

# HybridSPE™-Precipitation Technology

Bridging the Gap Between Simplicity and Selectivity in Pharma Bioanalytical Sample Prep

In pharmaceutical bioanalysis, researchers develop and run various assays to quantitate drugs, pharmaceutical candidates, and their metabolites in biological fluids such as serum and plasma. As analysts strive for faster analyses, shorter run times, and lower limits of detection, ion-suppression due to inadequate removal

## Features & Benefits:

- Merges both Protein PPT & SPE
- Offers simplicity & generic nature of protein PPT

### PLUS

- Selectivity approaching SPE via the targeted removal of phospholipids
- 2-3 step generic procedure
- 100% removal of phospholipids & precipitated proteins
- Minimal to no method development
- Available in 96-well and 1 mL cartridge dimensions
- Proprietary technology

endogenous sample interferences has become of great concern. It is well documented that one of the principle causes of ion suppression is phospholipid contamination. Not only is signal suppression often evident in the positive ion electrospray mode (+ESI), phospholipids can often remain on

the analytical column after sample analysis, and elute uncontrollably in a given LC run sequence.

HybridSPE-Precipitation combines the simplicity of protein precipitation (2-3 steps) with the selectivity of SPE for the targeted removal of phospholipids and proteins in biological plasma/serum. The technology utilizes a proprietary zirconia-coated particle, and exhibits a selective affinity towards phospholipids while remaining non-selective towards a range of basic, neutral and acidic compounds.

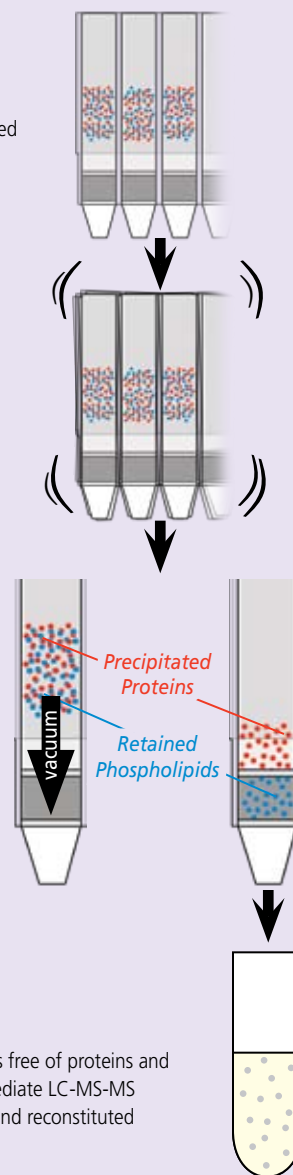
## HybridSPE-PPT "In-Well" 96-well Precipitation Method and Phospholipid Removal

1) **Precipitate Proteins** by adding 100  $\mu$ L plasma or serum to the HybridSPE-PPT plate followed by 300  $\mu$ L 1% formic acid in acetonitrile. Add I.S. as necessary.

2) **Mix** by vortexing/shaking HybridSPE-PPT plate or by aspirating/dispensing with 0.5-1 mL pipette tip (e.g., TOMTEC Quadra liquid handler)

3) **Apply vacuum.** The packed-bed filter/frit assembly acts as a depth filter for the concurrent physical removal of precipitated proteins and chemical removal phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.

4) **Resulting filtrate/eluate** is free of proteins and phospholipids and ready for immediate LC-MS-MS analysis; or it can be evaporated and reconstituted as necessary prior to analysis



## Comparative Extraction and LC-MS-MS of 10 ng/mL Clenbuterol (R(-) and S(+)) in Rat Plasma

In this application example, rat plasma samples were spiked with clenbuterol (R(-) and S(+)) enantiomers) at the level of 10 ng/mL and extracted using three different procedures: HybridSPE-PPT, Protein PPT, and a 9-step SPE procedure optimized for trace level clenbuterol analysis. The analysis was performed using a chiral stationary phase containing a macrocyclic glycopeptide covalently bound to silica and detection via MS-MS. Comparisons of sample preparation methods were made in terms of the amount of phospholipids in the sample extract and the overall effect on signal response of clenbuterol enantiomers. Absolute recovery was determined against an external standard.

