

Sample Prep

Perspectives

Ron Majors describes solid-phase extraction devices that provide new levels of convenience, improved performance, and automation possibilities. He discusses cartridges, pipette tips, disks, 96- and 384-well plates, large-volume cartridges, and flash chromatography columns.

New Designs and Formats in Solid-Phase Extraction Sample Preparation

Solid-phase extraction (SPE), as practiced in its modern form, is more than 25 years old. It has become one of the most common sample cleanup and concentration techniques used by analytical chemists. Over the years, SPE has undergone steady growth driven by analysts' need to find sample preparation procedures that were simple and relatively inexpensive, provided good analyte recovery and adequate selectivity, reduced the use of organic solvents, and could be automated when the need arose. More recently, SPE had another steep increase in its use driven by the high-throughput sample analysis requirements of the pharmaceutical industry with techniques such as liquid chromatography–mass spectrometry (LC–MS) and LC–MS–MS techniques. The increasing popularity of the technique has spurred the influx of many manufacturers into the commercial side of the technology, and the last *LCGC* Buyers' Guide listed no fewer than 54 companies that sell SPE columns (cartridges), disks, and packings and myriad other companies that sell peripheral accessories and hardware (1). Four new companies entered the SPE manufacturing market and displayed products at this year's Pittsburgh Conference (2).

LCGC has covered technical developments in SPE over the years, including a special issue in May 1998 that was devoted to advancements in SPE and had contributions from many experts in the field (3). The purpose of this "Sample Preparation Perspectives" column is to update readers about new SPE designs that provide higher levels of convenience, improved performance, and automation possibilities.

Cartridges

Classic SPE always has been performed with the sorbent-packed, medical-grade polypropylene syringe barrels, and today this format still is the most popular. *LCGC*'s last sample preparation survey found that SPE cartridges with 500 mg of

packing in 3- and 5-mL syringe barrels were the most popular configurations (4). However, some recent data indicates a movement toward cartridges that have smaller mass and volumes — 100 mg of sorbent in a 1-mL syringe barrel now is becoming popular (5). Some manufacturers have introduced even smaller packed beds with only 10, 25, and 50 mg of sorbent.

Part of this movement has been driven by a decrease in available sample, as is most common in clinical, biological, and biopharmaceutical analyses. However, improvements in instrument sensitivity also have allowed the use of smaller quantities of sample with subsequent needs for a lower mass SPE sorbent in the cartridge. Using smaller bed masses also reduced the amount of solvent needed for conditioning, washing, and eluting and the time required for each of these steps. With lesser amounts of solvent to evaporate from collected analytes before concentration, solvent exchange, or reconstitution steps, the overall sample preparation time is decreased even more. Smaller solvent volumes reduce the risk of concentrating potential impurities that can be contributed by large-volume solvent reduction.

Many variations of the syringe barrel format have been introduced, especially when analysts had large numbers of samples that required SPE cleanup. Automated systems — wide-mouthed SPE cartridges for robotic systems, cartridges for automated *xyz* liquid-handling systems, and cartridges for autosampler-based SPE workstations — were developed.

SPE Pipette Tips

The automation of SPE began to drive new formats to cope with the automated systems already present in many laboratories. The robotic systems were the first to mimic the SPE experiment, and robotics companies developed somewhat crude representations of the manual approach in the early 1990s. Next, the automated *xyz* liquid-

handling systems were adapted to handle SPE cartridges' syringe barrel designs. However, so many different shapes of popular SPE products didn't adapt so easily, and many of the liquid-handling systems were based upon using pipette tips to rapidly dispense liquid samples and solvent. It was an obvious extension of the technology to investigate using sorbent-filled pipette tips for SPE. Ansys Technologies (Lake Forest, California) introduced the sorbent-impregnated disk SPE pipette tip format in 1998 (6). This introduction came at roughly the same time as the advent of the 96-well SPE plates (see below), which also were designed with automation and high-throughput sampling in mind.

