MERCK

fast clean efficient

Efficiency in Analysis for Your Clinical LC-MS Workflow

Supelco®
Analytical Products

LC-MS Analysis for Clinical Testing

Sample Prep

Reference Standards

Sample Control Matrix

Mobile Phase

Chromatography

Enabling LC-MS in Clinical Testing

Sigma-Aldrich® – Supelco® – Cerilliant®

Clinical laboratories are reaping the benefits of LC-MS for Laboratory Developed Tests (LDTs) in terms of its sensitivity, specificity, throughput and potential to reduce cost per sample. Our aim has been to develop analytical tools to help LC-MS users maximize their efficiency and validate their choice of adopting the technology. To that end, we have focused our R&D efforts on addressing the challenges specific to LC-MS in clinical settings.

In this brochure, we provide a premier selection of proven tools and consumables for the LC-MS workflow in clinical laboratories.

Table of Contents

Sample Preparation

Eliminate Ion Suppression from Contaminants in Plasma Samples

HybridSPE® Products for Consistent LC-MS Ionization

When using positive ion electrospray (+ESI) the presence of phospholipids in biological fluids is one of the major causes of ion-suppression. Removing these phospholipids with HybridSPE® is a rapid and reliable means to improve identification and quantification of compounds in biological matrices.

- Maximize sensitivity by minimizing ion suppression
- Generic 2-3 step procedure
- With both 100-300 µl and 20-40 µl (small volume) sample size capacities, the 96-well plate format is ideal for high-throughput pre-clinical and clinical applications

How HybridSPE® Works

HybridSPE® 96-well Plate Protocol

Featuring an “in-well” precipitation procedure for both proteins and phospholipids

Add Sample

Pipette 100 µL plasma or serum to the HybridSPE® plate followed by 300 µL precipitation solvent. Add internal standards as necessary.

Mix

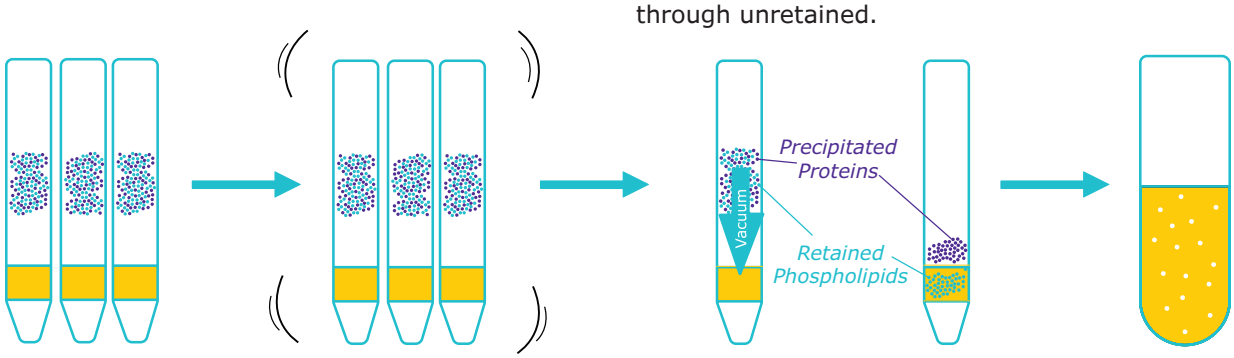
By vortexing/shaking HybridSPE® plate or by aspirating/dispensing with 0.5–1 mL pipette tip.

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.

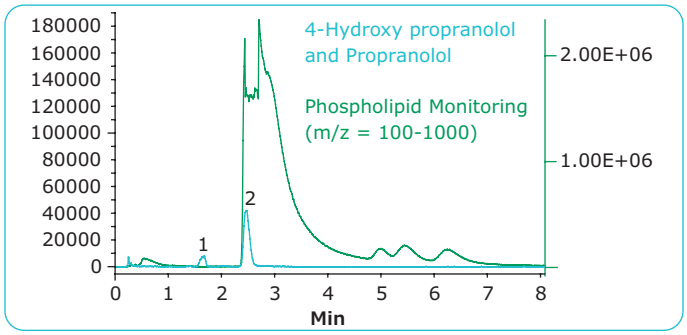
Collect Sample

Resulting filtrate/eluate is free of proteins and phospholipids and ready for immediate LC-MS/ MS analysis.

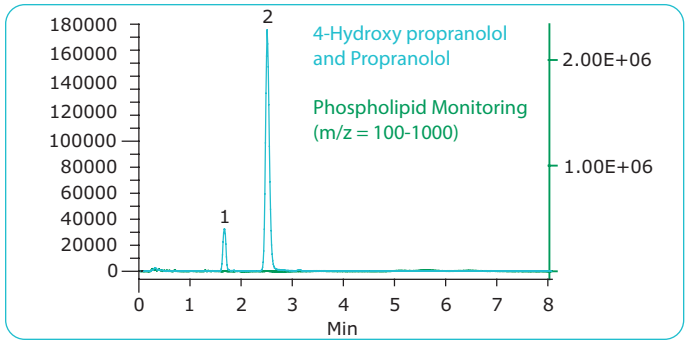


HybridSPE® Sample Preparation Products

Standard Protein Precipitation Technique (Note suppression of propranolol signal)



HybridSPE® Technique



Description	Qty.	Cat. No.
Well Plates		
HybridSPE®-PLus 96-well Plate, 50 mg/well	1	575659-U
	20	575673-U
HybridSPE®-PL, Small Vol. 96-well Plate, 15 mg/well	1	52794-U
	20	52798-U
HybridSPE®-PLus 96-Well Plate Essentials Kit (contains: 96-well Plate, 50 mg/well, 1 cap mat , sealing film, and collection plate)	1	52818-U
SPE Cartridges		
HybridSPE®-PL Ultra Cartridge, 30 mg/1 mL	100	55269-U
HybridSPE®-PL Cartridge, 30 mg/1 mL	100	55261-U
	200	55276-U
HybridSPE®-PL Cartridge, 500 mg/6 mL	30	55267-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
PlatePrep Vacuum Manifold	1	57192-U
96-well Protein Precipitation Filter Plate (for offline protein precipitation)	1	55263-U

For more information, visit
SigmaAldrich.com/hybridspe

Analysis of 9 Vitamin D Metabolites from Serum using HybridSPE Cleanup

The unique combination of column selectivity using Ascentis® Express F5 and phospholipid removal using HybridSPE®-PLus provides a robust and accurate LC-MS method for accurate and sensitive analysis of vitamin D metabolites, including isobaric critical pairs.

Introduction: Interest in Vitamin D

Analysis of vitamin D metabolites has continued to be a topic of interest in recent publications, primarily as biomarkers for possible disease states and vitamin deficiency. While vitamin D is present in two forms, vitamin D3 and vitamin D2, current ELISA methods demonstrate different cross-reactivities and cannot distinguish between D2 and D3 forms of the vitamin metabolites resulting in erroneous reporting of total 25-hydroxyvitamin D concentrations. Further, there is interest in an analytical means to differentiate the D2 and D3 forms from the D2 and D3 epimers because of their different degrees of bioactivity.

The epimers are isobaric to the D2 and D3 metabolites rendering them indistinguishable in the MS and thus requiring chromatographic separation to resolve them. Traditional reversed-phase chemistries, e.g., C18, do not resolve the critical epimeric pairs of vitamin D metabolites. In this application, we show our Ascentis® Express F5 column accomplishes efficient resolution of the Vitamin D metabolites shown in **Figure 1**.

