

## Increase Productivity by Minimizing Processing Time Between Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC) Analysis

### Abstract

The use of 96-well SPE plates allows bio-analytical chemists to process up to 96 samples at once in a single extraction. Some methods allow for the direct analysis of SPE eluant extracts. We present various tactics in this report to assist SPE users. Proper attention to these details result in faster methods.

### SPE Eluant – HPLC Compatibility

In reversed phase SPE, retention of the solute typically occurs in the presence of an aqueous sample matrix. A strong solvent (e.g., methanol or acetonitrile) then elutes the compound. Severe peak splitting, tailing, and early elution can occur if the analyst directly analyzes these extracts on a HPLC system without further treatment. (See Figure A). This is because the sample solvent is of higher strength than the HPLC mobile phase.

### Post 96-Well Plate Processing

A post 96-well plate solvent exchange step is required to avoid the chromatography problem discussed above. First, evaporate the eluant's strong solvent. Then, dissolve the residue with a weaker solvent (e.g., mobile phase) more suitable for the HPLC system. This is normally the most time-consuming step of the SPE procedure. There are a number of evaporation systems designed for 96-well collection plates. Their collection plate bases are heat-controlled. The 96 pins blow nitrogen into each individual well at the same time. Zymark Corporation (Hopkinton, MA) and Apricot Designs (Monrovia, California) sell these evaporation units. A pierceable cap mat or laminate heat sealer seals the collection plates, preventing contamination and/or evaporation. Most HPLC instrument providers now offer optional auto sampler racks, adapters, and software designed to accept 96-well platforms.

### No Post 96-Well Plate Processing

You can develop methods that skip the evaporation step. One strategy is to use pH (instead of solvent strength) to oppose the hydrophobic attractive force. For example, if the analyte of interest is a basic compound, decreasing the pH of this sample's solvent to two units below its pKa ionizes the compound. This weakens the hydrophobic interaction that

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Figure A. Effect of Sample Solvent on Peak Shape & Retention of n-Butylaniline

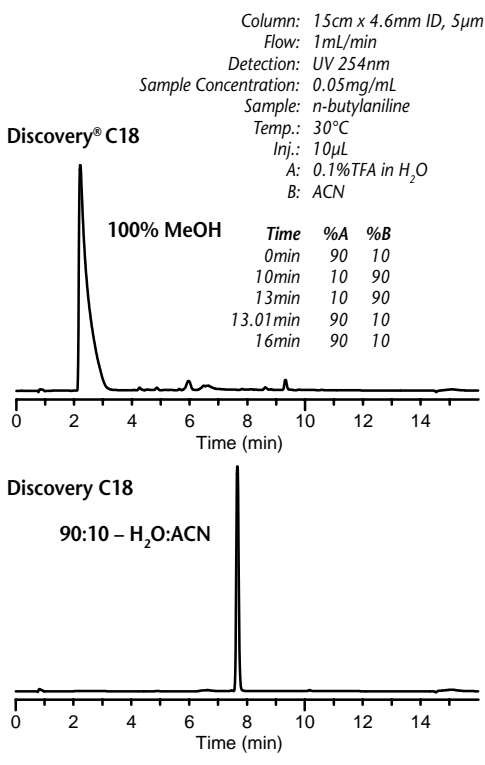
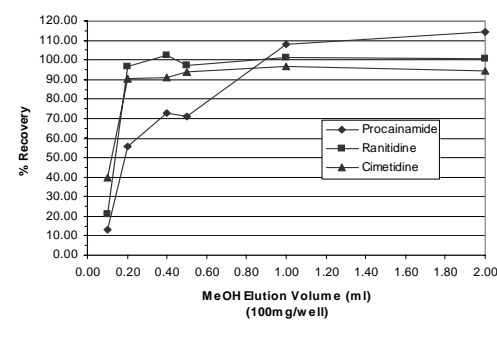


Figure B. Comparison of Elution Volume vs. Recovery of Pharmaceutical Compounds on Discovery DSC-18 SPE-96 Well Plates



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3µm Discovery HS C18 HPLC Column

## NEW PRODUCTS

### 3µm Discovery HS C18

In the fast-paced world of analytical chromatography, LC/MS stands above all others in popularity for quick and useful results. Typically, most LC/MS columns are nothing more than a pre-existing 3µm C18 bonded silica packed in short narrow dimensional columns. Now, an LC/MS column specifically made for LC/MS use is available.

