NEWS ON CLINICAL

2022 Volume 1

Special LC-MS Analysis

From sample preparation and separation, to our industry-leading Cerilliant® Certified Reference Materials (CRMs), explore our complete offering for your clinical diagnostic (vitamins, steroids, hormones etc.) and toxicology drug testing needs.

Highlights of this edition:

- Plasma Protein Binding Determination and Free Fraction Analyses
- Sample Preparation
  - HybridSPE® Technology
  - NEW: Supel™ Swift HLB SPE cartridges
- Selecting HPLC and UHPLC Columns for LC-MS
- NEW: Supel™ Carbon LC Column
- LC-MS Grade Solvents and Reagents
- New Analytical Standards for Clinical Applications

Plasma Protein Binding Determination and Free Fraction Analyses

With the NEW Supel™ BioSPME 96-Pin Device

Supel™ BioSPME 96-Pin Devices were developed to target the stringent specifications needed by bioanalytical laboratories while greatly improving upon speed and simplicity compared with current sample preparation techniques for plasma protein binding studies.

Primary Benefits of Supel™ BioSPME Devices:

- More than 3x throughput and time savings compared to rapid equilibrium dialysis workflows
- Completely removes phospholipids, unlike rapid equilibrium dialysis methods
- Simple workflow can be fully automated
- Accuracy and precision comparable to equilibrium dialysis and rapid equilibrium dialysis data
- Proven reproducibility across batches as well as across each 96-sample device

Automation and manual methods are available for download at SigmaAldrich.com/biospme with a full list of recommended products and accessories to successfully run your Supel™ BioSPME workflow.

Sample Preparation: How to Eliminate Ion Suppression by Phospholipids in Plasma Samples

Phospholipids (PLs) are abundantly (at the mg/mL level) present in biological fluids such as blood, plasma, serum, and cerebrospinal fluids, among others. PLs are often co-extracted with a broad range of analytes of interest during sample preparation. Phospholipids are notorious for causing various issues in LC/MS-based bioanalysis. PLs may cause ion suppression or, in rarer cases, ion enhancement, during MS detection. They also tend to build up on a reversed-phase (e.g., C18 and C8) column, negatively affecting the chromatographic separation and ultimately shortening the column lifetime. As a result, the accuracy, reproducibility, and sensitivity of LC/MS bioanalyses may be compromised if PLs are not removed.

HybridSPE®-Phospholipid technology has been developed for selective and rapid depletion of phospholipids from biological samples prior to LC/MS analysis of small molecules. The technology utilizes the affinity of zirconia particles for selective binding and removal of phospholipids. The technology was introduced in different product formats:

- 96-well filter plates for high throughput sample preparation
- Individual cartridges for low throughput applications
- On-line cartridge
- Tips

LC-MS/MS Analysis Using On-line Cartridges for Phospholipid Removal from Protein-Precipitated Biological Fluids

A summary of the experiments is below; for the full article, visit our webpage **SigmaAldrich.com/article1**

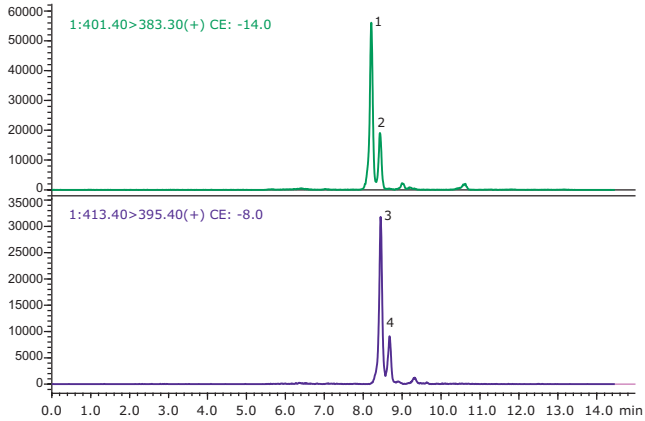
Three sets of compounds with different physicochemical properties (Table 1) were used to demonstrate the applicability of a system including the on-line cartridges with a LC/MS column and the efficiency for phospholipid removal from protein-precipitated plasma samples.

