

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
- Patent pending 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 patent pending zirconia-coated particle, and exhibits a selective affinity towards phospholipids while remaining non-selective towards a range of basic, neutral and acidic compounds.

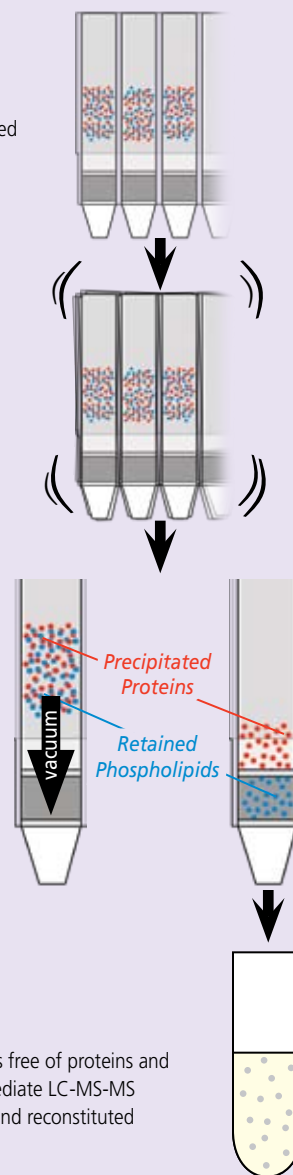
HybridSPE-PPT "In-Well" 96-well Precipitation Method and Phospholipid Removal

1) **Precipitate Proteins** by adding 100 μ L plasma or serum to the HybridSPE-PPT plate followed by 300 μ 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(+)) enantiomers 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.

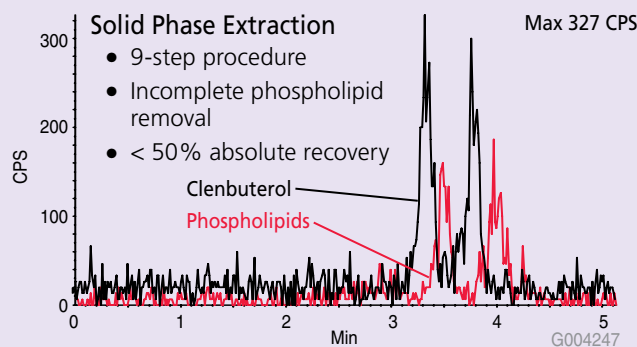
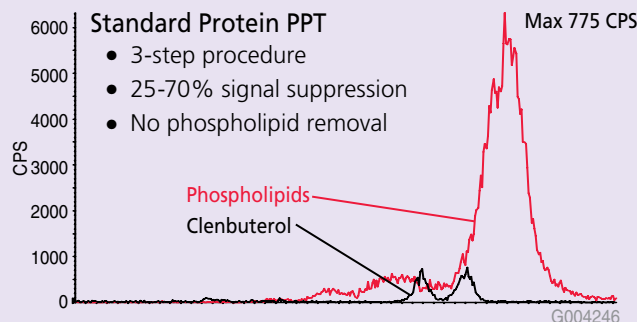
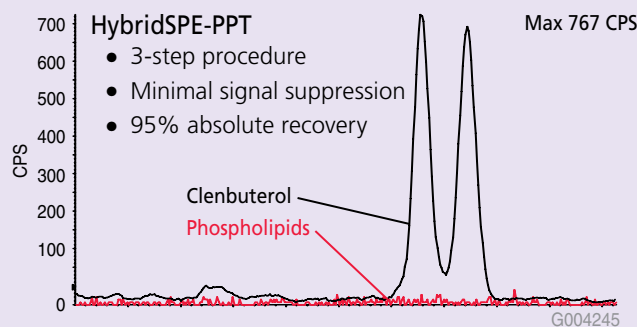
HybridSPE-PPT Plate and Vacuum Manifold



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 Cartridge, 30 mg/1 mL	100	55261-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/hybridspe-ppt

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