HybridSPE® Phospholipid Removal Technology for Biological Matrices

Remove phospholipids and proteins for accurate and reproducible LC-MS analysis

NEW - DPX® 96-tip dSPE

NEW - Supel™ Genie Online SPE

LC-MS Workflow Solutions

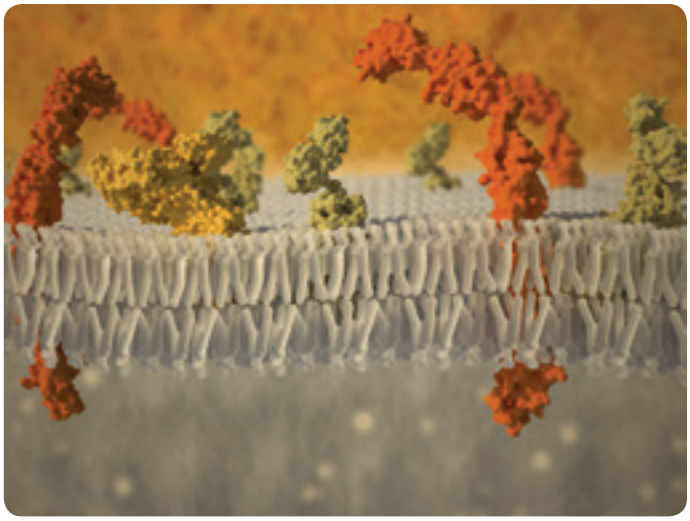
Plates, Cartridges and Accessories

Supelco®
Analytical Products

HybridSPE® Phospholipid Removal Technology

Key Features and Benefits

- More accurate results
  - Unique chemistry that can effectively separate hydrophobic analytes (such as vitamins) from phospholipids, unlike competitive product chemistries
  - Elimination of proteins and phospholipid-induced ion suppression
- Simultaneous removal of proteins and phospholipids
  - Simple, standardized methodology, analogous to traditional protein precipitation
  - Alternative to complex traditional SPE method development
- Reproducible, consistent performance reduces need for reprocessing
- Less instrument downtime and longer column life
- Decreased run times by eliminating the need for gradients to clean columns between samples
- High throughput processing that is automatable and compatible with common robotic systems
- Ready-to-use, no preconditioning required



Phospholipids: A Concern for LC-MS Analysis of Small Molecules in Biological Matrices

Phospholipids are present as a major component of all cell membranes. They are therefore present in all biological sample matrices including serum, plasma and whole blood and can be a problem in LC-MS analysis of small molecules because they often co-elute and ionize along with the analytes of interest. This co-elution results in ion suppression (an erroneous decrease) of the mass spec signal that can cause variability and impact LC-MS result accuracy (**Figure 1**).

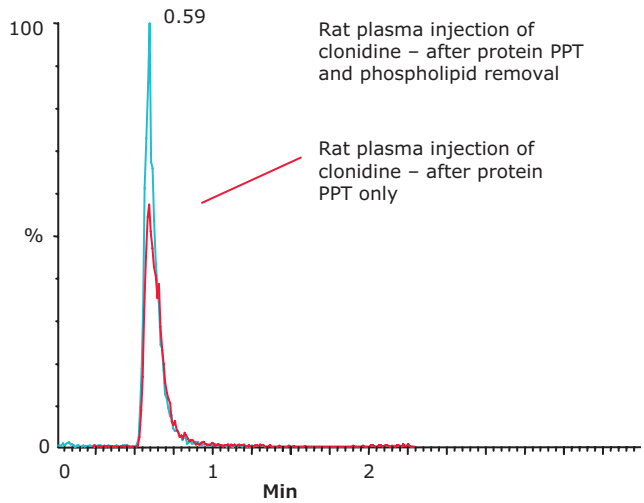
Importance of Accurate Results and Fast Answers in Bioanalysis

We understand that the results of your analyses can have a significant impact on the lives of many others. This puts pressure on you to ensure the data you produce is as accurate as possible. Not only do you need quick answers, you need answers you can trust. The complexity of the types of samples with which you work does not make your job any easier. Proteins and phospholipids inherently present in varying quantities in your samples, can add variability to your analytical results when using sensitive techniques such as LC-MS or LC-MS/MS. We have industry leading chromatography expertise to help you navigate the complexity of your sample prep options.

Limitations of Traditional Biological Sample Cleanup Methods

Most clinical and biological researchers use traditional methods such as protein precipitation and liquid-liquid extraction to clean up samples prior to analysis. While these techniques allow for inexpensive and quick removal of proteins, they completely fail to address the problem of phospholipid-induced ion suppression.

