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The Reporter

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Pharmaceutical Applications Using Discovery® DSC-18 SPE-96 Well Plates

Over the last 15 years, Solid Phase Extraction (SPE) has become the most powerful technique currently available for rapid and selective sample preparation. By selectively adsorbing a compound or analyte from a liquid phase to a solid, one can extract, purify, trace enrich, and exchange an analyte's matrix environment (aqueous to organic sample matrix) for subsequent analysis. The end result is a reproducible, safe, convenient, time-saving alternative to liquid-liquid extraction.

Speed is the Key

Historically, seasoned operators can process up to 12-24 samples concurrently using a multi-port vacuum manifold. However, with the emergence of new combinatorial techniques and faster analytical tools such as LC/MS/MS, the pace of new drug development has increased dramatically. As thousands of samples can be generated routinely, more drugs are being forwarded through the development pipeline resulting in the need for mass drug screening, higher sample throughput, and faster analysis. In response, Supelco has developed and further expanded our product line to include Discovery SPE Phases in a 96-well format.

96 Samples Prepared in 30 Minutes

In this report, we demonstrate the utility and quality of our Discovery DSC-18 SPE-96 well plates by delineating the recoveries and relative standard deviations for a number of acidic, basic, and neutral pharmaceuticals (Figure 1) extracted from serum. Figure 2 shows recovery data of the compounds listed in Figure 1 using Discovery DSC-18 SPE-96 plates with low relative standard deviations (RSDs) (≤ 5%) and high recoveries (≥ 90%). These compounds were processed in parallel using the "Universal Method" shown in Table 1. This method is the simplest

(continued on page 4)

Figure 1. Structures of Compounds Studied

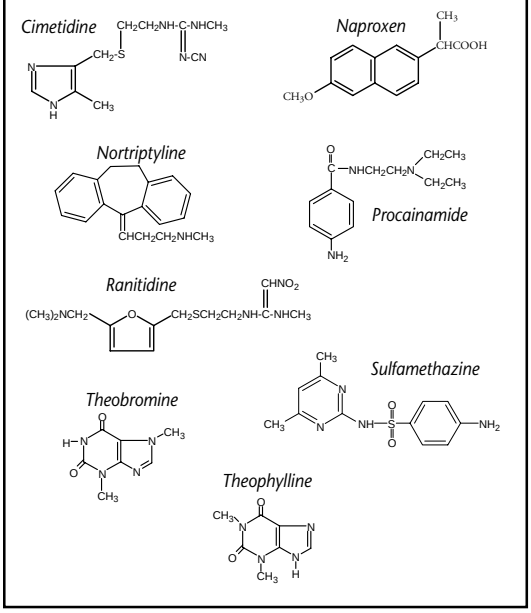
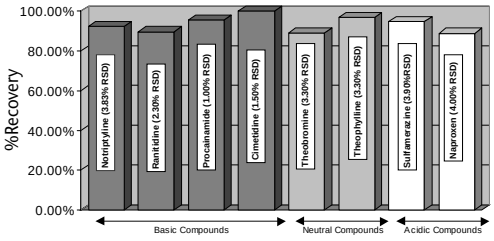


Figure 2. Recoveries for 8 Pharmaceutical Compounds (0.5µg) on Discovery DSC-18 SPE-96 Plates (100mg/well)



n = 8 for each individual compound.

Table 1. "Universal Method"

	Conditioning	Condition each well w/2mL MeOH & 2mL DI water.	
	Sample Load	Load each well with sample (1mL). In the case of serum, dilute 1:1 or 1:2 with water. If serum sample, then preparation dependent on drug type. Load speed: 1 drop/sec.	*Serum Preparation:* **Neutral:** No conditioning; sample pH7.5. **Basified:** Adjust sample pH to pH9 w/3-6µL 10N KOH/mL sample. **Acidified:** Adjust sample pH to pH3.5 w/6-24µL phosphoric acid/mL sample.
	Wash	Wash each well w/2mL 5% MeOH.	
	Drying	Vacuum dry with manifold for ~5-15 min.	• This is to remove any excess water from the sorbent. The presence of water in the final eluent may prolong eluent evaporation.
	Elution	Elute drug w/2mL MeOH. Elution speed: 1 drop/sec.	
	Drying	Dry eluate with Nitrogen purge (40°C; 15-20 min.) Reconstitute w/200µL mobile phase.	