The 3µm Discovery HS C18 LC/MS column has two features that set it apart from traditional LC/MS columns. First, it is a no bleed phase as tested by an independent laboratory. This feature allows trouble-free analyses without worry of bleed spectra peaks co-eluting with solutes of interest. It also simplifies the total ion chromatogram (TIC) for quantitative studies.

Second, the 3µm Discovery HS C18 LC/MS packing is a high surface (hence, "HS") area packing that provides several benefits. The HS phase is able to retain more polar compounds that other C18 phases cannot retain. This also can help in providing higher resolution between critical pairs. More solvent can be used in the mobile phase, which increases the signal to noise ratio in esi LC/MS runs. The greater LC/MS sensitivity obtained by the proper choice of the LC/MS column is significant. Do not overlook this simple way of getting your desired detection levels.

In summary, the 3µm Discovery HS C18 LC/MS column is an ideal LC/MS column and an excellent general use HPLC column.

#### Discovery HS C18 HPLC Columns

ID (mm)	Length (cm)	Cat. No.
2.1	5.0	569253-U
2.1	7.5	569254-U
2.1	15.0	569255-U
4.6	5.0	569250-U
4.6	7.5	569251-U
4.6	15.0	569252-U

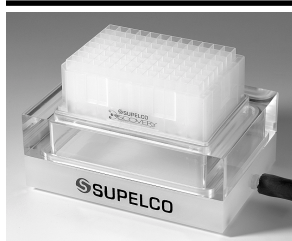
#### 2cm Supelguard™ Cartridges with 3µm Discovery HS C18 Packings

Description	Cat. No.
4.0mm ID Cartridges <sup>2</sup>	
kit	569275-U
pk of 2	569274-U
2.1mm ID Cartridges	
kit	569277-U
pk of 2	569276-U

<sup>2</sup> For 4.0mm ID or 4.6mm ID analytical columns.

For more information request T401026 - Discovery HS C18 HPLC Columns.

Discovery SPE-96 Well Plate & PlatePrep Vacuum Manifold



## FEATURED PRODUCTS

### Discovery SPE-96 Well Plates and Accessories

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.

#### Discovery SPE-96 Well Plates

Description	Bed Weight	Cat. No.
DSC-18 SPE-96 Plate	25mg/well	575601-U
DSC-18 SPE-96 Plate	50mg/well	575602-U
DSC-18 SPE-96 Plate	100mg/well	575603-U
DSC-18Lt SPE-96 Plate	25mg/well	575604-U
DSC-18Lt SPE-96 Plate	50mg/well	575605-U
DSC-18Lt SPE-96 Plate	100mg/well	575606-U
DSC-Si SPE-96 Plate	25mg/well	575607-U
DSC-Si SPE-96 Plate	50mg/well	575608-U
DSC-Si SPE-96 Plate	100mg/well	575609-U
DSC-PS/DVB SPE-96 Plate	25mg/well	575610-U
DSC-PS/DVB SPE-96 Plate	50mg/well	575611-U

#### 96-Well Plate Accessories

Description	Qty.	Cat. No.
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

Description	Cat. No.
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

For more information request T400171 - Discovery DSC-18 SPE-96 Plate; T400172 - Discovery DSC-18Lt SPE-96 Plate; T400173 - Discovery DSC-Si SPE-96 Plate; or T400174 - Discovery DSC-PS/DVB SPE-96 Plate.