Figure 1. Phospholipid Effect on Ionization of Clonidine



Even if phospholipids do not co-elute with the analyte of interest, they can accumulate on your analytical column and elute from the column sporadically in downstream analyses. This can cause unpredictable ion suppression and poor reproducibility, thereby putting the accuracy of your results at risk (**Figure 2**).

Phospholipid Removal Techniques

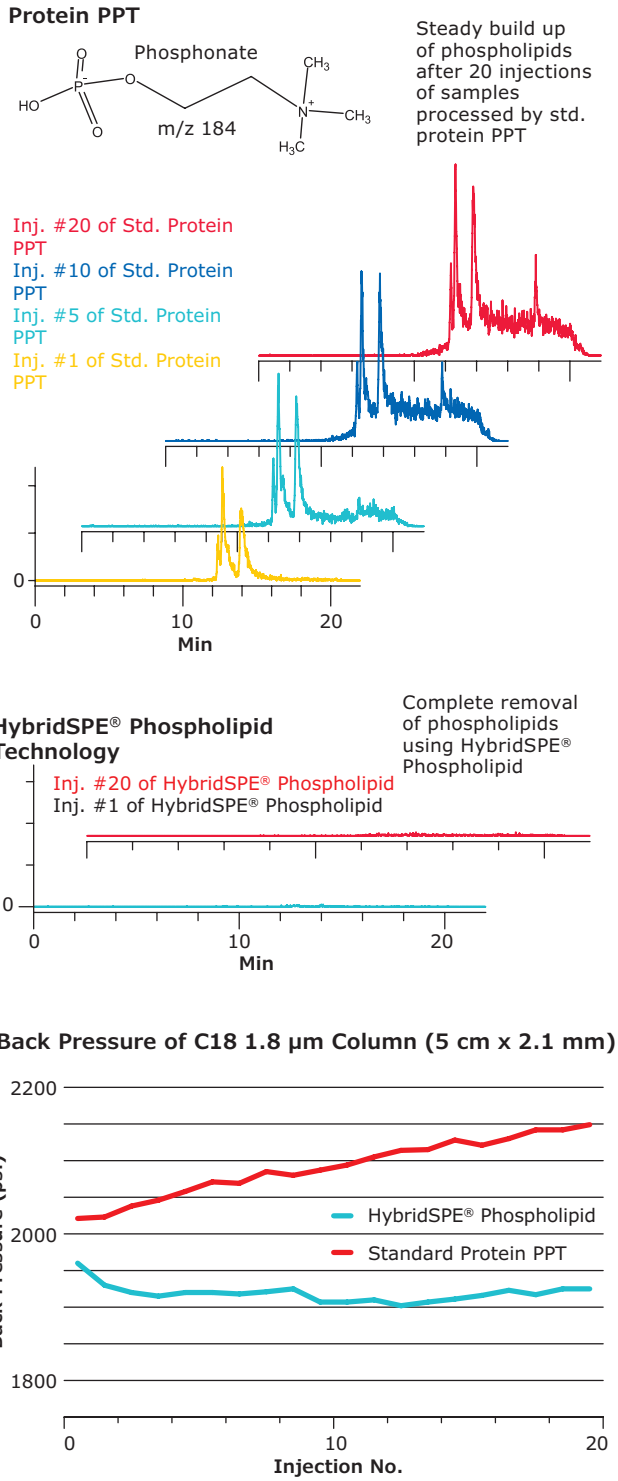
To overcome the problem of phospholipid-induced ion suppression, some analysts try traditional SPE. Traditional SPE often requires time-consuming and complex method development, but still only removes nominal amounts of phospholipids. Remaining phospholipids can impact the accuracy of your results, accumulate on your analytical column to impact future analyses, add to column replacement costs and increase instrument downtime.

A variety of products designed specifically for the removal of both proteins and phospholipids are now commercially available, including HybridSPE® plates and cartridges. Most of these products are simple, fast and easy-to-use, offering fairly standardized methods with minimal method development. Most, however, use a hydrophobic retention mechanism to separate phospholipids from analytes of interest in the sample. This poses a problem if the analytes of interest are also hydrophobic because they will be retained and removed along with the hydrophobic phospholipids. This results in decreased analyte recovery and inaccurate results. HybridSPE® Phospholipid Technology is different in that it completely removes both proteins and phospholipids from the sample without retaining other hydrophobic compounds.

How Is HybridSPE® Phospholipid Removal Technology Different from Other Methods?

The first of its kind, our HybridSPE® technology was introduced in 2008. It fuses the simple, standardized methodology of traditional protein precipitation with the specificity of solid phase extraction (SPE) for the simultaneous removal of proteins and phospholipids from biological samples prior to LC-MS analysis. Unlike other phospholipid removal products that use a hydrophobic retention mechanism to remove phospholipids from biological samples, the HybridSPE® system uses a unique retention mechanism (**Figure 3**). This allows it to separate phospholipids from even very hydrophobic analytes, such as vitamins which are often retained along with phospholipids on competitive products.

