

Application Note 165

Discovery SPE-96 Well Plates

The accelerated pace of new drug development, mass drug screening, and clinical investigations require higher sample throughput and faster analysis. Sample preparation has become the rate limiting step for analysis. In response, Supelco has introduced the Discovery SPE-96 Well Plates.

Discovery SPE-96 Well Plates are ideal for extracting active ingredients and metabolites from 96-different biological samples concurrently, in parallel. Also, uniform vacuum dynamics inherent with 96-well processing allow for more consistent flow characteristics, which in turn yield more reproducible recoveries.

Our Discovery SPE-96 Well Plates are quality controlled for accurate and reproducible results for pharmaceutical 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. We adhere to stringent guidelines regarding packing procedures to ensure consistent flow rates and sorbent bed weights.

We studied a number of acidic, basic and neutral test analytes extracted from serum using the 96 well plate format and the "Universal Method" for sample clean-up for all the SPE (solid phase extraction) applications. Following sample preparation, HPLC analysis was done on the clean sample.

Table 1 below shows the "Universal Method" for sample clean-up. Note that for basic compounds the sample was basified and for acidic compounds the sample was acidified.

Figure 1 shows a number of acidic, basic, and neutral compounds along with their recoveries and %RSD. These analytes were recovered from porcine serum at the level of 0.5µg. Samples were quantitated via HPLC analyses against external standards. Note that all recoveries are at least 90% with all %RSD at 4% or less. Excluding the final eluent evaporation step, up to 96 samples can be processed in less than 30 minutes.

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.	

In another study, we looked at the recovery of Cimetidine in porcine serum at sixteen different spiked concentrations ranging from 0.050-350µg/mL. **Figure 2** shows the results of this study. In this case, we basified the sample for extraction. This data shows excellent recovery over a wide range of sample concentrations with the average value

Figure 1. Recoveries for 8 Pharmaceutical Compounds (0.5µg) on Discovery DSC-18 SPE-96 Plates (100mg/well)

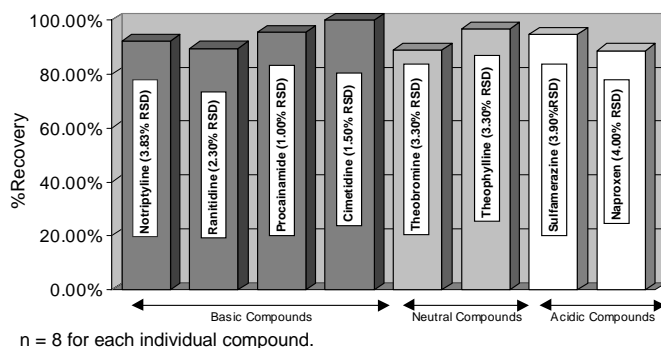
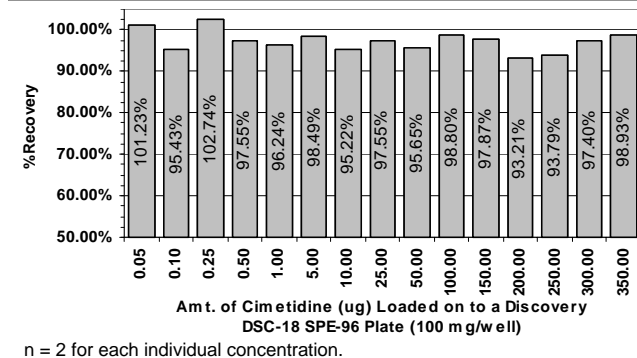


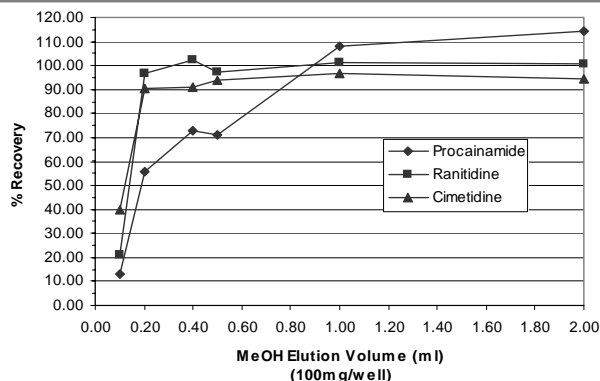
Figure 2. Average Recovery of Sixteen Spiked Concentrations of Cimetidine



at 95.5%. As in the above example, HPLC using an external standard was used to calculate recoveries.