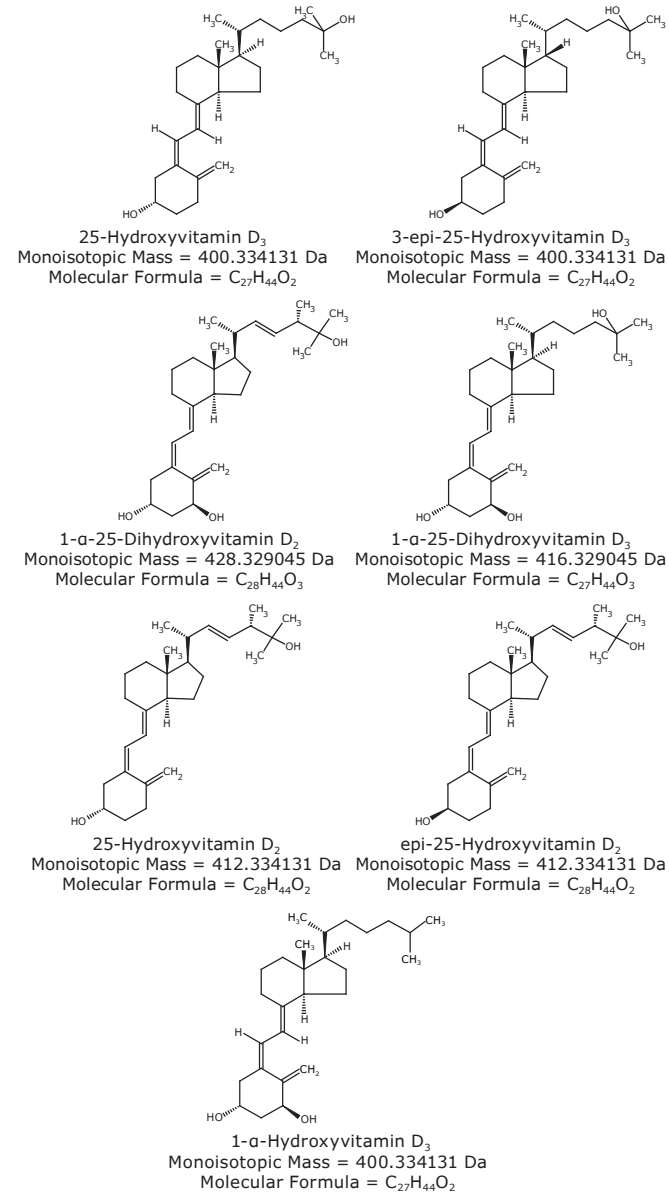
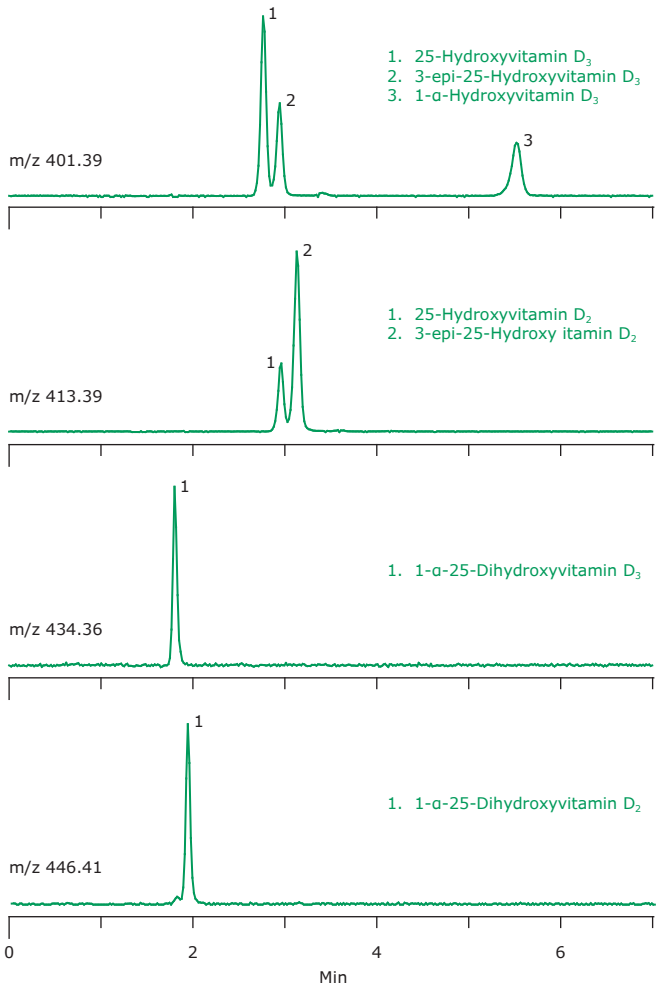


Figure 1. Structures of Vitamin D Metabolites

Figure 2. Separation of Vitamin D Metabolites on Ascentis® Express F5



column:	Ascentis® Express F5, 15 cm x 2.1 mm I.D., 2.7 µm particles (53571-U)
flow rate:	0.4 mL/min
column temp:	40 °C
injection:	2.0 µL
mobile phase:	5 mM Ammonium Formate, (A) water; (B) methanol, (23:75, A:B)
detection:	ESI+, 100-1000m/z
instrument:	Agilent 1290, 6210 TOF

Comparison of Sample Prep Techniques to Reduce Serum Matrix Effect

Particular attention was paid toward sample preparation and its impact on analytical results. Serum sample preparation requires protein precipitation with organic solvents or strong acids. This approach results in gross depletion of proteins from the sample, but leaves high levels of matrix interference from coextracted phospholipids. Coextracted phospholipids can cause quantitative irregularities and decrease sample throughput due to gradient column washing requirements.

Human serum samples were spiked with vitamin D metabolites and processed using two sample preparation techniques: standard protein precipitation alone and standard protein precipitation followed by phospholipid depletion with HybridSPE®-PLus plates. (Note that protein precipitation can also be performed directly in the HybridSPE® plates for a truly one-step sample prep method. However, users may prefer to carry out the protein precipitation as a separate step.) Results obtained from sample processed using the two sample preparation techniques were evaluated to determine analytical impact of phospholipid matrix interferences, both in terms of chromatographic overlap and impact on analyte response.

Sample Prep Methodologies

Human serum samples were spiked at 25 ng/mL with vitamin D metabolites. Protein precipitation was performed offline by adding 100 µL of spiked serum followed by 300 µL of 1% formic acid acetonitrile to a 96-well collection plate. Samples were thoroughly mixed by performing five 300 µL draw/dispense cycles using a digital pipette. Samples were then left to sit for 5 minutes before transferring 200 µL of the resulting supernatant into a HybridSPE® PLus 96-well plate. Samples were passed through the HybridSPE® PLus plate by applying 10” Hg vacuum for 4 minutes and analyzing the resulting filtrate directly. As a comparison, spiked human serum was also processed using standard protein precipitation alone by adding 100 µL of serum followed by 300 µL of 1% formic acid acetonitrile into 2 mL centrifuge vials. Samples were then vortexed and centrifuged, and the resulting supernatant was analyzed directly.

Improved Analyte Response after Phospholipid Removal

Figure 3 depicts the phospholipid selected ion chromatograms from samples processed via standard protein precipitation (red trace) and samples processed using the HybridSPE®-PLus plate (black trace). A significant amount of phospholipid matrix from samples processed via protein precipitation alone was observed eluting in the 2–4 minute window where several vitamin D metabolites also elute. Conversely, samples processed by the HybridSPE®-PLus plate displayed no detectable phospholipid matrix. Phospholipid coelution with analytes of interest has the potential to cause sensitivity and reproducibility issues resulting in irregularities in quantitation. **Table 1** compares analyte recovery of the two sample preparation techniques. The direct overlap in the elution windows of vitamin D metabolites and the phospholipid matrix interference for samples processed with standard protein precipitation resulted in a 40% reduction in signal response for several of the metabolites. As a result, samples processed using the HybridSPE®-PLus plate, which demonstrated no co-eluting matrix interference, demonstrated a higher analyte response.

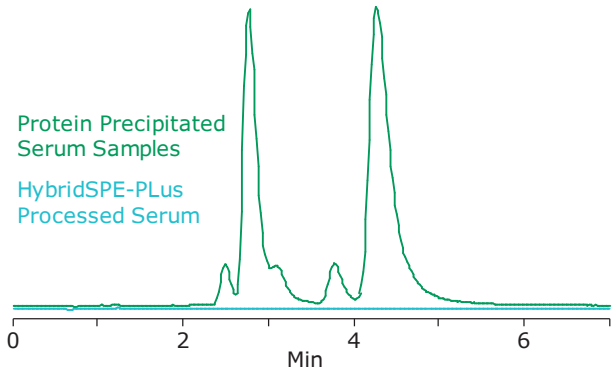


Figure 3.

Table 1. Summary of Minimum Isobaric Resolution by Column Phase and Organic Modifier

Sample	HybridSPE®-PLus Processed Serum 25 ng/mL Average n=16	Protein Precipitated Serum 25 ng/mL Average n=8
1-α-25-Dihydroxyvitamin D3	21.1	17.1
1-α-25-Dihydroxyvitamin D2	19.7	19.0
25-Hydroxyvitamin D3	24.3	15.5
3-epi-25-Hydroxyvitamin D3	21.3	15.3
25-Hydroxyvitamin D2	29.8	21.4
3-epi-25-Hydroxyvitamin D2	24.5	23.0
1-α-Hydroxyvitamin D3	27.7	21.0

For more information, visit:
SigmaAldrich.com/hybridspe

Summary

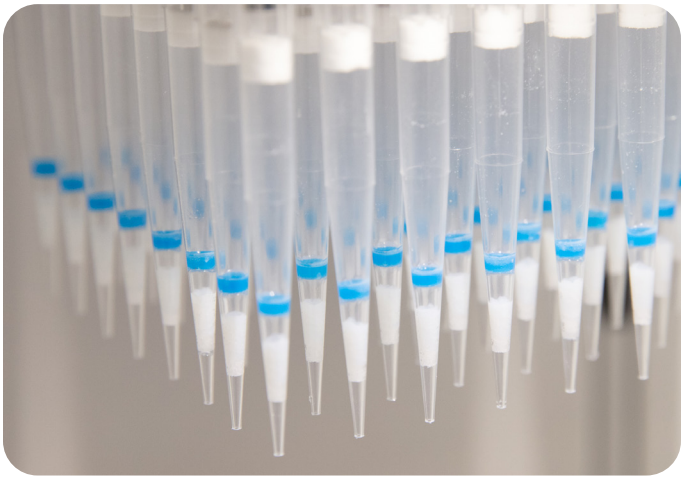
Chromatographic resolution still plays an important role in LC-MS applications when dealing with isobaric compounds. The unique selectivity of the Ascentis® Express F5 column gave a fast and efficient analytical method for 25-hydroxyvitamin D and related forms from serum samples. In addition, phospholipid depletion using the HybridSPE®-PLus 96-well plate enabled efficient sample cleanup increasing method reproducibility and accuracy. As demonstrated in this study, this novel sample prep technique coupled with the unique selectivity of the Ascentis® Express F5 column enables a fast and simplified bioanalytical method for associated vitamin D metabolites. Likewise, this approach demonstrates how selectivity, in both chromatographic and sample preparation steps, allows for efficient analysis that would otherwise be unattainable with traditional reversed-phase approaches.