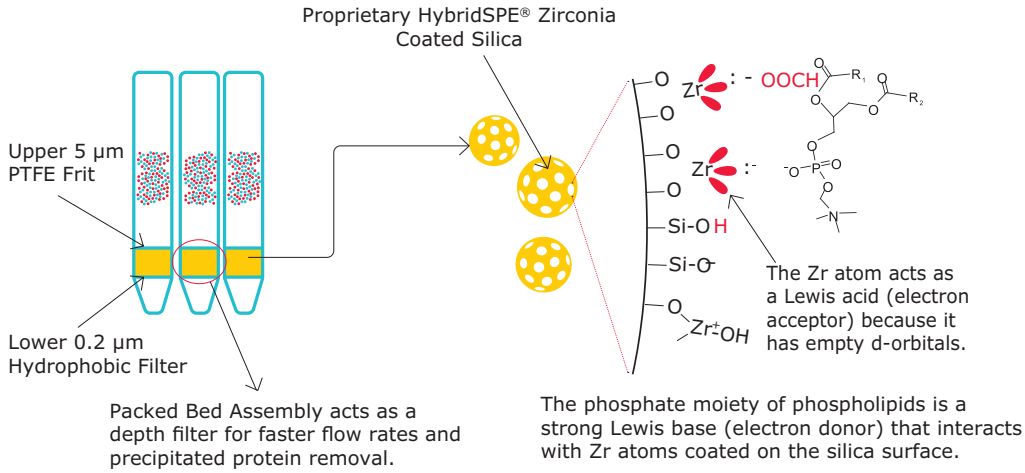
Figure 2. Gradient RP LC-MS of Blank Plasma Samples Prepared by Standard Protein PPT vs. HybridSPE® Phospholipid



To learn more about HybridSPE® Phospholipid and view a video of the product in action, visit

SigmaAldrich.com/hybridspe

Figure 3. Unique Retention Mechanism of HybridSPE® Phospholipid Technology Allows for Separation of Even Very Hydrophobic Analytes from Phospholipid Contaminants in Biological Sample Matrices



A Newer, Better "Go-to" Sample Prep Method for Phospholipid Removal

Labs working with biological samples often choose to perform protein precipitation prior to LC-MS analysis, using it as their "go-to" sample prep method. Phospholipid removal is viewed as more costly, more time-consuming and unnecessary if the specific analytes of interest do not co-elute with the phospholipids in the sample. This is not the case.

Reduce overall costs and increase overall throughput while generating more accurate data by using HybridSPE® Phospholipid technology to remove both proteins and phospholipids from all biological samples prior to small molecule LC-MS analysis.

Simple and Fast Phospholipid Removal

The 96-well plate protocol involves just a few simple steps (**Figures 4** and **5**), and plates can be used right out of the package with no pre-conditioning step required. The sample (containing internal standards if desired) and a precipitation solvent are first added to the well plate, followed by a mixing step and vacuum application to collect the sample. Collected samples are then ready to be analyzed.

Figure 4. Depiction of Basic HybridSPE® Phospholipid Sample Prep Workflow

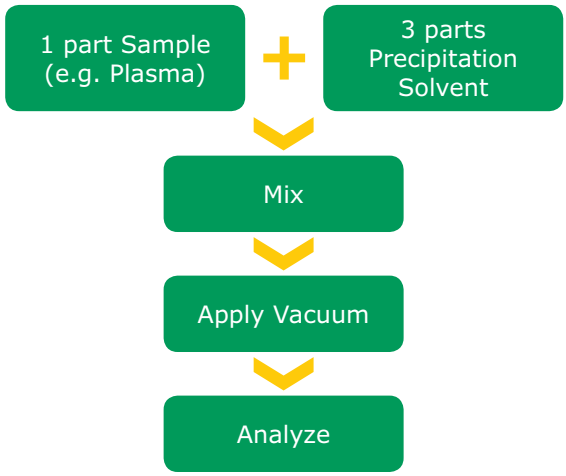
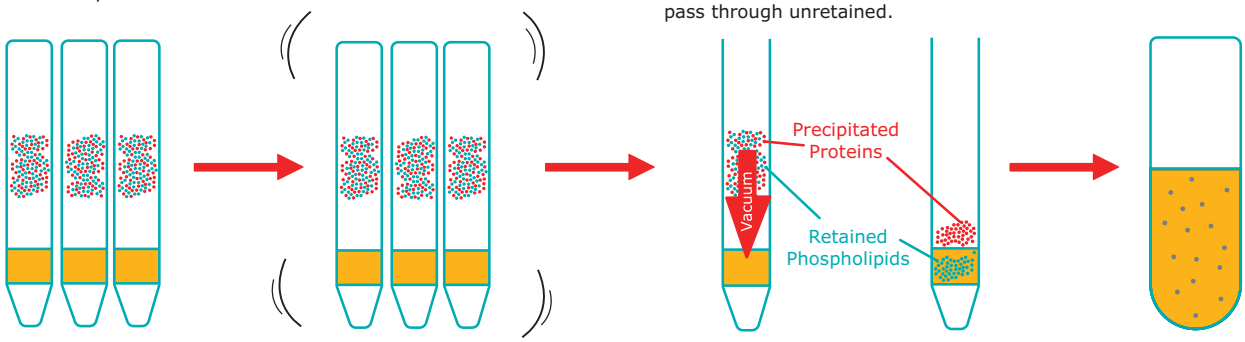