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

## SEMINARS

### PittCon 2001 Seminar Presentations

#### Improve Performance of Solid Phase Microextraction (SPME) Fibers and Applications

The seminar describes the improvements in SPME fiber chemistry and mechanical strength. The presentation focuses on Stableflex fibers with improved flexibility, minimization of bleed, and reproducibility.

For more information, request T401042.

#### Strategy For Faster, Easier Methods Development Using Reverse Phase Columns of Varying Selectivity

The seminar describes the new Discovery HS HPLC column. Discovery HS phase has high surface area and low bleed and is specifically designed for LC/MS applications. Fast gradients and rapid analyses are possible.

For more information, request T401043.

#### Improved Performance for Cyanopropylphenyldimethylpolysiloxane, (SPB-1701) Capillary Columns

The seminar describes the improvements in the SPB-1701 capillary column. The presentation focuses on the low bleed performance of the new SPB-1701.

For more information, request T401044.

#### High Throughput Sample Preparation of Acidic, Basic, and Neutral Drugs in Serum Using 96-Well SPE Plates

The seminar describes an overview of solid phase extraction (SPE) and the emergence of 96-well SPE. The presentation focuses on various pharmaceutical applications for bio-analytical analysis using Discovery SPE-96 well plates.

For more information, request T401045.

## LITERATURE

#### The 2001 Solid Phase Microextraction (SPME) Application Guide 3<sup>rd</sup> Edition

This NEW 66-page SPME Application Guide is now available electronically in a searchable CD format. The guide includes over 750 applications, which are specific to analyte and matrix. There are 151 new references in the 2001 3<sup>rd</sup> edition. The guide helps the analytical chemist to choose the SPME fiber to use for their sample preparation problems. Most entries list the SPME conditions and instrumentation used for the application.

For more information request T199925.

## SAMPLE PREPARATION PERFORMANCE TIP

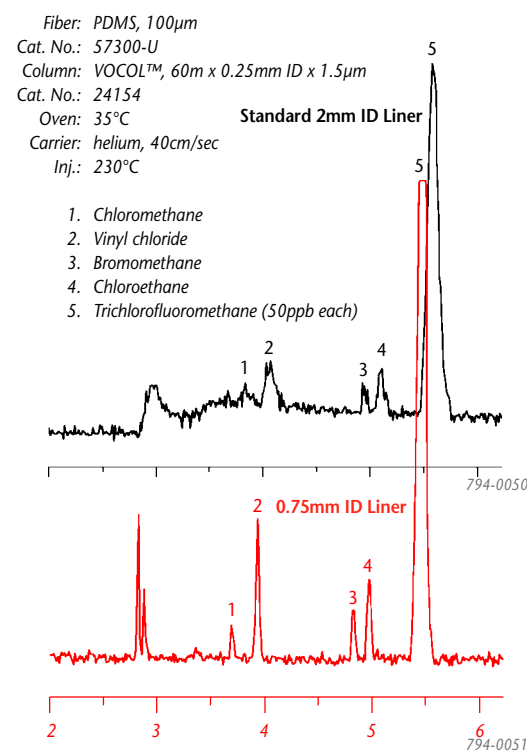
#### Proper GC Liner Selection Will Improve Your SPME Analyses

The proper GC inlet liner for an application reduces the dead volume and improves peak symmetry. Desorption of an analyte from a Solid Phase Microextraction (SPME) fiber depends on a number of variables. First is the boiling point of the analyte. Volatile compounds require lower injection port temperatures. Second, the thickness of the coating used on the fiber limits the removal of the analytes. Thin coatings remove analytes faster. Third, the temperature of the injection port increases volatility of the analyte. Injection port temperatures should not exceed the documented maximum temperature of the fiber coating.

Cryogenic cooling will focus slowly desorbed compounds on the capillary column. Alternatively, a narrow internal diameter inlet liner (0.75mm) will also sharpen the peaks and eliminate the need for cooling (Figure C). Sharper peaks improve integration, quantitation, and lower detection limits. Choose the correct 0.75mm inlet liner for your GC model for optimal performance when using SPME.

For more information, request T397136 - Reduce Inlet Liner ID for Sharper Peaks by SPME/GC.

