This Data Sheet Contains Important Information About The Product.

Instructions & Troubleshooting for HybridSPE® DPX® Tips

Product Description

It is well-known that phospholipid contamination is one of the principal causes of ion-suppression when analyzing small molecules in biological matrices via LC/MS/MS. Due to the inherent chemical nature of phospholipids (hydrophobic tail + zwitterionic polar head group), phospholipids are often co-extracted with analytes of interest during sample preparation and can be difficult to resolve during LC/MS analysis. This is especially true as shorter analytical LC/MS run times and ballistic gradients become increasingly mainstream.

HybridSPE®-PL technology is a simple and generic sample prep 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. 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 phosphonate moiety consistent with all phospholipids. The resulting eluent is ready for immediate LC/MS or LC/MS/MS analysis. There are two versions of the DPX® tips available for the HybridSPE®-PL; the standard HybridSPE® DPX® 50 mg tips are designed for use with biological sample volumes between 100-300 µL. The HybridSPE® DPX® 30 mg tips are designed for use with biological sample volumes between 30-100 µL (see Table 1).

HybridSPE® DPX® Tips

1. Spike biological plasma/serum sample and add I.S. as necessary.
2. Transfer plasma/serum sample to the proper centrifugation tube or 96-deep well collection/reservoir plate and precipitate proteins by combining the appropriate plasma/serum sample with a precipitating agent consisting of 1% formic acid in acetonitrile (1:3, v/v) according to the volumes in Table 1. Note: consistent results have been obtained by combining 100 µL plasma/serum + 300 µL 1% formic acid in acetonitrile.

Table 1. HybridSPE®-PL Sample and PPT Agent Guidelines

	30 mg	50 mg
Plasma/serum	30-100 µL	100-300 µL
Precipitating agent	90-300 µL	300-900 µL

3. Facilitate precipitation by agitating/vortexing for 1-3 minutes; and remove precipitated protein by centrifugation (3000 rpm for 2-5 minutes).
4. Transfer the resulting supernatant or an aliquot of the resultant supernatant to the proper 96-deep well collection/reservoir plate.
5. Condition the HybridSPE® DPX® tips by aspirating and dispensing 1% formic acid in acetonitrile (using the same volume that was used for protein precipitation). Discard eluent.
6. Aspirate and dispense supernatant (from steps 1-4) three times with the HybridSPE® DPX® tip.
7. Collect the resulting eluent and directly analyze via LC/MS. No further processing (evaporation/reconstitution) of the sample is necessary unless concentration of the eluent is desired prior to LC/MS analysis.

Important: Please do not use phosphate-containing buffers, including phosphoric acid.

Featured Products

Description	Pkg. Qty.	Cat. No.
HybridSPE® DPX® tip, 30 mg, Tecan® 200 µL	96	52973-U
HybridSPE® DPX® tip, 50 mg, Tecan® 1 mL	96	52974-U
HybridSPE® DPX® tip, 30 mg, Hamilton® 300 µL	96	52977-U
HybridSPE® DPX® tip, 50 mg, Hamilton® 1 mL	96	52978-U
HybridSPE® DPX® tip, 30 mg, INTEGRA 300 µL	96	52979-U
HybridSPE® DPX® tip, 50 mg, INTEGRA 1250 µL	96	52980-U
HybridSPE® DPX® tip, 30 mg ,Universal 1 mL	96	52981-U
HybridSPE® DPX® tip, 50 mg, Universal 1 mL	96	52982-U

Related Products

Description	Pkg. Qty.	Cat. No.
HybridSPE®-PL 96-well Plate, 50 mg/well	1	575656-U
	20	575657-U
HybridSPE®-PL Small Vol. 96-well Plate, 15 mg/well	1	52794-U
	20	52798-U
HybridSPE®-PL 96-well Plate Essentials Kit	1	52813-U
HybridSPE®-PL Cartridge, 30 mg/1 mL	100	55261-U
	200	55276-U
HybridSPE®-PL Cartridge, 100 mg/3 mL	54	52797-U
HybridSPE®-PL Cartridge, 500 mg/6 mL	30	55267-U
HybridSPE®-PL Ultra Cartridge, 30 mg/1 mL	100	55269-U

©2017 Sigma-Aldrich Co. LLC

Troubleshooting & Frequently Asked Questions

1. **Can I use HybridSPE® DPX® tips with smaller plasma volumes (e.g., <100 µL plasma)?**

   Yes, the HybridSPE® DPX® 30 mg tips are designed for use of plasma/serum volumes between 30-100 µL. Larger sample volumes of 100-300 µL should be used for the HybridSPE® DPX® 50 mg tips.

2. **Why is acetonitrile and formic acid used as a precipitating agent in the HybridSPE® DPX® tip method?**

   Acetonitrile is a commonly used protein precipitation agent when prepping plasma samples for LC/MS analysis. The resulting precipitated protein forms pellets easily when using centrifugation.