LC issue

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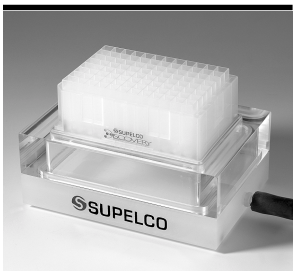
T200012

NEW PRODUCTS

Discovery SPE-96 Well Plate & PlatePrep Vacuum Manifold



Discovery SPE-96 Well Plates

Discovery SPE-96 well plates are extensively tested and quality controlled for pharmaceutical and clinical applications. Included with each plate is a certificate of analysis that describes tests used to ensure reproducible raw silica and bonded silica properties. Each lot is tested for consistent carbon loading, cleanliness, hydrophobic selectivity, and capacity, as well as efficiency for extracting model acidic, neutral, and basic pharmaceuticals. Stringent guidelines regarding packing procedures have also been imposed to ensure consistent flow rates and sorbent bed weights.

Supelco 96-Well Plates and Accessories

96-Well Plates

Discovery DSC-18 SPE-96 Plate, 25mg/well **575601-U**
Discovery DSC-18 SPE-96 Plate, 50mg/well **575602-U**
Discovery DSC-18 SPE-96 Plate, 100mg/well **575603-U**
Discovery DSC-18Lt SPE-96 Plate, 25mg/well **575604-U**
Discovery DSC-18Lt SPE-96 Plate, 50mg/well **575605-U**
Discovery DSC-18Lt SPE-96 Plate, 100mg/well **575606-U**
Discovery DSC-Si SPE-96 Plate, 25mg/well **575607-U**
Discovery DSC-Si SPE-96 Plate, 50mg/well **575608-U**
Discovery DSC-Si SPE-96 Plate, 100mg/well **575609-U**
Discovery DSC-PS/DVB SPE-96 Plate, 25mg/well **575610-U**
Discovery DSC-PS/DVB SPE-96 Plate, 50mg/well **575611-U**

96-Well Plate Accessories

96 Sq. Well Collection Plates, 0.35mL, PP, 50/pk **575651-U**
96 Sq. Well Collection Plates, 1mL, PP, 50/pk **575652-U**
96 Sq. Well Collection Plates, 2mL, PP, 50/pk **575653-U**
Disposable Reservoir/Waste Tray, PVC, 25/pk **575654-U**
96 Sq. Well Piercable Cap Mats, 50/pk **575655-U**
Reagent Reservoir ..... **R9259 - 100ea.**
Cluster Tube Rack ..... **Z372226 - 1pak**

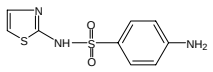
PlatePrep Manifold and Manifold Replacement Parts

96 Well Plate Starter Kit with Manifold **575650-U**
Contents of kit:
1 Plate Prep Manifold
1 96 Sq. Well Collection Plates, 2mL, PP
2 Disposable Reservoir/Waste Trays, PVC
1 96 Sq. Well Piercable Cap Mats
5 Reagent Reservoirs
1 Cluster Tube Rack

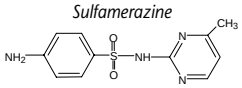
PlatePrep Vacuum Manifold **57192-U**
Acrylic Clear Top for Manifold **57193-U**
Polypropylene Base for Manifold **57194-U**
Gasket Kit for Manifold **57195-U**
Vacuum Gauge/Bleed Valve for Manifold **57161-U**

Figure 4. Chemical Structures of Compounds Studied

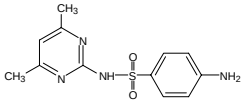
Sulfathiazole



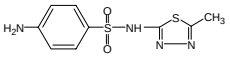
Sulfamerazine



Sulfamethazine



Sulfamethizole



*All literature mentioned in this issue can be obtained from the website, **www.sigma-aldrich.com/TheReporter**, by completing the Literature Request section on the reply card, or by calling our Technical Service Department.*

NEW APPLICATIONS

SPE Tubes: A Classic Alternative to 96 Well Plates

Even though Supelco now offers 96 well SPE plates in a variety of chemistries, we still have the more traditional SPE tubes available in many chemistries, tube configurations and bed weights. In addition, Supelco has a number of applications available using SPE tubes. We discuss one of these applications below.