Figure 5. HybridSPE® 96-well Plate Protocol

Featuring an "In-well" Precipitation Procedure for both proteins and phospholipids

1. **Add Sample**
   Pipette 100 µL plasma or serum to the HybridSPE® plate followed by 300 µL precipitation solvent. Add internal standards as necessary.
2. **Mix**
   by vortexing/shaking HybridSPE® plate or by aspirating/dispensing with 0.5–1 mL pipette tip.
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 of phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.
4. **Collect Sample**
   Resulting filtrate/eluate is free of proteins and phospholipids and ready for immediate LC-MS/MS analysis.



Produce More Accurate Data, Save Time and Reduce Overall Costs

With advances in LC-MS technology, many analysts seek to decrease LC run times by incorporating ballistic HPLC gradients and columns with sub-2 µm particles. Ballistic gradients are often inadequate at purging the column of the phospholipids that remain after standard protein precipitation techniques and sub-2 µm HPLC columns are more prone to clogging than larger particle size columns. In addition, because contaminating phospholipids are often very strongly retained on the analytical column, they can take more than 10 minutes to elute. When using short run times, phospholipids are more likely to accumulate on the column unless the analyst takes the time to allow the phospholipids to elute before beginning the next injection. This can dramatically decrease laboratory throughput (**Figures 6** and **7**).

Figure 6. Phospholipid Contamination from Standard Protein PPT Requires Increased Gradient Run Time (>10 min.)

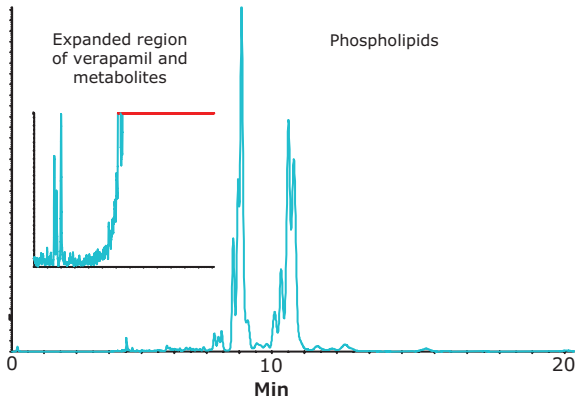
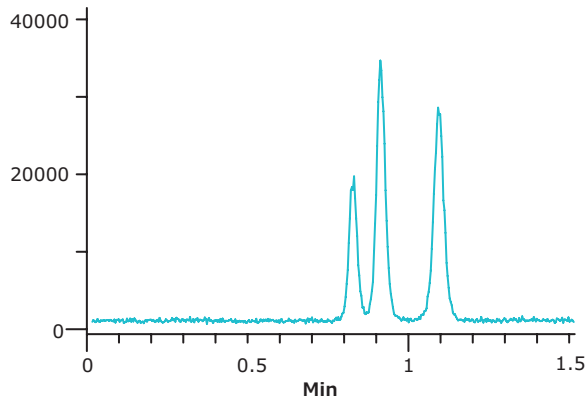


Figure 7. Less than 90 Second Run Time Achieved Using HybridSPE® Phospholipid and Ascentis® Express C18 (isocratic) for Verapamil and Metabolites in Rat Plasma



By removing phospholipids as part of the standard sample prep process, analysts can avoid issues with phospholipids building up on the analytical column, eluting unexpectedly and causing unpredictable ion suppression and poor reproducibility in later runs. Reduce the frequency of column replacement, and therefore consumable costs and downtime associated with column replacement. Finally, phospholipid removal can help achieve the increased throughput to get answers as quickly as possible.