Table 1: Sets of Analytes

Set-1: Basic analytes	Set-2: Polar neutral analytes	Set-3: Non-polar neutral analytes (Figure 1 & Table 2)
Risperidone	Digoxin	25-Hydroxyvitamin D_2
Clomipramine	Digitoxin	3-*epi*-25-Hydroxyvitamin D_2
Tamoxifen		25-Hydroxyvitamin D_3
		3-*epi*-25-Hydroxyvitamin D_3

Three applications were established using on-line HybridSPE® with LC/MS detection. Analyte recovery was 94%-102%, with reproducibility of 1%-5%. For all tested analytes, peaks were narrow and symmetric, peak width at half height was <6 s, and tailing factors were 0.9-1.3.

Figure 1: Representative LC/MS chromatogram of non-polar neutral analytes (Set-3)



Peak	Analyte	Peak width at 50% height (s)	Tailing factor
1	25-OH D3	4.74	0.9
2	3-*epi*-25-OH D3	4.80	0.9
3	25-OH D2	4.62	1.0
4	3-*epi*-25-OH D2	5.10	0.9

- All peaks are narrow: <6 s peak width at half height
- Both peaks are symmetric, with tailing factors of 0.9-1.0
- Baseline is low and clean: no interference peaks

Table 2: HybridSPE®-LC/MS conditions for Set-3 analytes

Instrument:	Shimadzu™ LCMS-8030 with 2DLC setup
HPLC Column:	Ascentis® Express F5 10 cm x 2.1 mm (53569-U)
Mobile Phase:	(A) Water/10 mM ammonium formate; (B) methanol
Gradient:	0% B% for 4 min, to 75% B in 0.5 min, held for 8 min
Flow:	0.3 mL/min
Column Temperature:	45 °C
Sample loading flow:	0.1 mL/min
Sample loading solvent:	methanol with 10 mM ammonium formate
Injection volume:	1µL
Detection:	MS, ESI(+), MRM mode

Summary

The Supel™ Genie HybridSPE® on-line cartridges are designed for removal of phospholipids from more than 100 injections of 1 µL of protein precipitated plasma samples.

Featured Products

Description	Cat.No.
Sample preparation	
Supel™ Genie HybridSPE® On-line Starter Kit	**55324-U**
Supel™ Genie HybridSPE® On-line SPE Cartridge, pk. of 2	**55326-U**
Supel™ Genie HybridSPE® On-line SPE Cartridge, pk. of 6	**55327-U**
LC	
Ascentis® Express C18 5 cm x 2.1 mm, 2.7 µm	**53749-U**

LC-MS Applications

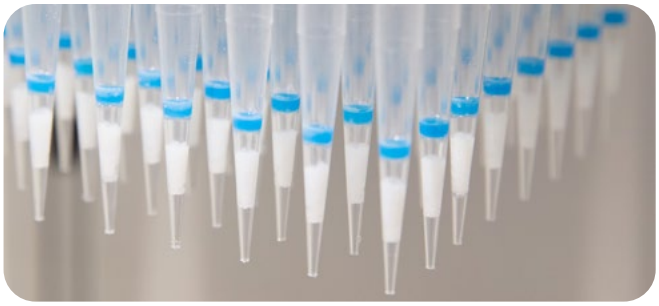
Applications using HybridSPE® Technology for sample preparation.

Examples include:

- Warfarin™ in plasma
- Digoxin and Digitoxin in serum or plasma
- Clenbuterol enantiomers in plasma
- Antiarrhythmic drugs and metabolites
- Vitamin D metabolites
- Steroid hormones

DPX® Tips for Automated Solid Phase Extraction (SPE) with HybridSPE® Technology

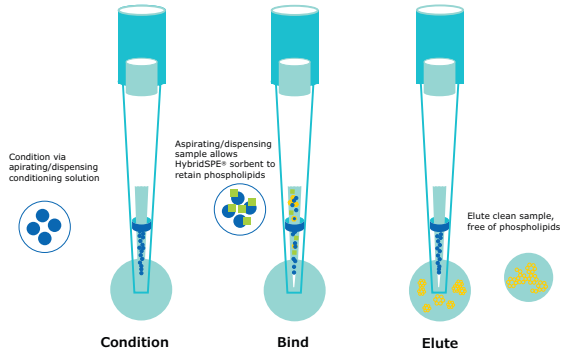
DPX® Dispersive Pipette Extraction pipette tips incorporate loosely-contained HybridSPE® sorbent material that is mixed with sample solution for solid phase extraction.



