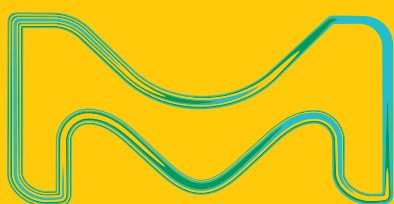


# LC-MS Resource Guide

A Comprehensive Portfolio for Consistent Results in Routine and Advanced LC-MS applications



The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

**Supelco®**  
Analytical Products

# Maximize the Performance of Your LC-MS Using Proven Consumables

In this guide, we provide a premier selection of proven Supelco® tools and consumables that meet the requirements of scientists who primarily use the LC-MS technique for analysis of drugs, biomolecules separation and analytical assays.

- Ascentis® Express Fused-Core® HPLC/ UHPLC Columns improve throughput and sensitivity, process more samples and get drugs to market faster
- BIOshell columns with Fused-Core® technology for faster peptide, protein, glycan, ADC and MAb Separations
- Chromolith® HPLC columns with revolutionary monolithic silica technology for fast separation at low backpressure and long column life time even for Matrix-rich samples
- Purospher™ STAR columns with outstanding batch-to-batch reproducibility and extended pH stability
- Astec® CHIROBIOTIC® chiral stationary phases for enantiomer separations under RP and LC-MS conditions
- HybridSPE® Phospholipid Cartridges and 96-well Plates remove or enrich phospholipids from plasma, serum and tissue samples and improve LC-MS sensitivity
- Supel™-Select SPE Tubes and Well Plates for sample prep needs
- SeQuant® ZIC-HILIC™ technique for separation of polar and hydrophilic compounds
- Smplicity® G2 filtration system for HPLC samples
- Clean, low background LiChrosolv® solvents and LiChropur® mobile phase additives specifically designed for UHPLC and LC-MS analysis
- Standards & Certified Reference Materials for Accurate LC-MS Analyses
- LC-MS Accessories that maximize performance
- Key applications for Bio-Analytical, Pharma, Food, Beverage, and Environmental industries

Discover the LC-MS workflow solutions at  
[SigmaAldrich.com/lcms](https://SigmaAldrich.com/lcms)



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# LC-MS Columns

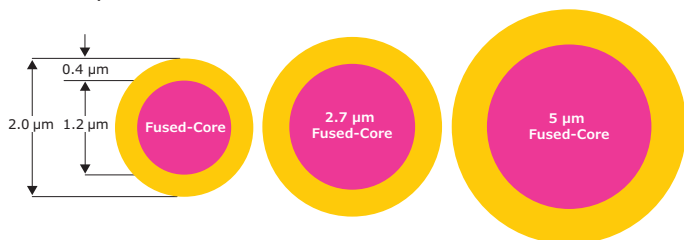
## Fast Ascentis® Express Fused-Core® Columns

### Key Features and Benefits

- Novel Fused-Core® technology (Superficially Porous Particles; SPP)
- Maximum speed and efficiency on both UHPLC and HPLC systems (2  $\mu\text{m}$ , 2.7  $\mu\text{m}$  and 5  $\mu\text{m}$ ).
- 40% more efficiency in comparison to Fully Porous Particles; FPP of same particle size
- UHPLC columns 2  $\mu\text{m}$  (pressure stable 1000 bar)
- Extremely broad range of Column chemistries

### Fused-Core® Technology for HPLC and UHPLC

Ascentis® Express columns provide a breakthrough in HPLC column performance. Based on Fused-Core® particle technology, Ascentis® Express columns provide the benefits of high speed and high efficiency of sub-2  $\mu\text{m}$  particles and at a backpressure low enough for use in traditional HPLC systems. The Fused-Core® particle consists of a solid silica core and a porous silica shell allowing for a shorter diffusion path compared to conventional fully porous particles. Compared to totally porous sub-2  $\mu\text{m}$  particles typically used in UHPLC, Ascentis® Express Fused-Core® 2.7  $\mu\text{m}$  particles generate approximately half the backpressure without loss of resolution. This permits both longer columns, for more resolving power, and faster flow rates, for higher throughput. Demonstrating this point, the separation shows a steroid mixture on Ascentis® Express (top) and a sub-2  $\mu\text{m}$  UHPLC column (lower) of the same dimensions. Because higher flow rates on Ascentis® Express—double in this example—generate similar backpressure, hyper fast separations are possible that have efficiency and resolution equal to the sub-2  $\mu\text{m}$  UHPLC particle column.



#### Best Fused-Core® UHPLC Column

An optimized solution for high-throughput small molecule analysis

#### Fast HPLC on ANY System

A practical solution that delivers UHPLC performance from any HPLC

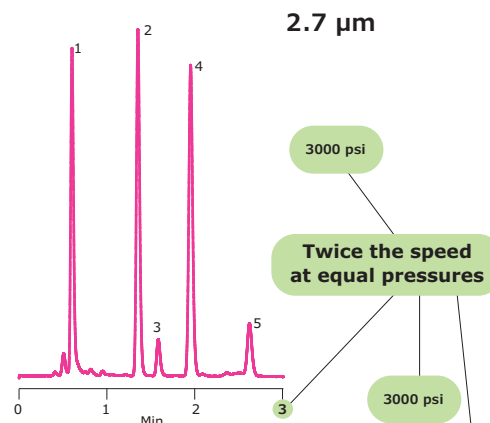
#### The Lab Work-Horse Column

True plug and play solution for improving existing 3 or 5  $\mu\text{m}$  porous particle HPLC columns

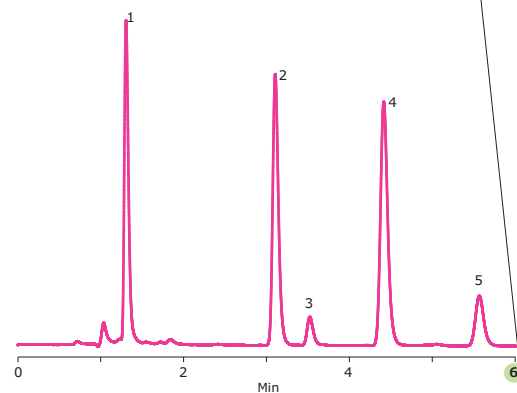
### Hyper-Fast Separations on Ascentis® Express Columns: Twice the Speed at Equivalent Pressure vs. Sub-2 $\mu\text{m}$

Columns	Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 $\mu\text{m}$ particles (53823-U) and sub-2 $\mu\text{m}$ particle column (same dimensions)
Mobile phase	49:51 or 55:45, water:acetonitrile
Flow rate	0.4 or 0.2 mL/min
Column temp	ambient
Detector	UV, 200 nm
Injection	1 $\mu\text{L}$

#### Ascentis® Express C18 0.4 mL/min flow rate



#### C18 Sub-2 $\mu\text{m}$ 0.2 mL/min flow rate



1. Estriol (Cerilliant® E-074)
2. 17 $\beta$ -Estradiol (Cerilliant® E-061)
3. Unknown

4. Estrone (Cerilliant® E-075)
5. Estrone degradant



# Guide to Selecting Ascentis® Express LC-MS Columns

## Start with C18

Since their introduction in 2007, Ascentis® Express columns have solved many separation problems by providing column efficiencies that were previously only possible with much smaller particles at much higher column backpressure. However, as was true with other technological breakthroughs in HPLC, not all separation problems can be solved with the same C18 column, not even an Ascentis® Express C18 column. Still, the Ascentis® Express C18 column is the first choice when starting a new LC-MS method.

## The General Elution Problem

A typical challenge when separating two or more compounds occurs when at least one of the compounds is not sufficiently retained under conditions where the other compound(s) elute from the column within the desired time window. This is known as the “general elution problem”, which can often be resolved by running a gradient from a weak starting solvent to a stronger final solvent. If this strategy is not successful, it may be time to select another Ascentis® Express stationary phase, one that will cause polar compounds to be retained and hydrophobic compounds to elute earlier.

## When Bands Overlap

A related challenge arises when the compounds are retained within the desired time window, but two or more compounds elute in the same position. The solution to this problem may be as simple as changing the mobile phase composition, be that the type of organic solvent, the buffer or buffer composition, the pH, a mobile phase additive, operating temperature, or, less likely, a change in flow rate. Generally, changing the type of stationary phase is the most expedient way to change selectivity (and retention) to separate co-eluting compounds.

## When C18 is Not Good Enough

The decision tree shown on the following page helps guide you in the selection of an alternative Ascentis® Express phase based on the particular compound type or separation challenge. All options displayed are relative to the C18 column that started your separation journey. We provide a wide selection of stationary phase options.

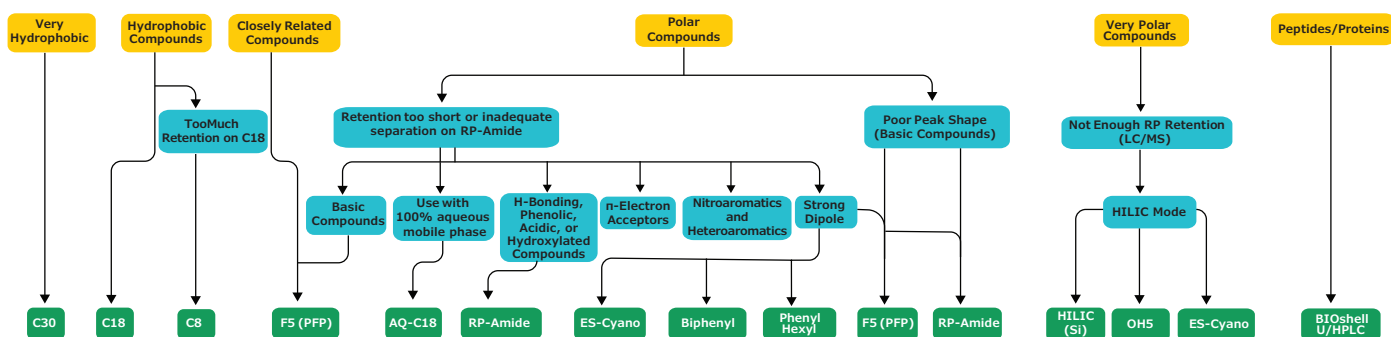
For more information on Ascentis® Express Applications, see page 51.

## Ascentis® Express UHPLC and HPLC Columns

Designed to deliver speed and resolution on all UHPLC and HPLC systems, Ascentis® Express with Fused-Core® technologies exceeds the benefits of fully porous sub-2, 3 and 5 µm particles. Ascentis® Express 2.7 µm delivers more resolving power per unit pressure than even sub-2 µm particles on any HPLC system (including UHPLC). Ascentis® Express 5 µm columns are able to achieve greater speed and efficiency than

any other 5 µm particle-based column. This means that Ascentis® Express 5 µm can be the standard column for all of your fully porous 5 µm-based methods. With the addition of 2.0 µm Ascentis® Express UHPLC columns, we now offer three U/HPLC Fused-Core® particle columns, making the Ascentis® Express column line truly scalable from UHPLC to legacy HPLC systems.

## Guide to Selecting Ascentis® Express HPLC/UHPLC Columns



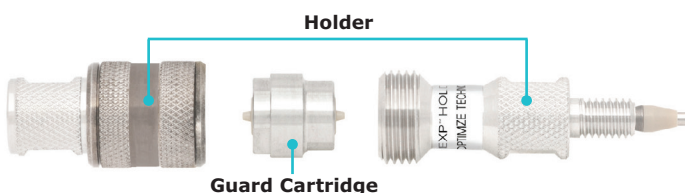
## Ascentis® Express Columns for LC-MS

Particle Size (µm)	I.D. (mm)	Length (cm)	C30	Phenyl-Hexyl (160Å)	AQ-C18	C18	C8	Biphenyl	RP-Amide	Phenyl Hexyl	HILIC (Si)	F5	ES-Cyano	OH5
Ascentis® Express Columns														
2.0	2.1	2				50805-U	51652-U		51567-U	51600-U	51403-U	50857-U	51709-U	50951-U
2.0	2.1	3				50809-U	51654-U		51568-U	51601-U	51404-U	50858-U	51712-U	50952-U
2.0	2.1	5			565349-U			584600-U						
2.0	2.1	7.5				50812-U	51657-U		51571-U	51605-U	51408-U	50861-U	51721-U	50958-U
2.0	2.1	10			565351-U			584601-U						
2.0	2.1	15			565362-U			584602-U						
2.0	2.1	25			565363-U			584603-U						
2.0	3.0	3			565364-U			584604-U						
2.0	3.0	5			565365-U			584605-U						
2.0	3.0	7.5				50817-U	51672-U		51587-U	51616-U	51424-U	50876-U	51729-U	50965-U
2.0	3.0	10			565365-U	50819-U	51673-U		51588-U	51617-U	51428-U	50879-U	51732-U	50967-U
2.0	3.0	15			577160-U			584606-U						
2.7	2.1	2			577320-U	53799-U	53795-U		53797-U	53798-U	—	53592-U	53494-U	53779-U
2.7	2.1	3			577321-U	53802-U	53839-U		53910-U	53332-U	53933-U	53566-U	53468-U	53748-U
2.7	2.1	5	577100-U	584609-U	577322-U	53822-U	53831-U		53911-U	53334-U	53934-U	53567-U	53470-U	53749-U
2.7	2.1	7.5			577323-U	53804-U	53843-U		53912-U	53335-U	53938-U	53568-U	53472-U	53755-U
2.7	2.1	10	577101-U	584610-U	577324-U	53823-U	53832-U		53913-U	53336-U	53939-U	53569-U	53473-U	53757-U
2.7	2.1	15	577102-U	584611-U	577325-U	53825-U	53834-U		53914-U	53338-U	53946-U	53571-U	53475-U	53764-U