To learn more about HybridSPE® Phospholipid removal technology and view a video of the product in action, visit

SigmaAldrich.com/hybridspe

DPX® Tips for Automated SPE with HybridSPE® Technology

Extraction in Seconds

DPX® Dispersive Pipette Extraction pipette tips incorporate loosely contained HybridSPE® sorbent material that is mixed with the sample solution to accomplish solid phase extraction. HybridSPE® technology is a simple and generic sample preparation platform designed for the gross level removal of endogenous protein and phospholipid interferences from biological plasma and serum prior to LC-MS or LC-MS/MS analysis.

The unique mixing technique provides numerous advantages:

- Minimal elution solvent volumes
- Rapid extraction times (less than 3 minutes/sample)
- High extraction efficiencies
- Easy to perform extractions
- Lower costs
- Higher throughput
- Minimal training requirements
- Better for the environment

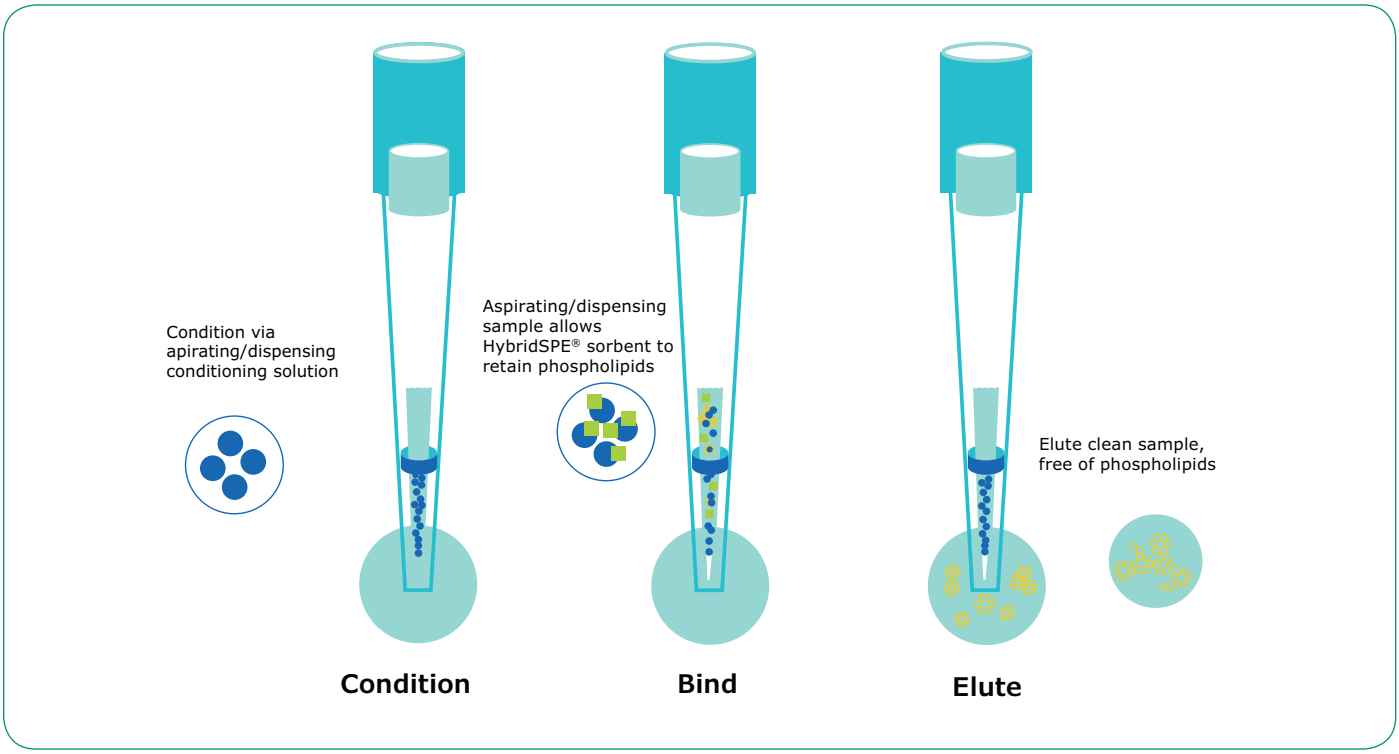
In this simple technique, biological plasma or serum is first subjected to protein precipitation via the addition and mixing of acidified acetonitrile. Precipitated proteins are then removed by centrifugation and the resulting supernatant is extracted using the HybridSPE® DPX tip which acts as a chemical filter that specifically targets the removal of endogenous sample phospholipids.

The phospholipid retention mechanism is based on a highly selective Lewis acid-base interaction between the proprietary zirconia ions functionally bonded to the HybridSPE® stationary phase and the phosphate moiety consistent with all phospholipids. The resulting eluent is ready for immediate LC-MS or LC-MS/MS analysis.