The unique mixing technique has numerous advantages:

- Minimal elution solvent volumes
- Rapid extraction times (less than 3 minutes/sample)
- High extraction efficiencies
- Easy-to-perform extractions
- Lower cost
- Higher throughput
- Minimal training requirements
- Environmentally friendly

We offer an array of tips that are compatible with the most common liquid handling platforms and pipettors on the market: Tecan, Hamilton, INTEGRA, as well as a universal format.



For additional information, visit SigmaAldrich.com/hybridspe to download our technical resources or ask for a sample.

NEW: Supel™ Swift HLB SPE cartridges - Solid Phase Extraction Made Easier

Supel™ Swift HLB SPE is a polymeric stationary phase for solid phase extraction prior to instrumental analysis. It has both hydrophilic and lipophilic functional groups for extraction of a broad range of compounds from aqueous samples. Its hydrophilic and lipophilic balance (HLB) property allows it to retain analytes having different polarities and Log P values. Benefits of Supel™ Swift HLB SPE cartridges include:

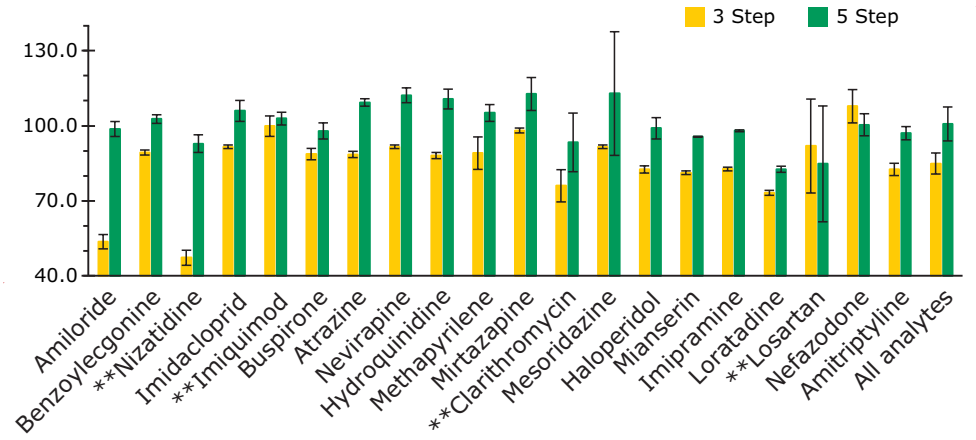
- Suitable for generic methodology
- Wide applicability
- Sorbent designed to provide fast and efficient flow rates (20% faster flow rates compared to another broadly-marketed fast-flow HLB cartridge in diluted plasma SPE)
- Ideal for LC-MS and other workflows (reduced ion suppression/enhancement)

SPE with Supel™ Swift HLB SPE cartridges can also reduce the number of steps for extraction of your analyte from 5 to 3. You can load samples directly onto the Supel™ Swift HLB SPE cartridge bed and potentially eliminate the need for cumbersome pre-conditioning steps. This feature of Supel™ Swift HLB SPE cartridges reduces the number of errors in sample processing and simplifies sample preparation with only a small reduction in recovery.

