

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

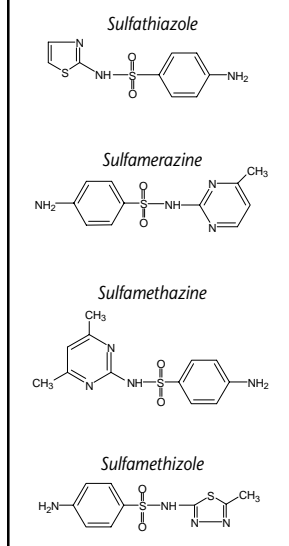
96 Well Plate Starter Kit with Manifold

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



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.

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 H₃PO₄.

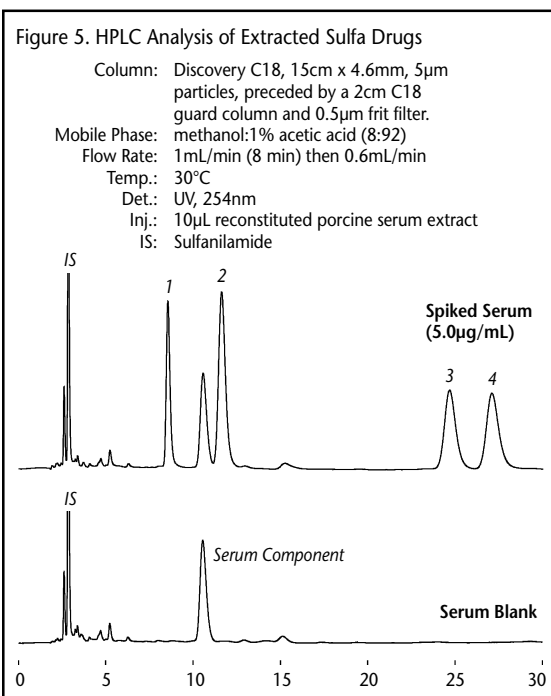
^B Eluent evaporated to dryness with a nitrogen stream at 40°C, using a Zymark TurboVap[®] LV Workstation, then reconstituted with 1mL mobile phase containing 3.0µg/mL sulfanilamide (IS).

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 (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.



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.