Automated SPE with HybridSPE® DPX Tips

Extraction in Seconds

HybridSPE® Sample Prep Workflow Using DPX® Tips



What size tips do I need?

HybridSPE®-PL Sample and PPT Agent Guidelines		
	30 mg tips	50 mg tips
Plasma/serum	30-100 µL	100-300 µL
Precipitating agent	90-300 µL	300-900 µL

Ordering Information

Product Description	Cat. No.
HybridSPE® DPX® tip, 30 mg, Tecan® 200 uL (96-tip box)	**52973-U**
HybridSPE® DPX® tip, 50 mg, Tecan® 1 mL (96-tip box)	**52974-U**
HybridSPE® DPX® tip, 30 mg, Hamilton® 300 uL (96-tip box)	**52977-U**
HybridSPE® DPX® tip, 50 mg, Hamilton® 1 mL (96-tip box)	**52978-U**
HybridSPE® DPX® tip, 30 mg, Integra 300 uL (96-tip box)	**52979-U**
HybridSPE® DPX® tip, 50 mg, Integra 1250 uL (96-tip box)	**52980-U**
HybridSPE® DPX® tip, 30 mg, Universal 1 mL (96-tip box)	**52981-U**
HybridSPE® DPX® tip, 50 mg, Universal 1 mL (96-tip box)	**52982-U**

Go Hands-free with Online SPE

Supel™ Genie Online SPE Cartridges

Supel™ Genie HybridSPE® online cartridges have been developed as an alternative option of phospholipid removal and sample preparation. Our HybridSPE® online cartridges have been designed for online phospholipid removal allowing direct injection of protein precipitated samples for LC-MS analysis.

When the samples flow through the HybridSPE® online traps, the phospholipids are retained while the analytes of interest flow through. HybridSPE® online cartridges are engineered to remove >95% phospholipids from protein precipitated biological samples in a direct, flow-through format.

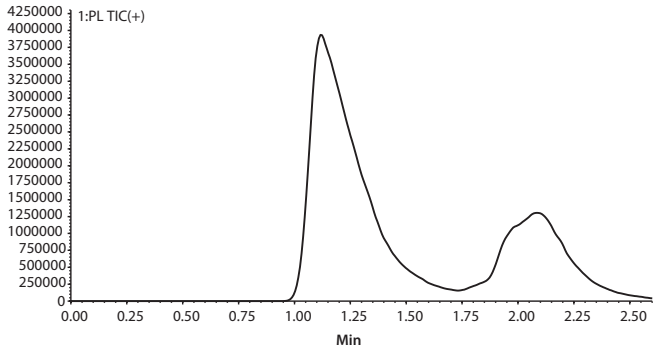
Supel™ Genie Online SPE cartridges enable online sample cleanup, concentration, and separation with excellent reproducibility and performance. Coupled with mass spectrometry for detection, our Supel™ Genie Online SPE cartridges provide a simple, automated method for sample analysis and are ideal for use in the LC-MS/MS analysis of biological fluids such as plasma and serum.

How will Online SPE help you?

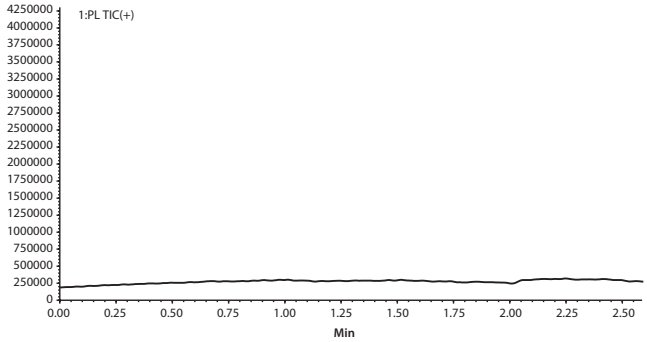
- Hands-free workflow
- Elimination of human error
- Decreased cost per sample
- Automation results in rapid throughput and greater reproducibility
- Clean samples =
  - Greater column life
  - Less instrument downtime
  - More accurate and reproducible data

Our HybridSPE® phase offers complete phospholipid removal from biological samples:

Phospholipids in Plasma Sample without Supel™ Genie HybridSPE® Online Cartridge (1st injection)



Phospholipids in Plasma Sample with Supel™ Genie HybridSPE® Online Cartridge (120th injection)



Supel Genie HybridSPE® Online Starter Kit (55324-U)