The SPE pipette tip format had some advantages: it could be used with multi-channel pipettors, and the liquid flow could be bidirectional. Liquid samples or eluents could be pulled from the bottom or dispensed from the top. No modification of the *xyz* automated systems was necessary. The pipette tip format was disposable, and carryover and cross contamination was less common than with early 96-well plate designs. However, plugging was problematic because of the small cross-sectional area and the presence of the SPE disk.

Several companies began to manufacture pipette tips, each with a different twist. Millipore (Bedford, Massachusetts) introduced the ZipTip C4 pipette tip for desalting as little as 1 μ L of 100–500 fmol amounts of peptides and small proteins before LC–MS analysis. More recently, the company introduced the ZipTip_{MC} pipette tip with an immobilized metal affinity phase for the trace enrichment of phosphopeptides from protein digests before matrix-assisted laser-desorption ionization–time-of-flight MS. Amika's ProTip pipette tips (Columbia, Maryland) and the Nest Group's SuproTip pipette tips (Southbor-

ough, Massachusetts) have hydrophilic or hydrophobic polymers coated onto the interior walls of the pipette tip. With this more-open structure, plugging was minimized with protein samples.

EST Analytical's (Cincinnati, Ohio) introduction of its DPX disposable pipette extraction tips provided a pipette tip designed to extract drugs of abuse from small volumes of urine or serum (see Figure 1). In this configuration, the packing is placed loosely between two frits inside the pipette tip; sample is drawn and mixed with the stationary phase. The matrix is sent to waste, and the adsorbed drugs are eluted with a small amount of solvent and analyzed by gas chromatography (GC)–MS.

Figure 2 depicts a GC separation of the so-called NIDA-5 drugs of abuse (National Institute on Drugs of Abuse, now called Substance Abuse and Mental Health Services Administration [SAMHSA]) in 100 μ L of whole blood after centrifugation and cleanup using EST Analytical's DPX disposable pipette extraction tips. After washing away interferences with water, the extraction required approximately 2 min.

Disks

The disk format was first popularized in 3M's Empore disk (Minneapolis, Min-

nesota). The disk format had a much greater cross-sectional area than did cartridges; subsequently, workers could use higher flow rates for large volumes of low-concentration sample, as might be encountered in environmental analyses. The disk was a packing-impregnated polytetrafluoroethylene (PTFE) flexible disk that had approximately 8- μ m particles and a packing-to-PTFE ratio of roughly 90%. Later, Ansys Technologies introduced the Spec disk, which was fiberglass-based and more rigid. Both disks eliminated channeling, which sometimes occurred in packed cartridges because of inadequate packing structure. Other companies introduced their own versions of SPE disks, including disk cartridges that provided the convenience of cartridge operation with the absence of channeling. Because disks tend to plug with dirty samples that contain particulates, I recommend using a prefilter or filter bed before the membrane.

Fixed 96-Well SPE Plates

The 96-well plate format of 8 \times 12 wells spaced 9.0 mm from center-to-center was established in microtiter plate applications. When the need arose for greater degrees of automation and sample throughput, SPE flowthrough well plates were available. In

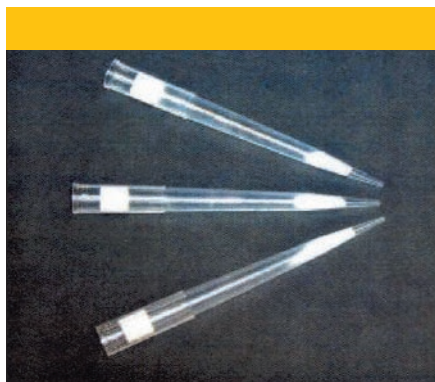


Figure 1: EST DPX disposable pipette extraction columns. (Courtesy of EST Analytical.)

