

CDS Analytical, LLC

Empore™

High Performance Extraction Disk Plates

High Throughput Pharmaceutical Bioanalysis

Introduction

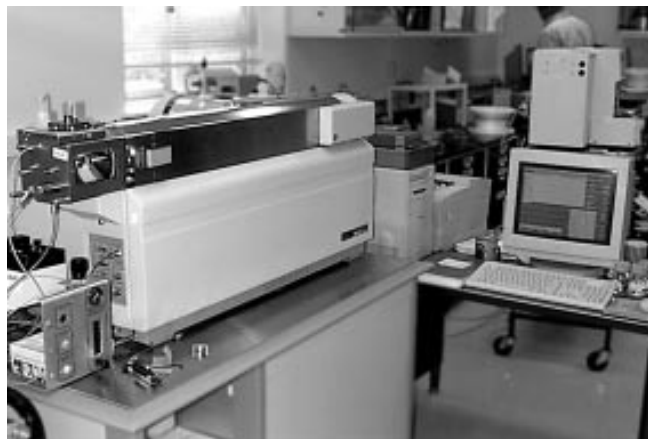
New drug development within the pharmaceutical industry is undergoing dramatic changes as a result of two technological advancements—combinatorial chemistry techniques and LC/MS/MS analytical instrumentation.

Combinatorial Chemistry

Combinatorial chemistry allows for the rapid synthesis (commonly in microtiter plate format) of large libraries of compounds which are evaluated for pharmacological activity. Lead compounds are identified via receptor assays and “hits” are moved into drug metabolism studies to obtain pharmacokinetic data.

LC/MS/MS Instrumentation

Utilization of LC/MS/MS has grown rapidly and is now widely recognized as an ideal, extremely sensitive technique for the analysis of drugs in biological fluids. LC/MS/MS assay run times can be as short as 1-3 min, compared with traditional chromatographic techniques requiring 10-30 min per sample.



Combinatorial chemistry is placing a greater number of compounds into the development process. LC/MS/MS is providing the ability to analyze these compounds, extracted from biological fluids, more quickly and efficiently than ever before. 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.

Sample Preparation Techniques

Empore Disk Technology

A patented process transforms loose SPE sorbent particles into thin (0.75 mm), particle-loaded membranes (disks). These disks consist of particles (e.g., bonded silica C2, 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 recent development of 96-well microtiter plates containing CDS Analytical, LLC Empore SPE disks. The membrane approach has become an ideal complement to LC/MS/MS for bioanalysis.

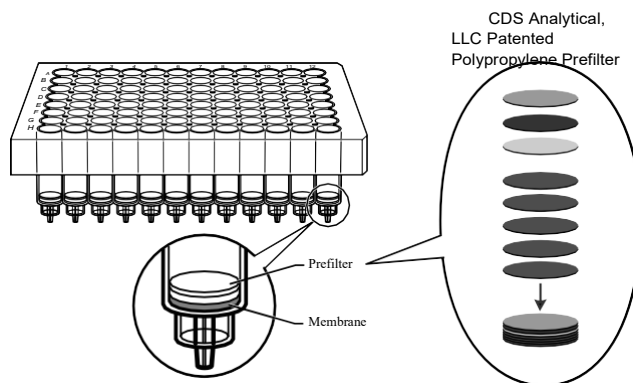


Micrograph of a cross section of a standard density (SD) Empore disk (40-60 µm particle size)

Empore 96-Well Disk Plates

Description

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 microtiter format. One disk plate can replace four separate runs on a conventional SPE manifold handling 24 individual cartridges. Each well of the Empore Extraction Disk Plate has an effective membrane diameter of 5.5 mm and a reservoir volume of 1.2 mL. Each disk contains about 14 mg of particle mass and is secured in place with a sealing ring. Above each ring is a patented prefilter (graded density polypropylene) that improves flow with challenging sample matrices.



Advantages

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

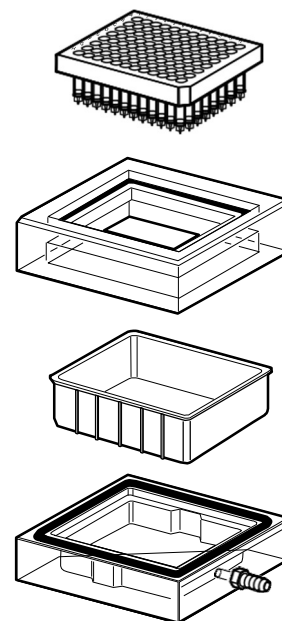
- Reduced solvent volumes
- Smaller elution volumes
- Ability to eliminate the evaporation step
- Higher throughput
- Improved precision and efficiency
- Channeling effects reduced or eliminated

Disk Plate Sample Processing

Processing liquid through the disk plate can be achieved by use of a vacuum manifold system designed specifically for the small volumes used with CDS Analytical, LLC Empore Disk Plates.