In a similar study using Cimetidine, Procainamide and Ranitidine, we wanted to study the effect of elution volume on recovery. The trend has been to elute with a minimum volume (~0.2mL). A minimum volume is desirable to allow for direct analysis of the final eluent without evaporation-reconstitution. **Figure 3** shows the recovery of 0.5µg of the above mentioned drugs in 1mL of 1:1 porcine serum: DI water for analyses using decreasing volumes of eluent. This demonstrates that for some applications, one can effectively elute an analyte from 100mg of Discovery DSC-18 with as little as 200µL.

Figure 3. Elution Volume vs. Recovery



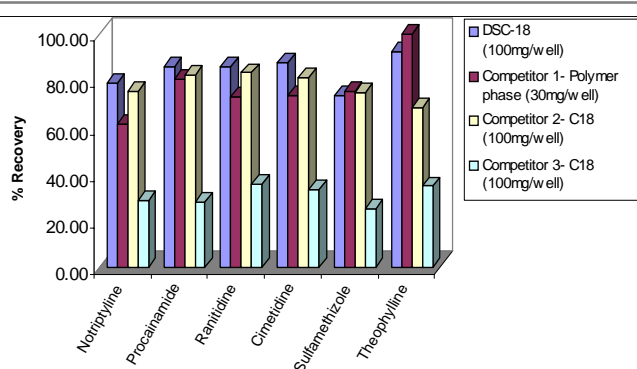
Our final study in this application note looked at the recovery data for a number of compounds on competitors extraction plates. We demonstrated that 100mg of Discovery DSC-18 seems to perform equally as well as a 30mg polymeric material and two other C18 materials when extracting 6 different drugs from porcine serum. **Figure 4** shows the recovery data. Note that all the recoveries in Figure 4 were obtained using the Universal Method.

The data presented in this report shows good recovery and low RSDs for the sample preparation of a number of drugs spiked in porcine serum using the Discovery DSC-96 Well Plates. The Universal Method used in conjunction with these plates can speed sample clean-up to enable faster sample analysis. In addition, we found that we were able to obtain excellent recoveries over a wide range of analyte concentrations. We have shown that Discovery DSC-96 Well Plates also permit low elution volumes with no appreciable loss of sample. Finally, we demonstrated that Discovery DSC-96 Well Plates show recoveries as good or better than competitors' plates under the same clean-up conditions.

To view the SPE phases Supelco currently stocks, visit our web site at www.sigma-aldrich.com.

To order, call Supelco at 800-247-6628 or fax your request to 800-447-3044.

Figure 4. Discovery DSC-18 vs. Competitor's Extraction Plates



Discovery SPE-96 Products & Accessories

Description		Cat. No.
SPE -96 Well Plates	DSC-18 SPE-96	100mg/ well 575603-U
		50mg/ well 575602-U
		25mg/ well 575601-U
DSC-18 Lt SPE-96	100mg/ well	575606-U
	50mg/ well	575605-U
	25mg/ well	575604-U
DSC-PS/DVB SPE-96	50mg/ well	575611-U
	25mg/ well	575610-U
DSC-Si SPE-96	100mg/ well	575609-U
	50mg/ well	575608-U
	25mg/ well	575607-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, 100 each	R9259
Cluster Tube Rack, 1/pk	Z372226

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
Supelco PlatePrep Vacuum Manifold,each	57192-U

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UNITED STATES · Supelco · Supelco Park · Bellefonte, PA 16823-0048 · Phone 800-247-6628 or 814-359-3441 · Fax 800-447-3044 or 814-359-3044 · email: supelco@sial.com

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