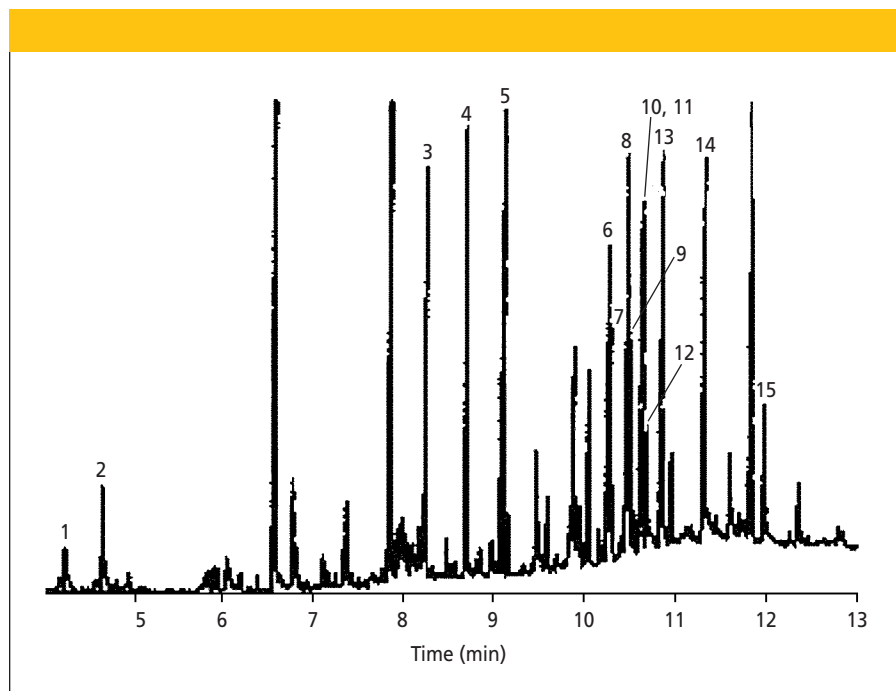


Figure 2: GC–MS chromatogram of an extract of a 0.5-mg/L drug mix in whole blood. A 0.1-mL sample of whole blood was centrifuged with 0.1 mL of water and the supernatant was extracted in 2 min following five water wash steps. Peaks: 1 = amphetamine, 2 = methamphetamine, 3 = meperidine, 4 = glutethimide, 5 = phencyclidine, 6 = methadone, 7 = methaqualone, 8 = amitriptyline, 9 = cocaine, 10 = imipramine, 11 = doxepin, 12 = desipramine, 13 = pentazocine, 14 = codeine, 15 = oxycodone. (Courtesy of EST Analytical and W.E. Brewer, Clemson Veterinary Diagnostic Center, Columbia, South Carolina.)

1999, David Wells published two excellent reviews of the state of the art in 96-well plate formats (7) and of accessories used with the 96-well SPE plates (8).

With the great interest in drug discovery, combinatorial chemistry, high-throughput analysis, and LC-MS, the 96-well SPE plate market is the most rapidly growing area of SPE. Consequently, many companies have entered the 96-well marketplace since the 1999 review (7). Figure 3 shows a typical fixed 96-well plate. Each of the 96-wells has a small 1- or 2-mL SPE column with 10–100 mg of packing material. As in an SPE cartridge, a bottom frit or membrane and a top frit contain the packing material. Recently, Waters Corp. (Milford, Massachusetts) introduced a 96-well plate with only 5 mg of polymeric sorbent per well (9).

Disk plates also are available; these flexible disks require some support, but the fiberglass disks are free standing. Liquids are moved through the plates by gravity, constant flow or pressure, vacuum, or centrifugation.

