Drug Discovery and Development

Introduction:

Bioanalysis is the quantitation and determination of drugs and their metabolites in biological fluids for the evaluation and interpretation of bioequivalence, pharmacokinetic (PK), and toxicokinetic (TK) studies in animals and then humans.

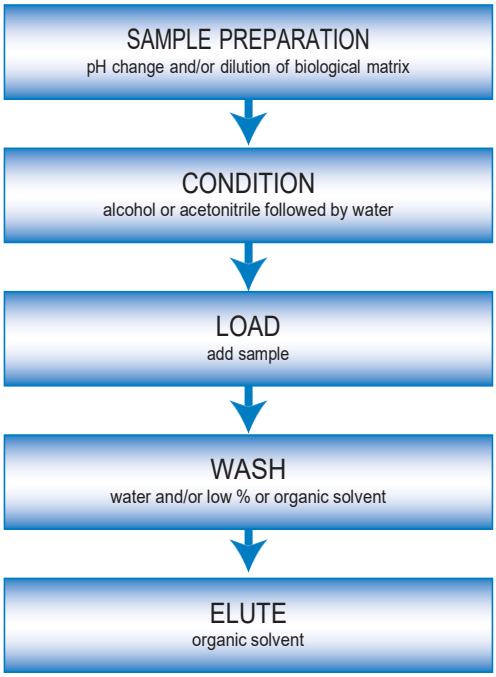
After a drug candidate has been selected, a number of tests must be performed and reported to the FDA before approval is given for clinical trials in humans. Almost all of these preclinical tests are performed using validated bioanalytical methods. There are specific rules and guidelines that have been set up for bioanalytical method validation. Once the method is validated, it must be followed exactly or additional validation is needed for any changes made to the procedure or equipment.

Solid phase extraction is often used to remove interfering biological matrix material from samples and/or to concentrate the analytes of interest. Preparation of the sample for analysis is often the slowest step in the process. This is why sample preparation is often run using the 96-well plate format which can then be automated with one of the many sample handling platforms available. The drive toward decreasing drug development time has placed significant demands on bioanalytical laboratories to "do more, faster." Sample preparation has now become the rate limiting step to achieving higher throughput in bioanalysis.

Drug development process outline:

- Identify likely candidate (high throughput screening, library of compounds and classes)
- Efficacy and safety tests
- ADME (Absorption distribution metabolism elimination) in animals/ human
- Pharmacokinetic tests in animals, maybe humans with micro-dosing
- Phase I clinical trials, first in man (most of the time), dose response, side effects

Solid Phase Extraction process or removing contaminants and concentrating compounds of interest.



Elution can be performed into one of several types of collection devices: Deep well plate, shallow well plate, or rack of micro tubes. Plates can be processed manually using a multichannel pipetter, or by semi-automatic mode using a liquid handling workstation. Alternate processing methods include centrifugation with appropriate plate carriers and customized rotors. Centrifugation times and speeds should be optimized for individual samples and analytes.

The Process:

Sample preparation goals:

- Separate compound(s) form sample matrix (matrix is any body fluid or tissue).
- Eliminate unwanted contaminants that interfere with analysis (salts, proteins, enzymes).
- Concentrate compound(s) to meet analytical instrument detection limits.
- Analyze a large number of samples in the least amount of time.

Problem:

Significant advancements are occurring within pharmaceutical bioanalytical laboratories. LC/MS/MS instrumentation allows greater selectivity, sensitivity and speed than ever envisioned. Along with this progress comes a demand for improvement in sample preparation throughput, presently the rate-limiting step in the bioanalytical extraction process. Traditional sample preparation techniques using 96-well packed column plates can have problems with reduced volume of the well due to the volume of the packing, channeling from shifting particles, and fines that can be washed through during elution.

The challenge is to develop methods for the simultaneous quantification of a drug and metabolites that can meet the criteria for an FDA method validation in the shortest amount of time and with the least number of problems.

Solution:

A process transforms loose SPE sorbent particles into thin (0.75 mm), particle-loaded membranes (disks). These disks consist of particles (e.g., bonded silica C8, C18 and mixed-phase cation, and various copolymers) tightly held together within an inert PTFE matrix (90% particles: 10% PTFE, w/w). Empore disks are unique in achieving dense packing with uniform particle distribution. The result is improved mass transfer kinetics with more reliable efficiency in solid phase extraction methods. A significant advance in high throughput sample preparation is the development of 96-well plates containing CDS Analytical, LLC Empore SPE disks. The membrane approach is an ideal complement to LC/MS/MS for bioanalysis.