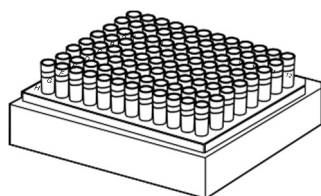
An illustration of the vacuum manifold system shows that liquids are collected into a waste tray during the condition, load and wash steps of extraction process. Elution can be performed into one of several types of collection devices: deep well plate, shallow well plate, or rack of microtubes. Plates can be processed manually using a multichannel pipettor, and in semi-automatic mode using a liquid handling workstation. Alternate processing methods include centrifugation with appropriate microplate carriers and customized rotors. Centrifugation times and speeds should be optimized for individual samples and analytes. Some general guidelines for getting started are:

Sample	Sample Extraction	Elution
Buffer	100g/2minutes	100g/2minutes
Serum diluted 1:3	100g/2minutes	100g/2minutes
Serum diluted 1:1	250g/2minutes	100g/2minutes
Undiluted serum	250g/2minutes	100g/2minutes

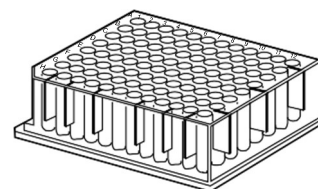


Collection Plate Options

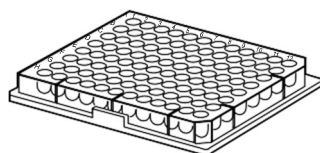
The illustration below represents the collection plate options available from CDS Analytical, LLC for use with the Empore Extraction Disk Plate. Selection of the appropriate collection device will be defined by individual assay conditions, elution volumes and subsequent eluate handling procedures. Additional options for collection plates exist from other vendors, such as smaller volume (0.65 mL) microtubes, and larger volume (2.0 mL) square deep well plates. Refer to the Accessory Products Guide from CDS Analytical, LLC for details.



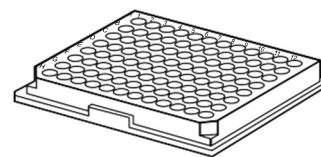
*Microtubes
1.2 mL*



*Deep Well
1.0 mL*



*Shallow Well, U-bottom
0.3 mL*



*Shallow Well, V-bottom
0.3 mL*

Optimizing an Extraction Method

The reduced solvent volumes 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.

Condition and Wash Steps

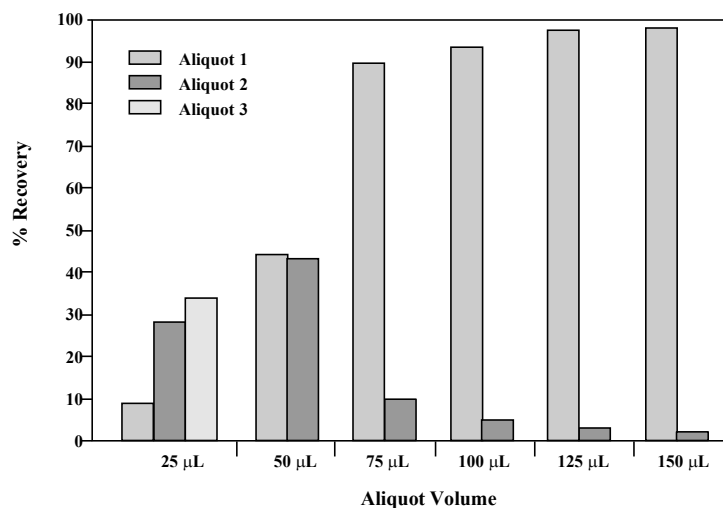
As an example of optimizing a method, consider the extraction of tritium-labeled diazepam from serum using C18-SD Empore Disk Plates. Conditioning of the disk is effectively performed with only 100 μL methanol. The methanol quickly soaks into the prefilter and disk, making a vacuum step unnecessary.

Displacement of the methanol is accomplished with 200 μL water, drawn through the disk using a vacuum of 15 in Hg. A serum volume of 250 μL (diluted by 250 μL ammonium dihydrogen phosphate 0.1M) is then passed through the disk using vacuum. A wash volume of 500 μL water is added. At this point, it is important for the vacuum to remove as much residual water from the disk as possible before eluting with organic solvent. This vacuum should be applied for a slightly longer period of time than in the previous steps.