Particle Size (µm)	I.D. (mm)	Length (cm)	C30	Phenyl-Hexyl (160Å)	AQ-C18	C18	C8	Biphenyl	RP-Amide	Phenyl Hexyl	HILIC (Si)	F5	ES-Cyano	OH5
2.7	2.1	25	577103-U	584612-U										
2.7	3.0	3	577104-U	584613-U	577327-U	53805-U	53844-U		53915-U	53341-U	53964-U	53574-U	53476-U	53766-U
2.7	3.0	5	577105-U	584614-U	577328-U	53811-U	53848-U		53916-U	53342-U	53967-U	53576-U	53478-U	53767-U
2.7	3.0	7.5			577329-U	53812-U	53849-U		53917-U	53343-U	53969-U	53577-U	53479-U	53768-U
2.7	3.0	10	577106-U	584615-U	577330-U	53814-U	53852-U		53918-U	53345-U	53970-U	53578-U	53481-U	53769-U
2.7	3.0	15	577107-U	584616-U	577331-U	53816-U	53853-U		53919-U	53346-U	53972-U	53579-U	53483-U	53771-U
2.7	4.6	2			577332-U									
2.7	4.6	3	577108-U	584617-U	577333-U									
2.7	4.6	5	577134-U	584618-U	577334-U									
2.7	4.6	7.5			577335-U									
2.7	4.6	10	577135-U	584619-U	577336-U									
2.7	4.6	15	577136-U	584620-U	577337-U									
5.0	2.1	2			581363-U	50507-U	50362-U		50732-U	50442-U	50255-U	50603-U	50557-U	50313-U
5.0	2.1	3			582702-U	50508-U	50363-U		50733-U	50443-U	50256-U	50604-U	50558-U	50314-U
5.0	2.1	5			584572-U	50509-U	50364-U	584585-U	50734-U	50446-U	50257-U	50605-U	50559-U	50317-U
5.0	2.1	7.5			584573-U	50511-U	50367-U		50735-U	50451-U	50258-U	50607-U	50562-U	50321-U
5.0	2.1	10			584574-U	50517-U	50368-U	584586-U	50737-U	50454-U	50260-U	50612-U	50563-U	50322-U
5.0	2.1	15			584575-U	50518-U	50372-U	584587-U	50739-U	50455-U	50261-U	50613-U	50564-U	50327-U
5.0	2.1	25			584576-U	50521-U	50373-U	584588-U	50747-U	50456-U	50262-U	50614-U	50566-U	50328-U
5.0	3.0	3			584577-U	50522-U	50376-U	584590-U	50749-U	50459-U	50264-U	50615-U	50567-U	50329-U
5.0	3.0	5			584578-U	50523-U	50377-U	584589-U	50751-U	50464-U	50265-U	50616-U	50568-U	50335-U
5.0	3.0	7.5				50525-U	50378-U		50752-U	50466-U	50268-U	50619-U	50569-U	50336-U
5.0	3.0	10			584579-U	50526-U	50381-U	584591-U	50753-U	50469-U	50269-U	50622-U	50570-U	50338-U
5.0	3.0	15			584580-U	50527-U	50382-U	584592-U	50758-U	50470-U	50270-U	50623-U	50574-U	50339-U
5.0	3.0	25			584581-U	50528-U	50385-U		50759-U	50472-U	50276-U	50624-U	50575-U	50341-U
5.0	4.6	5			584578-U			584593-U						
5.0	4.6	3			584579-U			584594-U						
5.0	4.6	10			584580-U			584595-U						
5.0	4.6	15			584581-U			584596-U						
Ascentis® Express Guard Cartridges, Package of 3														
2.0	2.1	0.5			577161-U	50822-U	51676-U	584607-U	51594-U	51619-U	51430-U	50884-U	51736-U	50969-U
2.0	3.0	0.5			577162-U	50823-U	51679-U	584608-U	51595-U	51623-U	51433-U	50886-U	51739-U	50973-U
2.7	2.1	—		584621-U	577338-U	53501-U	53509-U		53514-U	53524-U	53520-U	53594-U	53495-U	53780-U
2.7	3.0	—		584622-U	577339-U	53504-U	53511-U		53516-U	53526-U	53521-U	53597-U	53496-U	53781-U
2.7	4.6	0.5	577139-U	584623-U	577340-U									
5.0	2.1	—			584582-U	50539-U	50395-U	584597-U	50776-U	50496-U	50295-U	50633-U	50592-U	50349-U
5.0	3.0	—			584583-U	50541-U	50396-U	584598-U	50777-U	50497-U	50297-U	50634-U	50593-U	50350-U
5.0	4.6	0.5			584584-U			584599-U						
Ascentis® Express Capillary Columns														
2.7	75	5				53982-U	53983-U		—	—	—	—	—	—
2.7	75	15				54219-U	54229-U		—	—	—	—	—	—
2.7	100	5				53985-U	53987-U		—	—	—	—	—	—
2.7	100	15				54256-U	54260-U		—	—	—	—	—	—
2.7	200	5				53989-U	53991-U		—	—	—	—	—	—

## Guard Cartridge Holder

Description	Pkg. Size	Cat. No.
Universal Guard Holder		
Holder w/EXP®Titanium Hybrid Ferrule (cartridge not included)	1	53500-U





## BIOshell™ Fused-Core® Columns for Faster Peptide, Protein, Glycan, ADC, and MAb Separations

BIOshell™ HPLC and UHPLC columns deliver maximum speed and efficiency for the separation of biomolecules on both HPLC and UHPLC systems. The novel, superficially porous silica particles (SPP), with pore sizes from 90 Å up to 1000 Å, allow the separation of glycans as well as of very large proteins. In particular,

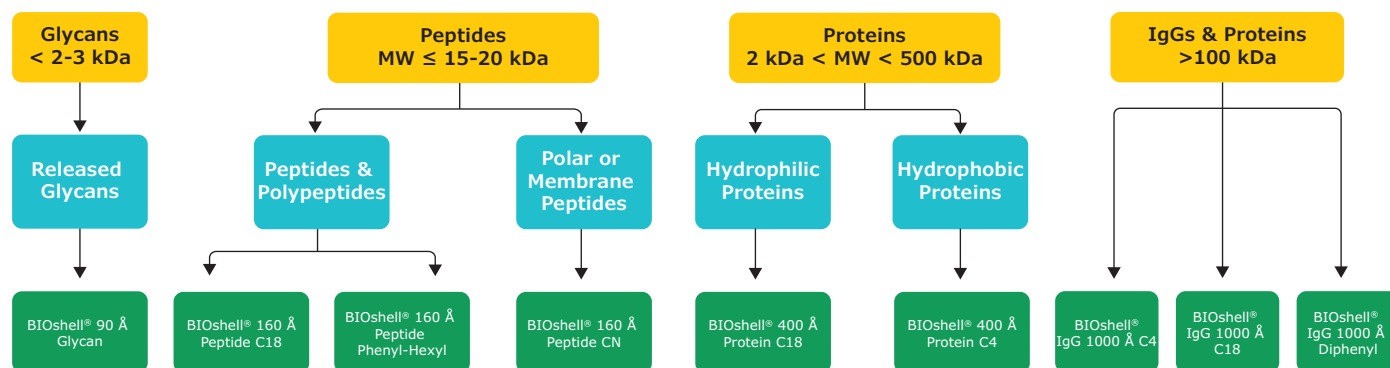
- The efficiency of core-shell particles is about 40% higher than that of fully porous particles of the same size.
- Dialkyl silane reagents provide extra bonded phase stability.
- High operating temperatures increase throughput and improve peak shape and efficiency of strongly hydrophobic peptides and proteins.

a pore size of 1000 Å shows clear advantages over common 300 Å pores for the separation of very large proteins in biotherapeutic drug development such as monoclonal antibodies (mAbs) or proteins with molecular weights greater than 100 kDa.

- Each column type was tested up to 600 bar pressure to allow operation at high flow rate.
- Narrow particle size distributions allow the use of 2 micron porosity frits, even for 2.7 micron particles\*.
- BIOshell™ Fused-Core® columns are rugged, robust and reliable.

\*While the use of 2 micron porosity frits means that BIOshell™ Fused-Core® columns do not plug easily, the user is still advised to clean samples before injection with a 0.2 micron syringe filter.

### Guide to Selecting BIOshell™ UHPLC and HPLC Column Phases



## BIOshell™ Fused-Core® Columns

	Pore Size (Å)	Particle Size (µm)	I.D. (mm)	L (cm)	C4	C18	CN	Diphenyl	Phenyl-Hexyl	Glycan
Use	BIOshell™ Fused-Core HPLC Columns									
mAb	1000	2.7	2.1	5		581362-U		577419-U		
mAb	1000	2.7	2.1	10		582701-U		577420-U		
mAb	1000	2.7	2.1	15		582703-U		577421-U		
mAb	1000	2.7	2.1	25		582704-U		577422-U		
mAb	1000	2.7	3	3		582705-U		577423-U		
mAb	1000	2.7	3	5		582706-U		577424-U		
mAb	1000	2.7	3	10		582707-U		577425-U		
mAb	1000	2.7	3	15		582708-U		577426-U		
mAb	1000	2.7	4.6	3		582709-U		577427-U		
mAb	1000	2.7	4.6	5		582710-U		577428-U		
mAb	1000	2.7	4.6	10		582713-U		577429-U		
mAb	1000	2.7	4.6	15		581348-U		577430-U		
mAb	1000	3.4	2.1	5	63283-U					
mAb	1000	3.4	2.1	10	63288-U					
mAb	1000	3.4	2.1	15	63289-U					
mAb	1000	3.4	4.6	3	63325-U					
mAb	1000	3.4	4.6	5	63328-U					
mAb	1000	3.4	4.6	10	63329-U					
Proteins	400	3.4	2.1	5	66824-U	67459-U				
Proteins	400	3.4	2.1	10	66825-U	67463-U				
Proteins	400	3.4	2.1	15	66826-U	67469-U				
Proteins	400	3.4	4.6	5	66827-U	67473-U				
Proteins	400	3.4	4.6	10	66828-U	67475-U				
Proteins	400	3.4	4.6	15	66829-U	67477-U				
Proteins	160	2.7	2.1	5		66902-U	66966-U			
Proteins	160	2.7	2.1	10		66904-U	66968-U			
Proteins	160	2.7	2.1	15		66905-U	66969-U			
Proteins	160	2.7	4.6	5		66913-U	66974-U			
Proteins	160	2.7	4.6	10		66915-U	66975-U			
Proteins	160	2.7	4.6	15		66917-U	66976-U			
Proteins	160	5	2.1	5		67002-U	67062-U			
Proteins	160	5	2.1	10		67004-U	67064-U			
Proteins	160	5	2.1	15		67006-U	67065-U			
Proteins	160	5	4.6	5		67013-U	67071-U			
Proteins	160	5	4.6	10		67014-U	67080-U			
Proteins	160	5	4.6	15		67015-U	67081-U			
Peptide	160	2.7	2.1	2					577523-U	
Peptide	160	2.7	2.1	3					577524-U	
Peptide	160	2.7	2.1	5					577525-U	
Peptide	160	2.7	2.1	7.5					577526-U	
Peptide	160	2.7	2.1	10					577527-U	
Peptide	160	2.7	2.1	15					577528-U	
Peptide	160	2.7	3	2					577529-U	
Peptide	160	2.7	3	3					577530-U	
Peptide	160	2.7	3	5					577531-U	
Peptide	160	2.7	3	7.5					577532-U	
Peptide	160	2.7	3	10					577533-U	
Peptide	160	2.7	3	15					577534-U	
Peptide	160	2.7	4.6	2					577535-U	
Peptide	160	2.7	4.6	3					577536-U	
Peptide	160	2.7	4.6	5					577537-U	
Peptide	160	2.7	4.6	7.5					577538-U	
Peptide	160	2.7	4.6	10					577539-U	
Peptide	160	2.7	4.6	15					577540-U	
Glycans	90	2.7	2.1	5						50991-U
Glycans	90	2.7	2.1	10						50993-U
Glycans	90	2.7	2.1	15						50994-U
Glycans	90	2.7	4.6	5						50997-U
Glycans	90	2.7	4.6	10						50998-U
Glycans	90	2.7	4.6	15						50999-U
Use	BIOshell™ Fused-Core® Peptide and Protein Capillary Columns									
Proteins	400	3.4	75	15	67032-U	67490-U	—			
Proteins	400	3.4	100	15	67034-U	67493-U	—			
Proteins	400	3.4	200	15	67037-U	67495-U	—			
Proteins	400	3.4	300	15	67039-U	67497-U	—			