   The addition of 1-2% formic acid to the acetonitrile precipitating agent is critical because: 1) formic acid is a stronger Lewis base than most carboxyl (-COOH) groups found in acidic pharmaceutical compounds (inhibiting analyte retention on the HybridSPE® phase) but not as strong a Lewis base as the phosphate moiety found in phospholipids; and 2) the low pH environment neutralizes residual silanol activity on the silica surface thereby eliminating secondary cation-exchange interaction with basic compounds of interest.

3. **What if my analyte(s) of interest are not soluble in acetonitrile?**

   Although some analytes may not be soluble in acetonitrile, after protein precipitation, the HybridSPE® eluent will consist of 75% acetonitrile (w/ formic acid) and 25% aqueous (from the biological sample). The aqueous content of the sample should provide adequate solubility prior to LC/MS analysis.

   Alternatively, 1% ammonium formate in methanol may be used in place of 1% formic acid in acetonitrile. Ammonium formate in methanol provides increased solubility of polar compounds and precipitates proteins as well as acetonitrile

4. **Can I increase assay sensitivity by either increasing sample volume and/or concentrating (evaporation and reconstitution) of the HybridSPE® eluent?**

   It is not recommended to use >100 µL of biological sample for the HybridSPE® DPX® 30 mg tips, however biological sample volumes of up to 300 µL can be used with the HybridSPE® DPX® 50 mg tips. Some phospholipid breakthrough may occur with analytes of interest. 98-100% of biological phospholipids are removed when <300 µL plasma is applied to the HybridSPE® DPX® 50 mg tips. When increasing sample volume be sure to increase the volume of the precipitating agent accordingly. A 1:3 (v/v) plasma:precipitating agent ratio is necessary for optimal performance.

   Another strategy for increasing sensitivity is through evaporation of the HybridSPE® eluent followed by reconstitution in a smaller volume of LC/MS mobile phase. The acetonitrile portion of the HybridSPE® eluent greatly aids the evaporation process. On average it takes less than 10 minutes to evaporate 300-400 µL of HybridSPE® eluent under nitrogen at 37 °C.

5. **Why is ion-suppression still evident during LC-MS analysis after HybridSPE®-PL?**

   HybridSPE® technology will only remove phospholipids and gross levels of precipitated protein from biological samples. Other chemical entities common to biological samples can lead to ion-suppression if not removed prior to LC/MS/MS analysis. It is important to identify the ion-suppression causing component to facilitate troubleshooting. It may be necessary to adjust chromatographic conditions to separate analytes of interest from interfering matrix components. Examples of non-phospholipid chemicals that can lead to ion-suppression include:

   - sodium citrate which is an anti-coagulant used to prepare plasma from blood
   - phthalates, plasticizers and other mold release agents found in plastic ware
   - polyethylene glycol which is a common dosing vehicle for many drugs
   - extractables from o-rings, plastic ware, and seals used to store biological samples

6. **Why should I condition the HybridSPE® DPX® tips?**

   Conditioning is performed in order to avoid large solvent and subsequent analyte losses. The dead volume for the HybridSPE® DPX® 50 mg tips is ~120 µL. The dead volume for the HybridSPE® DPX® 30 mg tips is ~90 µL. As long as the conditioning solvent is fully dispensed, there should not be a dilution effect.

   Nevertheless, addition of an I.S. is recommended prior to HybridSPE® processing (which is standard for most sample prep techniques).

   If increasing signal response is necessary during LC/MS analysis, we recommend evaporating the eluent and reconstituting in a smaller known volume of LC mobile phase prior to LC/MS analysis.

7. **Why am I experiencing low absolute recovery of <50%?**

   The primary protein precipitation procedure using formic acid and acetonitrile as a precipitating agent will work well for ~80% of the applications encountered. However, ~20% of the analytes will co-retain with phospholipids under these conditions resulting in absolute recoveries of <50%. On the next page, strategies are described on how to deal with low recovery compounds.

8. **How fast should I aspirate the sample using the DPX® tip?**

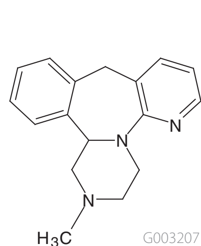
   On an automated platform, aspirate and dispense speeds can be easily controlled and are recommended to be approximately 100-200 µL/second. For manual use, relatively slow aspiration and dispense speeds are needed to ensure efficient mixing of the sorbent and sample solution for phospholipid removal. The number of aspirate/dispense cycles may need to be optimized depending on the analytes of interest.

9. **I've never used DPX® tips before, how do they work?**

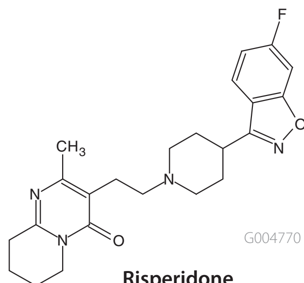
   DPX® stands for Disposable Pipette EXtraction. It is a patented solid-phase extraction (SPE) device that is unique from all other SPE devices. DPX® uses pipette tips that incorporate loosely contained sorbent material (e.g., HybridSPE®) that is mixed with the sample solution via aspirating and dispensing. DPX® tips can be done manually with single or multi-channel pipettors or on an automated platform such as Hamilton®, Tecan®, and INTEGRA. DPX® tips do not require additional hardware such as vacuum or positive pressure manifolds. For more information about DPX®, please visit dpxtechnologies.com.