In this example, we show the extraction of four Sulfa drugs, Sulfathiazole, Sulfamerazine, Sulfamethazine and Sulfamethizole (Figure 4). The sulfonamide moiety is acidic and readily forms salts while the aromatic amino group imparts basic properties to the drugs. These polar functionalities make the sample preparation a challenge and HPLC separation difficult.

We were able to extract these four anti-bacterial compounds from serum using Discovery DSC-18, 500mg/3ml tubes using a simple methanol/water SPE method. Table 2 shows the methodology used for the extraction. In this case we used the Zymark® Rapid Trace® SPE Workstation. Table 3 shows the recovery data for the four compounds at the 1 and 5µg/ml levels of each compound spiked into porcine serum. Note that recoveries ranged from 88.7% to 98.3% with corresponding %RSDs listed after each drug and concentration. Figure 5 shows the resulting chromatograms for an extracted serum blank and the extracted sulfa drugs. Note that all samples were filtered prior to HPLC introduction and a guard column was used in the analysis.

The above example illustrates that there still is a place in many labs for SPE tubes and not just 96 well plates. The large range of chemistries and bed weights make SPE tubes

Table 3. Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Sulfathiazole	1.0µg/mL	90.1	±2.7
	5.0µg/mL	97.7	±2.1
2. Sulfamerazine	1.0µg/mL	91.8	±2.8
	5.0µg/mL	97.2	±2.4
3. Sulfamethazine	1.0µg/mL	91.9	±2.8
	5.0µg/mL	96.5	±2.2
4. Sulfamethizole	1.0µg/mL	88.7	±3.2
	5.0µg/mL	98.3	±2.5

Table 2. SPE Procedure, Using a Zymark® RapidTrace® SPE Workstation

Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	methanol	2.0	5.0	Conditions sorbent
2. Condition	water	2.0	5.0	Conditions sorbent
3. Load	spiked porcine serum	2.0[A]	0.75	Applies serum sample
4. Rinse	5% methanol in water	2.0	5.0	Washes sorbent
5. Purge-Cannula	water	4.0	30.0	Cleans sample cannula
6. Rinse	vent	0.1	2.0	Positions SPE tube over waste port
7. Dry	nitrogen	Time = 10 min		Dries sorbent
8. Purge-Cannula	methanol	4.0	30.0	Cleans sample cannula
9. Collect	methanol	1.0	1.0	Elutes analytes into collection vessel
10. Collect	vent	6.0	3.0	Pushes residual eluent into collection vessel[B]
11. Purge-Cannula	water	4.0	30.0	Cleans sample cannula

[A] 1mL porcine serum spiked with 1.0µg/mL or 5.0µg/mL each analyte, diluted with 1mL water, then acidified with 40µL H_3PO_4.
[B] Eluent evaporated to dryness with a nitrogen stream at 40°C, using a Zymark TurboVap® LV Workstation, then reconstituted with 1mL mobile phase containing 3.0µg/mL sulfanilamide (IS).

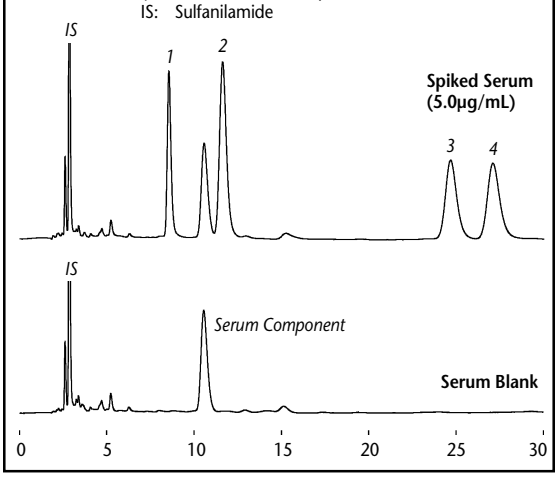
NEW APPLICATIONS (contd.)

a good first choice when developing methods before scaling down to the smaller 96 well plates. Supelco will continue to investigate other phases and applications for this time-tested methodology.