	Pore Size (Å)	Particle Size (µm)	I.D. (mm)	L (cm)	C4	C18	CN	Diphenyl	Phenyl-Hexyl	Glycan
Proteins	400	3.4	500	15	67041-U	67502-U	—			
Proteins	400	3.4	1.0	15	67045-U	67504-U	—			
Peptides	160	2.7	75	15	—	67086-U	67152-U			
Peptides	160	2.7	100	15	—	67088-U	67155-U			
Peptides	160	2.7	200	15	—	67091-U	67158-U			
Peptides	160	2.7	300	15	—	67093-U	67160-U			
Peptides	160	2.7	500	10	—	67096-U	—			
Peptides	160	2.7	500	15	—	67097-U	67163-U			
Peptides	160	2.7	1.0	15	—	67099-U	67165-U			
Peptides	160	5	75	15	—	67202-U	67307-U			
Peptides	160	5	100	15	—	67204-U	67312-U			
Peptides	160	5	200	15	—	67206-U	67315-U			
Peptides	160	5	300	15	—	67208-U	67324-U			
Peptides	160	5	500	15	—	67212-U	67326-U			
Peptides	160	5	1.0	15	—	67219-U	67329-U			
<b>BIOshell™ Fused-Core® Peptide and Protein Guard Columns, pk. of 3</b>										
Proteins	400	3.4	2.1	0.5	66830-U	67505-U				
Proteins	400	3.4	4.6	0.5	66831-U	67508-U				
Peptide	160	2.7	2.1	0.5		66918-U	66977-U		577541-U	
Peptide	160	2.7	3.0	0.5		66919-U	66978-U		577542-U	
Peptide	160	2.7	4.6	0.5		66921-U	66979-U		577543-U	
Peptides	160	5	2.1	0.5		67016-U	67082-U			
Peptides	160	5	3.0	0.5		67017-U	67083-U			
Peptides	160	5	4.6	0.5		67018-U	67084-U			
mAb	1000	2.7	2.1	0.5		581349-U		577431-U		
mAb	1000	2.7	3.0	0.5		581360-U		577432-U		
mAb	1000	2.7	4.6	0.5		581361-U		577433-U		

## Related Product

Description	Cat. No.
Universal Guard Holder	
BIOshell™ Guard Cartridge Holder	66841-U

## Other U/HPLC Columns for Proteins and Peptides Available from Supelco® Analytical Products

Supplier, Manufacturer	dp (µm)	Matrix	Size Exclusion	Ion Exchange	Reversed Phase	HILIC	HIC	Affinity
GE Life Sciences	8, 13	dextran-agarose	Superdex™					
	>10 (dry)	dextran	Desalting					
	3, 10	PS/DVB		MiniBeads™, MonoBeads™				
Sepax Technologies	15	PS/DVB		SOURCE™	SOURCE™		SOURCE™	
	1.9	silica	Unix, Unix-C					
	3	silica	Zenix®, Zenix-C					
	5	silica	SRT®, SRT-C					
	1.7, 3, 5, 10	PS/DVB		Proteomix®				
Supelco® Columns	1.7, 3, 5, 10	PS/DVB		Antibodix®				
	2.7, 3.4, 5	silica			BIOshell™			
	3, 5, 10	silica			Discovery® BIO			
	5	methacrylate		Discovery® BIO PolyMA				
Tosoh Bioscience®: TSKgel®	2, 3, 4, 5, 10	silica	UltraSW, SuperSW, SWxl, SW, UP-SW					
	2.5, 5, 10	methacrylate		NPR, STAT, 5PW				
	2.5, 10	methacrylate			NPR, 4PW, 5PW			
	3	silica			Peptide C4			
	3, 5	silica				Amide-80		
	2.5, 10	methacrylate					NPR, 5PW	
	10	methacrylate						5PW

For more information, visit [SigmaAldrich.com/bio-hplc](https://www.sigmaaldrich.com/bio-hplc)



# Purospher™ STAR

## HPLC and UHPLC columns

### High-purity HPLC columns

The key component for modern RP-HPLC sorbents is a high purity silica as starting material. Purospher™ HPLC columns are based on high purity, metal-free silica for outstanding separations with excellent peak symmetry. The base silica for Purospher™ high purity HPLC columns is made from tetraalkoxysilane in a sol-gel process. Due to the absence of metals in the silica matrix and combined with an optimized surface coating and shielding process, Purospher™ columns provide tailing-free separations of acidic, basic, and chelating compounds. This is of particular advantage for any kind of method development in Research and Development (R&D) and Quality Control (QC) laboratories.

### Consistent results

The success of any method depends on the quality of the stationary phase. Precise, long-term reproducibility is a key factor in achieving reliable results. The base silica of Purospher™ STAR columns is 99.999% pure. Furthermore, meticulous care is given to quality control over all aspects of silica structure and chemistry.

These factors ensure that the columns will always perform consistently, resulting in method reproducibility you can trust.

### Perfect peak shape

Accurate results rely on two important chromatographic Properties of the stationary phase: resolution and peak shape. With Purospher™ STAR columns, high efficiency and bonded phase surface coverage produce sharp, symmetrical peaks for acidic, basic and chelating compounds. This makes Purospher™ STAR RP-18 endcapped and RP-8 endcapped columns the optimal choice for USP methods as well as for general method development.

### Enhanced pH stability

Thanks to their outstanding performance and stability, Purospher™ STAR RP-18 endcapped, RP-8 endcapped and Phenyl columns offer maximum flexibility in method development.

Robust methods can be developed over the entire pH range from 1.5 to 10.5. This high pH-stability allows the separation of strongly basic compounds with alkaline eluents.

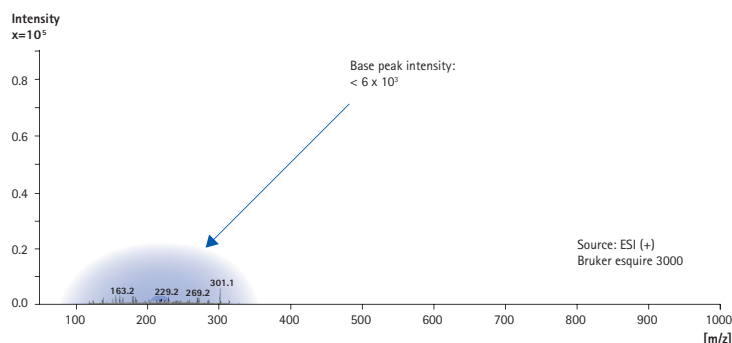
### Compatible with aqueous mobile phases

Standard reversed phase columns, particularly RP-18 columns, often suffer from phase collapse when used in combination with highly aqueous mobile phases. In contrast, Purospher™ STAR RP-18 endcapped, RP-8 endcapped and Phenyl columns still perform perfectly with 100% aqueous mobile phase.

## Excellent for LC-MS

## Purospher™ STAR HPLC and UHPLC columns

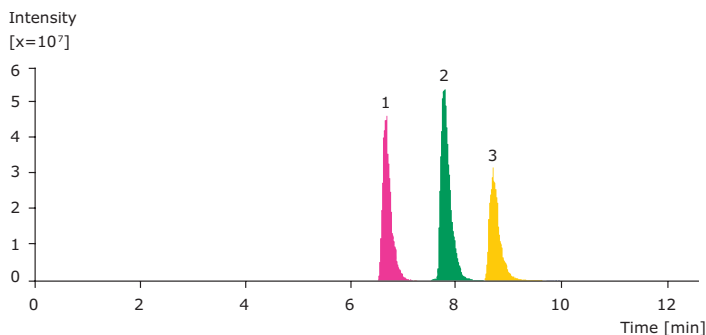
Mass spectrometric (MS) detection is rapidly growing in popularity thanks to its ease of use, better compatibility with liquid chromatography, and cost-efficiency. It enables positive analyte identification, and the possibility to discriminate between co-eluting peaks in specific ion monitoring modes.



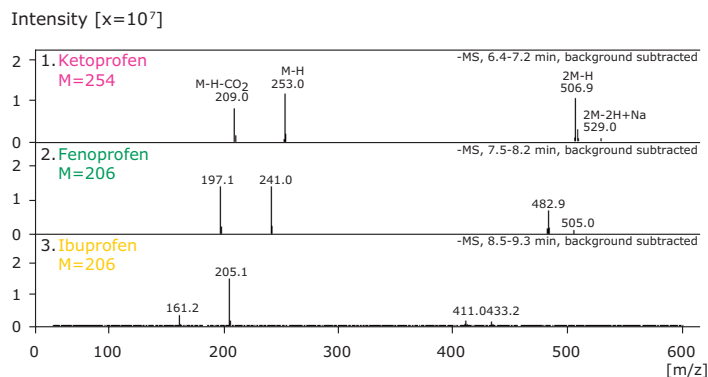
In order to obtain sensitive results with LC-MS, it is essential to avoid trace impurities in the column and solvents.

Purospher™ STAR HPLC and UHPLC columns are highly suitable for LC-MS. To ensure low and stable background signals, it is recommended to wash columns with an eluent of isopropanol and 0.1% formic acid.

### Extracted ion chromatograms of profens in negative ion mode separated on Purospher™ STAR RP-18 endcapped



EIC 209; 253; 507 – All MS  
EIC 197; 241; 483 – All MS  
EIC 161; 205; 411 – All MS



### Chromatographic conditions

<b>Column:</b>	Purospher™ STAR RP-18 endcapped, 3 µm LiChroCART® 55-2	
Mobile phase A:	0.1% Acetic acid in Acetonitrile	
Mobile phase B:	0.1% Acetic acid in Water	
Gradient:	From 25% A to 50% A in 3 min, then isocratic	
Flow Rate:	300 µL, without split	
Detection:	UV 220 nm, Ion Trap MS	
Temperature:	ambient	
Injection volume:	1 µL	
Sample:	1. Ketoprofen	0.1 µg/µL
	2. Fenoprofen	0.1 µg/µL
	3. Ibuprofen	0.1 µg/µL

### MS conditions

Nebulizer:	36 psi
Dry gas:	8.5 L/min
Dry temperature:	330°C
Smart mode optimization:	Target mass 205
Ion charge control:	Target 50,000, max 50 ms
Scan mode:	Standard/Normal
Scan range:	50 – 600 m/z

Ketoprofen, Fenoprofen and Ibuprofen (100 ng) give ghost-peak-free MS spectra using LiChrosolv® Acetonitrile hypergrade and Purospher™ STAR RP-18 endcapped columns.

## Ordering Information

### Purospher™ STAR in stainless steel LiChroCART® cartridges

#### LiChroCART® cartridge 2 mm i.d.

Modification	Particle size	30-2	55-2	100-2	125-2	150-2	250-2
RP-18 endcapped	3 µm	1.50238.0001**	1.50241.0001**	-	-	-	-
RP-18 endcapped Set*	3 µm	1.50237.0001*	1.50240.0001*	-	-	-	-
RP-18 endcapped	5 µm	1.50229.7185	1.50234.7185	1.50623.0001	1.50255.0001	1.50624.0001	1.50256.0001
RP-8 endcapped	3 µm	1.50229.7220	1.50234.7220	-	-	-	-
RP-8 endcapped	5 µm	-	-	-	1.50274.0001	-	1.50275.0001

#### LiChroCART® cartridge 3 mm i.d.

Modification	Particle size	30-3	55-3	100-3	125-3	150-3	250-3
RP-18 endcapped	3 µm	1.50233.7184	1.50236.7184	-	-	-	-
RP-18 endcapped	5 µm	1.50233.7185	1.50236.7185	1.50625.0001	1.50253.0001	1.50626.0001	1.50254.0001
RP-8 endcapped	5 µm	-	-	-	1.50038.0001	-	1.50237.0001

#### LiChroCART® cartridge 4 mm i.d.

Modification	Particle size	4-4 (10 guard columns)	30-4	55-4	75-4	125-4	250-4
RP-18 endcapped	3 µm	-	1.50225.0001**	1.50231.0001**	1.51460.0001	-	-
RP-18 endcapped Set*	3 µm	-	1.50239.0001*	1.50242.0001*	-	-	-
RP-18 endcapped	5 µm	1.50250.0001	1.50302.7185	1.50228.7185	-	1.50251.0001	1.50252.0001
RP-8 endcapped	5 µm	1.50270.0001	-	-	-	1.50271.0001	1.50272.0001
NH <sub>2</sub>	5 µm	1.50267.0001	-	-	-	1.50244.0001	1.50245.0001
Si	5 µm	1.50249.0001	-	-	-	1.50268.0001	1.50269.0001

#### LiChroCART® cartridge 4.6 mm i.d.

Modification	Particle size	4-4 (10 guard columns)	100-4.6	150-4.6	250-4.6
RP-18 endcapped	3 µm	-	1.51448.7184	-	-
RP-18 endcapped	5 µm	1.50250.0001	1.50627.0001	1.50358.0001	1.50359.0001
RP-8 endcapped	5 µm	1.50270.0001	-	1.50031.0001	1.50032.0001
Phenyl	5 µm	-	-	1.51922.0001	1.51921.0001
NH <sub>2</sub>	5 µm	1.50267.0001	-	1.50247.0001	1.50248.0001
Si	5 µm	1.50249.0001	-	1.50356.0001	1.50357.0001

#### LiChroCART® cartridge 10 mm i.d.

Modification	Particle size	10-10 (guard column)	75-10	100-10	125-10	150-10	250-10
RP-18 endcapped	5 µm	1.50178.7185	1.51449.7185	1.51445.7185	1.51443.7185	1.51444.7185	1.50257.0001
RP-8 endcapped	5 µm	-	-	-	-	-	1.50276.0001

\* One set contains: 1 LiChroCART® cartridge and one holder

\*\* 3 cartridges in one pack

The LiChroCART® columns (75, 125, 150 and 250 mm length) in the list above (2, 3, 4 and 4.6 mm i.d.) require part number 1.51486.0001 manu-CART® cartridge column holder, which can be used to hold one cartridge column with or without a 4-4 mm guard column. LiChroCART® columns 250-10 mm require part number 1.51419.0001 manu-CART® 10. The short LiChroCART® columns (30 and 55 mm length) can be ordered as a set including the corresponding cartridge holder and one cartridge, or as a pack of 3 cartridges without cartridge holder. The separate part numbers for the cartridge are as follows: 1.50227.0001 LiChroCART® cartridge holder for 30 mm cartridge and 1.50226.0001 LiChroCART® cartridge holder for 55 mm cartridge.