Figure C. A Reduced Inlet Liner Volume Improves SPME/GC Analyses of Volatile Analytes



Bob Shirey - SPME R&D Chemist

**Trademarks and Registered Trademarks:**

Discovery, SPB, VOCOL - Sigma-Aldrich  
Microseal - Merlin Instrument Company  
Teflon - E.I. du Pont de Nemours & Co., Inc.

**Patents:**

SPME - Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #523092.

▼Merlin Instrument Co, US Patent #4,954,149

## Increase Productivity by Minimizing...

(continued from page 1)

causes retention on a reversed phase sorbent. Therefore, use pH instead of organic solvent strength to elute compounds of interest.

In another strategy, dilute a portion of the organic eluant with a buffer or weaker mobile phase component. Avoid diluting the eluant beyond the limit of detection of the analytical system. Use lower SPE bed weights ( $\leq 15$ mg/well) to reduce eluant solvent volume. With lower bed masses, however, there is greater risk of channeling and well-to-well bed mass variation. We found those bed weights of 25mg or more give the most reproducible results. Just 200 $\mu$ L of methanol effectively elutes an analyte from 100mg of Discovery DSC-18 sorbent.

## Conclusion

Minimize processing time between SPE elution and HPLC analysis. Skip the evaporation/reconstitution steps by pH and solvent strength control. Where this is not possible, 96-well plate accessories designed to reduce the time are commercially available. Research efforts at Supelco continue towards this goal.

☎ For more information request T700002, T100826, & T300165.

## CASE STUDY 2

### Where are Those Peaks Coming From?

**The Problem:** A customer using the Solid Phase Microextraction (SPME) technique in a headspace application was detecting extraneous peaks. During routine quality control, the customer would extract water blanks. The blank analysis produced several peaks as shown in Figure D. He conditioned the SPME fiber again as recommended in the product instructions and repeated the blank analysis. However, the extraneous peaks continued to appear!

The customer contacted Supelco Technical Service for assistance. The Technical Service chemist inquired if the customer had inspected the GC inlet liner for septum particles or impurities. The customer had recently installed a new 0.75mm ID inlet liner and was using a Merlin Microseal™ septumless injection port. Technical Service instructed the customer to condition the fiber again, skip the headspace extraction step, and immediately desorb the fiber by inserting it into the GC injection port. A clean chromatogram resulted with no extraneous peaks. This test effectively eliminated the SPME fiber as the source of the extra peaks. Where were the peaks coming from?

**The Solution:** The customer was using a Teflon® lined silicone septum on the sample vial. The vial septum was the source of the contaminants detected in the headspace analysis. Verification by mass spectrometry confirmed the extraneous peaks as siloxane compounds introduced when the fiber assembly punctured the vial septum during sampling. When the customer switched to a vial septum pre-

conditioned overnight in an oven at 150°C, the extraneous peaks disappeared. Figure E shows the result.

*Mystery solved!*

☎ For more information request T198923 - Solid Phase Microextraction: Theory and Optimization of Conditions.

Figure D. Customer Evaluation of SPME Fiber

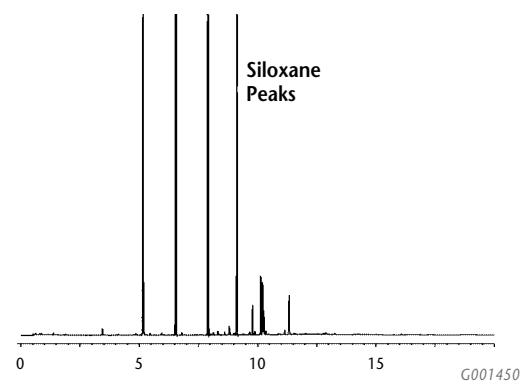
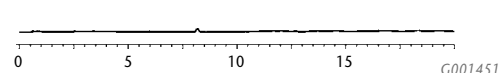


Figure E. Result of Installing the Preconditioned Vial Septa



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