The plates are called *fixed* because most of them have a fixed volume and fixed amount of sorbent. They are most useful in an existing SPE method developed for the 96-well plate format. Transferring a method from a typical syringe barrel cartridge to a 96-well plate rarely is straight-

forward. Compared with typical SPE cartridges, these small packed bed wells have different flow characteristics, masses, and volumes, so they require some adjustment of the SPE conditions to downsize an SPE method. Vacuum control and the alignment of the SPE flowthrough and collection plates are especially critical parameters. Because the exits of the 96 tiny SPE cartridges are in such close proximity and the amount of packing is so small, smooth and homogenous flow through the individual wells is necessary. Any uneven flow caused by fluctuation in the vacuum or differential flow through any of the 96 wells because of plugging, channeling, or uneven well permeability could result in cross contamination or carryover. If some wells dry out before others are drained, the SPE sorbent can be affected and analyte recovery can be variable.

Method development in SPE is accomplished by investigating different stationary phases and their masses; volumes of conditioning, sample load, wash, and elution solvents; and amounts of sample used in the experiments. In a cartridge format, these mechanical operations can be performed easily on a manual basis. This approach to method development on fixed 96-well plates requires a number of plates each with different stationary phases and masses. Because these plates are rather

costly and a given test may use only a few of the 96 wells, laboratories must invest a considerable expense in method development experiments. Analysts can store a partially used 96-well plate and later use the remaining unused wells, but this approach requires keeping track of used and unused portions of plates, which could disrupt the automation approach and require manual intervention of data entry. Thus, this approach is time consuming, expensive, and wasteful.

Initially, some 96-well SPE plate suppliers sold custom-packed method development plates, but these plates soon went out of favor because of the expense and inconvenience of waiting for plates to be customized.

Flexible 96-Well SPE Plates

To overcome some of the disadvantages of performing method development on fixed 96-well SPE plates, manufacturers have developed a more-flexible array configuration. The flexible well plates have removable small round or square plastic SPE cartridges that fit tightly into separate 96-hole base plates. Each individual cartridge has top and bottom frits and a stationary-phase packing, as used in the fixed 96-well SPE plate design. Because many of the individual cartridges can be placed as needed in the base plate, the 96-well plate can be customized to meet the needs of individual users. Figure 4 shows an example of this design.

This flexible approach makes the idea of method development on the 96-well plate more feasible and affordable. Users can place individual cartridges in as many positions as needed. The individual cartridges can contain different stationary phases or different amounts of the same phase. In some cases, different cartridge volumes (1 or 2 mL) are available and can be used to