Figure 2. Summary of Recovery for 3- and 5-Step Processes using Supel™ Swift HLB

5-Step method

3-Step method Recovery



** Analytes did not have an internal standard

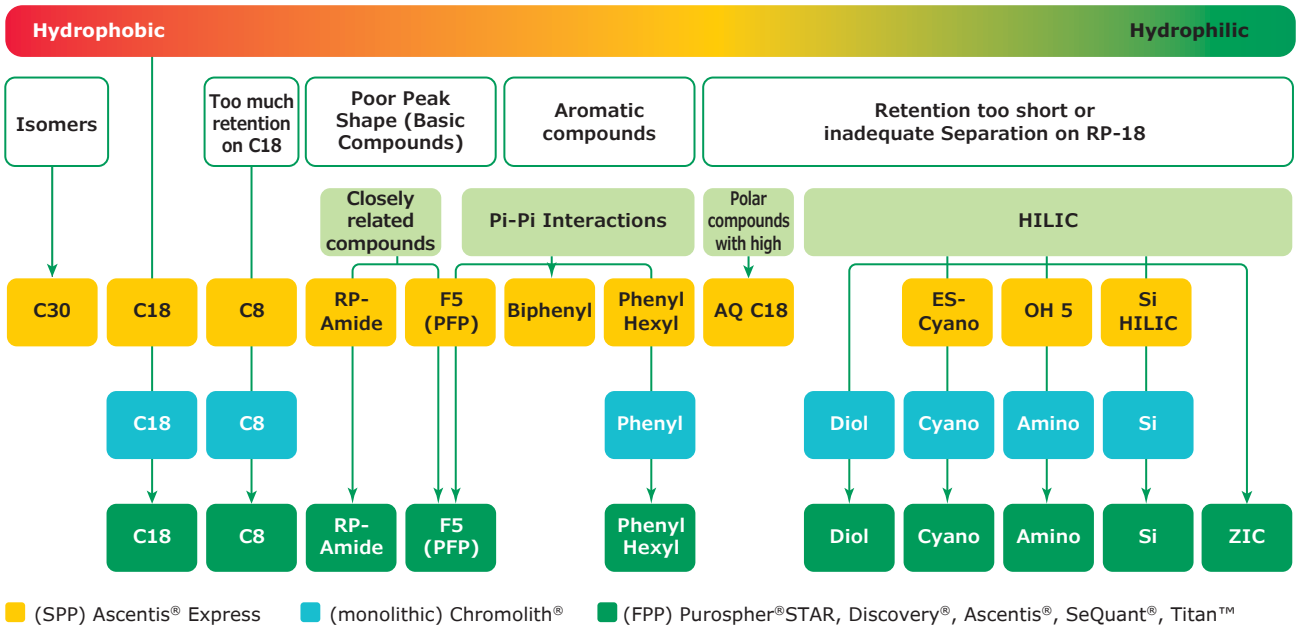
Ordering Information

Description	Cat.No.
SPE cartridges	
Supel™ Swift HLB SPE Tubes weight 200 mg (bed), volume 6 mL, pk of 30 ea	**57491-U**
Supel™ Swift HLB SPE Tubes weight 60 mg (bed), volume 3 mL, pk of 54 ea	**57492-U**
Supel™ Swift HLB SPE Tubes weight 30 mg (bed), volume 1 mL, pk of 108 ea	**57493-U**
Supel™ Swift HLB SPE 96-Well Platebed weight 30 mg, pk of 1 ea	**57494-U**
Supel™ Swift HLB SPE 96-Well Platebed wt. 10 mg, pk of 1 ea	**57495-U**
96-well plates (available in Q2 2021)	

See our complete offering of Supel™ Swift HLB SPE products at SigmaAldrich.com/SupelSwiftHLB

Selecting HPLC and UHPLC Columns for LC-MS: Valuable Information and Guides

Selection Guide for Small Molecules



NEW: Supel™ Carbon LC Column

Porous Graphitic Carbon (PGC) particle packed column for separating poorly retained, polar compounds.

Supel™ Carbon LC particles offer a unique retention mechanism for polar compounds under reversed-phase conditions. This same retention mechanism can also lead to resolution of geometric isomers of compounds

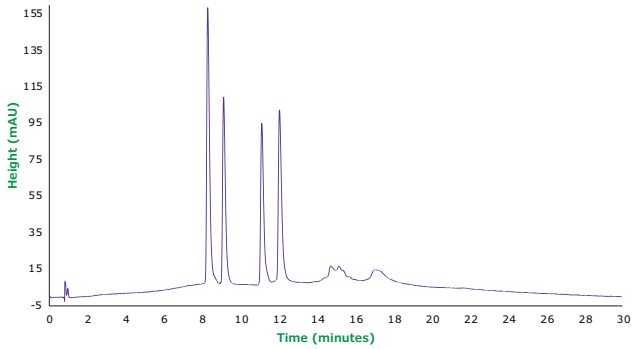
Supel™ Carbon LC Specifications

Particle Platform	Porous Graphitic Carbon (PGC)
Particle Size	2.7 µm
Pore Size	200 Å
Surface Area	155 m^2/g
pH range	1 - 14
Maximum Temperature	250 °C