DPX® Tips for Automated SPE with HybridSPE® Technology

Extraction in Seconds

DPX stands for Dispersive Pipette Extraction, a patented technology that introduces the benefits of solid phase extraction into a revolutionary, easy-to-use pipette tip. This device is unique from all other SPE devices because HybridSPE sorbent material is loosely contained within a tip. The HybridSPE material inside is a patented sorbent designed for selective retention and removal of endogenous phospholipid interferences from biological matrices for LC-MS or LC-MS/MS analysis.

Dispersive pipette extraction provides an INTip solution for complete sample preparation that can be easily automated. The loose sorbent mixes with a sample solution during aspirate and dispense steps and enables a highly efficient interaction of the sorbent and analyte of interest for accurate downstream analysis. This technology is ideal for bind – wash – elute and/or cleanup protocols.



The unique mixing technique provides numerous advantages over traditional SPE formats:

	Dispersive Pipette Extraction	SPE Cartridge	96-Well plate
Customization	✓	✗	✗
High efficiency	✓	✗	✗
Low sample volumes	✓	✗	✓
Reduced solvent volumes	✓	✗	✓
Scalable Process 1-96	✓	✗	✗
Automation	✓	✗	✓
No hardware required	✓	✗	✗

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.

What size tips do I need?

HybridSPE® 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

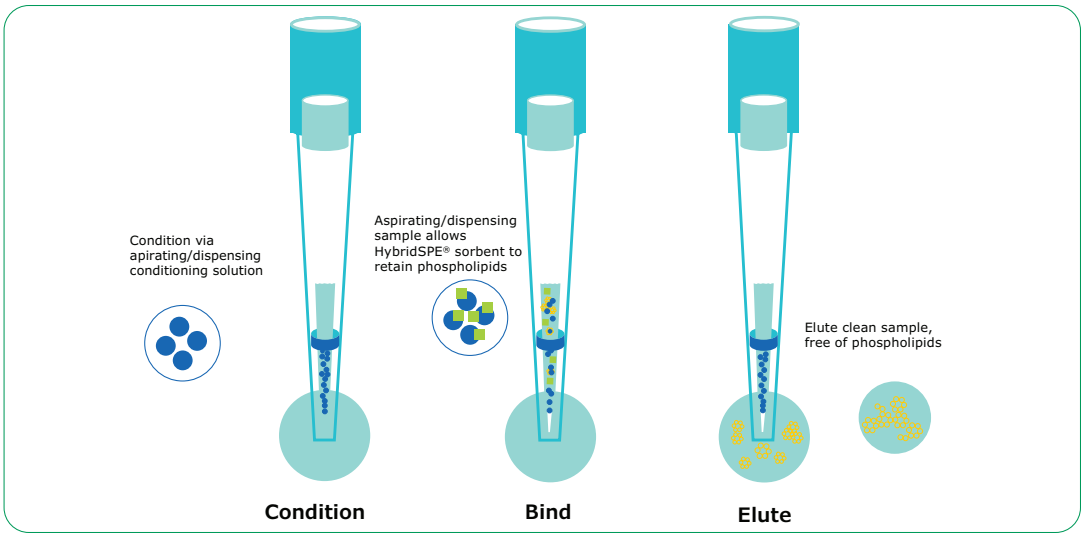
Ordering Information

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

Automated SPE with HybridSPE® DPX Tips

Extraction in Seconds

HybridSPE® Sample Prep Workflow Using DPX® Tips



To learn how to do extraction in seconds, visit: **SigmaAldrich.com/DPX**

Establish a Hands Free LC-MS Workflow

Supel™ Genie Online SPE Cartridges

Supel™ Genie Online SPE cartridges offer a sample preparation solution for a seamless workflow from start to finish performed entirely "online" the LC instrument. Your samples are directly injected onto the SPE cartridge located on the analytical instrument (LC instrument) for a simple and efficient, hands free solution.

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

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

We currently offer 3 phase chemistries:

- HybridSPE® - for complete removal of phospholipids (a leading cause of matrix effects) from biological samples
- C8 - for reversed-phase extraction of hydrophobic or nonpolar to moderately polar compounds
- RP-Amide - for reversed-phase extraction of nonpolar to polar compounds and offer improved performance for polar analytes

Our Starter Kits come with reusable hardware that will fit any Supel Genie cartridge, as well as 1 cartridge of selected phase chemistry. Additional cartridge packs include cartridges only.

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**
Supel Genie RP-Amide Online Starter Kit	**55516-U**
Supel Genie RP-Amide Online SPE Cartridge, pk. of 2	**55519-U**
Supel Genie RP-Amide Online SPE Cartridge, pk. of 6	**55522-U**
Supel Genie C8 Online Starter Kit	**55274-U**
Supel Genie C8 Online SPE Cartridge, pk. of 2	**55512-U**
Supel Genie C8 Online SPE Cartridge, pk. of 6	**55515-U**

For more information or to order, visit **SigmaAldrich.com/onlinespe**

Supel™ Swift HLB SPE cartridges

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 the extraction of a broad range of compounds from aqueous samples. It retains analytes having different polarities and Log P values due to its hydrophilic and lipophilic balance (HLB) property. Benefits of *Supel™ Swift HLB* SPE cartridges include:

- Suitable to the generic methodology
- Wide applicability
- Ideal for LC-MS and other workflows

The possibility of 3-step SPE

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

5-Step Method (Standard)		3-Step Method (Simplified)
Prime with 300 µL MeOH	❶ Condition	
Condition with 300 µL H_2O	❷ Equilibrate	
Load 200 µL Diluted Serum	❸ Load	Load 200 µL Diluted Serum
Wash with 200 µL 5% MeOH in H_2O	❹ Wash	Wash with 200 µL 5% MeOH in H_2O
Elute with 300 µL 50/50 ACN/MeOH, 2x	❺ Elute	Elute with 300 µL 50/50 ACN/MeOH, 2x

Figure 1. General processing of samples (serum 1:1 diluted) with *Supel™ Swift HLB cartridges* (30 mg/1 mL) using a 5-step method and a 3-step method

Excellent recovery for a wide range of compounds having different polarities and Log P values

Supel™ Swift HLB SPE cartridges offers good recovery for a wide range of compounds and polarities. **Figure 2** presents absolute recoveries of compounds ranging in log P from -0.9 to 4.8 using *Supel™ Swift HLB SPE cartridges* with both the 3-Step and 5-Step methods from plasma.

All-in-all, the 5-Step method shows better recoveries as compared to the 3-Step process. In the 5-Step process, all twenty analytes had recoveries between 80% and 120%. However, eighty percent of the analytes still showed recoveries in the 80% to 120% range by the 3-Step process.

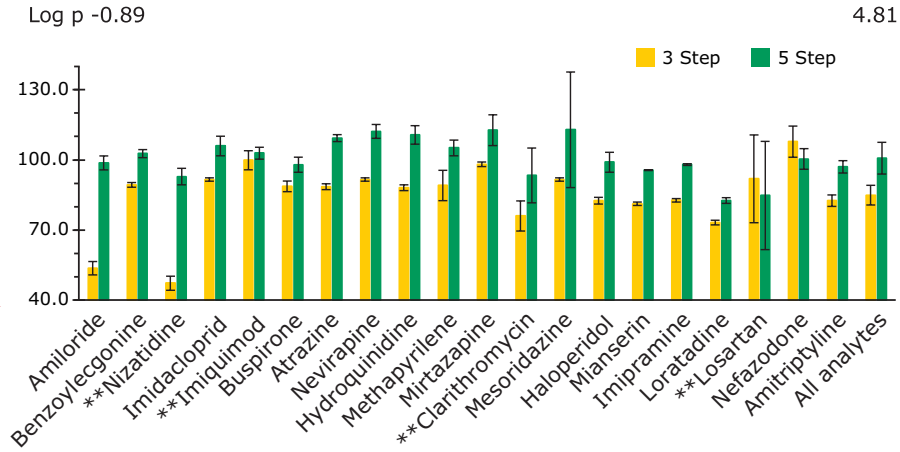


Figure 2: Summary of Recovery for the 3- and 5-Step Process using *Supel™ Swift HLB SPE cartridges*. Analytes are ordered by increasing log P values.

