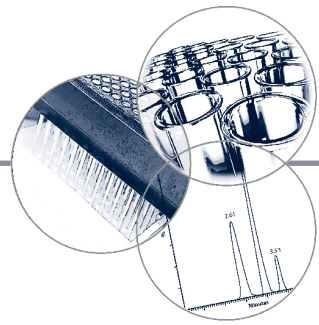

Revolutionizing SPE for improved bioanalysis

"SPE is an indispensable tool for the bioanalyst and recent developments have allowed it to continue to offer effective sample preparation solutions for modern laboratories."

Keywords: sample preparation ■ SPE

Today's high-throughput bioanalytical laboratories desire a combination of high throughput and also high levels of robustness in their analytical processes. This has led to the routine use of LC–MS/MS technology within bioanalytical laboratories. Early aspirations of this technology eluded to the concept of 'dilute and shoot' where the high degree of specificity of the MS/MS technology would ensure high-throughput and robustness; however, these initial hopes were not realized and, as a result, the use of sample pretreatment in the field of bioanalysis is now commonplace, with sample preparation being required to:

- Ensure that detection is not compromised by: ion suppression in atmospheric pressure ionization LC–MS; protein binding; or matrix interferences;
- Ensure system robustness by: providing longer column lifetimes; reducing detector maintenance; reducing contamination/carryover;
- Concentrate samples: improves sensitivity but relies on large sample volumes.

Bioanalytical laboratories are now looking for sample preparation techniques that will not only allow them to achieve these goals, but to do so as quickly and cost effectively as possible [1]. Another key driver for the bioanalytical industry at present is the move towards reduced sample volumes, which is part of the three R's policy that the pharmaceutical industry is engaged in, to refine, replace and reduce the use of animal models. The ability to reduce the amount of sample taken from an animal model allows serial sampling from small animals, making the scientific data substantially more robust and removing the need for satellite groups with concomitant reduction in the number of animals used. A reduced sample volume also has extra benefits as it allows for pediatric studies to be performed, where low sample volumes are obligatory. However, this reduced sample size does present a number of analytical challenges.

A wide range of sample preparation techniques are now available in bioanalytical laboratories. Chief among these are SPE and protein precipitation. Each technique offers the user different benefits in terms of:

- Cost
- Method development time
- Assay robustness
- Analysis time
- Assay sensitivity

It is important to match the technique used to the specific analytical problem, thus achieving the desired outcome.

Protein precipitation offers a rapid and generic approach to removing high-concentration proteins from blood-based samples, however, it offers very little clean-up of other endogenous materials. As a consequence, the large number of remaining components can interfere with the analysis. This can manifest itself in a number of ways, but most commonly appears as chromatographic interference, variability and/or compromised sensitivity from modification of ionization. By comparison, while requiring greater investment in development time and a more complex protocol, SPE allows specific and targeted methods to be developed leading to cleaner sample extracts and higher quality analytical results

SPE has evolved in a number of ways leading to improvements in ease of use and quality of results. Early polymeric resins developed in the 1960s by Rohm and Hass were based on styrene-divinylbenzene [2] and were used to extract polar contaminants from aqueous samples [3]. These resins were packed in a cartridge format and represent the first commercial introduction of

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SPE. SPE cartridges then moved on to the use of functionalized silica, initially C18 for reversed-phase extractions, which were followed by a number of other chemistries broadening users' selectivity choice and allowing analysts to develop increasingly more specific and cleaner methods.

A return to the use of polymeric SPE sorbents based on functionalized divinylbenzene allowed for the production of more robust SPE methods by improving pH stability and removing secondary interactions. These polymeric resins are often formed with a hydrophilic group such as vinylpyrrolidone, which improves the water wettability of the phase improving robustness and reducing the impact of drying the sorbent bed during extraction procedures. They also provide higher loading capacity than silica materials, particularly for polar compounds [4]. This increased capacity offered bioanalysts the ability to reduce the bed weight of the cartridge used without affecting the ability of the cartridge to perform the desired clean-up and maintaining high recoveries. By reducing the sorbent bed it became possible to reduce the volume of elution solvent used and, therefore, utilize smaller sample volumes without losing the ability to preconcentrate samples on the cartridge or having to introduce lengthy dry down stages into work flows.