## Purospher™ STAR in Hibar® RT columns

### Hibar® RT column 2 mm i.d.

Modification	Particle size	50-2	100-2	125-2	150-2	250-2
RP-18 endcapped	5 µm	1.50593.0001	1.50595.0001	1.50596.0001	1.50597.0001	1.50598.0001

### Hibar® RT column 3 mm i.d.

Modification	Particle size	50-3	100-3	125-3	150-3	250-3
RP-18 endcapped	3 µm	1.50393.0001	1.50398.0001	1.50413.0001	1.50414.0001	1.50427.0001
RP-18 endcapped	5 µm	1.50607.0001	1.50612.0001	1.50615.0001	1.50617.0001	1.50620.0001
RP-8 endcapped	3 µm	-	-	-	1.50750.0001	-
RP-8 endcapped	5 µm	-	-	-	1.50644.0001	-
Phenyl	3 µm	-	-	-	1.50631.0001	-
Phenyl	5 µm	-	-	-	1.51920.0001	-

### Hibar® RT column 4 mm i.d.

Modification	Particle size	50-4	125-4	250-4
RP-18 endcapped	3 µm	1.50428.0001	1.50431.0001	1.50468.0001
RP-18 endcapped	5 µm	1.50621.0001	1.50036.0001	1.50037.0001
RP-8 endcapped	5 µm	-	1.50033.0001	1.50035.0001

### Hibar® RT column 4.6 mm i.d.

Modification	Particle size	100-4.6	125-4.6	150-4.6	250-4.6
RP-18 endcapped	3 µm	1.50469.0001	-	1.50470.0001	1.50471.0001
RP-18 endcapped	5 µm	1.50622.0001	1.51914.0001	1.51455.0001	1.51456.0001
RP-8 endcapped	5 µm	1.51917.0001	1.51916.0001	1.51453.0001	1.51454.0001
Phenyl	5 µm	-	-	1.51919.0001	1.51918.0001
NH <sub>2</sub>	5 µm	-	-	-	1.51913.0001
Si	5 µm	-	-	-	1.51911.0001

### Hibar® RT column 10 mm i.d.

Modification	Particle size	250-10
RP-18 endcapped	5 µm	1.51915.0001
Si	5 µm	1.51912.0001

The Hibar® RT columns are complete with endfittings. When using a guard column with a Hibar® RT column, we recommend part number 1.51487.0001 guard column cartridge holder for 4-4 mm guard column cartridges LiChroCART®.



### Purospher™ STAR in Hibar® HR UHPLC columns 2.1 mm i.d.

Modification	Particle size	30-2.1	50-2.1	100-2.1	150-2.1	250-2.1
RP-18 endcapped	2 µm	1.50645.0001	1.50646.0001	1.50648.0001	1.50649.0001	-
RP-18 endcapped	3 µm	1.50650.0001	1.50651.0001	1.50653.0001	1.50654.0001	1.50655.0001
RP-8 endcapped	2 µm	-	1.50630.0001	1.50629.0001	-	-
RP-8 endcapped	3 µm	-	1.50674.0001	1.50675.0001	-	-
Phenyl	2 µm	-	1.51013.0001	1.51014.0001	-	-
Phenyl	3 µm	-	1.50672.0001	1.50673.0001	-	-

The Hibar® HR UHPLC columns are designed for use in UHPLC instruments. The pressure stability is set at 1000 bar.

# Chromolith® LC-MS Columns for Extended Lifetime with Demanding Applications

Thanks to our patented monolithic silica technology, Chromolith® HPLC columns allow you to race through separations with maximum robustness - at minimal backpressure.

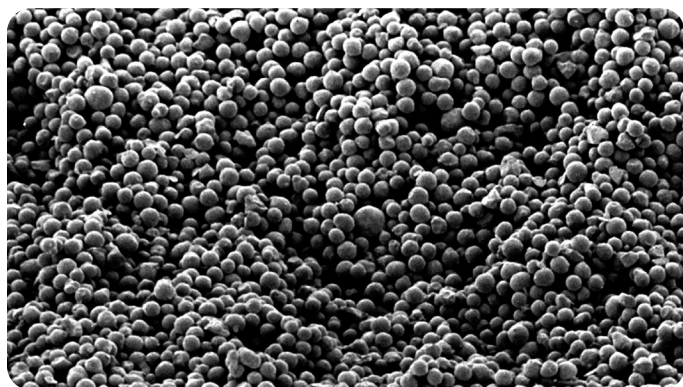
## Characterization of Chromolith® HPLC columns

The use of conventional HPLC columns containing small silica particles often results in high backpressure. This reduces column lifetime, system robustness and the operational range of flow rates.

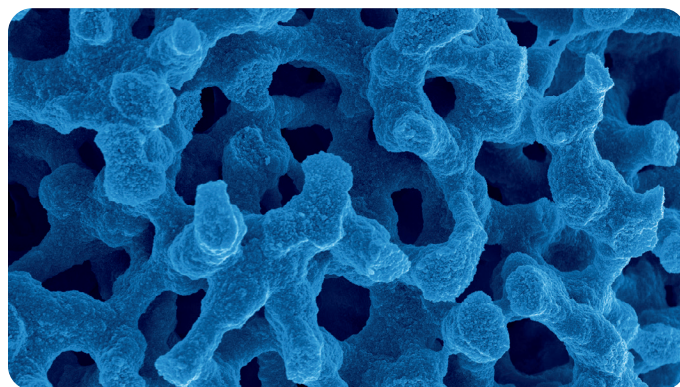
A column packed with tiny particles is relatively easy to block since the space between particles is directly

proportional to particle size (approx. one sixth of particle diameter). The smaller space leads to higher backpressure and blockage.

A thin skeleton ensures fast mass transfer, while the large channels allow unobstructed flow through the column.



SEM image: Silica Particles



SEM image: Cross section of a silica monolith. Total porosity > 80%.

## Long-term stability

Besides lower backpressure and greater flow rate flexibility, Chromolith® columns achieve faster equilibration after gradient elution than particle packed

columns of similar dimensions. These features enable high-throughput analysis-without loss of separation efficiency or peak capacity.

## Column robustness

Chromolith® columns offer excellent robustness with unsurpassed column lifetime. This not only ensures maximum reliability and versatility, but also minimizes maintenance on the HPLC system. As a result, Chromolith® columns reduce costs per analysis while enhancing data integrity.

The optimal solution is a column that offers faster throughput without the risk of plugging the Chromolith® column. In contrast to conventional HPLC columns, Chromolith® columns are not packed with small silica particles. Instead, each column consists of a single rod of high-purity silica gel with a bimodal pore structure of macro and mesopores. This unique construction enables highly efficient separations at unbeatable speeds.

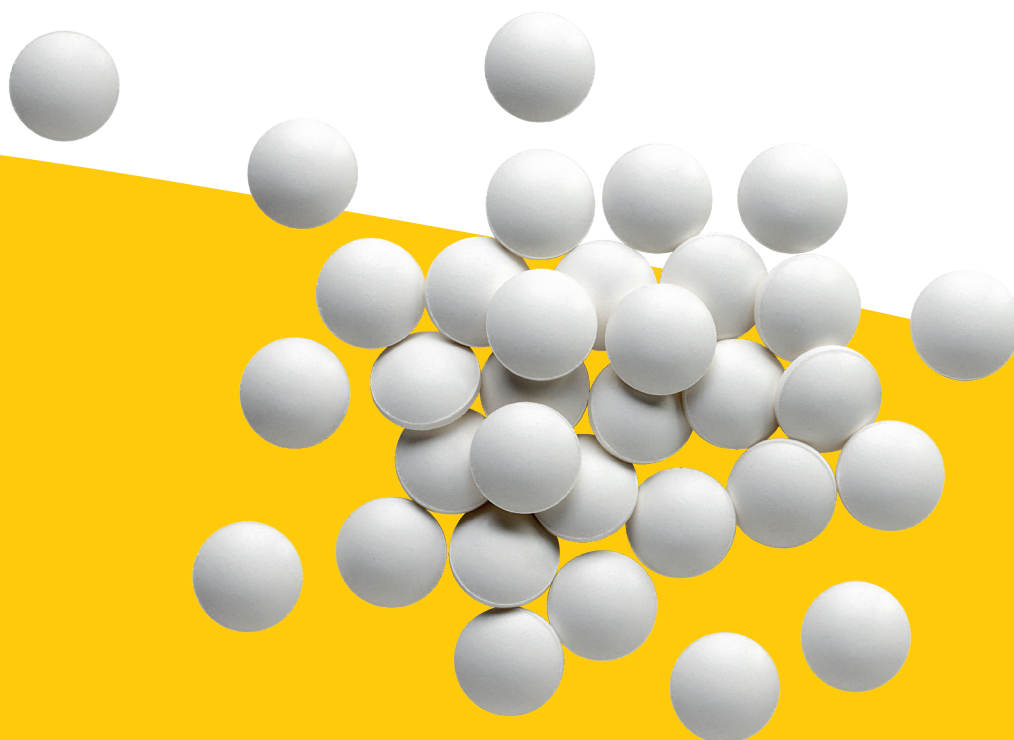
## Why choose analytical Chromolith® columns?

- Fast, high-performance results
- Substantially longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability
- Compatible with all low dead volume LC instruments (UHPLC, UPLC, HPLC)
- Possibility of flow gradients
- Increased column performance by column coupling

## Column robustness and lifetime

Exceptional robustness or lifetime of a column – described as number of injections – directly affects the economic situation in a laboratory in a positive way. But there is also an indirect effect: Understanding robustness as high matrix tolerance decreases tedious sample preparation steps, speeds up all processes and allows for fast and simple HPLC analyses.

Two examples display the utilization of Chromolith® columns in a pharma R&D as well as in a high throughput screening (HTS) everyday lab environment. In pharma R&D small molecules (products or intermediates) synthesized during drug development have to be analyzed via fast and robust methods without shutdown periods caused by column failure. Typical run times for these experiments are in the range of 2–3 minutes. In combination with standard HPLC equipment, columns showing low backpressures are desirable to perform these quick runs. In this example an LC-MS system is utilized for separation and detection. The performance of the setup is checked for consistency by the injection of a standard mixture of theophylline, caffeine and 2-amino-5-chlorobenzophenone every workday morning. In this environment the monolithic silica column allows for more than 10,000 chromatographic runs on standard HPLC equipment within approximately half a year (**Figure 2**) before it is replaced routinely. All test runs performed during this period display consistent peak shape and retention as well as chromatographic resolution and performance without any increase in column backpressure.