Description	Cat. No.
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**

For more information visit: **SigmaAldrich.com/SupelSwiftHLB**

Filtration

Amicon® Ultra Centrifugal Filters for Cleaner Results

Amicon® Ultra Centrifugal Filters

- High recovery Ultracel® regenerated cellulose membrane in a range of molecular weight cut-offs
- High retentate recovery of >90%
- Vertical membrane reduces concentration polarization for ultra-fast spin times (as fast as 10–15 minutes)
- True dead stop – provides predictable concentration factor, avoids spinning to dryness
- Broad chemical compatibility – Amicon® Ultra filters are made of materials that are compatible with solutions with diverse chemical properties and pH ranging from 1 to 9

Centrifree® Ultrafiltration Device

Free/Bound Separation Now Easy

- A better alternative to the time-consuming equilibrium dialysis technique for free/bound separation is ultrafiltration with our Centrifree® micropartition device
- Centrifree® employs a membrane filter with controlled porosity which retains more than 99.9% of serum protein and lets free drugs readily pass the membrane for collection and analysis
- Separation of free from bound microsolute in serum, plasma, and other biological samples
- Determine free concentration of steroid hormones such as testosterone, thyroid hormones including thyroxine, anticonvulsants such as Phenytoin (Dilantin), heart medications such as digoxin, and free copper
- Binding studies
- Deproteinization

Cat. No.	Product	Description
UFC901008D	Amicon® Ultra (IVD Registered)	Amicon® Ultra-15 Centrifugal Filter Unit with Ultracel-10 membrane
UFC801008D	Amicon® Ultra (IVD Registered)	Amicon® Ultra-4 Centrifugal Filter Unit with Ultracel-10 membrane
UFC501008	Amicon® Ultra	Amicon® Ultra-0.5 Centrifugal Filter Unit with Ultracel-10 membrane
ACS500024	Amicon® Pro	Amicon® Pro 24 Pack (excluding Amicon® Ultra-0.5 filter)
4104	Centrifree® (IVD Registered)	Centrifree® Ultrafiltration Device with Ultracel® PL membrane
4304	Centriprep® (IVD Registered)	Centriprep® Centrifugal Filter Unit with Ultracel®-10 membrane
4306	Centriprep® (IVD Registered)	Centriprep® Centrifugal Filter Unit with Ultracel®-30 membrane
UFC30GV00	Ultrafree®-MC	Ultrafree®-MC GV Centrifugal Filter unit with a pore size of 0.22 µm
UFC30LG25	Ultrafree®-MC	Ultrafree®-MC LG Centrifugal Filter unit with a pore size of 0.2 µm
UFC40HV00	Ultrafree®-CL	Ultrafree®-CL HV Centrifugal Filter unit with a pore size of 0.45 µm
UFC40LH25	Ultrafree®-CL	Ultrafree®-CL LH Centrifugal Filter unit with a pore size of 0.45 µm
9031	Minicon® (IVD Registered)	Minicon® B15, 8 cells/unit

Why Amicon® Ultra? – Heat sealed membrane technology minimizes extractables being added to sample

Applications

- Concentration of biological samples containing antigens, antibodies, enzymes, nucleic acids, or microorganisms
- Protein removal prior to HPLC
- Desalting, buffer exchange and protein dialysis

MultiScreen® plates

- High throughput for drug and protein bioassays
- Specifically developed for high-throughput use with automated work stations
- Custom Multiscreen® filter plates available
- MultiScreen® HTS Vacuum manifold was designed specifically to work with MultiScreen® HTS filter plates. The combination results in optimal filtration characteristics and seamless automation integration
- New MultiScreen® HTS+ Hi flow filter plate design lessens non-specific binding and reduces variability in both background and signal intensities specifically for biochemical screening assays

Protect your results with HPLC-certified Millex® hydrophilic PTFE syringe filters

- Millex®-LG and Millex®-LCR filters contain hydrophilic PTFE membranes and are HPLC-certified for low levels of UV-absorbing extractables.
- Hydrophilic PTFE membranes exhibit low analyte binding and broad solvent compatibility, enabling filtration of aqueous and organic solvents.

For ordering information, visit
SigmaAldrich.com/OneMillex

Broad organic solvent compatibility with hydrophobic PTFE Millex® syringe filters.

- Millex®-FG and Millex®-FH filters contain hydrophobic PTFE membranes that are compatible with a broad range of pure organic solvents.

Best recovery of protein samples with Durapore® PVDF Millex® syringe filters.

- Millex®-GV and Millex®-HV filters contain hydrophilic Durapore® PVDF membranes which exhibit the lowest protein binding of all Millex® syringe filters.*

*Lowest protein binding based on binding to IgG “

Applications: Sample Filtration Prior to UHPLC, HPLC and Mass Spec; Solvent Filtration; Filtration of Biological Samples and Protein Solutions

MultiScreen® Plates

Efficient Hydrolysis - Achieve More Sensitive Detection of Low abundance Glucuronide Compounds

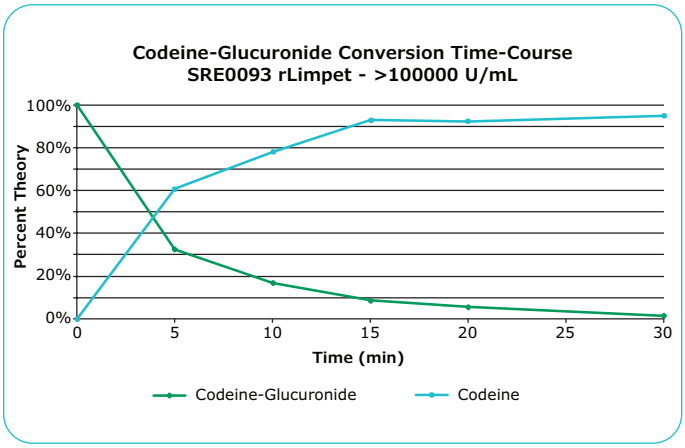
β-Glucuronidase

β-Glucuronidases are routinely used for the enzymatic hydrolysis of glucuronides from urine, plasma and other fluids prior to analysis by LC-MS for better sensitivity.

Now available Recombinant β-Glucuronidase from Limpet source:

Cat. No.	Form	Product Description	Glucuronidase Activity	Sulfatase Activity
SRE0093	Liquid	β-Glucuronidase from limpets (Patella vulgata) – **Recombinant** NEW	100,000-200,000 units/mL	Little to no activity

Codeine-6-β-D-glucuronide

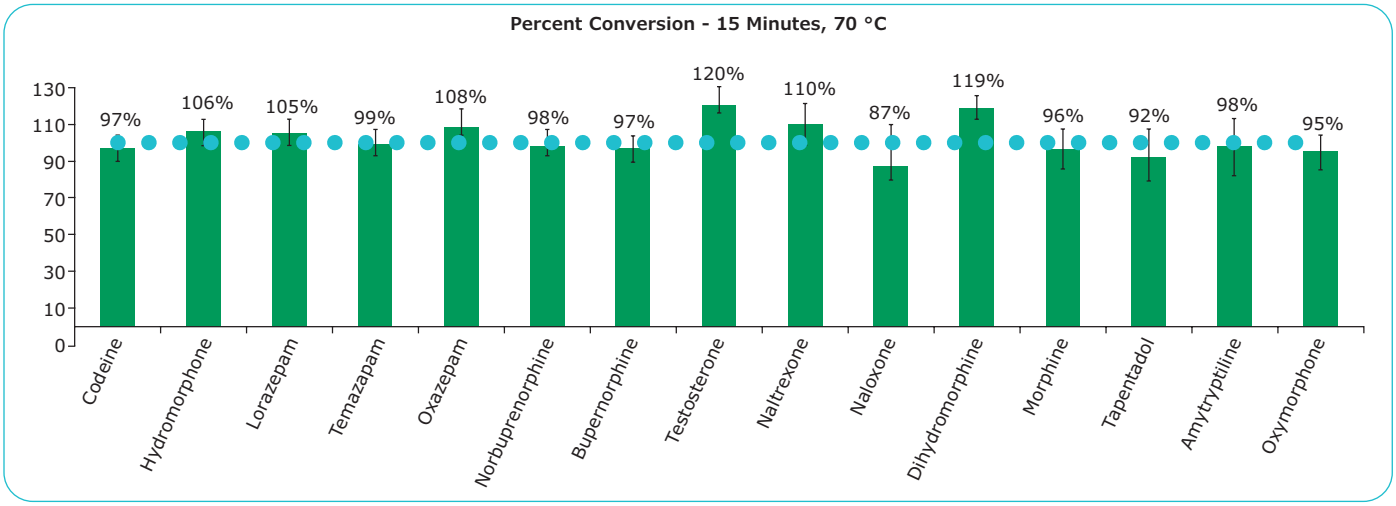


Beta-Glucuronidase from Multiple Sources:

- Limpet
- Abalone
- Purified Abolone
- *E. Coli*
- Helix Pomatia

For more information, visit
SigmaAldrich.com/betagluc

15 minute hydrolysis



Certified Reference Materials

Accurate reference, accurate results
Cerilliant® Certified Reference Materials for your clinical applications

- Our clinical portfolio of reference standards and Certified Reference
- Materials for analytical testing applications includes parents, metabolites, impurities, degradants, endogenous biomarkers & internal standards including stable isotope labeled.
- Our wide portfolio also includes OEM in addition to custom products and services.
- Our reference material manufacturing sites are at a minimum double accredited to the highest achievable quality level for reference material producers:
- ISO 17034 / Guide 34, ISO/IEC 17025

Consistency is our standard.

For more information, visit
SigmaAldrich.com/standards

Extensive Cerilliant product portfolio includes:

- Acylcarnitines
- Alcohol Standards
- Amphetamines
- Analgesics (Non-Opiates)
- Anesthetics
- Anticonvulsants/ Antiepileptics
- Antidepressants
- Antifungals
- Antihistamines
- Antipsychotics
- Barbiturates
- Benzodiazepines
- Benzyl & Phenyl Piperazines
- Bile Acids
- Cannabinoids
- Cardiac Drugs
- Catecholamines
- Cathinones
- Certified Reference Materials in Matrix
- Cocaine Analogs
- Fatty Acids
- Fentanyls
- Hallucinogens
- Immunosuppressants
- Multi-Component Drug Standards and Kits
- Non-benzodiazepines
- NSAIDs
- Opiates
- Other Drugs
- Pharmaceutical Impurities
- Phenethylamines
- Proteins
- Skeletal Muscle Relaxants
- Steroids/Hormones
- Stimulants
- Synthetic Cannabinoids
- Thyroid Hormones
- Vitamins
- Weight-Loss-Drugs

same products new labels

As part of the Supelco® portfolio of analytical products from Merck, we are updating our packaging and making vibrant science a reality by introducing smarter labels and more sustainable materials for all our Cerilliant® Certified Reference Materials (CRMs).