Elution Step

The 96-well format allows different elution volumes to be used across rows or columns within the same plate. The percent recovery versus elution volume can be quickly determined in a single experiment. In this same example, methanol is used as the elution solvent. The following volumes of methanol are added to the plate in columns one through six: 25, 50, 75, 100, 125 and 150 μL , and vacuum is applied. The replicate samples in a column ($n=8$) are collected and counted for radioactivity recovered. To determine if analyte remains on the disk, a second elution from the same wells of the same plate is performed using the scheme above. If desired, a third elution can be performed. Note that clean collection plates are used for each of the elution steps.

Recovery Vs. Elution Volume



N = 8
Diazepam; C18-SD Disk Plate; Methanol Elution

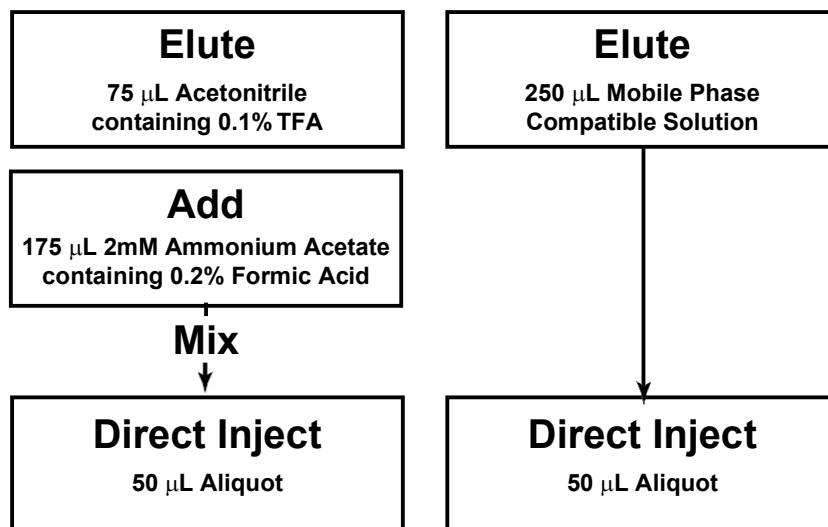
Recovery Vs. Elution Volume

(Continued)

The graph of [³H]diazepam recovery vs. elution volume (opposite page) provides the necessary information to define optimal elution conditions. Presented here as a guideline, this model shows that a single experiment can provide detailed information on a variety of elution schemes. Solvent volume and the required number of aliquots may be evaluated. Ultimately, it is the decision of the analyst to prioritize the aspects of the assay to be optimized. Elution data show that recovery of diazepam can be achieved with as little as 25 μL , though working with such small volumes may prove difficult. This small volume also requires three sequential elutions to obtain maximal recovery, creating additional processing time. Elution using 50 μL or 75 μL aliquots is more practical, both yielding complete recovery after only two aliquots. While total recovery is similar between 2 x 50 μL and 2 x 75 μL , a comparison of the first aliquots from each approach shows that elution with 75 μL yields a more concentrated fraction than does elution with 50 μL . In some cases it may be preferable to use only the first fraction (75 μL) and dilute that volume with aqueous solution for direct injection. Such an approach allows more mass injected on column for greater sensitivity. If a single elution giving the greatest total recovery is preferred, 125 μL or 150 μL may yield sufficient results. By performing such experiments early in the method development process, elution optimization can improve performance for each assay.

Eliminating the Evaporation Step

A unique feature of the Empore Extraction Disk format is the ability to directly inject eluates onto the 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 microtiter collection plate, using either of the two approaches above, the injection can be done directly from that same plate. Autosamplers are commercially available that are designed to inject from microtiter plates.



Automation of Empore Disk Plates

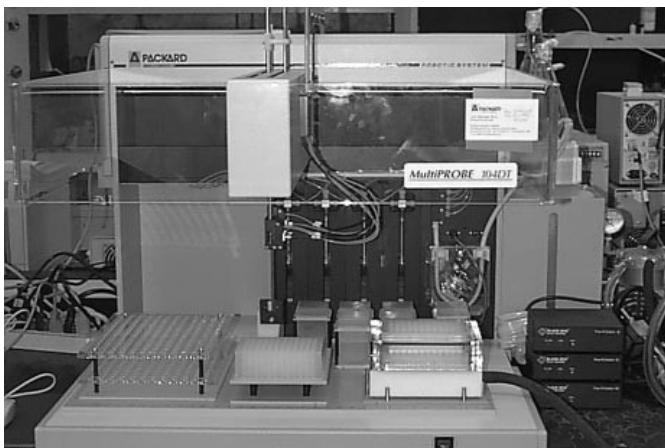
The throughput gains provided by the Empore Extraction Disk Plate attain an even higher level of performance when the extraction is automated, thereby eliminating the possibility for human pipetting error and/or procedural error. Commercially available liquid handling workstations provide a variety of automation solutions since many are designed to work with the microtiter plate specifically. At this time, two of the more established automated systems for SPE in 96-well format include the Packard MultiPROBE and the Tomtec Quadra 96. Liquid handling and robotic processing of microtiter plates is a rapidly expanding field, and additional vendors continue to develop new solutions.