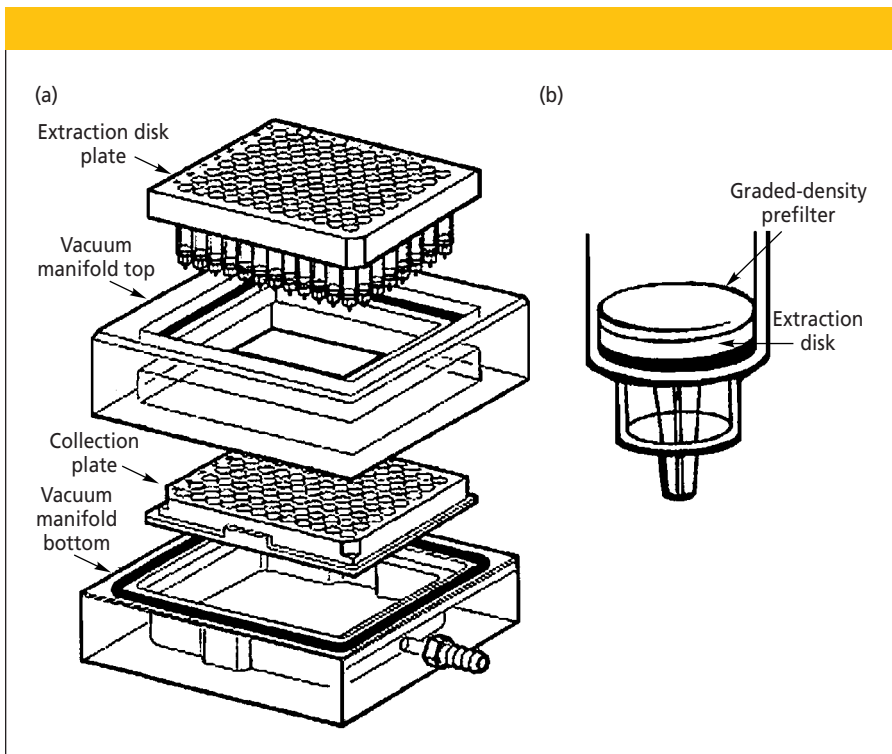


Figure 3: Diagrams of (a) a fixed 96-well SPE plate and (b) a single extraction disk. (Courtesy of 3M Co.)



Figure 4: Photograph of a flexible 96-well SPE plate with removable cartridges and plug strips. (Courtesy of Agilent Technologies.)

accommodate larger sample, wash, and elution volumes. A vacuum manifold often is used to pull liquids through a flexible well plate, and users can plug the unused holes of the base plate with plug strips if only a portion of the 96-wells is needed (Figure 4).

Examples of flexible 96-well SPE plates currently available include Zorbax (Agilent Technologies Inc., Wilmington, Delaware), Isolute Array SPE (International Sorbent Technology, Tucson, Arizona), Speedisk 96 (J.T. Baker–Mallinkrodt Baker, Phillipsburg, New Jersey), Chromegabond Multi 96 (Macherey-Nagel Inc., Easton, Pennsylvania), MultiSep (Thermo Hypersil, Needham Heights, Massachusetts), and Versa-plate (Varian Inc., Palo Alto, California) plates. In most cases, the round or square individual cartridges are sold separately from the base plates, which can be used repeatedly. The individual removable cartridges can be used in regular vacuum manifolds in place of larger SPE cartridges if users desire to perform experiments on individual units. In some cases, workers must use a Luer adapter to interface a flexible cartridge with a Luer fitting on a typical SPE vacuum manifold.

The bed sizes or masses of the removable cartridges are the same as those for fixed 96-well SPE plates. After a stationary phase is selected, the bed mass and volumes are optimized, and the automated SPE method is developed and validated, users can confidently transfer the entire method to a fixed plate for routine analysis and expect exactly the same performance. An advantage of automated method development is that analysts can expect improved method precision and accuracy over manual SPE methods because they generally have better control over the sample and solvent manipulation in an automated environment (10).

384-Well SPE Plates

The 384-well format of a 16 × 24 array with a 86 mm × 128 mm footprint and 4.5-mm center-to-center well spacing already is available for automated liquid-handling systems and being used for high-throughput screening, liquid-phase reactions through combinatorial methods, and storage of synthesized compounds. However, these plates are not being used in flowthrough configurations as would be necessary for SPE or filtration plates. These 384-well plates have the same external dimensions as the current 96-well plates, so SPE wells would be very tightly sandwiched together.

In his report about 96-well SPE plates, Wells (7) speculated that 384-well SPE plates could be around the corner if manufacturers could overcome certain mechanical obstacles and make accessories available. Two years later, no 384-well SPE products have been introduced, which indicates that some of these obstacles have yet to be conquered. In addition to the volumetric measurement concerns expressed by Wells, an anticipated problem associated with these plates is the reproducibility and homogeneity of liquid flow through these tiny packed beds that may contain only a few milligrams of stationary phase. The other problem is associated with the collection of well effluents in 384-well plates and the possible nebulization of liquids spraying from the tiny orifices of the well exits. If nebulization occurs and an aerosol forms, cross contamination of samples collected in adjacent wells is sure to occur. Stay tuned for updates about 384-well SPE plate development.

Large-Volume SPE Cartridges and Flash Chromatography Columns

SPE in convenient cartridge designs traditionally has been used for cleaning smaller size samples; 88% of all usage has been performed on cartridges containing 1 g of sorbent or less (4). Commercially available SPE cartridges have ranged from 1 mL to almost 70 mL. Sorbent masses have ranged from 100 mg to 10 g, the latter being for environmental samples in which the higher capacity often is necessary because of dirty sample matrices. Recently, however, the use of large-volume SPE cartridges has been growing, and the driving force appears to be the purification needs of combinatorial chemists who want to use phases other than silica gel as their stationary phases.

