001 (%)	Respondents in 1996 (%)
Less than 1 ppb	10.2	3.7
1–100 ppb	18.0	16.8
100 ppb–1 ppm	18.8	12.1
2 ppm–100 ppm	26.4	32.7
100 ppm–1 ppt*	16.4	10.4
Greater than 1 ppt	10.2	24.3

* ppt = Parts per thousand (10^3).

Automation

With the increased sample loads noted in Table II, readers might surmise that respondents would turn to automation to help increase sample throughput. However, the current survey showed that the percentage of respondents who used automation compared with those who used it in 1996 is essentially the same (26% in 2001 and 26.6% in 1996). Of those who don't use automated methods, 34% explained that their sample throughput does not justify it. Considering that 60% of the survey respondents process fewer than eight samples per day per instrument on average (see the "Sample Loads" section above), this response is not surprising. Nearly one-third of the respondents considered automation unnecessary. Thus, only 5.8% of the respondents are reviewing the possibility of using automated sample preparation devices or plan to use these devices within the next 12 months.

Automated instrumentation: Of those who indicated that they use automated equipment, 43% use autosamplers. Strictly speaking, autosamplers allow only direct injection of liquid samples but do not perform automated sample preparation functions. Some autosamplers have limited sample preparation capability, but I observed only a slight decrease in their use in the present survey compared with the last survey.

Full laboratory robots that can automate many manual tasks are found in many high-throughput laboratories. However, in this survey the use of full laboratory robots dropped by 50% (12.3% of respondents in 1996 and 6.0% in 2001). Apparently, the automation laboratories are finding more cost-effective productivity enhancement approaches than laboratory robots. Many users have found that integrating full laboratory robots into their analytical automation calls for more attention and maintenance than they anticipated. In fact, a marked growth was noted in use of automated liquid-handling systems such as the xyz devices reviewed by Wells (11). In 1996, 8.4% of the respondents using automation employed xyz devices, and 13.4% reported that they now use this type of automation. Many of these devices that formerly only performed liquid-handling tasks have been adapted to handle other sample preparation techniques such as SPE (both cartridges and well plates), solvent evaporation, and filtration. In fact, some laboratories now call these xyz systems laboratory robots.

The other area of automation that has gained acceptance is the use of dedicated sample preparation workstations. Examples of dedicated automated instruments include pressurized-fluid and matrix-assisted sorbent extraction instruments, 96-well plate evaporation stations (11), automated Soxhlet extractors (12), and headspace and purge-and-trap units, which are used in gas sampling and injection.

In the last survey, users were asked which sample preparation techniques that they would like to see automated (3). For the most part, since this 1996 survey, all of the techniques on the users' wish list have been automated, either partially or fully. For example, solid-phase microextraction, Soxhlet extraction, SPE, trace enrichment, digestion (by the microwave approach), and the myriad liquid-handling tasks (for example, solvent exchange, dilution, reconstitution, and liquid-liquid extraction) all have automation equipment.

Solid-Phase Extraction

SPE has been around since the introduction of the first packed cartridges in the late 1970s. The technique has seen a slow but steady growth during this time. In this survey, 38% of the respondents use SPE, down slightly from 40% in the last survey (3). Now, users have a choice of cartridges, disks, and 96-well SPE plates. SPE cartridges still maintain their lead (62% of the respondents used them), but disks (24%) have seen strong growth since the 1996 survey. The biggest advantage of the disk format is its ability to accommodate higher flow rates (and therefore shorter extraction times), which is most important in the enrichment of trace organic compounds in water. The flow rate that can be passed through cartridges with their smaller cross-sectional areas generally is lower than the flow rates used for the disks. The 96-well SPE plates had been shown for the first time in 1996, so the fact that 14% of the respondents are using them now is a tribute to their fairly rapid acceptance. The 96-well SPE plates have proven to be popular in high-throughput laboratories that perform combinatorial chemistry experiments and high-throughput drug assays.

In the pharmaceutical survey (4), the breakdown of users of cartridges (72.7%), disks (6.6%), and 96-well plates (20.1%) was quite different than it was in the *LCGC* survey. Relative to other industries surveyed in the *LCGC* study (Figure 1), the pharmaceutical industry has increased needs for high-throughput sample prepara-

tion, which explains the higher percentage of 96-well plates being used. Because nearly one-third of the *LCGC* respondents were from pharmaceutical laboratories, the 14% reported who use SPE plates probably was skewed by this high percentage. The *LCGC* survey was not broken down to indicate the percentage of pharmaceutical respondents using 96-well plates. The decreased use of SPE disks in the pharmaceutical industry can be explained by the automation requirements, smaller sample requirements, and long-standing acceptance and familiarity of the cartridge format. The SPE disks are favored in the environmental market.