Go Hands-free with Online SPE

Supel™ Genie Online SPE Cartridges

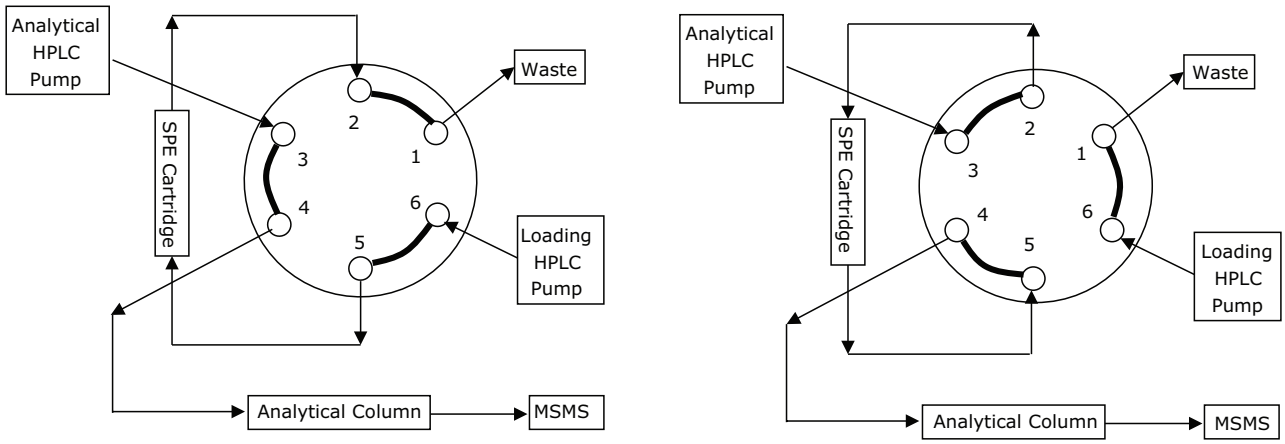
If protein removal is desired, protein precipitation should be performed prior to injecting the sample into the online SPE cartridge.

Our starter kits come with reusable hardware that will fit any Supel™ Genie cartridge, as well as one cartridge of selected phase chemistry. Additional cartridge packs include cartridges only.

Recommended Configuration of Online SPE with LC-MS

For optimum results of both sample cleanup and HPLC separation, we recommend to configure the Supel™ Genie Online SPE cartridges with an LC-MS system as shown in **Figure 8**. The setup consists of two pumps, one pump for loading samples onto the cartridge and washing the samples afterwards in one direction, while the other pump serves to elute the samples from the cartridge to the HPLC column in a reversed direction.

Figure 8. Recommended Setup of Online SPE with LC/MS



Description	Cat. No
Supel™ Genie HybridSPE® Online Starter Kit	**55324-U**
Supel™ Genie HybridSPE® Online SPE Cartridge, pk. of 2	**55326-U**
Supel™ Genie HybridSPE® Online SPE Cartridge, pk. of 6	**55327-U**

Ordering Information

Standard Sample Volume (100–300 µL) Phospholipid Removal Plates and Cartridges:

Our HybridSPE® technology is available in both a 96-well plate format (**Figure 9**) and a cartridge format (**Figure 10**). 96-well plates are sold individually and in 20 plate packs. Cartridges are sold in various pack sizes, depending on cartridge size (see ordering information).

Like the 96-well plate, our HybridSPE® Phospholipid Ultra cartridge is capable of removing both proteins and phospholipids in an online format. If an offline protein precipitation is desired, the 1 mL or 6 mL HybridSPE® Phospholipid cartridges, which do not contain a protein precipitation filter, can be used for phospholipid removal following a separate offline protein precipitation (using a product such as Cat. No. 55263-U, 96-well protein precipitation filter plate).

Figure 10. HybridSPE® Phospholipid ULTRA Cartridge

Small Sample Volume (20–40 µL) Phospholipid Removal Plates:

The HybridSPE® Phospholipid Small Volume 96-well Plate is designed for processing plasma/serum volumes between 20-40 µL. It is available in both single and 20 plate pack sizes.