Rest assured, we're not changing the high standards of our comprehensive range of products or services that you've come to expect with Cerilliant® CRMs. The same Cerilliant® quality, just with a new label and packaging.

Why Supelco®? Merck has created the Supelco portfolio of analytical products, built on providing accuracy and reliability, and developed for analytical chemists, by analytical chemists.

The rebranding of our product packaging and labeling is in conjunction with the realignment of our products into our six portfolio brands. To learn about each please visit: **SigmaAldrich.com/Packaging**

Reference Materials you can rely on

Our high standards match yours

Reference materials are particularly important in analytical chemistry to ensure test accuracy. They are essential for validation of methods, accurate calibration of measurement systems, and effective quality control. Research grade working standards are not fully characterized and certified, which is why we recommend reference materials (RMs) and certified reference materials (CRMs) to ensure accuracy and reproducibility. Results are only as accurate as your reference.

Consistency is our standard

Our analytical chemists have developed organic, inorganic, and microorganism reference materials which can be used to calibrate simple measurements like pH, all the way to testing applications involving complex diagnoses such as thyroid cancer. Our reliable Cerilliant®, TraceCERT® and Certipur® product lines deliver high-quality standards and certified reference materials to ensure accuracy and reliable results while supporting each laboratory's requirements.

Compliance, always ensured

Regulatory compliance is a critical component of a reference material. With a meticulous supply-chain compliance and regulatory documentation—all our products are tested to industry-specific protocols to ensure the highest standards.

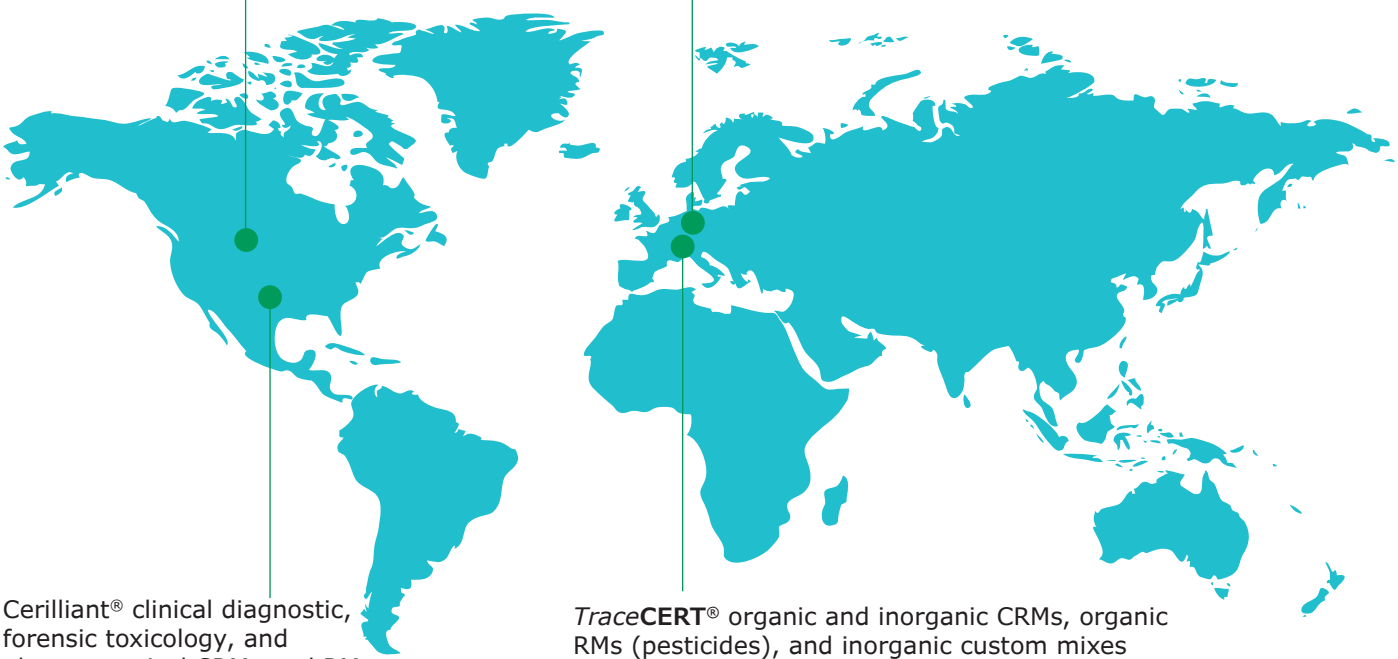
Our systems & processes are inspected and certified by the same regulatory agencies and accreditation bodies that audit your work, including the FDA and the EPA. We develop innovative products in collaboration with national metrology institutes, pharmacopeias and governmental agencies.

Whatever your field of analysis is, we want to help you provide accurate, precise and consistent results. Make sure your decisions are based on accurate and reliable data achieved with Supelco® reference materials and certified reference materials.

Reference Materials Production Sites

Pharmaceutical secondary standards, matrix environmental CRMs and proficiency testing schemes
Laramie, USA

Certipur® inorganic and elemental CRMs
Darmstadt, Germany



Cerilliant® clinical diagnostic, forensic toxicology, and pharmaceutical CRMs and RMs
Round Rock, USA

*Trace***CERT**® organic and inorganic CRMs, organic RMs (pesticides), and inorganic custom mixes
Buchs, Switzerland

ACCURATE RESULTS FOR TESTING LABORATORIES

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Whether you use IVD products in your hospital or reference lab, or create your own Lab Developed Tests (LDTs), cost per test, sensitivity, and the need to analyze novel markers with a high degree of control and transparency are important considerations.

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1 SAMPLE COLLECTION

- Labware (bottles, vials, tubes, lids, bags, syringes)

2 SAMPLE PREPARATION

- Biologicals and enzymes
  - New Recombinant Limpet β-Glucuronidase
- SPE cartridges and 96-well plates (e.g. HybridSPE®, Supel™-Select SPE)
- Amicon® protein centrifugal filters
- Milli-Q® Lab Water Solutions

3 ANALYSIS AND INTERPRETATION

- HPLC accessories
- HPLC & UHPLC columns
- Hardware
- Solvents and Buffers for HPLC and UHPLC
- Milli-Q® Lab Water Solutions
- Cerilliant® Certified Reference Materials (CRMs)

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Solvents for Brighter Analysis

Solvents for accurate, brilliant results – Chromatography is only as reliable as the quality of solvents used.

Expertise

A global network of R&D, Manufacturing, and Quality Assurance combined with centuries of experience gives you the most up-to-date method development and documentation support for solvents & inorganics.

Efficiency – Go Green

Programs like the recycler program allow delivery of solvents in containers dedicated to a single customer – creates consistency and minimizes waste.

HPLC and LC-MS Grade Solvents

Cat. No.	Description
UHPLC-MS Solvents	
900667	Acetonitrile, for UHPLC-MS
900688	Methanol, for UHPLC-MS
900682	Water, for UHPLC-MS
900686	ACN with 0.1% formic acid, for UHPLC-MS
900687	Water with 0.1% formic acid, for UHPLC-MS
632546	Methanol with 0.1% formic acid for UHPLC-MS
LC-MS Solvents	
AX0156	Acetonitrile LC-MS OmniSolv®
MX0486	Methanol LC-MS,OmniSolv®
WX0001	Water LC-MS Grade OmniSolv®
HPLC Plus Solvents for HPLC, GC, and residue analysis	
34998	Acetonitrile
646377	Methanol
34877	Water
650447	2-Propanol
650498	Chloroform contains amylenes as stabilizer
650471	Chloroform contains 0.5-1.0% ethanol as stabilizer
650463	Dichloromethane contains 50-150 ppm amylene as stabilizer
650536	Heptane
650552	Hexane
650420	Hexane, mixture of isomers
650579	Toluene
650439	2,2,4-Trimethylpentane
650528	Ethyl acetate
650560	tert-Butyl methyl ether
650501	Acetone

UHPLC-MS Solvents:

- Highest solvent purity for low detection limits
- Extremely low MS baseline noise
- Exemplary lot-to-lot reproducibility
- Microfiltered (0.1 µm)

LC-MS Solvents:

- Low gradient baseline drift
- Trace metals analysis
- Very low levels of particles

HPLC Plus Solvents:

- Low UV impurity profile for use near a solvent's UV cutoff
- Very low levels of particles and non-volatile impurities
- Low impurity gradient LC baseline

For more information, visit
SigmaAldrich.com/solvents

LiChropur® LC-MS Reagents

It is common practice in LC-MS to add certain chemicals to the mobile phase or introduce them post-column prior to the interface to influence analyte ionization. Most often, an improvement in the analyte signal is the goal. However, some additives may be used to suppress unwanted signals or selectively enhance the signal of particular compounds in a mixture, for example glycosidic species in a mixture of peptides.

We offer a wide range of high purity mobile phase additives for LC-MS applications. Our offer includes the most commonly used acids, bases and volatile salts. All are of high purity and are rigorously tested for LC-MS application suitability, offering many advantages for both small and large molecule analysis.