Many solid phases are available in the cartridge format, but fewer are available in the disk and plate formats. Figure 4 provides data from the current survey in terms of relative phase usage (4). Not surprisingly, the data resemble the HPLC modes usage data (13) and are similar to the 1996 survey results (3). Reversed-phase chromatography is the most widely used separation mode in HPLC; likewise, the C8 and C18 phases are the top two SPE phases, with silica gel being a close third.

Silica gel is used more as an SPE cleanup phase than as a separation phase in HPLC, and it always has been popular as a cleanup media in low-pressure column and flash chromatography. Silica gel-based SPE is performed in the normal-phase mode using organic solvents; samples are eluted with organic solvents that are concentrated to dryness with reconstitution in a solvent that is compatible with the mobile phases of reversed-phase chromatography. Florisil and alumina also are used for normal-phase SPE cleanup, particularly for pesticides and food samples.

Ion-exchange chromatography always has been a popular technique for the rapid cleanup and concentration of ionic and ionizable compounds and both cation and anion SPE phases were very popular with respondents in the present survey as well. The pharmaceutical survey results (4) are shown alongside the *LCGC* survey results and show a similar pattern, but, on a relative basis, C18 phases are used much more in pharmaceutical laboratories than in general chromatography laboratories.

When chromatographers were asked to rate the relative importance — very important, somewhat important, important, and not important — of the criteria used to select SPE cartridges, disks, and plates for sample isolation, batch-to-batch repro-

ducibility came out at the top of the list, with 71% of the respondents rating this factor as very important. Obviously, nobody wants to develop an SPE method only to find out that the next box of cartridges cannot repeat the sample cleanup without reworking or tweaking the method. Reproducibility was followed by labor time (55%) and cost (50%). Until now, cost always has been lower on the list, but perhaps the current economic climate has caused users to look more closely at their bottom lines.

When users were asked how much they pay for their cartridges, disks, and plates, of those who actually use them, 39% of the cartridge users didn't know how much they pay, and 50% of the users of disks or 96-well plates were unaware of the costs of these products. Perhaps these expenses are coming out of a laboratory budget that only the laboratory manager or purchasing department documents. Other very important selection factors included literature methods, validated protocols and applications bibliographies (all of which can help

reduce method development time), required solvent volume (environmental, disposal, and cost concerns), and accessories and range of chemistries available. Low on the list of selection criteria were availability of free samples, company seminars, and brand names.

An important parameter in SPE is the mass of available stationary phase. The weight of the phase in the cartridge, disk, or SPE well plate determines the overall sample capacity that is dictated by the amount of analyte and the level of matrix interferences. On the other hand, the smaller the SPE sorbent mass, the lower the amount of sample and the volumes of conditioning, washing, and elution solvent that are required. One of the biggest savings favoring smaller SPE sorbent mass is that the total cycle time is shortened, particularly the solvent evaporation step that sometimes can limit sample throughput.

Table IV shows that in the present surveys, respondents reported that the amount of sorbent in the cartridges that they use now has decreased dramatically since the 1996 survey (1,3,4). The use of cartridges with masses greater than 500 mg/cartridge has decreased, and the use of cartridges with masses of 100 mg/cartridge or less has increased strongly. In 96-well plates, the amount of sorbent generally is lower than 100 mg/well, and 50- and 10-mg/well plates are the most popular sorbent masses. In the pharmaceutical survey (4), the results are similar to those of the *LCGC* survey (Table IV). Larger bed masses are used even less, and smaller bed masses are in favor.

Emerging Sample Preparation Techniques

The present survey asked respondents to provide three sample preparation techniques that they felt could be commonplace or for which they saw a need in the next five years. Although not everybody answered this question, several respondents provided some interesting predictions or needs. Several predicted that molecularly imprinted polymers could provide one-step sample preparation by providing affinity-like selectivity (14). Mixed-mode SPE is beginning to attract attention because analysts can accomplish multiple extraction techniques in a single SPE cartridge, and this technique was suggested as a favored approach in the future. One extraction technology mentioned was the use of subcritical water for extraction of solids. When heated above its boiling point in a closed