MS intensity

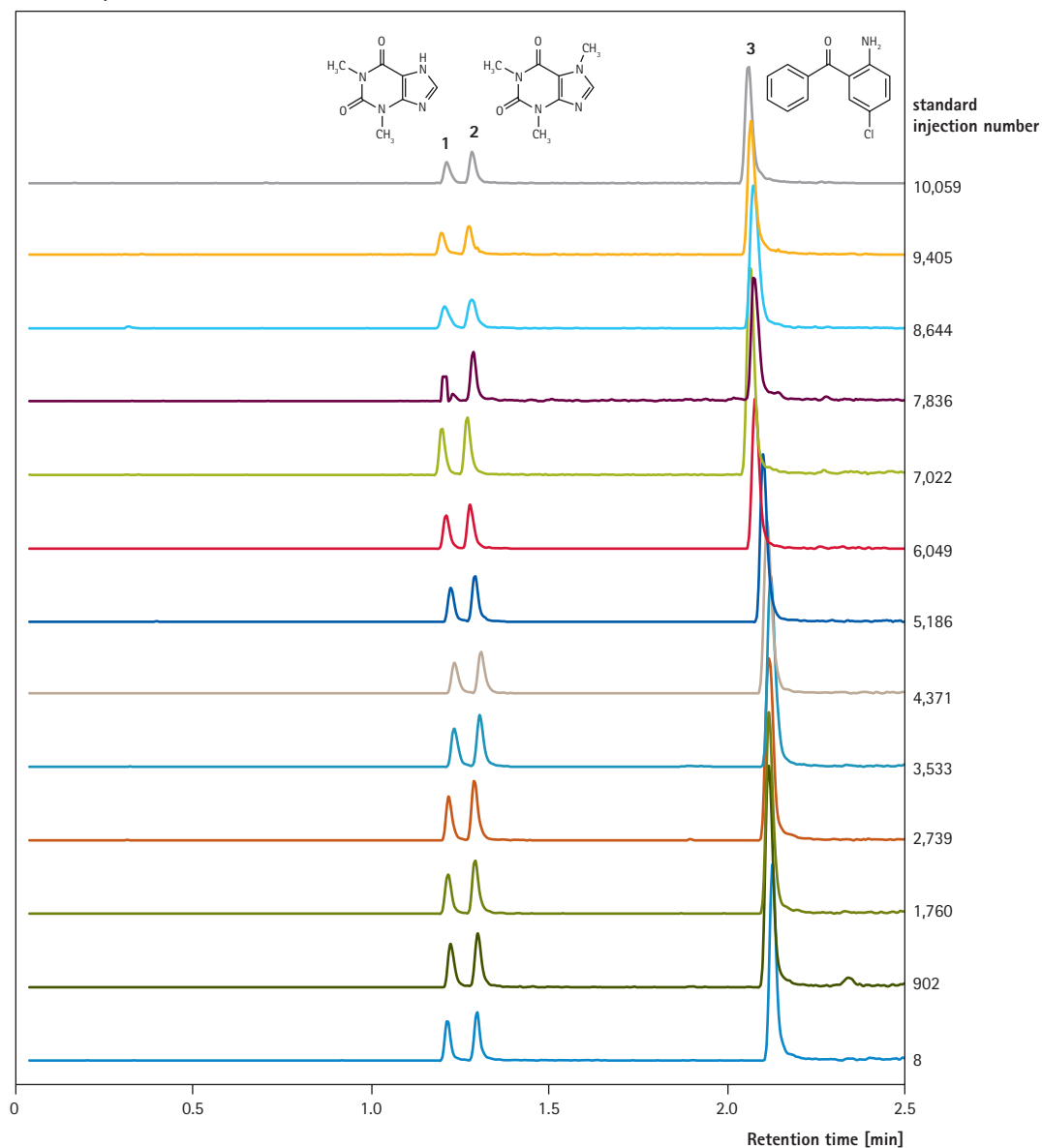


Figure 1

#### Chromatographic conditions

Column	Chromolith® Performance RP-18 endcapped 100-3 mm (Cat. No. 152001)
Injection volume	1 µL
System	Agilent 1100 HPLC and Waters single quadrupole MS
Detection	Pos. API-MS, m/z range 85 – 800
Flow rate	0.4 mL/min (MS; HPLC flow 2 mL/min, post-column split)
Mobile phase	A: Milli-Q® water from water purification system + 0.05 % formic acid (Cat. No. 100264)
Gradient	0 min 99 % A, 1.8 min 0 % A, 2.5 min 0 % A
Temperature	25°C
Sample	1 theophylline, 2 caffeine, 3 2-amino-5-chlorobenzophenone (1 mmol each) dissolved in methanol / water 50:50 (v:v).

Pharma R&D small molecule analysis, results of everyday standard runs testing the performance of the utilized column.

High throughput screening (HTS) is with pharma libraries containing numerous small molecules for their suitability as an active ingredient. In a similar way as for pharma R&D, fast separations on standard HPLC equipment is an important issue in HTS that can best be addressed by columns showing low backpressures. Robust and reliable column technology is prerequisite to avoid any system failure and shutdown. In this example, an HTS system with four columns as well as four evaporative light scattering detectors (ELSD) and UV-MS detectors operating in a parallel setup was used.

The performance of this setup was checked for consistency by the injection of a standard (Sarizotan) every 50 runs.

Within one year, more than 40,000 fast chromatographic runs were performed on each of the four monolithic silica columns (**Figure 2**). During this period all relevant chromatographic criteria (performance, peak shape, retention time) remained constant and no degradation of the column was observed.

### Extreme robustness of Chromolith® columns

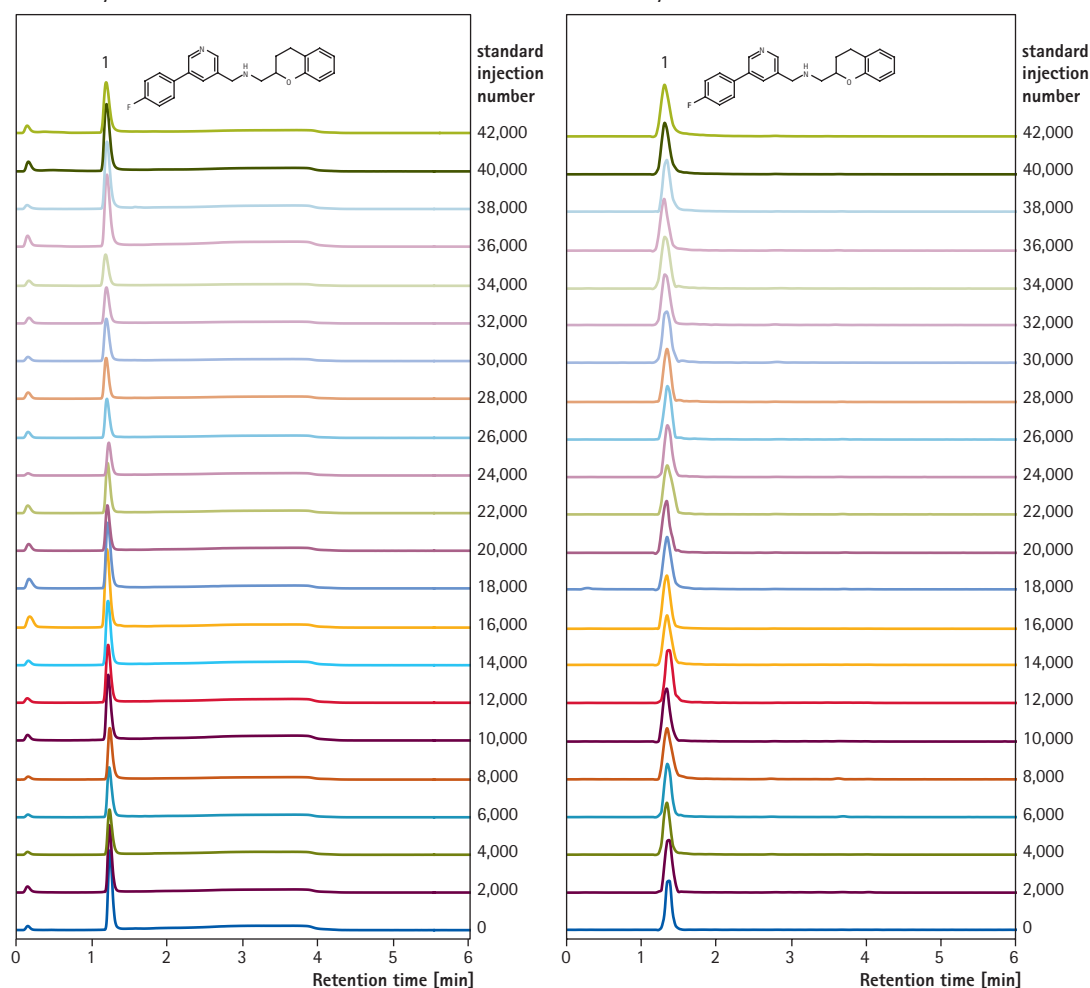
The two application examples display the extreme robustness of Chromolith® columns in a lab environment, where time of analysis has to be low and speed, reproducibility and a long column lifetime are required. Chromolith® delivers these features on standard HPLC systems without the need for upgrading to high-priced LC solutions.



Chromolith® columns

## Column robustness and lifetime

Figure 2



### Chromatographic conditions

Column	Chromolith® Flash RP-18 endcapped 25-2 mm (Cat. No. 152014)
Injection volume	5 µL
System	Waters 2777 sample manager, 2488 MUX-UV detector, 4 2420 ELS detector, ZQ-MUX
Detection	Left: pos. ESI-MS, m/z range 160 – 1000, base peak chromatogram (BPC); right: UV 254 nm, ELSD
Flow rate	0.8 mL/min
Mobile phase	A: Milli-Q® water from water purification system + 0.1% formic acid (Cat. No. 100264) B: Acetonitrile (Cat. No. 100029) + 0.1% formic acid
Gradient	0 min 95% A, 1.7 min 0% A, 3 min 0% A, 3.01 min 100% A, 6.25 min 95% A
Temperature	25 °C
Sample	Sarizotan (1 mmol) dissolved in dimethyl sulfoxide

High throughput screening experiment, results of the control runs testing column performance.

## Sensitivity and column selection

Samples such as those of biological origin are often only available in small amounts and the concentration of analytes is low. Typical analytes are proteins or digested proteins, peptides and numerous types of metabolites. Under this precondition, a setup consisting of both highly sensitive separation and detection techniques is necessary for proper identification of the target molecules. The sensitivity of a separation is influenced by the internal diameter (i.d.) of the column used, e.g. when changing from a 4.6 mm i.d. column to a 0.1 mm i.d. capillary column, sensitivity increases by a factor of approximately 2000. Hence, a combination of chromatography utilizing capillaries coupled to mass spectrometry is the best combination for high sensitivity analysis.

Two gradient separation methods were developed describing the analysis of a tryptic digest of cytochrome c and of a peptide mixture on nano-LC equipment. The chromatographic separation was performed on a small i.d. analytical monolithic silica capillary column directly coupled to the source of a mass spectrometer.

The amino acid sequence of cytochrome c from cattle (*bos taurus*) was analyzed and a set of nine tryptic fragments was identified within a run time of approximately 10 minutes (**Table 8** and **Figure 3**). Utilization of a monolithic silica capillary column and a two-step gradient enabled the elution of narrow peaks and baseline separation of all peptides except for the critical peak pair No. 5/6. The amino acid sequence of all fragments was identified via the obtained MS data and a library search.

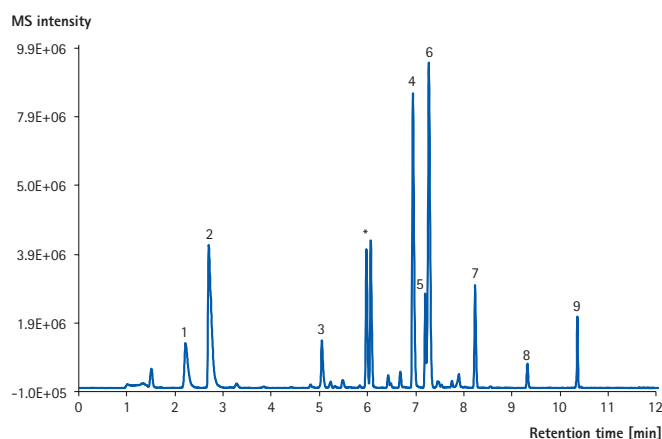
**Table 8**

Peak no.	Amino acid sequence	Retention time (min)	Detected mass m/z (g/mol)
1	YIPGTK	2.21	339.7
2	IFVQK	2.70	317.7
3	KTGQAPGFSYTDANK	5.05	528.9
*	unidentified compounds	5.98 / 6.07	n.a.
4	TGPNLHGLFGR	6.95	390.2
5	GEREDLIAYLKK	7.20	478.9
6	MIFAGIK	7.28	390.2
7	EDLIAYLK	8.24	482.8
8	IFVQKCAQCHTVEK	9.33	545.3
9	GITWGEETLMEYLENPKK	10.37	713.4

Peak number, amino acid sequence, retention time and detected molar mass of fragments obtained after tryptic digestion of cytochrome c

In a second experiment a mixture of five peptides was analyzed utilizing a monolithic silica capillary column and gradient elution conditions (**Figure 4**). The analysis of all five compounds was achieved within less than seven minutes, including the baseline separation of the critical peaks of Met-enkephalin, angiotensin II and Leu-enkephalin.