On the other hand, flash chromatography — traditionally performed with self-packed glass or plastic columns that contain large-particle silica-gel adsorbent (11) — has been receiving increased attention from organic chemists who do not want to prepare their own flash columns. Instead, they prefer to purchase prepacked large-volume SPE columns or flash columns that are specially prepared to meet their needs. These large-particle, relatively low-efficiency preparative columns packed primarily with silica and reversed-phase bonded silicas have experienced a renaissance in recent years. The columns are sufficiently inexpensive to be treated as disposables. Recent introductions of

prepacked silica-based bonded-phase flash columns using dynamic axial compression system with bottom upward flow (to remove air bubbles) borrowed some of the concepts used in more expensive large-scale preparative systems but at a more affordable price. A wider variety of phases, including ion-exchange phases and normal bonded phases such as amino and diol, have become available in flash chromatography configurations.

Formerly, flash columns were used with pipette injection, gravity feed, and simple

fraction collection but not with on-line detection. Recent market introductions include flash chromatography instruments that provide a low level of automation compared with a modern high performance liquid chromatograph but increased levels of automation compared with a manually operated fraction collector using a low-pressure, self-packed column. Modern flash chromatographs include built-in ultraviolet absorption detectors, low-pressure pumps, and integrated fraction collectors.

The manufacturers of SPE columns also have approached flash chromatography from the column end and have manufactured columns and column racks to conveniently perform flash chromatography using the same sorbents, and sometimes the same columns, that they have been selling for years by using 60- or 70-mL large-volume syringe barrel cartridges packed with 10 g of sorbent. Some manufacturers even sell custom-packed columns at a premium price.

Future of SPE Column Design

Undoubtedly, the needs of high-throughput automation will continue to spur new SPE designs that will allow increased levels of automation with even smaller bed masses; perhaps 384-well SPE flowthrough plates may come about. An anticipated advantage of the smaller bed masses associated with the 384-well format is that the elution volume of isolated analytes would be in the 20–30 μL range. Thus, collected effluent could be directly injected into an MS system, and the current evaporation and reconstitution steps could be eliminated, thereby greatly improving sample throughput (12). The need for some sample cleanup before analysis using microfluidic separation devices will require sub-microliter-volume SPE devices that will allow on-line concentration of analytes similar to the devices recently described for capillary electrophoresis (13). The laboratory-on-a-chip concept with in situ generated monolithic phases and microfluidic channel switching would provide solid-phase sample cleanup in nanoliter volumes before separation. Column switching is having a renaissance driven by the high resolution needs of proteomics, and the short columns used in these systems resemble SPE columns very closely



Figure 5: Photograph of a regular-length HPLC column (25 cm) and short, fast 1- and 3-cm HPLC columns. (Courtesy of Agilent Technologies.)

but are considered reusable because they are on-line.

In fact, one on-line instrument, the Prospekt-2 automated SPE system from Spark Holland (Emmen, The Netherlands), already incorporates a disposable flowthrough SPE cartridge that is part of a column switching system. Several manufacturers sell their phases packed into these small cartridges. A general trend in HPLC is to move to shorter columns, as depicted in Figure 5, particularly when coupled with MS and MS-MS detection systems. The difference between a short HPLC column of 10 mm or less in length and an SPE cartridge is getting rather gray.

Summary

New formats for SPE provide reduced bed masses, high-throughput capabilities, and greater convenience for method development. Small-bed-mass SPE devices allow faster method development, reduced solvent usage, and a shorter overall sample preparation times. The transfer of manual methods to automated methods has been improved by the advent of removable, flexible cartridges that can be used manually or in an automated environment.

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