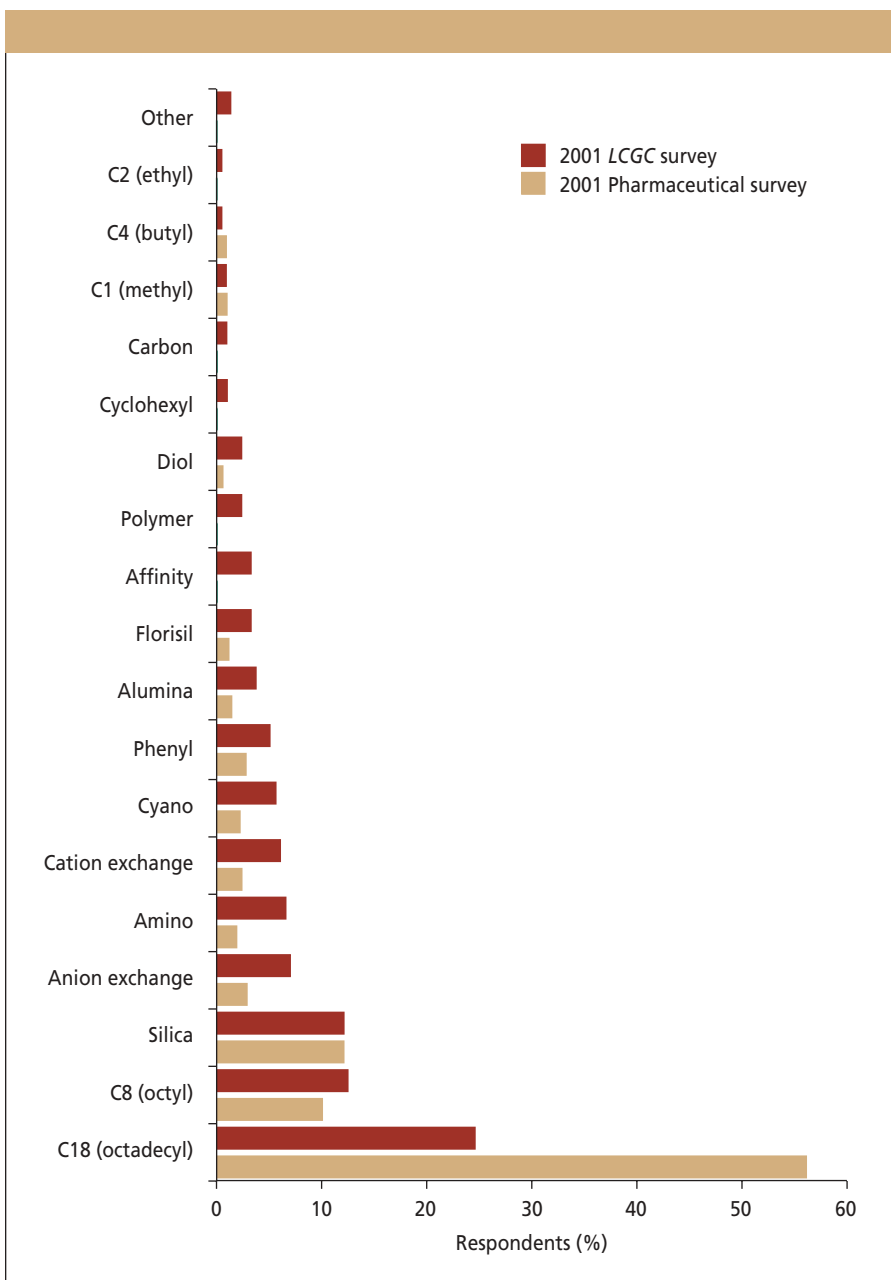


Figure 4: SPE chemistries currently in use (1,4). The data have been normalized to 100%.

Table IV: Mass of sorbent in SPE cartridges in current use

Sorbent Mass in Cartridge	Respondents in 2001 (%) (1)	Respondents in 1996 (%) (3)	Pharmaceutical Survey 2001 Respondents (%) (4)
Less than 50 mg	18.4	7.8	15.1
100 mg	32.7	18.2	34.2
300 mg	N/A*	N/A	13.6
500 mg	26.5	38.1	24
1 g	6.1	23.3	5.5
2 g	8.2	3.5	3.6
Greater than 2 g	6.1	5.9	2.5
Other	2.0	3.4	2.5

*N/A = not applicable.

container, water becomes a more powerful solvent with organic solvent-like extraction power. One respondent predicted that pressurized-fluid extraction could become more popular and that subcritical water extractions could be accomplished using this technique.

Several respondents requested automated SPE. Because several automated instruments already are on the market, perhaps these respondents were referring to an improved generation of instruments dedicated to SPE technology. One respondent suggested the introduction of 384-well SPE plates could be the next major advance in sample preparation for high-throughput, sample-limited laboratories. Liquid-handling systems capable of precisely delivering tiny volumes of solvents needed for 384-well plates already are available. In these tiny footprints, only a few milligrams of sorbent per well would be required. The combination of sonication with SPE was proposed, presumably to allow a more rapid mass transfer of analytes or matrix components that could speed SPE experiments.

Further miniaturization of sample preparation was mentioned, with one respondent specifically predicting that MS-MS on-line chip extraction with direct interfacing to the MS ion source could be useful in improving throughput. Turbulent-flow column switching was suggested as an approach for the rapid preparation of drugs in biological fluids for liquid chromatography-MS-MS analysis. In turbulent-flow chromatography, large porous particles packed in small-inner diameter columns are used at extremely high flow rates. Large, slowly diffusing biomolecules such as proteins don't have time to sufficiently penetrate pores of the packing, so they pass through the column rapidly and transfer to waste while faster-diffusing small drugs of interest penetrate the pores and are held by hydrophobic or ionic forces.

