Sample Preparation

Approaches to Automation for SPE

Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.
Aims and Objectives

Aims

- Introduces students to the principles automation for solid phase extraction
- Present fundamental concepts regarding molecular imprinted polymer sorbents

Objectives

At the end of this Section you should be able to:

- Recognise advantages and opportunities of implementing automatic systems for SPE
- Select the correct sample preparation for your analysis
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Automation via On-line Solid Phase Extraction

The most common SPE approach used involves preparing the sample extract as a separate operation from the analytical procedure. There is, however, an additional approach in which the SPE is intimately integrated with the analytical technique. This approach is known as On-Line SPE.

The principle of on-line SPE is quite simple. Instead of the sample being prepared on a relatively low-performance extraction column, in on-line SPE the sample is typically prepared on a high-performance chromatographic cartridge, which is plumbed into the analytical chromatograph in such a way that the final step of analyte elution allows for the analytes to be eluted directly into the analytical column. In most cases this is accomplished by connecting the cartridge into the analytical system in the same place normally occupied by the sample loop of an injection valve. This allows the cartridge to be processed through the SPE protocol when the cartridge is off-line to the analytical system flow, and eluted into the analytical system by the LC mobile phase when the cartridge is switched into the system flow. Higher performance packing materials are required than in off-line SPE, since the cartridge becomes part of the analytical system.

On-line SPE is typically practiced in one of two ways:

1) reusing the same cartridge over and over, washing extensively between samples to prevent carryover, and

2) using a system that employs changeable cartridges, such that each new sample uses a pristine, new cartridge.
Mechanisms for On-line SPE

The first of these techniques has been in use for decades, and is commonly referred to as valve switching. The benefits of valve switching are that it is relatively inexpensive to implement, and the user often can fashion their own switching device using off-the-shelf commercial hardware. The significant downside to valve-switching is the problem of carryover from the previous sample. This is an especially important problem when working with widely varying analyte concentrations — a very low-level sample following a high-level sample will often exhibit falsely high values due to residue from the high sample.

The second technique, employing changeable extraction cartridges, is more sophisticated (and expensive), and is primarily manifested through devices such as the Prospekt or Symbiosis, manufactured in the Netherlands by Spark Holland BV.

These devices are designed for on-line SPE, and interface readily with most commercial LC/MS systems. The principle of operation is essentially the same as in valve-switching, but the extraction cartridges are not typically re-used sample-to-sample, which reduces the carryover problem immensely, thus making the approach highly feasible for modern LC/MS.

On-line SPE offers a number of advantages over off-line SPE, including significantly enhanced sensitivity and complete automation of the overall SPE/analysis process.
Turbulent Flow Chromatography

Turbulent flow chromatography is another novel approach that is currently used for sample clean-up prior to LC and LC/MS. Turbulence is a phenomenon that occurs in HPLC when very high linear velocities are reached when flowing through a column of relatively large silica particles (~50μm). Under turbulent conditions, large biomolecules (for example, protein interferences) are swept through the analytical column, while smaller molecules (like typical pharmaceuticals) are retained normally, yet exhibit very high chromatographic efficiency in their retention behavior. The composite effects of these phenomena allow direct application to the analytical column of raw biological samples, with the primary large molecule interferences eluting near the void volume, thus separating these interferences from the analytes, which elute later. This process is accomplished via high flow rates and chromatographic packings that have larger particle sizes than conventional HPLC packings, allowing higher flow rates at acceptable backpressures.

Turbulent flow offers the benefits of simplicity, high throughput, and relatively clean analyte chromatography. The most widely reported downside of turbulent flow is sample-to-sample carryover, since the analytical column is the actual device to which the raw samples are applied and, of course, is re-used time and time again for each successive sample.

Turbulent flow chromatography is a patented technique and the patent is the property of Cohesive Technologies, Inc. (For more information on turbulent flow, contact Thermo Scientific.)
Molecular Imprinted Polymer Sorbents (MIPS)

A recent development in creation of highly selective sorbents for specific applications, involves sorbents known as molecular-imprinted polymers. These materials are manufactured using a highly-sophisticated synthetic scheme whereby “template” molecules similar to the target analytes are present in the synthetic reaction as the polymer sorbent is formed. The presence of these templates creates chemically and physically-specific pockets, which are highly selective for retention of the desired analytes.

Manufacture of MIPS sorbents

Since creation of a MIPS material is a custom process, most MIPS applications involve situations where very large numbers of samples must be analyzed, thus justifying the expense of creating the custom products. For these applications, MIPS offer perhaps the most powerful sample preparation possible, providing extracts of extraordinary selectivity and cleanliness.
Selecting a Sample Prep Technique

Armed with the detailed information presented up to this point, the user is faced with the task of selecting an appropriate sample preparation technique for any given situation. This brings us full circle back to the beginning and it is appropriate to list once again the most common techniques for sample preparation, including:

- Dilution
- Filtration/centrifugation (particulate removal)
- Protein precipitation
- Liquid/liquid extraction
- Support-assisted liquid/liquid extraction (SALL)
- Solid phase extraction

Common techniques for sample preparation
Each of these techniques has value in its own right, and each may be the most suitable choice for a particular application. There are many factors that can influence the choice of one technique over the other, and only the user can weigh these factors to arrive at the optimum decision. Some of the more common factors include:

- Simplicity — how skilled must the user of the technique be?
- Speed — how fast can samples be processed?
- Selectivity — how clean an extract is required?
- Sensitivity — how small a final extract volume is required?
- Complexity of method development — what skill levels exist in the lab?
- Automatability — must the technique be run unattended?
Summary of Sample Prep Technique Features

The following commentary summarizes the most important features of the different techniques presented above. These comments are meant to serve as guidelines, and must be viewed in the context of each individual laboratory environment. As an example, “complexity of method development” is very subjective — in some labs an SPE development may be a challenging enterprise; in others the same development may be trivial.