For more information, request T499127, T498364 and T197910.

Figure 5. HPLC Analysis of Extracted Sulfa Drugs

Column:	Discovery C18, 15cm x 4.6mm, 5µm particles, preceded by a 2cm C18 guard column and 0.5µm frit filter.
Mobile Phase:	methanol:1% acetic acid (8:92)
Flow Rate:	1mL/min (8 min) then 0.6mL/min
Temp.:	30°C
Det.:	UV, 254nm
Inj.:	10µL reconstituted porcine serum extract
IS:	Sulfanilamide



LITERATURE

Discovery SPE-96 Well Plates

Four product profiles are available from Supelco on the Discovery DSC SPE-96 Plates. These profiles describe four of the phases currently available, how and for what compounds they may be used with, and the product specifications of each phase.

Discovery DSC-Si SPE-96 Plate - An acid washed silica. Our 96 well format enables you to quickly and effectively clean samples or remove baseline impurities in combinatorial chemistry applications.

For more information, request T400173.

Discovery DSC-PS/DVB SPE-96 Plate - A polystyrene-divinylbenzene material that retains hydrophobic compounds which contain some hydrophilic functionality, especially aromatics.

For more information, request T400174.

Discovery DSC-18 SPE-96 Plate - A polymerically bonded trifunctional C18 silica used to extract, isolate, purify and concentrate pharmaceuticals from biological fluids and other aqueous media.

For more information, request T400171.

Discovery DSC-18Lt. SPE-96 Plate - A monomerically bonded C18 used to extract moderately polar to non-polar compounds from aqueous media, such as biological fluids.

For more information, request T400172.

LC PERFORMANCE TIP

Conditioning and Equilibration on 96-Well Plates

When performing Solid Phase Extraction (SPE) on a silica-based reversed-phase sorbent, a conditioning and equilibration step must be employed prior to sample addition. Conditioning with an organic solvent solvates the bonded phase which ensures proper and uniform interaction between the solid phase and sample matrix. Equilibration with an aqueous solution similar in solvent content, pH, or salt concentration to the sample matrix promotes better retention of the appropriate compounds onto the sorbent. Over-drying during the conditioning and equilibration steps will typically lead to low and variable recoveries. Traditionally, single cartridge SPE users are advised to leave a thin film of liquid on top of the sorbent after each of the pre-sample addition steps. However, in 96-well SPE where the growing trend involves an overall reduction in volume (< 1ml) for each step and a decreased bed weight (< 50mg), over-drying becomes a concern. In single cartridge SPE, each SPE tube is controlled individually with its own flow control valve; therefore, it is relatively easy to leave a thin film of liquid after each process step. In 96-well SPE, a single valve controls all 96-wells, and the slight packing variations between each well that is inherent to all extraction plates may promote slight differences in flow rate. Also, because most extraction plates are made of polypropylene, (which is opaque), it becomes very difficult to visibly determine liquid heights within the inner wells during a 96-SPE process step. In our labs, we have discovered that a 96-well SPE user can pass a conditioning solvent through a reversed-phase 96-well SPE plate completely (100mg/well) without greatly affecting recovery. In our studies, we passed 1mL of methanol through the well plate, and left the vacuum on at full draw for increasing lengths of time. We observed that less than 5% loss in recovery incurred after drying for 1 minute. However, after 2 minutes, the loss in recovery and increase in variation were significant. In a similar study measuring the loss in recovery vs. drying time for the equilibration step (DI water), we observed that recovery was not affected after 2 minutes of vacuum drying, and less than a 5% loss in recovery was observed after 7 minutes.

For more information, request T300165.

Pharmaceutical Applications…

(continued from page 1)

method possible for reverse phase SPE applications. Recovered eluent extracts were then quantified via Discovery C18 HPLC analyses (15cm x 4.6mm). Excluding the final eluent evaporation step, up to 96 samples can be processed in less than 30 minutes.