A single Empore Extraction Disk Plate is essentially 96 individual disk cartridges assembled into one compact, molded unit. The plates allow for high throughput SPE by processing up to 96 samples in a standard 8 x 12 (96-well) format. One disk plate can replace four separate runs on a conventional SPE manifold handling 24 individual cartridges. Each well of the Empore

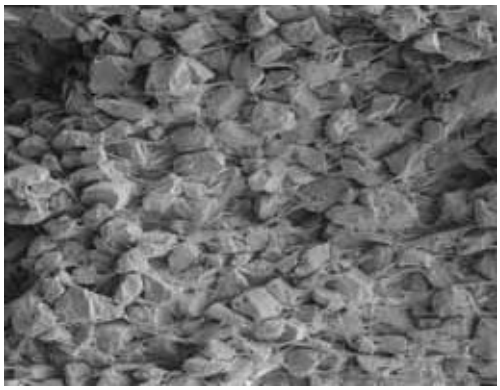


Figure 1 — Silica-based Particles

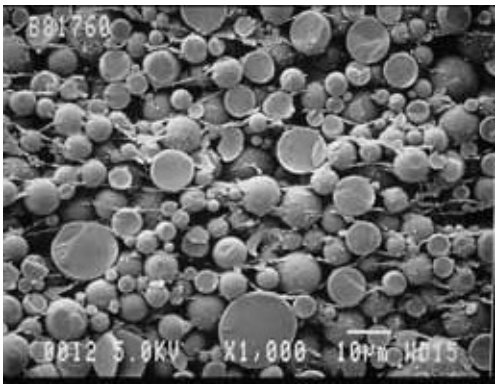


Figure 2 — Resin-based Particles

extraction disk contains a graduated layered polypropylene prefilter, the Empore membrane, and a polypropylene support. A collar around plate tip helps prevent contamination from sample to sample during processing.

The distinct advantages of this membrane format over traditional loosely packed solid phase extraction material in columns are:

- Reduced solvent volumes
- Smaller elution volumes
- Ability to eliminate the evaporation step
- Higher throughput
- Improved precision and efficiency
- Channeling effects reduced or eliminated

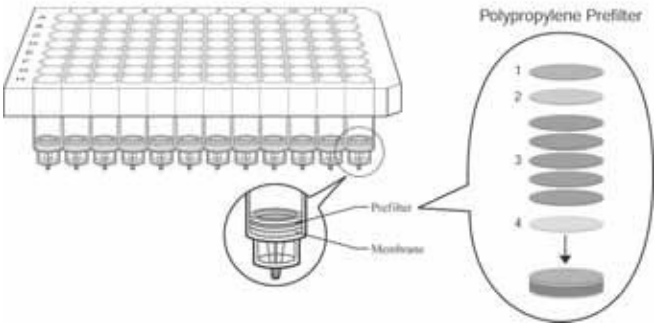


Figure3 — Polypropylene Prefilter

The reduced solvent volumes for elution allowed by the disk format can yield great gains in throughput. These smaller volumes require less time to pass through the sorbent bed. Note that the elution volume should be closely examined to ensure maximal recoveries using the smallest practical volume. Elution volume will not always be constant for every assay, but will vary slightly depending on the particular analyte, its affinity for the chosen sorbent, and the strength of the elution solvent.

A feature of the Empore Extraction Disk format is the ability to directly inject eluates onto an LC/MS/MS system. The elimination of dry-down and reconstitution can improve throughput and may avoid analyte thermal instability problems. These gains are possible by the small solvent volume requirements of the Empore disk format. A common approach is to elute with a small volume of organic solvent, then add a volume of aqueous liquid to the eluate so that the composition of the resulting solution is compatible with mobile phase. Another approach is to elute using a solvent with sufficient organic content which is also compatible with mobile phase for direct injection. When elution is performed into a 96-well collection plate, using either of the two approaches above, the injection can be done directly from that same plate.

Conclusion and Summary

Beyond the gains achieved by the 96-well format and parallel processing of 96 samples, the Empore disk technology provides the means for ultimate improvement in throughput. The Empore disk generates eluates free of particle fines that can foul LC systems. These eluates can be directly injected onto the LC/MS/MS when processed with small volumes of mobile phase compatible solution. The injection can be done out of that very same collection plate when configured with the appropriate autosampler. The CDS Analytical, LLC™ Empore Extraction Disk Plate system provides a solution to the sample preparation bottleneck. In the drive toward decreased drug development time, the Empore Disk Plate is an answer to doing more, faster.

The key advantages to the Empore disk membrane are:

- Dense particle packing, no void space and uniform particle distribution
- Improved efficiency & reproducibility
- Better mass transfer efficiency which reduces variability and improves performance
- More consistent results
- Saves time

Additional Literature

Empore 96-Well SPE Plates; Instructions for Use

Empore 96-Well SPE Plates; Method Optimization Guide

Empore 96-Well SPE Plates; Technical Information on C8 (Octyl) and C18 (Octadecyl)

Empore 96-Well SPE Plates; Technical Information on Mixed Phase Cation-Exchange (MPC)

Empore 96-Well SPE Plates; Technical Information on Universal Resin (UR)

Empore 96-Well Filter Plates; Technical Information

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