Secondary Procedure for Low Recovery Basic Compounds (contains amine functional groups):

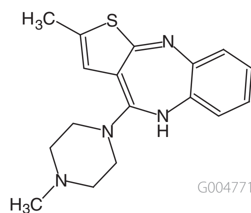
- Some basic compounds may experience low recovery when employing the primary method (1:3 plasma:1% formic acid in acetonitrile). Example compounds include:



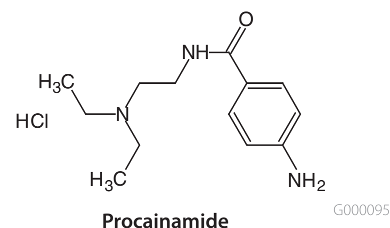
Mirtazapine



Risperidone



Olanzapine



Procainamide

- Low recovery of such basic compounds are caused by: 1) secondary weak cation exchange interactions between HybridSPE® silanol groups (Si-O-); and 2) secondary HILIC interactions between HybridSPE® silica surface and analytes

- We recommend combining: 1:3 plasma:1% ammonium formate in methanol followed by HybridSPE®-PL processing as described in the standard recommended procedure(s).

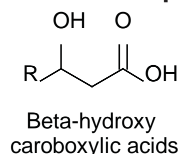
Note:

- Recovery of basic and neutral compounds can improve from <40% to >89%
- NH4+ (ammonium formate) is a stronger counter-ion than H+ (formic acid) inhibiting most basic compounds from interacting with HybridSPE® silanol groups (Si-O-).
- Methanol is a more polar solvent than acetonitrile further inhibiting any potential secondary HILIC interactions between the analyte and HybridSPE® silica surface.
- Note that ammonium formate in methanol is an excellent protein precipitation agent.

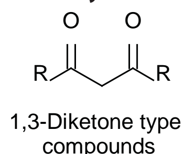
Secondary Procedure for Low Recovery Acidic Chelator & Chelator Compounds:

- Some acidic chelator and chelator compounds may experience low recovery when employing the primary method (1:3 plasma:1% formic acid in acetonitrile).

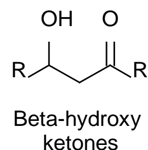
Chelation Functional Groups That Can Lead to Low HybridSPE®-PL Recovery:



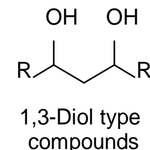
Beta-hydroxy caroboxylic acids



1,3-Diketone type compounds

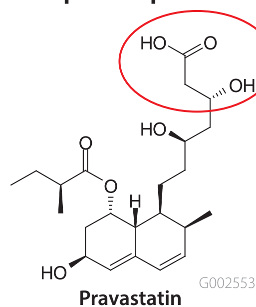


Beta-hydroxy ketones

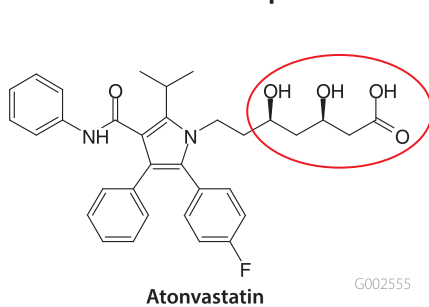


1,3-Diol type compounds

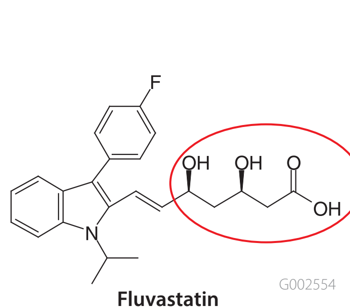
Example Compounds with Chelation Functional Groups:



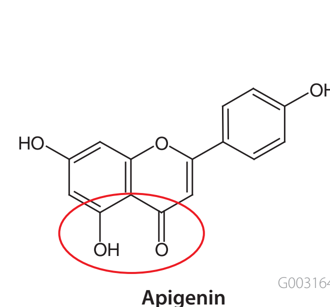
Pravastatin



Atonvastatin



Fluvastatin



Apigenin

- In such cases, using citric acid (a stronger Lewis base than formic acid) in acetonitrile as the precipitating agent will inhibit analyte retention while still allowing phospholipids to retain (be removed).

- When experiencing low recovery for such compounds, we recommend to first condition the HybridSPE® phase with 400 µL 0.5% citric acid in acetonitrile. Combine 1:3 plasma:0.5% citric acid in acetonitrile followed by HybridSPE®-PL processing on the conditioned phase.

Note:

- Recovery of chelator compounds can improve from <40% to 65-95%
- Citric acid is a stronger Lewis base than formic acid inhibiting the retention of chelator compounds.
- Citric acid is not a strong enough Lewis base to inhibit phosphates (phospholipids) from retaining on the HybridSPE® phase.

MilliporeSigma, the vibrant M, Supelco, and HybridSPE are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.