**Dilution** — this technique is simple, fast, and involves almost no method development. The major downside of simple dilution is that it does not result in actual sample simplification or cleanup; i.e., whatever interferences were present in the original sample will still be present after dilution. However, there may be situations involving very clean matrices (for example, an ophthalmic solution) where dilution is all that is required. Another downside of dilution is that it offers no concentration benefit (actually, a negative concentration benefit) so when high sensitivity is needed the technique is not ideal.

**Filtration / centrifugation** — these techniques have features very similar to dilution; they are fast, simple, and involve no method development. Again, however, they offer no inherent sample cleanup other than particulate removal. Like dilution, they are applicable as the sole cleanup procedure only in situations with very simple matrices.

**Protein precipitation** — from a speed standpoint protein precipitation is somewhat slower than the first two techniques, since it requires both steps (i.e., dilution followed by filtration or centrifugation). Protein precipitation is relatively simple to execute, but does require nominal method development, since there are many possible ways to execute the technique. Protein precipitation is not an especially selective technique, and extracts often suffer from significant ion-suppression. From a sensitivity standpoint, again, the technique involves sample dilution rather than concentration, and with aqueous samples there is no easy evaporation/reconstitution approach. Finally, protein precipitation can not be readily automated, although minimal human intervention may be required if the proper consumables are employed (such as 96-well filter plates which can be centrifuged directly after precipitation).

**Liquid / liquid extraction** — this technique’s strongest point is the degree of selectivity possible. The technique is also reasonably quick, but is somewhat user-skill dependent, especially during phase separations. For sensitivity, liquid/liquid extraction typically does not offer a direct concentration benefit, although most organic solvents used for extraction may be easily evaporated. Liquid/liquid extraction does require method development time, although the empirical approach works quite well, so not a great deal of development skill is required. Finally, liquid/liquid extraction is not readily automated, although pipeting and evaporation operations may be facilitated by commercially available equipment.

**Support-assisted liquid / liquid extraction** — this technique is very similar to standard liquid/liquid extraction in selectivity, degree of method development required, and concentration. However, the approach is less influenced by technician skill (simpler), is faster, and is more highly automatable using robotic platforms.

**Solid phase extraction** — SPE is fast, requires little technician skill, and offers a built-in concentration benefit when executed properly. SPE has the potential to offer the highest selectivity of all (when methods are developed properly), and is the most automatable of all the techniques. The biggest disadvantage of SPE is the degree of method development skill required, although this can be significantly offset by the use of generic methods.
In the modern pharmaceutical laboratory, high sample throughput is often one of the greatest demands on the scientist. There are typically limitations to how fast any particular sample preparation approach may be executed, so an optimum alternative to improve throughput is automation. Automation devices range from the simple to the highly complex, with commensurate expense.

One of the most important questions to be asked when considering automation is, “How much automation is required?” It is a well-understood paradigm that the greater the degree of automation required, the greater the cost and complexity. These curves are exponential, so selecting just the right amount of automation can save large amounts of money and resource time.

From a sample preparation perspective, there are a range of solutions available, starting with simple, single-function devices that improve human efficiency (such as multi-channel pipettors), through attended workstations and up to fully automated, unattended robotic platforms and dedicated instruments. These items are addressed below from the simplest to the most complex.

Multi-channel pipettors — the most common versions of these devices accommodate 4 or 8 channel pipetting simultaneously. They are typically used in dilution or pipetting operations for any of the common techniques. The most compatible consumable format for these devices is the 96-well plate.

Evaporation stations — these devices perform unattended, batch evaporations of sample extracts. They are used primarily in liquid/liquid extractions, SALL, and solid phase extraction.

Centrifuges — these are used primarily in protein precipitation and liquid/liquid extraction (to promote phase separations). Some centrifuges offer 96-well plate capability, helping to streamline the overall sample prep procedure (e.g., facilitating use of multi-channel pipettors.

96-well pipettors — these are dedicated workstations that can pipette liquids simultaneously into all 96-wells of a plate. Compared to simple handheld multi-channel pipettors, these workstations can reduce pipetting time by as much as 20 times.

Vacuum manifolds — these devices facilitate flow through a column format consumable, including the 96-well formats of filters, SALL columns, or SPE columns.

Multi-function workstations — these instruments range widely in capability from semi-automated to fully automated. Common functions include pipetting and liquid transfer, vacuum or pressure processing of column consumables, evaporation of sample extracts, and injection into analytical instruments.

Dedicated platforms for automation — the Prospekt or Symbiosis are good examples of this category of device, specifically designed to automate one technique (in this case solid phase extraction).