Chromatographic separation of Vitamin D2 and D3 metabolites on Supel™ Carbon LC

Peaks	Compound	Ret. Time (Min)
1	3-epi-25-hydroxyvitamin D3 (50 µg/mL)	8.294
2	25-hydroxyvitamin D3 (25 µg/mL)	9.125
3	3-epi-25-hydroxyvitamin D2 (50 µg/mL)	11.126
4	25-hydroxyvitamin D2 (100 µg/mL)	12.052

Vitamin D2 & D3 Separation on Supel™ Carbon LC column



Conditions: Column: Supel™ Carbon LC, 10 cm x 2.1 mm I.D., 2.7 µm; Mobile Phase: [A] 2-Propanol; [B] Tetrahydrofuran; Gradient: 0% B to 70% B in 15 min; hold at 70% B for 5 min; Flow Rate: 0.3 mL/min; Column Temp.: 25 °C; Detector: UV, 275 nm; Injection: 2.0 µL; Sample: Vitamin D2 and D3 metabolites mix, varied concentration, ethanol

Visit SigmaAldrich.com/carbonLC to learn more.

Download the Clinical LC-MS Workflow brochure

LC-MS Grade Solvents and Reagents

The popularity of LC-MS led to many improvements in various aspects of the technique: instrumental, chemical, and database methods have been developed, increasing the sensitivity, specificity and analysis speed of this invaluable technique. New ion sources, high-resolution LC systems, and rapid mass spectrometers with enhanced ion optics and detectors have lowered the limits of detection, but raised the bar on the purity expectations of reagents used for sample preparation, mobile phases, and as additives.

Some compound classes that can be problematic are alkali ions, plasticizers, and surfactants, as they are widespread and interfere significantly with LC-MS by forming adducts, causing higher background noise, and through signal suppression. Because of the integral part that chemistry plays in a successful LC-MS analysis, we have developed and introduced a broad portfolio solvents, additives, and reagents designed specifically to meet the requirements of high purity and consistency.

Download our **brochure** for informative articles on LC-MS additives and the advantages of high purity solvents for both small and large molecule analysis.

For more information, visit
SigmaAldrich.com/lcms_solvent

New Analytical Standards for Clinical Applications

Acyl-carnitines and Fatty Acids for Newborn Screening Programs

Measuring different acyl-carnitines can be used to detect more than 40 different inborn errors of metabolism. If these diseases are not diagnosed, the resulting metabolic disorders can lead to severe and irreversible harm to newborns within their first few days of life. Newborn screening programs aim to detect congenital metabolic disorders early, before they become symptomatic.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has expanded the screening possibilities in newborn screening programs, and is an efficient and robust methodology for analyzing acyl-L-carnitines.

MilliporeSigma has a comprehensive portfolio of metabolites for newborn screening programs including:

- Acyl-carnitines
- Fatty Acids
- Aliphatic Acyl-Carnitines
- Dicarboxylic Acyl-L-Carnitines
- Unsaturated Acyl-L-Carnitines
- 3-Hydroxy alkyl Acyl-L-Carnitines
- Unsaturated hydroxy alkyl Acyl-L-Carnitines
- Aromatic Acyl-L-Carnitines
- Isotope labelled Metabolites

Accurate Reference, Accurate Results: Cerilliant® Certified Reference Materials for Your Clinical Applications

Our clinical portfolio of reference standards and certified reference materials (CRMs) for analytical testing applications includes parents, metabolites, impurities, degradants, endogenous biomarkers, and internal standards including stable isotope-labelled standards. Our reference material manufacturing sites are double-accredited at a minimum to the highest achievable quality level for reference material producers: ISO 17034 / Guide 34, ISO/IEC 17025.

For more information, visit
SigmaAldrich.com/standards

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