Impurities, such as alkali ions, plasticizers and surfactants, found in lower-grade reagents are particularly problematic as they interfere strongly with LC-MS, resulting in higher background noise and formation of adducts. Only highly pure reagents allow high signal-to-noise ratios.

Features:

- LC-MS application tested for consistent quality
- Improves ionization and resolution
- Extremely low levels of inorganic and organic impurities
- Manufactured specifically for accurate and fast LC-MS
- Highest quality acids, bases & salts

For more information, visit
SigmaAldrich.com/lcms-reagents

Cat No.	Product Name	Description	Package Size
5.33001.0050	Acetic acid	100% for LC-MS LiChropur®	50 mL
5.33002.0050	Formic acid	98-100% for LC-MS LiChropur®	50 mL
5.33003.0050	Ammonia solution	25% for LC-MS LiChropur®	50 mL
5.33004.0050	Ammonium acetate	for LC-MS LiChropur®	50 mL
5.33005.0050	Ammonium hydrogen carbonate	for LC-MS LiChropur®	50 mL

Milli-Q® Lab Water Solutions

Milli-Q® IQ Series Water Purification System – Ultrapure Water for HPLC and LC-MS

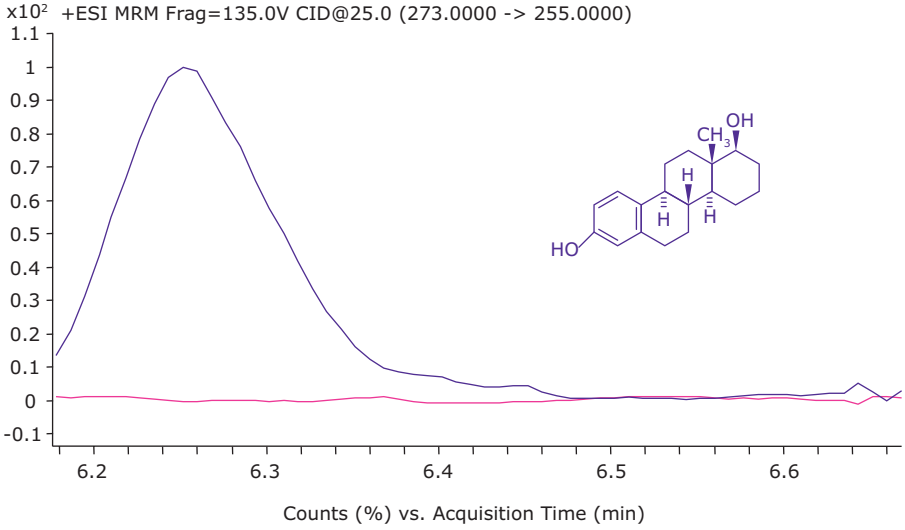
Invest in Lab Efficiency

Our Milli-Q® Lab Water Solutions portfolio offers a broad range of water purification systems designed to make your work in the lab as pleasant and efficient as possible.

Our new Milli-Q® IQ series systems offer:

- **Simple and intuitive dispensing:** Convenient and easy access to purified water with ergonomic Q-POD® ultrapure and E-POD® pure water dispensers.
- **Unparalleled ease of use:** Easy-to-navigate touchscreens on each POD dispenser give you quick access and control of essential system functions in just a few clicks.
- **Superior ultrapure water:** The combination of innovative purification technologies delivers consistently low TOC (Total Oxidizable Carbon) ultrapure water, optimal for HPLC and LC-MS analyses.

To achieve and maintain good chromatographic performance, it is recommended to prepare your mobile phases with freshly produced ultrapure water (low TOC, ≤ 5 ppb). For illustration purposes, Milli-Q® ultrapure water was analyzed for estradiol, a commonly tested hormone. The figure below demonstrates the absence of this hormone in Milli-Q® ultrapure water, which is crucial to reach low detection limits and is important for the unambiguous identification of peaks and for the quantitation of analytes.



Water Source	Milli-Q® Ultrapure Water
HPLC conditions (Agilent 1290 Infinity®)	
Column	Purospher® STAR RP-18e (2 µm) Hibar® HR 50-2.1 mm
Flow rate	0.5 mL/min
Injection volume	40 µL sample, 10 µL standards
Eluent A	1% acetic acid in Milli-Q® ultrapure water
Eluent B	Acetonitrile (LiChrosolv® Hypergrade)
Gradient (Time, min; %B)	0, 0%; 2, 0%; 5, 100%; 6, 100%; 9, 0%; 13, 0%
MS conditions (Agilent® 6420 Triple Quadrupole)	
Ionization mode	ESI+, MRM
Capillary	4000 V
Nebulizer	37 psi
Drying gas	N_2, 7.5 L/min, 300 °C

For more information, visit **SigmaAldrich.com/Milli-Q-IQ7003-05-10-15**

HPLC and UHPLC columns for LC-MS

For LC-MS, a wide range of HPLC columns, preferably based on high purity Type B silica, are widely used. Particulate HPLC columns are typically based on either fully porous particles (FPP) or superficially porous particles (SPP). Small particles deliver higher separation efficiency. They are best suitable for LC-MS of cleaner samples after removal of matrix components. Monolithic silica columns require less sample preparation due to their high matrix-tolerance which can be a significant cost and time saving factor.

Fully porous particulate columns:

- **Purospher® STAR HPLC and UHPLC columns** are available with particle sizes of 2 µm, 3 µm and 5µm in various column modifications providing high efficiency, extended pH stability (pH 10.5) for RP-18e and RP-8e and stability in aqueous mobile phases.
- **Ascentis® and Discovery® HPLC columns** with 3 µm and 5 µm particle sizes and a very broad range of column chemistries providing selectivity for the separation of almost every compound.
- **Titan® UHPLC columns** based on monodisperse particles of 1.9 µm particle size providing very high efficiency due to a more consistent packed bed.
- HILIC is superior for the separation of polar hydrophilic molecules, i.e., many of the endogenous molecules. **SeQuant® ZIC®-HILIC/cHILIC/pHILIC** bonded zwitterionic stationary phases combine perfectly with ESI-MS detection due to the applied solvents and additives. A significant increase in sensitivity in comparison with reversed phase chromatography can be achieved. Strongly retained polar analytes can be removed from HILIC columns by changing to a more polar eluent.

Superficially porous particulate columns for maximum resolution and speed:

Fused-core® columns feature narrower particle size distribution as well and shorter diffusion path compared to fully porous particles. The result is increased resolution, added sensitivity and faster runs.

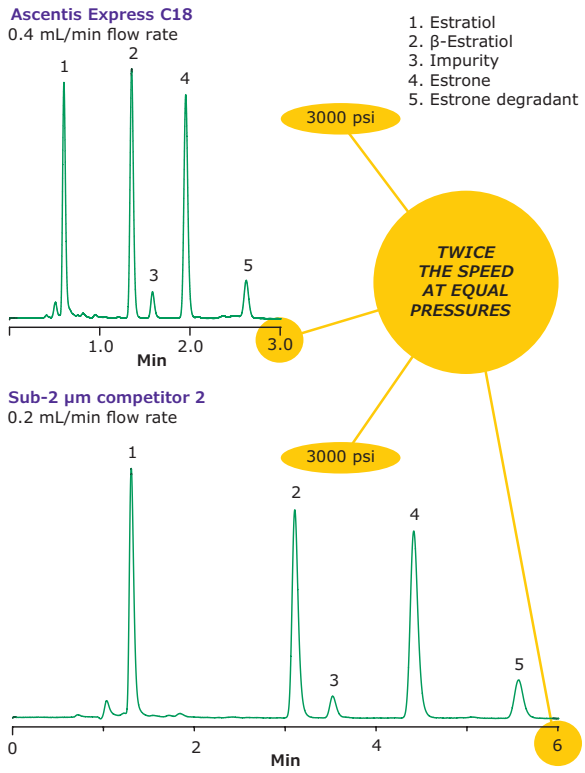
The advantages of Fused-Core® columns are:

- Maximum speed and efficiency on both UHPLC and HPLC systems (particle sizes: 2 µm, 2.7 µm and 5 µm)
- 40% more efficiency in comparison to Fully Porous Particles (FPP) of same particle size
- UHPLC columns with 2 µm particles (pressure **stable 1000** bar)
- Column dimensions from 0.075 mm ID (capillary columns) to 4.6 mm ID (analytical HPLC columns)
- Very broad range of column chemistries

Ascentis® Express HPLC and UHPLC columns provide about 40% more efficiency in comparison to columns with fully porous particles of the same size. This performance enhancement is applicable to all HPLC instruments (in addition to UHPLC systems).

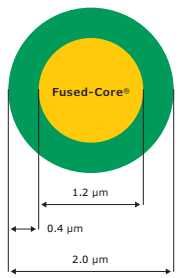
Due of the lower backpressure of the Ascentis® Express 2.7 µm particles in comparison to sub-2µm particles an increased flow rate (double in this case) can be applied providing the same back pressure, separation efficiency and resolution as on a sub-2 µm UHPLC, just with a 50% shorter runtime, increasing sample throughput.

Chromatographic conditions:	
Columns:	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
Mobile phase:	water/acetonitrile 49:51 (for Ascentis® Express); water/acetonitrile 55:45 (for sub-2 µm)
Column temp:	ambient
Detector:	UV, 200 nm
Injection:	1 µL



Particle sizes of 2 µm, 2.7 µm and 5 µm are available with a very broad range of modifications, all excellently suitable for LC-MS use.