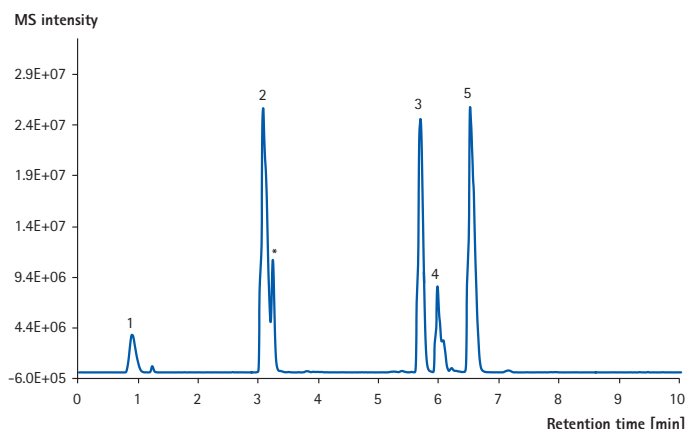
In combination with mass spectrometry detection the use of monolithic silica capillary HPLC columns offers maximum sensitivity analyses of low amounts of biological samples at overall low backpressure and minimized solvent consumption. Due to the rigidity and robustness of the fritless column bed no tedious sample preparation is necessary and clogging can be minimized.



**Figure 3** LC-MS analysis of a tryptic digest of cytochrome c utilizing a monolithic silica capillary column.

Chromatographic conditions	
Column	Chromolith® CapRod® RP-18 endcapped 150-0.1 mm capillary column (Cat. No. 150402)
Injection volume	1 nL
System	Bruker Esquire 6000plus
Detection	Pos. ESI-MS, m/z range 300 – 750, BPC
Flow rate	3.5 µL/min
Mobile phase	A: Milli-Q® water from water purification system + 0.1 % formic acid (Cat. No. 100264) B: Acetonitrile (Cat. No. 100029) + 0.1 % formic acid
Gradient	0 min 95 % A, 7 min 75 % A, 10 min 30 % A
Temperature	25°C
Sample	Lyophilized tryptic digest of cytochrome c (from cattle, <i>bos taurus</i> ) resuspended in ACN/water 5/95 (v/v)





**Figure 4** LC-MS analysis of a mixture of five peptides on a monolithic silica capillary column.

Chromatographic conditions	
Column	Chromolith® CapRod® RP-18 endcapped 150-0.1 mm capillary column (Cat. No. 150402)
Injection volume	1 nL
System	Bruker Esquire 6000plus
Detection	Pos. ESI-MS, m/z range 300 – 750, BPC
Flow rate	3.5 µL/min
Mobile phase	A: Milli-Q® water from water purification system + 0.1 % formic acid (Cat. No. 100264) B: Acetonitrile (Cat. No. 100029) + 0.1 % formic acid
Gradient	0 min 99 % A, 5 min 85 % A
Temperature	25°C
Sample	Lyophilized peptides: 1 Gly-Try, 2 Val-Tyr-Val, 3 Met-enkephalin, 4 Angiotensin II, 5 Leu-enkephalin, resuspended in water.

## Chromolith® HPLC Guard Cartridges and Kits

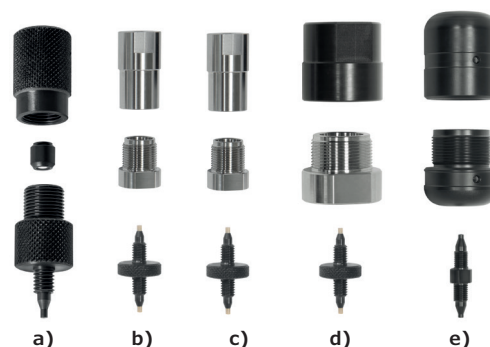
Although monolithic columns are well known for their robustness and longevity, our Chromolith® guard cartridges, cartridge holders and kits further enhance these advantages.

### Guard cartridges

Chromolith® HPLC guard cartridges are extremely easy to use. They are simply added directly in front of the main column to protect it from chemical or mechanical contamination. Due to the benefits of monolithic technology, and the convenience of Chromolith® guard columns, they are also popular for use with classical particulate columns. Moreover, guard columns can be used as trap columns when large sample volumes are to be injected. Guard columns should be changed frequently in order to avoid excessive accumulation of impurities.

#### Guard cartridge starter kit for 2 and 3mm i.d. columns

The Chromolith® guard cartridge kit includes everything needed to significantly enhance the lifetime of monolithic columns: a guard cartridge holder, and three guard cartridges.



#### Guard cartridge holders

Depending on your needs, we offer several different guard cartridge holders: made out of PEEK for 2 and 3 mm i.d. cartridges; bioinert PEEK lined stainless steel holder and standard stainless steel holder for 4.6 mm i.d. cartridges and holders for 10 and 25 mm i.d. cartridges.

Guard cartridge holder type	Material holder is made of	Max. back pressure	How to tighten holder	Guard cartridge i.d.	Guard cartridge length
a)	PEEK	200 bar (2,940 psi)	Finger-tight	2, 3 mm,	5 mm
b)	PEEK lined SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm, 10 mm
c)	SS	400 bar (5,880 psi)	Finger-tight + tool (not included)	4.6 mm	5 mm, 10 mm
d)	PEEK / SS	150 bar (2,205 psi)	Finger-tight + tool (not included)	10 mm	10 mm
e)	PEEK	100 bar (1,470 psi)	Finger-tight + tool (included)	25 mm	10 mm

For maximum convenience and flexibility, guard cartridges are available in five dimensions with corresponding holders made of PEEK, PEEK lined stainless steel, having different maximum back pressures.

PEEK = Poly Ether Ether Ketone, SS = Stainless Steel

# Ordering information

## Capillary columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® CapRod®	RP-18e	0.05 mm	150 mm		1 analytical column	1.50403.0001
Chromolith® CapRod®	RP-8e	0.1 mm	150 mm		1 analytical column	1.50400.0001
Chromolith® CapRod®	RP-18e Trap	0.1 mm	50 mm		1 trapping column	1.50426.0001
Chromolith® CapRod®	RP-18e	0.1 mm	150 mm		1 analytical column	1.50402.0001
Chromolith® CapRod®	RP-18e	0.1 mm	300 mm		1 analytical column	1.50424.0001
Chromolith® CapRod® HR	RP-18e	0.1 mm	150 mm		1 analytical column	1.50404.0001
Chromolith® CapRod®	RP-18e Trap	0.2 mm	50 mm		1 trapping column	1.50409.0001
Chromolith® CapRod®	RP-18e	0.2 mm	150 mm		1 analytical column	1.50405.0001
Chromolith® CapRod® HR	RP-18e	0.2 mm	150 mm		1 analytical column	1.50407.0001
Chromolith® HR	RP-18e	4.6 mm	150 mm		1 HPLC Column	1.52023.0001

## Analytical columns

Product	Modification	I.d.	Length	guard cartridge holder type	Content	Cat. No.
Chromolith® Validation Kit	RP-18e	2 mm	100 mm		3 columns from 3 different batches	1.52062.0001
Chromolith® Performance	RP-18e	2 mm	100 mm		1 HPLC column	1.52006.0001
Chromolith® FastGradient	RP-18e	2 mm	50 mm		1 HPLC column	1.52007.0001
Chromolith® Flash	RP-18e	2 mm	25 mm		1 HPLC column	1.52014.0001
Chromolith® Guard Cartridge Kit	RP-18e	2 mm	5 mm	a*	3 guard cartridges, 1 cartridge holder	1.52008.0001
Chromolith® Guard Cartridge	RP-18e	2 mm	5 mm	a*	3 guard cartridges	1.52009.0001
Chromolith® Validation Kit	RP-18e	3 mm	100 mm		3 columns from 3 different batches	1.52063.0001
Chromolith® Performance	RP-18e	3 mm	100 mm		1 HPLC column	1.52001.0001
Chromolith® FastGradient	RP-18e	3 mm	50 mm		1 HPLC column	1.52002.0001
Chromolith® Flash	RP-18e	3 mm	25 mm		1 HPLC column	1.52003.0001
Chromolith® Guard Cartridge Kit	RP-18e	3 mm	5 mm	a*	3 guard cartridges, 1 cartridge holder	1.52004.0001
Chromolith® Guard Cartridge	RP-18e	3 mm	5 mm	a*	3 guard cartridges	1.52005.0001
Chromolith® Performance	RP-18e	4.6 mm	100 mm		1 HPLC column	1.02129.0001
Chromolith® Validation Kit	RP-18e	4.6 mm	100 mm		3 columns from 3 different batches	1.51466.0001
Chromolith® SpeedROD	RP-18e	4.6 mm	50 mm		1 HPLC column	1.51450.0001
Chromolith® Flash	RP-18e	4.6 mm	25 mm		1 HPLC column	1.51463.0001
Chromolith® Guard Cartridge	RP-18e	4.6 mm	10 mm	b/c*	3 guard cartridges	1.51452.0001
Chromolith® Guard Cartridge	RP-18e	4.6 mm	5 mm	b/c*	3 guard cartridges	1.51451.0001
Chromolith® Validation Kit	RP-18e	4.6 mm	100 mm		3 columns from 3 different batches	1.52019.0001
Chromolith® HR	RP-18e	4.6 mm	100 mm		1 HPLC column	1.52022.0001
Chromolith® HR	RP-18e	4.6 mm	50 mm		1 HPLC column	1.52021.0001
Chromolith® HR	RP-18e	4.6 mm	25 mm		1 HPLC column	1.52020.0001
Chromolith® HR Guard Cartridge	RP-18e	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52025.0001
Chromolith® Performance	RP-8e	4.6 mm	100 mm		1 HPLC column	1.51468.0001
Chromolith® HR	RP-8e	4.6 mm	100 mm		1 HPLC column	1.52064.0001
Chromolith® Guard Cartridge	RP-8e	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52013.0001

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith®	Phenyl	4.6 mm	25 mm		1 HPLC column	1.52056.0001
Chromolith®	Phenyl	4.6 mm	50 mm		1 HPLC column	1.52057.0001
Chromolith®	Phenyl	4.6 mm	100 mm		1 HPLC column	1.52058.0001
Chromolith® Guard Cartridge	Phenyl	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52059.0001
Chromolith®	CN	4.6 mm	25 mm		1 HPLC column	1.52046.0001
Chromolith®	CN	4.6 mm	50 mm		1 HPLC column	1.52047.0001
Chromolith®	CN	4.6 mm	100 mm		1 HPLC column	1.52048.0001
Chromolith® Guard Cartridge	CN	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52050.0001
Chromolith®	DIOL	4.6 mm	25 mm		1 HPLC column	1.53170.0001
Chromolith®	DIOL	4.6 mm	50 mm		1 HPLC column	1.53171.0001
Chromolith®	DIOL	4.6 mm	100 mm		1 HPLC column	1.53172.0001
Chromolith® Guard Cartridge	DIOL	4.6 mm	5 mm	b/c*	3 guard cartridges	1.53175.0001
Chromolith® Performance	Si	4.6 mm	100 mm		1 HPLC column	1.51465.0001
Chromolith® Guard Cartridge	Si	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52011.0001
Chromolith® Performance	NH2	4.6 mm	100 mm		1 HPLC column	1.52028.0001
Chromolith® SpeedROD	NH2	4.6 mm	50 mm		1 HPLC column	1.52027.0001
Chromolith® Flash	NH2	4.6 mm	25 mm		1 HPLC column	1.52026.0001
Chromolith® Guard Cartridge	NH2	4.6 mm	5 mm	b/c*	3 guard cartridges	1.52030.0001
Chromolith® Guard Cartridge Holder	-	4.6 mm	5 mm	c*	1 cartridge holder	1.52032.0001
Chromolith® Guard Cartridge Holder	-	4.6 mm	10 mm	c*	1 cartridge holder	1.52033.0001
Chromolith® Column Coupler	-	-	-		1 column coupler	1.51467.0001