Of particular interest to bioanalysts are mixed mode chemistries introduced in the late 1980s that allow multiple interactions on a single cartridge (typically reversed phase and ion exchange). By utilizing both these functionalities it is possible to use much more aggressive washing protocols to remove interferences from biological matrices and improve sample cleanliness [5]. Mixed mode SPE also allows for the fractionation of ionizable and neutral components by using the ion exchange interaction to retain ionic compounds while neutral compounds are eluted, and then modifying the pH or counter ion to elute the ionic compounds.

For both silica and polymeric materials, advances in the materials and the methods of packing have been made, which has been driven by analyst requirements for greater recovery, reproducibility and efficiency. Materials have moved from irregular to uniform spherical particles with much tighter particle size distributions. Packing methods have been improved to allow for more consistent and robust packing of cartridges and plates. Both these factors have led to more reliable and consistent products viewed far less as a commodity and much more as another dimension of chromatography prior to analysis.

Despite the constant evolution of SPE products, the overall structure, whether in individual cartridge form or 96-well plates, has remained largely the same with loose sorbent material supported between two porous (typically polyethylene) frits. Historically, this format has been adequate for most analyses; however, it is prone to a number of problems that have a more significant effect on performance as the bed weight is lowered. Alternative formats that have been introduced, such as fiber-based disc products, have so far not been widely accepted by bioanalytical laboratories.

"Developments in fritless SPE formats are now offering significant performance advantages, whilst future advances will continue to keep sample preparation an integral part of bioanalytical workflows."

Using traditional cartridges and 96-well plate formats, bed weights are currently limited due to packing issues. However, many industries wish to move to smaller bed sizes. So far this has been achieved with a change in the format of the device used to house the SPE material. Typically for sub-5-mg SPE, a pipette-tip format has been used in either a single tip or a modified 96-well format. Moving to lower bed weights has required a number of changes to SPE products driven by both the need to have a practical way to handle small sample volumes and the need to ensure a consistent sample flow is achieved through the very small sorbent bed.

Most of the problems that occur with SPE extractions are a result of the limitations of the classical frit-based SPE design and can be grouped into three categories:

- Voiding
- Channeling
- Packing inconsistencies

Voiding is the formation of gaps between the sorbent and the frits and is typically caused by settling of the sorbent material, loss of sorbent material, or displacement of the upper frit. The result is increased hold-up volume and inconsistency in flow through the cartridge.

Channeling is the result of the formation of flow channels through the sorbent bed that offer a preferential flow path for sample and solvents,

reducing contact with the sorbent and decreasing the opportunity for interaction.

The final problem relates to inconsistencies in the packing process. Although the greatest care is taken to ensure each cartridge or well is packed with the same quantity of material, variation can occur and it becomes increasingly difficult to ensure consistent packing, particularly with smaller bed weights. This is particularly the case for polymeric media, especially mixed mode ion exchangers, which are more prone to electrostatic interactions that make reproducible packing a challenge. This is why mixed mode materials are quite often not available in low bed weight formats.

Other than holding the loose packing in place, the frits offer no other benefit to the SPE product and can potentially be a source of interferences as they provide a high surface area from which compounds such as plasticizers can be leached into samples during extraction. In addition, they increase the total hold-up volume, having a detrimental effect on the performance of the SPE at low elution volumes. As the ratio of frit volume to bed volume increases these effects are magnified.

With ever increasing regulatory demands bioanalysts require more robust and sensitive analytical methods. Modern high-sensitivity selective detection (for example Q-Exative LC–MS) and high-quality chromatographic separations offer a foundation for this, but SPE technology has lagged behind.

These requirements have driven the development of next-generation, low-bed-weight SPE designed specifically for the demands of the modern bioanalytical laboratory. Significant changes in the format of the SPE product were required to provide improved robustness and reproducibility. Removing the frits and supporting the sorbent in a single porous material has made it possible to eliminate the main issues currently experienced with low bed weight SPE. This has allowed the evolution of a consistent and robust SPE format that is immune to packing inconsistencies, channeling and voiding issues, even when subjected to physical stress. Once these problems were eliminated it became possible to consider moves towards production of currently unobtainable bed weights that maintain ease of use and the high performance expected from standard bed weight SPE products.

SPE is an indispensable tool for the bioanalyst and recent developments have allowed it to continue to offer effective sample preparation solutions for modern laboratories. As bioanalysis continues to develop, a new class of SPE product is available to meet the increasing demands of high-throughput, low sample volume applications. Developments in fritless SPE formats are now offering significant performance advantages, whilst future advances will continue to keep sample preparation an integral part of bioanalytical workflows.

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