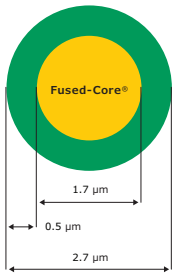
Best Fused-Core UHPLC column



An optimized solution for high throughput small molecule analysis

Pressure stability: 2 µm: 1000 bar

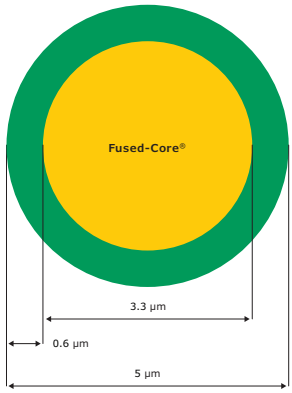
Fast on any System



A practical solution that delivers UHPLC performance from any HPLC

2.7 µm: 600 bar

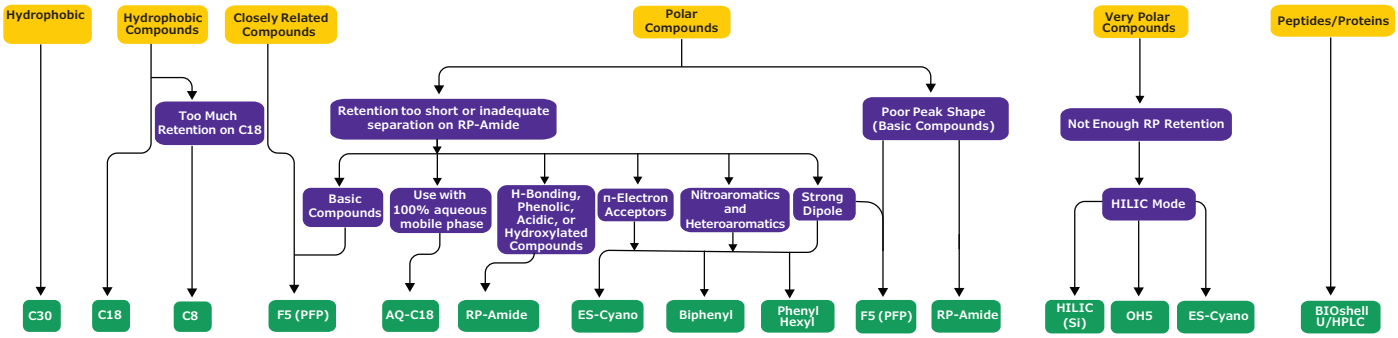
The Lab Work-horse Column



True plug and play solution for improving existing 3 or 5 µm porous particle HPLC columns

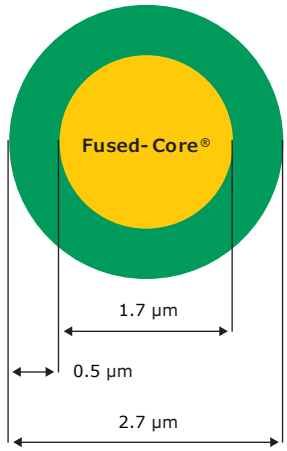
5 µm: 600 bar

Column selectivity has the highest influence on resolution in chromatography. Selection of the best suitable column chemistry for your target analytes is therefore an important selection parameter. C18 column chemistries are typically the first choice. Nevertheless, when a C18 doesn't give the desired separation or the sample contains compounds that are known to be difficult to retain or resolve on a C18, consider changing stationary phase early in method development for more optimal applications. The range of selectivity provided by Ascentis® Express makes this easy. The flowchart below helps to guide users in the selection of an Ascentis® Express phase, based on the particular compound type or separation challenge.



Bonded Phase	Chemistry	USP Designation	Chromatographic Properties / Use	Particle Size (s) (µm)	Pore Size (Å)
C30	Triacontyldimethyl	L62	Excellent selectivity for very hydrophobic compounds, long-chain and structurally related isomers	2.7	160
C18	Dimethyloctadecyl	L1	Outstanding performance for a broad range of analytes	2, 2.7, 5	90
Peptide ES-C18	Diisobutyloctadecyl	L1	Fast separation of peptides and polypeptides with high peak capacity	2.7	160
AQ-C18	Polar modified Octadecyl	L1	Resistant to dewetting; compatible to 100% aqueous mobile phase	2, 2.7, 5	90
C8	Dimethyloctyl	L7	Enhanced retention for less hydrophobic compounds or faster separation if retention on C18 is too long	2, 2.7, 5	90
RP-Amide	C16-Amide	L60	Complementary selectivity to alkyl phases	2, 2.7, 5	90
Phenyl-Hexyl	Dimethylphenyl-hexyl	L11	Enhanced selectivity for aromatic compounds; strong pi-pi donor	2, 2.7, 5	90 and 160
Biphenyl	Dimethylbiphenyl	L11	Enhanced selectivity for aromatic compounds	2, 2.7, 5	90
F5 (PFP)	Pentafluorophenylpropyl	L43	Outstanding selectivity for stereoisomers, strong pi-pi acceptor	2, 2.7, 5	90
ES-Cyano	Diisopropylcyanopropyl	L10	Enhanced retention for polar compounds and much less retention for hydrophobic compounds	2, 2.7, 5	90
OH5	Penta-hydroxy	L95	Ideal for the HILIC separation of very polar compounds with a LogP value close to 0 or less than 0	2, 2.7, 5	90
HILIC	Bare silica	L3	Enhanced separation of polar compounds; can be used in HILIC and normal-phase mode.	2, 2.7, 5	90

BIOshell™ IgG 1000 Å U/HPLC Columns: Maximizing Pore Diameter to Minimize Size Exclusion

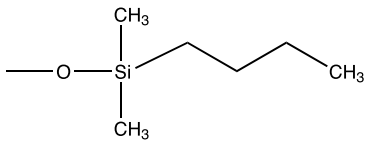


- A 1000 Å pore diameter allows unrestricted access of large biomolecules into the particles.
- Superficially porous particles (SPPs) provide narrower peak widths and improved resolution for characterization of biomolecules in comparison to fully porous particles (FPPs).
- Post-translational modifications (PTMs) of expressed proteins can lead to subtle differences in molecular structure and function of the protein. These minor variants can be resolved with BIOshell™ IgG 1000 Å columns.

Applications	Features
• mAbs • ADCs • Biosimilars • H/D Exchange • mAb Fragments	• Temperature stable up to 90 °C • Compatible with UHPLC, HPLC, and MS • Resolution of very large proteins with superior peak shape and efficiency as compared to separations on FPP-packed columns • Minimal to no LC-MS bleed

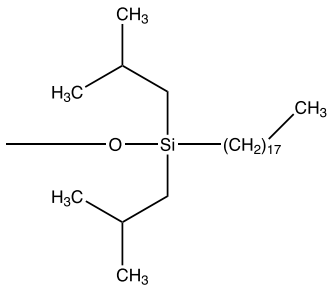
BIOshell™ IgG 1000 Å Phase Chemistry Portfolio

BIOshell™ IgG 1000 Å C4



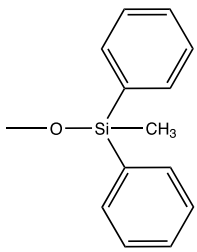
Ligand: Dimethylbutylsilane
USP Designation: L26
Available Particle Sizes: 2.7 µm
Pore Size: 1000 Å

BIOshell™ IgG 1000 Å C18



Ligand: Diisobutyloctadecylsilane
USP Designation: L1
Available Particle Sizes: 2.7 µm
Pore Size: 1000 Å

BIOshell™ IgG 1000 Å Diphenyl



Ligand: Diphenylmethylsilane
USP Designation: L11
Available Particle Sizes: 2.7 µm
Pore Size: 1000 Å

Monolithic silica columns have high matrix tolerance

The analysis of samples with high matrix load requires tedious and time-consuming sample preparation steps. For cost-effective investigations, sample handling has to be kept as short as possible and combined with robust LC columns displaying a high matrix tolerance and long lifetime.

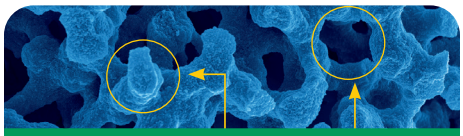
Chromolith® monolithic silica columns

The 50-2 mm monolithic silica column is well-suited for fast gradient run liquid chromatography, and the applied low flow rates make it the perfect choice for MS detection. Analysis of matrix-rich samples, such as food or tissue, can be performed on this robust column type without the need for a guard column or tedious and complex sample preparation procedures.

The advantages of Chromolith® monolithic silica columns are:

- Exceptional robustness or lifetime—described as number of injections—enabling cost savings.
- High matrix tolerance decreases tedious sample preparation steps, speeds up all processes and allows for fast and simple HPLC analyses.
- Very low column back-pressure, fast analytical speed and high reproducibility on standard HPLC systems as well as using UHPLC instruments.

Thanks to their patented monolithic silica technology, Chromolith® HPLC columns allow rapid separations with maximum robustness and selectivity—at minimal back pressure. The revolutionary bimodal pore structure of Chromolith® columns provides a unique combination of macropores and mesopores.