## Chromolith® WP 300 columns

Product	Modification	I.d.	Length	Type	Content	Cat. No.
Chromolith® WP 300 Protein A	Protein A	4.6 mm	25 mm		1 HPLC column	1.52258.0001
Chromolith® WP 300 RP18	RP-18	4.6 mm	100 mm		1 HPLC column	1.52270.0001
Chromolith® WP 300 RP18	RP-18	4.6 mm	50 mm		1 HPLC column	1.52271.0001
Chromolith® WP 300 RP18 Guard Cartridge	RP-18	4.6 mm	10 mm	b*	3 guard cartridges	1.52272.0001
Chromolith® WP 300 RP18 Guard Cartridge	RP-18	4.6 mm	5 mm	b*	3 guard cartridges	1.52273.0001
Chromolith® WP 300 RP8	RP-8	4.6 mm	100 mm		1 HPLC column	1.52265.0001
Chromolith® WP 300 RP8	RP-8	4.6 mm	50 mm		1 HPLC column	1.52266.0001
Chromolith® WP 300 RP8 Guard Cartridge	RP-8	4.6 mm	10 mm	b*	3 guard cartridges	1.52267.0001
Chromolith® WP 300 RP8 Guard Cartridge	RP-8	4.6 mm	5 mm	b*	3 guard cartridges	1.52268.0001
Chromolith® WP 300 RP4	RP-4	4.6 mm	100 mm		1 HPLC column	1.52260.0001
Chromolith® WP 300 RP4	RP-4	4.6 mm	50 mm		1 HPLC column	1.52261.0001
Chromolith® WP 300 RP4 Guard Cartridge	RP-4	4.6 mm	10 mm	b*	3 guard cartridges	1.52262.0001
Chromolith® WP 300 RP4 Guard Cartridge	RP-4	4.6 mm	5 mm	b*	3 guard cartridges	1.52263.0001
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	100 mm		1 HPLC column	1.52250.0001
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	50 mm		1 HPLC column	1.52251.0001
Chromolith® WP 300 Epoxy	Epoxy	4.6 mm	25 mm		1 HPLC column	1.52252.0001
Chromolith® WP 300 Epoxy Guard Cartridge	Epoxy	4.6 mm	10 mm	b*	3 guard cartridges	1.52253.0001
Chromolith® WP 300 Epoxy Guard Cartridge	Epoxy	4.6 mm	5 mm	b*	3 guard cartridges	1.52254.0001
Chromolith® Guard Cartridge Holder Bioinert	-	4.6 mm	5 mm	b*	1 cartridge holder	1.52255.0001
Chromolith® Guard Cartridge Holder Bioinert	-	4.6 mm	10 mm	b*	1 cartridge holder	1.52256.0001

## SeQuant® HILIC

**ZIC®-HILIC, ZIC®-cHILIC and ZIC®-pHILIC are the ideal columns for all classes of polar hydrophilic compounds**

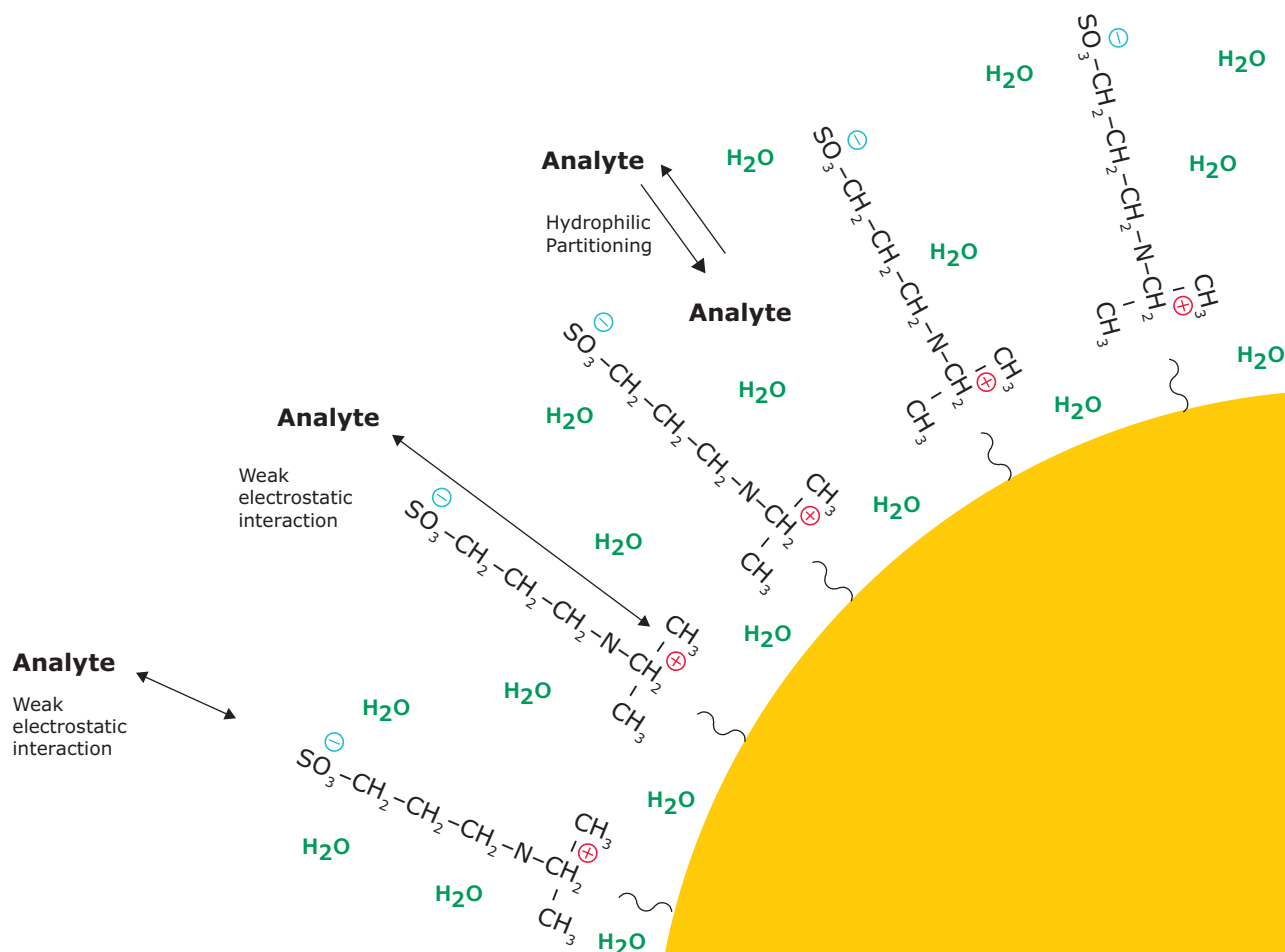
Your ideal choice for separation of all types of polar and hydrophilic compounds are the SeQuant® HILIC HPLC columns. Reproducible retention for compounds that have proved difficult to separate on reversed-phase. HPLC columns is ensured by the high-performance zwitterionic sorbents in these columns.

Straightforward separation of compounds such as acids and bases, anions and cations, carbohydrates, metabolites, metal complexes, amino acids, peptides, protein digests and oligonucleotides can therefore be achieved with a selectivity complementary to reversed-phase columns. Enhanced LC-MS sensitivity is an additional benefit of using these columns.

Columns are available in a wide range of formats from capillary to semi-preparative dimensions, and with several different particles sizes and pore sizes.

### SeQuant® HILIC benefits

- Improved separation of hydrophilic polar compounds
- Selectivity complementary to reversed phase
- Optimal design for HPLC and LC-MS
- Easy method development
- Excellent stability



## What is HILIC?

HILIC or Hydrophilic Interaction Liquid Chromatography is a straightforward chromatographic technique for separation of many types of polar and hydrophilic compounds. To put it simple one can say that HILIC is a normalphase (NPLC) type of separation but uses reversed-phase (RPLC) type eluents.

### In HILIC one uses:

- A column with a hydrophilic stationary phase
- An eluent with water, buffer and a high concentration of water-miscible organic solvent.

A typical HILIC application uses an eluent with 50 – 95% organic solvent in an aqueous buffer that has a high solubility in the solvent, for example acetonitrile in ammonium acetate. The elution order in HILIC is roughly the opposite of that in RPLC and retention increases with hydrophilicity and charge of the analyte. This enables

straightforward separation of compounds that would otherwise elute in the void volume on RPLC columns.

### For all classes of polar and hydrophilic compounds

With the SeQuant® HILIC columns, separation of polar and hydrophilic compounds is straightforward. The selectivity is complementary to reversed phase and therefore suitable for a wide variety of molecules containing hydrophilic or ionizable functional groups. This includes compounds such as carbohydrates, metabolites, acids and bases, organic and inorganic ions, metal complexes, amino acids, peptides, protein digests, plant and cell extracts, plus much more. These compounds are normally characterized by a small or negative log P value\* and have poor retention on reversed-phase columns. The high hydrophilicity and high retention per surface area of the SeQuant® HILIC columns enable separation of a very wide range of polar hydrophilic compounds.

## Ordering information

### SeQuant® ZIC®-HILIC analytical PEEK columns

Product	Ordering No.	Particle size	Porosity	Dimension length	Dimension i.d.	Contents of one package
ZIC®-HILIC PEEK HPLC column	1.50439.0001	3.5 µm	100 A	20 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50440.0001	3.5 µm	100 A	50 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50441.0001	3.5 µm	100 A	100 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50442.0001	3.5 µm	100 A	150 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50443.0001	3.5 µm	100 A	250 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50445.0001	3.5 µm	200 A	50 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50447.0001	3.5 µm	200 A	100 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50448.0001	3.5 µm	200 A	150 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50450.0001	5 µm	200 A	50 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50452.0001	5 µm	200 A	100 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK HPLC column	1.50454.0001	5 µm	200 A	150 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK validation kit (3 columns of 3 different sorbent batches)	1.504540.1003*	5 µm	200 A	150 mm	2.1 mm	3 pieces
ZIC®-HILIC PEEK HPLC column	1.50457.0001	5 µm	200 A	250 mm	2.1 mm	1 piece
ZIC®-HILIC PEEK fitting guard column (5-pak)	1.5434.001	5 µm	200 A	14 mm	1 mm	5 pieces
ZIC®-HILIC guard column (1-rd column (5-pak)	1.50435.0001	5 µm	200 A	20 mm	2.1 mm	1 piece
ZIC®-HILIC guard column incl. column coupler	1.5436.001	5 µm	200 A	20 mm	2.1 mm	3 pieces



## SeQuant® ZIC®-HILIC Nano, Capillary and Microbore columns

Product	Ordering No.	Particle size	Porosity	Dimension length	Dimension i.d.	Contents of one package
ZIC®-HILIC Microbore column	1.50487.0001	3.5 µm	100 A	150 mm	1 mm	1 piece
ZIC®-HILIC Microbore column	1.50478.0001	3.5 µm	200 A	30 mm	1 mm	1 piece
ZIC®-HILIC Microbore column	1.50480.0001	3.5 µm	200 A	150 mm	1 mm	1 piece
ZIC®-HILIC Nano column	1.50466.0001	3.5 µm	200 A	100 mm	100 µm	1 piece
ZIC®-HILIC Capillary column	1.50489.0001	3.5 µm	200 A	30 mm	300 µm	1 piece
ZIC®-HILIC Capillary column	1.50479.0001	3.5 µm	200 A	150 mm	300 µm	1 piece
ZIC®-HILIC Microbore column	1.50482.0001	5 µm	200 A	150 mm	1 mm	1 piece
ZIC®-HILIC Nano column	1.50465.0001	5 µm	200 A	150 mm	75 µm	1 piece
ZIC®-HILIC Capillary column	1.50491.0001	5 µm	200 A	30 mm	300 µm	1 piece
ZIC®-HILIC Capillary column	1.50481.0001	5 µm	200 A	150 mm	300 µm	1 piece
ZIC®-HILIC guard column (5-pak)	1.50492.0001	5 µm	200 A	5 mm	300 µm	5 pieces
ZIC®-HILIC guard column (5-pak)	1.50490.0001	5 µm	200 A	5 mm	1 mm	5 pieces

## SeQuant® ZIC®-cHILIC analytical PEEK columns

Product	Ordering No.	Particle size	Porosity	Dimension length	Dimension i.d.	Contents of one package
ZIC®-cHILIC PEEK HPLC column	1.50656.0001	3 µm	100 A	50 mm	2.1 mm PEEK	1 piece
ZIC®-cHILIC PEEK HPLC column	1.50657.0001	3 µm	100 A	100 mm	2.1 mm PEEK	1 piece
ZIC®-cHILIC PEEK HPLC column	1.50658.0001	3 µm	100 A	150 mm	2.1 mm PEEK	1 piece
ZIC®-cHILIC PEEK guard kit incl. column coupler	1.50664.0001	5 µm	100 A	20 mm	2.1 mm PEEK	3 pieces

## SeQuant® ZIC®-cHILIC Capillary columns

Product	Ordering No.	Particle size	Porosity	Dimension length	Dimension i.d.	Column hardware	Contents of one package
ZIC®-cHILIC Capillary column	1.50669.0001	3 µm	100 A	150 mm	300 µm	GL-SS	1 piece
ZIC®-cHILIC Capillary column	1.50670.0001	3 µm	100 A	150 mm	1 mm	GL-SS	1 piece
ZIC®-cHILIC Capillary guard	1.50665.0001	5 µm	100 A	5 mm	300 µm	GL-SS	3 pieces
ZIC®-cHILIC Capillary guard	1.50666.0001	5 µm	100 A	5 mm	1 mm	GL-SS	3 pieces

## Ordering information – SeQuant® ZIC®-pHILIC PEEK columns

Product	Ordering No.	Particle size	Dimension length	Dimension i.d.	Contents of one package
ZIC®-pHILIC PEEK HPLC column	1.50459.0001	5 µm polymeric	50 mm	2.1 mm	1 piece
ZIC®-pHILIC PEEK HPLC column	1.50462.0001	5 µm polymeric	100 mm	2.1 mm	1 piece
ZIC®-pHILIC PEEK HPLC column	1.50460.0001	5 µm polymeric	150 mm	2.1 mm	1 piece
ZIC®-pHILIC Guard column (1-pak)	1.50437.0001	5 µm polymeric	20 mm	2.1 mm	1 piece
ZIC®-pHILIC Guard column incl. column coupler (3-pak)	1.50438.0001	5 µm polymeric	20 mm	2.1 mm	3 pieces

# Chiral LC-MS Columns

## Astec® CHIROBIOTIC® chiral stationary phases

### Key Features and Benefits

- Versatile, robust chiral HPLC and LC-MS separations
- Amenable to aqueous samples and mobile phases
- Wide applicability, especially suited to polar and ionizable compounds
- Covalently bonded chiral selector for rugged operation

### Ideally Suited for LC-MS of Polar, Ionizable and Neutral Drugs and Biomolecules

Highly enantioselective Astec® CHIROBIOTIC® CSPs (chiral stationary phases) are based on macrocyclic glycopeptides that have been bonded through multiple covalent linkages to high-purity silica particles. CHIROBIOTIC® columns separate the enantiomers of many drugs and biochemical compounds, like amino acids, that cannot be separated by other CSPs. Their most relevant attribute to bioanalysis is the presence of ionic interactions. This allows CHIROBIOTIC® columns to be used with polar ionic (polar organic solvents containing salts) and reversed-phase mobile phases for sensitive LC-MS operation, where analyte ionization and detection sensitivity are of critical concern. Due to the fact that the stationary phase is covalently bonded to the silica surface means CHIROBIOTIC® columns have exceptional stability and long column life, even with repeated injections of biological samples.