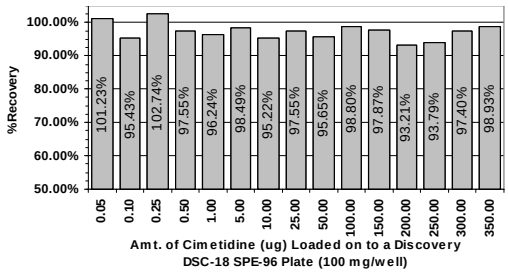
Wide Range of Concentrations Show Good Recoveries

In another study, 16 different spiked concentrations (0.050-350µg/mL) of the anti-arrhythmic compound Cimetidine were recovered from 1ml of porcine serum using a Discovery DSC-18 SPE-96 well plate in conjunction with the "Universal Method". Recoveries were determined against external standards via subsequent HPLC analyses. Figure 3 shows that excellent recoveries were obtained across a wide range of sample concentrations with an average value of 95.5%.

No More Sample Prep Bottleneck

The data presented in this report shows that Discovery DSC-18 SPE-96 well plates provide excellent recoveries and low

Figure 3. Average Recovery of Sixteen Spiked Concentrations of Cimetidine



n = 2 for each individual concentration.

RSDs for a number of compounds when extracted from a biological matrix. High recoveries were also observed when processing a wide range of analyte concentrations. Discovery SPE-96 Well Plates are ideal for higher throughput laboratories by eliminating the analytical bottleneck commonly inherent in sample preparation.

For more information, request T300165 and T499127.

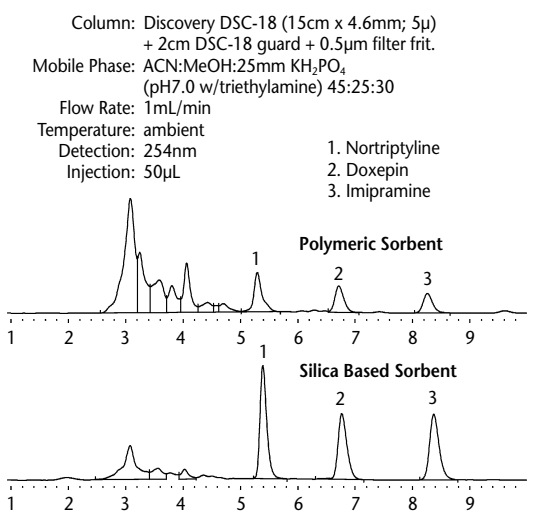
CASE STUDY 6

Background Concerns for Reversed-Phased Polymeric Sorbents

Manufacturers have developed new reversed-phase polymeric SPE sorbents that use nonpolar interaction as the mechanism of retention while displaying hydrophilic characteristics allowing for water wettability. In general, the newer generation of these polymeric SPE phases have greater binding capacity due to their larger surface area which allows for greater hydrophobic coverage. Also, these polymeric sorbents permit a broader range of selectivity, claiming universal extractability for both polar and non-polar acidic, basic, and neutral analytes. However, many SPE users have expressed dissatisfaction with the newer polymeric sorbents with regards to binding capacity and selectivity. Although retention is observed for a broader range of compounds, polymeric sorbents tend to co-extract interfering matrix components as well as the analyte(s) of interest. As a result, HPLC analyses of polymeric SPE extracts tend to yield higher background due to retention of matrix components.

Figures 6 shows two chromatograms run under identical conditions. Tricyclic Anti-Depressants analyzed in the top chromatogram were extracted from serum using a competitor's 96-well polymeric sorbent (30mg/well). The same sample was processed using a more traditional cyanopropyl bonded silica-based SPE sorbent (Supelco LC-CN) packed in a Supelco SPE-96 well plate (50mg/well). Chromatographic results are shown in the bottom chromatogram. Both samples were processed from the same lot

Figure 6. Analysis of Three Tricyclic Anti-Depressants from Serum Using a Polymeric Sorbent vs. a Silica Based Sorbent



of spiked porcine serum under identical SPE conditions. When comparing the chromatograms for the two divergent SPE chemistries, the extract obtained from the silica-based sorbent was significantly cleaner than the extract obtained from the polymeric SPE phase while still maintaining excellent recovery and low RSDs.

For more information, request T300165 and T499127.

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