Average pore size	Mesopores	Macropores
Chromolith® Performance	13 nm (130 Å)	2 µm
Chromolith® 2 mm ID	13 nm (130 Å)	1.5 µm
Chromolith® HR	15 nm (150 Å)	1.15 µm

Ultra-high performance and extremely low operating pressure make Chromolith® 2 mm columns truly unique. Excellent, ultra-fast results are obtained, not only in the new UHPLC and UPLC® instruments, but equally well in all standard HPLC systems with low dead volume. Chromolith® 2 mm columns have macropores of 1.5 µm in diameter, resulting in a column efficiency that exceeds 100,000 plates/meter. The mesopores are 13 nm (130 Å) in diameter, and the surface modification is octadecylsilane with full endcapping.

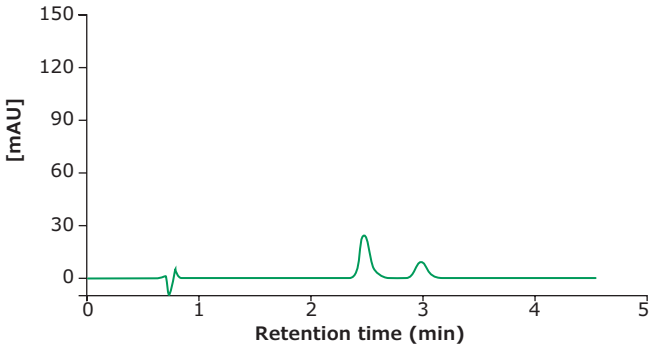
Increase sensitivity and save solvents with 2 mm i.d. Chromolith® RP-18 endcapped columns

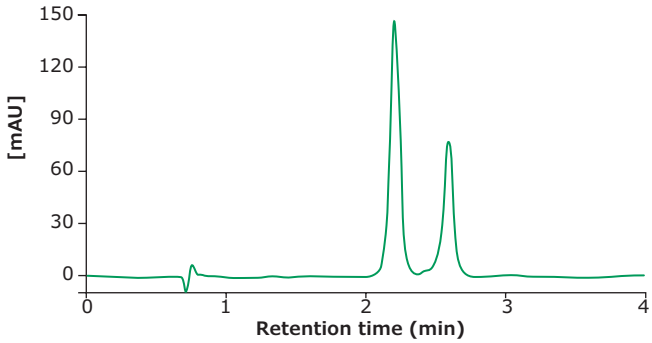
Chromolith® Performance RP-18e 100-4.6 mm

Column	Chromolith® Performance RP-18 endcapped 100-4.6 mm
Mobile phase	A: 100 % Acetonitrile B: 100 % Water + 0.1 % TFA (v/v) C: 100 % Methanol
Isocratic	Initial composition: A/B/C 30/60/10 (v/v/v)
Flow rate	2 mL/min
Pressure	45 bar (4.5 MPa, 65.3 psi)
Detection	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell	11 µL
Temperature	ambient
Injection volume	1 µL
Sample	Bimatoprost Bimatoprost free acid

Chromolith® Performance RP-18e 100-2 mm

Column	Chromolith® Performance RP-18 endcapped 100-2 mm
Mobile phase	A: 100 % Acetonitrile B: 100 % Water + 0.05 % TFA (v/v) C: 100 % Methanol
Isocratic	Initial composition: A/B/C 30/60/10 (v/v/v)
Flow rate	380 µL/min
Pressure	48 bar (4.8 MPa, 70 psi)
Detection	Dionex Ultimate 3000 VWD-3400, 2.5 Hz, Response time 0.1 s, UV = 210 nm
Vol. detector cell	1.4 µL
Temperature	ambient
Injection volume	1 µL
Sample	Bimatoprost Bimatoprost free acid





The same separation on a Chromolith® 2 mm i.d. column demonstrates improved sensitivity and solvent savings of 81 %.

Chromolith® WP 300 monolithic silica columns for Biomolecule separation

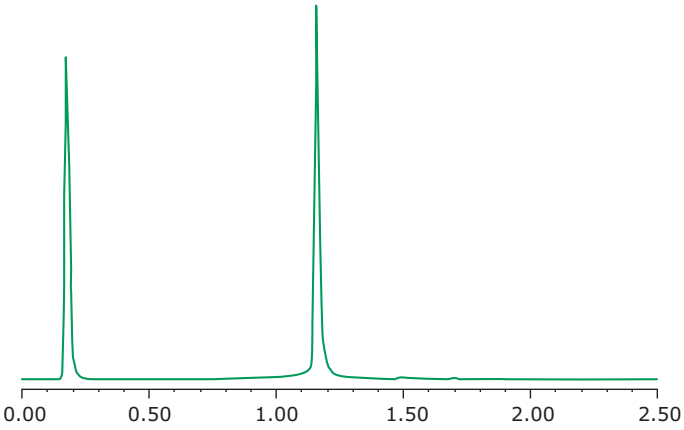
Chromolith® columns have shown great potential and superiority in comparison to standard silica. In contrast to conventional packed-particle columns, wide pore (300 Å) monolithic silica columns are made of a single continuous bed of high purity porous silica that is then bonded with C18, C8, C4 and Protein A depending on the use of the column.

Chromolith® WP 300 Protein A – Fast monoclonal antibody quantitation

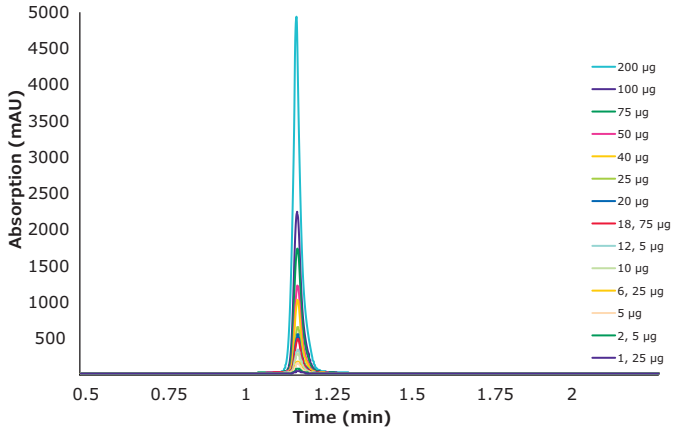
Affinity chromatography is a most selective technique which takes advantage of very specific molecular interactions, for example antigen and antibody. The Chromolith® WP 300 Protein A HPLC column is designed to monitor monoclonal antibody titer and yield determination from cell-culture supernatants. Analytical scale procedure helps to optimize the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody products. Chromolith® WP 300 Protein A column could be used for separation of all IgGs (except class 3). Columns provide extremely fast separations and could be used longer; minimizing analysis costs.

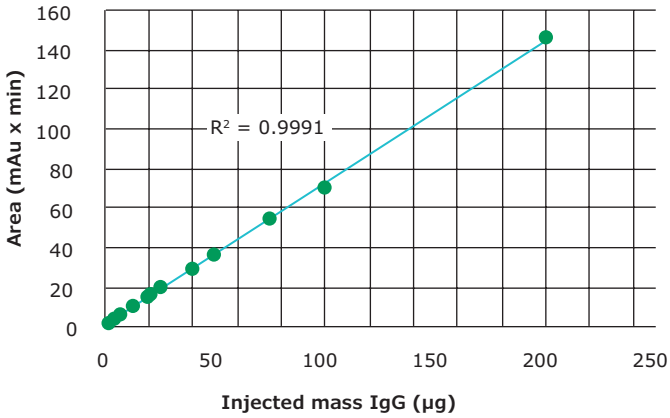
Separation of monoclonal antibodies

Eluent A	100mM sodium phosphate pH7.4		
Eluent B	100mM sodium phosphate pH2.5		
Flow rate	2.0 mL/min		
Detection	280 nm		
Temperature	25°C		
Injection volume	10µl		
Gradient	Time	%A	%B
	0.00	100	0
	0.25	100	0
	0.26	0	100
	1.25	0	100
	1.26	100	0
	2.50	100	0



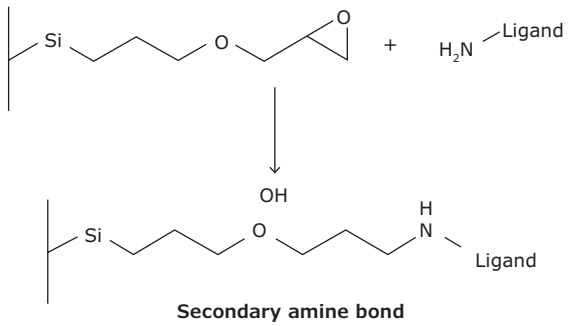
Linearity





Chromolith® WP 300 Epoxy - designed for the user-specific immobilization

Chromolith® WP 300 Epoxy columns are specially designed for the user-specific immobilization of ligands and their later application in HPLC. The unique bimodal pore structure of silica monoliths allows efficient coupling independent of molecule size. The wider mesopores also enable the use of proteins and antibodies as both ligand immobilized on the column, and later analyte separated by an immobilized column. Potential applications: attach Trypsin to obtain HPLC column-protein digestion reactor; attach protein and measure other protein interaction with the attached one; attach any chiral selector to obtain a chiral column attach any affinity ligand to obtain custom made affinity column etc.



Secondary amine bond

The epoxy ring system enables a nucleophilic attack through a ring opening process leading to a covalent bond between the nucleophilic functional group and the primary carbon atom. At the adjacent carbon atom, a hydroxyl group is formed. Epoxides can react with carboxyl, thiol, amine and hydroxyl groups depending on the pH of the medium.

Note

Note

Supelco®

Analytical Products

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

SigmaAldrich.com/LCMS

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