Packard MultiPROBE™

Packard MultiPROBE

The MultiPROBE (Packard Instrument Company, Meriden, CT) automates all of the necessary liquid handling steps for SPE and is used in a semi-automated mode. The flexible deck design allows the placement of vacuum manifold, sample tubes, and reagent troughs onto deck positions. The Empore Disk Plate is placed on top of the manifold. The Varispan™ four-tip liquid processing feature allows for parallel processing in the sample tubes, reagent troughs and the Empore Disk Plate, with the ability to adjust the tip spacing from source to destination to accommodate different widths. This feature allows for the MultiPROBE to easily transfer samples from standard test tubes to wells of microtiter plates. Additional features include a variety of available syringe sizes, independent liquid sensing capabilities, and the ability to check for blocked wells after sample processing. The MultiPROBE is also used to control the application of vacuum to the 96-well disk plate via power switching hardware.

Once the samples, reagents and Empore Disk Plate have been defined and placed onto the deck, the method is started. The MultiPROBE performs all liquid handling steps and controls the vacuum without user intervention. Just prior to elution, the instrument pauses and the user removes the waste tray and manually places a collection plate (or microtubes) into the vacuum manifold. As the vacuum is applied, the eluate from each well is collected. The collection plate is now ready to be sealed and placed into an autoinjector for analysis. The total time required for extracting 96 samples on the Empore Disk Plate using the MultiPROBE will vary depending on total volumes used, but generally is between 45 to 75 min.



Tomtec
Quadra 96™

The Quadra 96 (Tomtec, Hamden, CT) automates all of the liquid handling steps for SPE and is used in a semi-automated mode. Its 96 separate precision pipettes operate simultaneously to reduce the time necessary to process a plate. Each pipette delivers from 1 to 450 μL , and volumes larger than this amount require an additional delivery step. Extraction methods are programmed via its front panel or a PC using RS232 interface. Its deck is a six-position automated shuttle which accommodates the following: a vacuum manifold, samples, clean set of pipette tips, and three different solvent troughs (*e.g.*, for the conditioning, wash and elution steps). Note that all samples, before analysis, must be separately reformatted from test tubes (or vials) into the 8 x 12 microtiter array (deep well plate) in order to be processed by this system. The speed at which solvents are picked up and delivered can be varied to satisfy particular assay requirements. The instrument is traditionally attended by a user who manually controls vacuum conditions during plate processing.

After the plate has been conditioned and the samples have been loaded onto the disk plate and processed, a clean set of polypropylene tips is exchanged. The wash solution is then added and processed through the disk using vacuum. Just prior to elution, the user removes the waste tray from inside the vacuum manifold and inserts a collection plate or microtubes. The elution solvent is added to the plate and the Quadra 96 then completes the remainder of the method. The entire time required for extracting 96 samples on the Empore Disk Plate using the Quadra 96 commonly ranges from 15-25 minutes, depending on total volumes processed and the number of steps comprising the method.



Conclusions

A significant advancement is occurring within pharmaceutical bioanalytical laboratories. LC/MS/MS instrumentation is allowing 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 individual packed columns require from 5-6 hours to manually process 96 samples for analysis. Individual columns cannot satisfy the throughput needs of LC/MS/MS, not even with existing automation. The combination of CDS Analytical, LLC Empore disk technology and the

96-well microtiter format now offer the solution to the bottleneck in sample preparation. Manual processing of 96 samples using Empore Disk Plates requires only about 30-40 minutes. Certain automation approaches can reduce the total extraction time to as little as 15 minutes.

Beyond the gains achieved by the microtiter 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 appropriate autosamplers. The CDS Analytical, LLC Empore Extraction Disk Plate system provides the solution to the sample preparation bottleneck. In the drive toward decreased drug development time, the Empore Disk Plate is the answer to doing more, faster.

Visit Our Web Site

Detailed product and technical information, including *Instructions for Use* and *Answers to Frequently Asked Questions*, can be found on the Internet.

www.cdsanalytical.com/empore

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