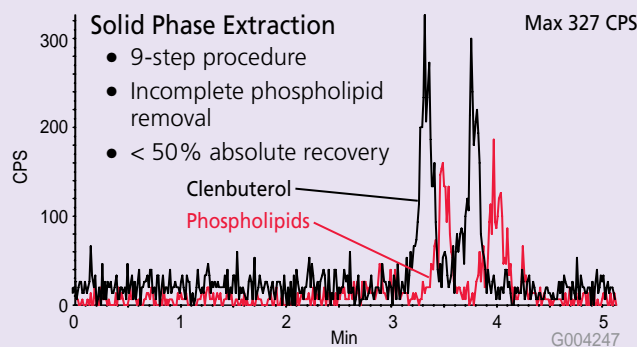
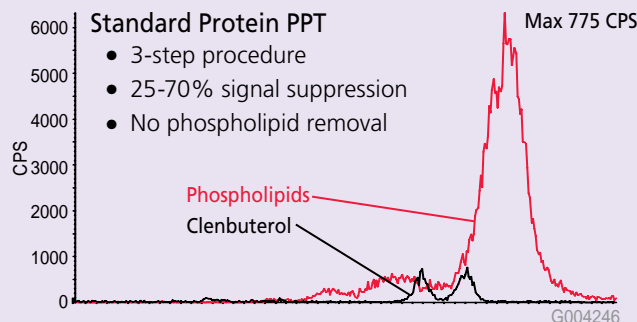
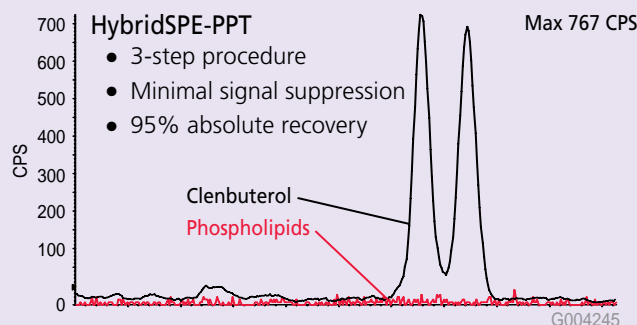
### HybridSPE-PPT Plate and Vacuum Manifold



E000967

## Comparative Extraction and LC-MS-MS of 10 ng/mL Clenbuterol (R(-) and S(+)) enantiomers) in Rat Plasma

column: Chirobiotic™ T, 10 cm x 2.1 mm, 5 µm (12018AST)  
 instrument: Agilent 1100  
 mobile phase: 10 mM ammonium formate in methanol  
 temperature: 30 °C  
 flow rate: 0.3 mL/min.  
 detection: ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids) and 277.2/203.1 (clonidine)  
 inj. vol.: 10 µL



Description	Qty.	Cat. No.
-------------	------	----------

#### HybridSPE Products

HybridSPE-Precipitation 96-well Plate, 50 mg/well	1	575656-U
HybridSPE-Precipitation 96-well Plate for Small Volume Samples, 15 mg/well	1	52794-U
HybridSPE-Precipitation Cartridge, 30 mg/1 mL	100	55261-U
HybridSPE-Precipitation Cartridge, 500 mg/6 mL	30	55267-U

#### Related Products

96-well Protein Precipitation Filter Plate	1	55263-U
Supelco PlatePrep Vacuum Manifold	1	57192-U
96 Square/Deep Well Collection Plates, 0.35 mL, PP	50	575651-U
96 Square/Deep Well Collection Plates, 1 mL, PP	50	575652-U
96 Square/Deep Well Collection Plates, 2 mL, PP	50	575653-U
96 Square Well Pierceable Cap Mats	50	575655-U

For more information, visit [sigma-aldrich.com/hybrid-spe-ppt](http://sigma-aldrich.com/hybrid-spe-ppt)

#### Trademarks

HybridSPE, CHIROBIOTIC — Sigma-Aldrich Biotechnology LP

*Accelerating Customers' Success through Innovation and Leadership in Life Science, High Technology and Service*

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052  
 Technical Service (800) 325-5832 • [sigma-aldrich.com/techservice](http://sigma-aldrich.com/techservice)  
 Development/Custom Manufacturing Inquiries **SAFC**® (800) 244-1173  
 Safety-related Information [sigma-aldrich.com/safetycenter](http://sigma-aldrich.com/safetycenter)

**World Headquarters**  
 3050 Spruce St.  
 St. Louis, MO 63103  
 (314) 771-5765  
[sigma-aldrich.com](http://sigma-aldrich.com)

©2010 Sigma-Aldrich Co. All rights reserved. SIGMA, SAFC, SIGMA-ALDRICH, ALDRICH, FLUKA, and SUPELCO are trademarks belonging to Sigma-Aldrich Co. and its affiliate Sigma-Aldrich Biotechnology, LP. Sigma brand products are sold through Sigma-Aldrich, Inc. Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.