Product Description	Qty.	Cat. No.
HybridSPE® PLus Plate Essentials Kit		
Includes HybridSPE® PLus 96-well plate (575659-U), plate cap mat (as in 575680-U), sealing film (as in Z721581) and collection plate (as in Z717266)	1	**52818-U**
HybridSPE® PLus 96-Well Plates		
50 mg/well	1	**575659-U**
	20	**575673-U**
HybridSPE® Phospholipid Small Volume 96-Well Plates		
15 mg/well	1	**52794-U**
	20	**52798-U**
HybridSPE® Phospholipid Cartridges		
HybridSPE® Phospholipid Ultra Cartridge, 30 mg/1 mL	100	**55269-U**
HybridSPE® Phospholipid Cartridge, 500 mg/6 mL	30	**55267-U**
HybridSPE® Phospholipid Cartridge, 30 mg/1 mL	100	**55261-U**
HybridSPE® Phospholipid Cartridge, 30 mg/1 mL	200	**55276-U**
Plate Accessories		
Round Well Cap Mat, Pierceable for HybridSPE® PLus	50	**575680-U**
96 Round/Deep Well Collection Plate, PP for HybridSPE® PLus	60	**Z717266**
96 Well-Plate Pre-cut Sealing Films	100	**Z721581**
Supelco® PlatePrep Vacuum Manifold	1	**57192-U**
96-well Protein Precipitation Filter Plate (for offline protein precipitation)	1	**55263-U**
Cartridge Accessories		
Visiprep™ DL Solid Phase Extraction Cartridge Manifold		
12 Port Model	1	**57044**
24 Port Model	1	**57265**
Visiprep™ Solid Phase Extraction Cartridge Manifold		
12 Port Model	1	**57030-U**
24 Port Model	1	**57250-U**
Disposable Valve Liners, PTFE (for Visiprep™ DL Manifold)	100	**57059**
Equipment		
KNF Laboport® Vacuum Pumps	1	**Inquire**
SPE Vacuum Pump Trap Kit	1	**57120-U**
SPE Manifold Gauge/Bleed Valve, Remote In-Line Design	1	**57161-U**
IKA® VORTEX 3, vortex mixer (230 V)	1	**Z654779**
IKA VORTEX 3, vortex mixer (115 V)	1	**Z654760**
Precipitation Solvents, Blends and Additives		
Acetonitrile, for UHPLC-MS	4x4L, 1L	**900667**
Methanol, for UHPLC-MS	4x4L, 1L	**900688**
Water, for UHPLC-MS	4x4L, 1L	**900682**
ACN with 0.1% formic acid, for UHPLC-MS	4x4L, 1L	**900686**
Water with 0.1% formic acid, for UHPLC-MS	4x4L, 1L	**900687**
Methanol with 0.1% formic acid for UHPLC-MS	4x4L	**632546**

We Provide the LC-MS Workflow....

The perfect complement of speed, selectivity and sensitivity for bioanalysis

- Increase sample prep and LC-MS speed
- Decrease sample prep method development time
- Increase sensitivity by reducing ion-suppression and increasing LC efficiency

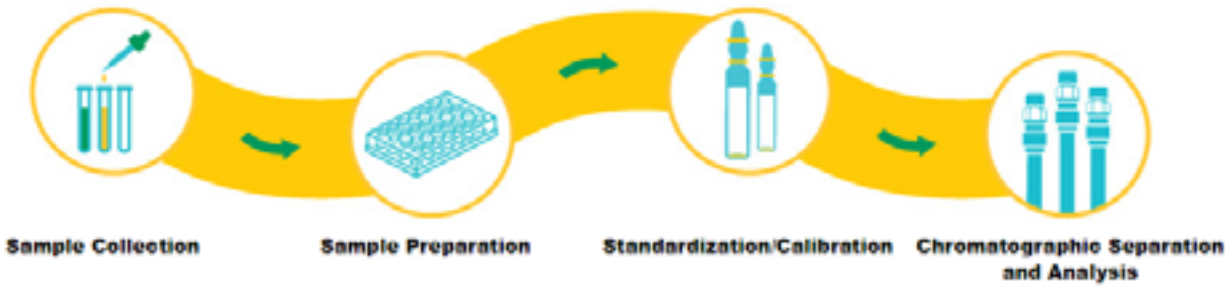
Cerilliant® Certified Spiking Solutions® and Certified Reference Materials are manufactured and tested specifically for use as reference standards for laboratories performing bioanalysis, therapeutic drug monitoring, diagnostic and toxicology testing

HybridSPE® Phospholipid Removal Technology

- Simple 2–3 step protocol
- Reduce ion-suppression through phospholipid and protein removal
- Minimal to no method development
- Available in 96-well plates and individual cartridges
- NEW - DPX® 96-tip dSPE format and Supel™ Genie Online SPE Cartridges

Increase Resolution with HPLC and UHPLC columns Ascentis Express® for small molecules and BIOshell for Biomolecule separation.

- Novel Fused-Core® technology (Superficially Porous Particles; SPP)
- Maximum speed and efficiency on both UHPLC and HPLC systems (2µm, 2.7µm and 5µm).
- 40% more efficiency in comparison to Fully Porous Particles; FPP of same particle size
- UHPLC columns 2µm (pressure stable 1000 bar)
- Extremely broad range of Column chemistries
- Wide range of pore sizes for perfect suitability for small and large molecules



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