Micro liquid-liquid extraction capability would enable existing methods to be miniaturized and further cut solvent usage and disposal. Someone requested a liquid-liquid extraction column, and several already are commercially available. In this technique, one of the two immiscible extraction solvents, which sometimes contain the sample, is immobilized on an inert sorbent, and the second solvent is passed over the immobilized liquid and extraction occurs in an efficient manner with emulsion formation a nonevent.

Several respondents requested improvements in environmental sample preparation methods. One respondent asked that all EPA drinking water methods have an SPE alternative, and another desired better sample preparation methods so that he or she could obtain better chromatography results. Dialysis was mentioned as a technique that could be more useful in the future. Dialysis is useful for enriching liquid samples and is one of several techniques in which membranes have been underutilized in sample preparation (15).

Summary

Sample preparation still gets attention in chromatography laboratories. Some definite trends in samples and their preparation were determined by this survey. Of 39 sample preparation techniques listed, some new extraction techniques for solid materials — microwave-assisted, pressurized-fluid, and matrix-assisted sorbent extraction — have made their appearance since the last survey.

Sample loads have been increasing; for liquid samples, the initial volumes available have decreased and the analyte concentrations in these smaller volumes have decreased. Automation has shifted from full laboratory robots to modified xyz liquid-handling systems and dedicated sample preparation stations. The introduction of 96-well SPE plates has accelerated sample throughput, especially in the pharmaceutical industry. As in HPLC, reversed-phase SPE dominates the phase technology with more than one-half of the pharmaceutical samples being cleaned up by this technique. Batch-to-batch reproducibility appears to be the most important parameter in the selection of an SPE device. The use of smaller sorbent masses in SPE cartridges and in 96-well SPE plates seems to be increasing.

New technologies have generated some interest. In addition to the new extraction technologies mentioned above, subcritical water needs more investigation as an environmentally friendly extraction solvent. More-selective sample preparation technologies — such as molecular-imprint phases and mixed-mode SPE products — were mentioned by respondents as future needs. Improvements in SPE automation and the possibility of 384-well SPE plates have excited users. The miniaturization of sample preparation and micro-extraction techniques could be of future interest. Turbulent-flow chromatography combined

with MS also can increase sample throughput and analysis.

I will continue to survey the sample preparation market in the future and report about new trends.

References

- (1) "2001 Sample Preparation Study," Advanstar Communications (Edison, New Jersey, June 2001).
- (2) R.E. Majors, *LCGC* **10**(12), 912–918 (1992).
- (3) R.E. Majors, *LCGC* **14**(9), 754–766 (1996).
- (4) "Sample Preparation Consumables Survey," Agilent Technologies (Wilmington, Delaware, January 2001).
- (5) *Understanding Environmental Methods, Analytical Methods Environmental Analysis, Guide to Environmental Methods* (Genium Publishing Corp., Schenectady, New York, 3rd ed. CD-ROM, 1997).
- (6) B.E. Richter, *Current Trends and Developments in Sample Preparation*, *LCGC* **17**(6S), S22–S28 (1999).
- (7) G. LeBlanc, *Current Trends and Developments in Sample Preparation*, *LCGC* **17**(6S), S30–S37 (1999).
- (8) S.A. Barker, *Current Trends and Developments in Sample Preparation*, *LCGC* **May 1998**, S37–S40 (1998).
- (9) R.E. Majors, *LCGC* **9**(1), 16–20 (1991).
- (10) R.L. Grob, *Modern Gas Chromatography* (Wiley-Interscience, New York, 1995).
- (11) D.A. Wells, *LCGC* **17**(9), 808–822 (1999).
- (12) S. Arment, *Current Trends and Developments in Sample Preparation*, *LCGC* **17**(6S), S38–S42 (1999).
- (13) "2002 HPLC Columns Survey," Advanstar Communications (Edison, New Jersey, November 2002).
- (14) K. Ensing, C. Berggren, and R.E. Majors, *LCGC* **19**(9), 942–954 (2001).
- (15) R.E. Majors, *LCGC* **13**(5), 364–373 (1995).

Ronald E. Majors

"Sample Prep Perspectives" editor
Ronald E. Majors is business development manager, consumables and accessories business unit, Agilent Technologies, Wilmington, Delaware, and is a member of LCGC's editorial advisory board.



Direct correspondence about this column to "Sample Prep Perspectives," *LCGC*, 859 Willamette Street, Eugene, OR 97401, e-mail lcgcredit@lccmag.com.