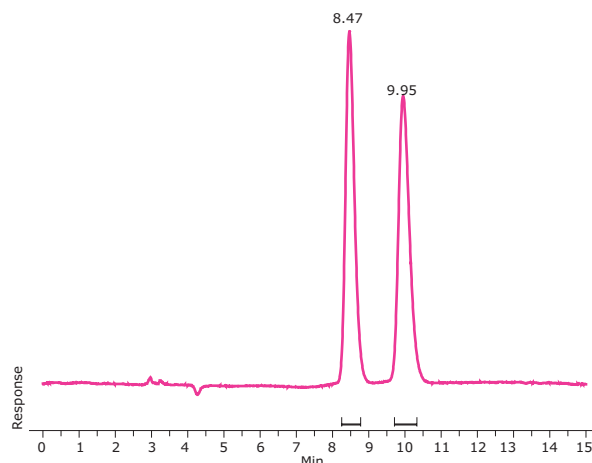
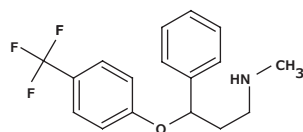
### Astec® CHIROBIOTIC® Application Areas

- **Drug Discovery** – High enantioselectivity, fast screening protocols, scalability to prep, reproducibility for reliable methods, effective for both polar and nonpolar analytes
- **Clinical, Bioanalytical, Drug Metabolism** – High throughput, MS-compatibility, aqueous samples, short run times, rugged columns
- **Amino Acid and Peptide Analysis** – Resolves underivatized natural and synthetic chiral amino acids and peptides

Want to learn more about chiral HPLC column selection and method development? Download our wall chart at [SigmaAldrich.com/chiral](https://www.sigmaaldrich.com/chiral)

### Separation of Fluoxetine Enantiomers on Astec® CHIROBIOTIC® V2

Column	Astec® CHIROBIOTIC® V2, 25 cm x 4.6 mm I.D., 5 µm (15024AST)
Mobile phase	15 mM ammonium acetate in methanol (LC-MS Ultra)
Flow rate	1 mL/min
Column Temp	25°C
Detector	UV, 230 nm
Injection	5 µL, 1 mg/mL fluoxetine in methanol (Cerilliant®, F-918)



### Chiral Column Selection

Astec® CHIROBIOTIC® CSPs are based on 5, 10 or 16 µm, high purity, porous silica gel. They differ in the nature of the bonded macrocyclic glycopeptide and resulting enantioselectivity.

- Astec® CHIROBIOTIC® V and V2 – Vancomycin
- Astec® CHIROBIOTIC® T and T2 – Teicoplanin

- Astec® CHIROBIOTIC® R – Ristocetin
- Astec® CHIROBIOTIC® TAG – Teicoplanin Aglycone

For additional information, request our “Chiral Method Development Wall Chart” at **SigmaAldrich.com/chiral**

### Astec® CHIROBIOTIC® Columns

Many more dimensions are available. Please call or consult **SigmaAldrich.com/chiral**

Particle Size	I.D. (mm)	Length (cm)	V	V2	T	T2	TAG	R
5 µm	2.1	10	11018AST	15018AST	12018AST	16018AST	14018AST	13018AST
5 µm	2.1	15	11019AST	15019AST	12019AST	16019AST	14019AST	13019AST
5 µm	2.1	25	11020AST	15020AST	12020AST	16020AST	14020AST	13020AST
5 µm	4.6	10	11022AST	15022AST	12022AST	16022AST	14022AST	13022AST
5 µm	4.6	25	11024AST	15024AST	12024AST	16024AST	14024AST	13024AST

### Method Development Kit

Contains one column each of Astec® CHIROBIOTIC® V2, T, TAG and R

Particle Size	I.D.	Length	Cat. No.
5 µm	4.6	10	10300AST
5 µm	4.6	25	10305AST

# LC-MS Sample Preparation

## HybridSPE® - Phospholipid for Phospholipid and Protein Removal

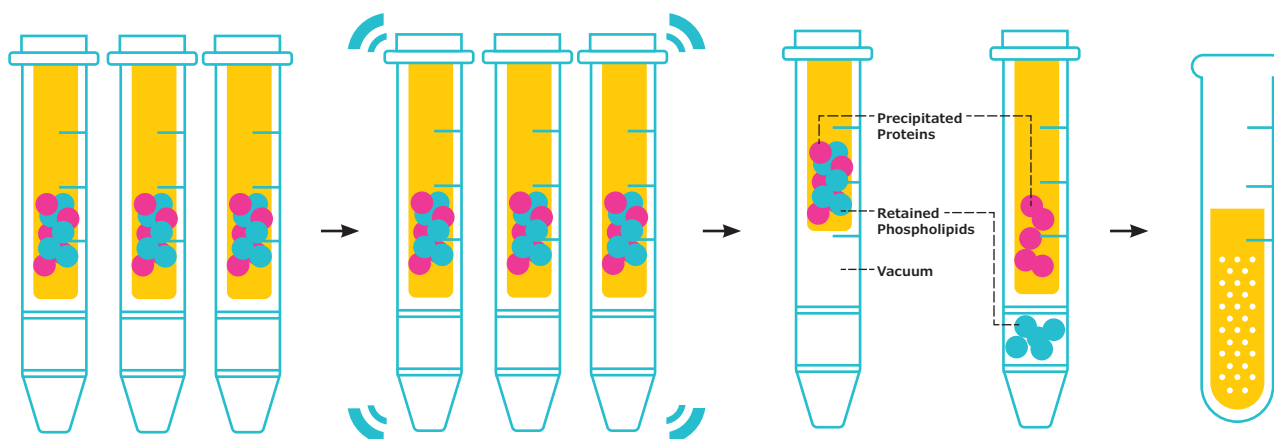
HybridSPE® - Phospholipid (HybridSPE® - PL) combines the simplicity of protein precipitation with the selectivity of solid phase extraction (SPE) for the targeted removal of phospholipids in biological plasma or serum. The technology utilizes a zirconia-coated particle, and exhibits selective affinity towards phospholipids while remaining non-selective towards a range of basic, acidic and neutral compounds. The phospholipid retention mechanism is based on a highly selective Lewis acid-base interaction between the proprietary zirconia coating (functionally bonded to the HybridSPE® stationary phase) and the phosphate moiety present in all phospholipids.



### Key Features and Benefits

- Merges both protein precipitation and SPE
- Offers the simplicity of protein precipitation
- Selectively removes phospholipids via Lewis acid-base interactions
- 2–3 step generic procedure
- Typically >98% removal of phospholipids and precipitated proteins
- Minimal to no method development required

### HybridSPE®-PL “In-well” Method



#### 1. Precipitate Proteins

By adding 100  $\mu$ L plasma or serum to the HybridSPE®-PL plate followed by 300  $\mu$ L 1% formic acid in acetonitrile. Add I.S. as necessary.

#### 2. Mix

By vortexing/shaking HybridSPE®-PL plate or by aspirating/dispensing with 0.5–1 mL pipette tip (e.g., TOMTEC Quadra liquid handler).

#### 3. Apply Vacuum

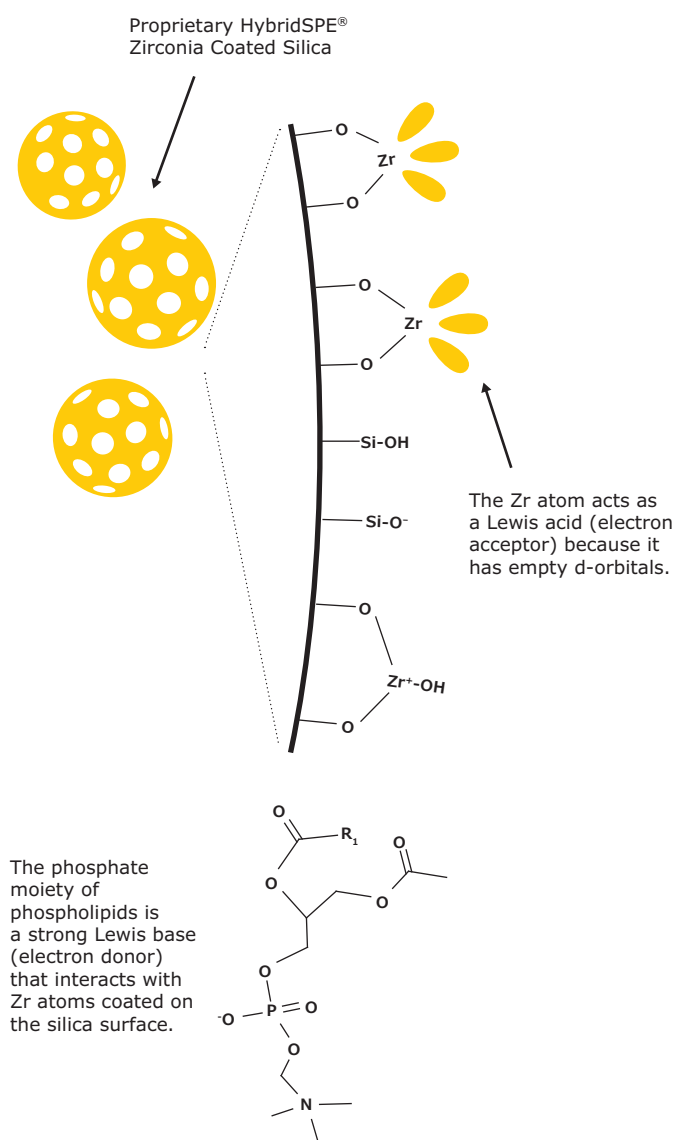
The packed-bed filter/frit assembly acts as a depth filter for the concurrent physical removal of precipitated proteins and chemical removal of phospholipids. Small molecules (e.g., pharma compounds and metabolites) pass through unretained.

#### 4. Resulting filtrate/eluante

Is free of proteins and phospholipids and ready for immediate LC-MS/MS analysis; or it can be evaporated and reconstituted as necessary prior to analysis.

Description	Qty.	Cat. No.
HybridSPE®-PLus Plate Essentials Kit	1	52818-U
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)		
<b>HybridSPE®-PLus 96-Well Plates</b>		
HybridSPE®-PLus 96-Well Plate, 50 mg/well	1	575659-U
HybridSPE®-PLus 96-Well Plate, 50 mg/well	20	575673-U
<b>HybridSPE®-Phospholipid Small Volume 96-Well Plates</b>		
HybridSPE®-Phospholipid Small Volume 96-Well Plate, 15 mg/well	1	52794-U
HybridSPE®-Phospholipid Small Volume 96-Well Plate, 15 mg/well	20	52798-U
<b>HybridSPE®-Phospholipid Cartridges</b>		
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	200	55276-U
<b>Plate Accessories</b>		
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
<b>Cartridge Accessories</b>		
<b>Visiprep™ DL Solid Phase Extraction Cartridge Manifold</b>		
12 Port Model	1	57044
24 Port Model	1	57265
<b>Visiprep™ Solid Phase Extraction Cartridge Manifold</b>		
12 Port Model	1	57030-U
24 Port Model	1	57250-U
Disposable Valve Liners, PTFE (for Visiprep™ DL Manifold)	100	57059
<b>Equipment</b>		
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

## Lewis Acid-Base Interactions Between HybridSPE® Zirconia and Phospholipids



# Automated SPE with HybridSPE® DPX® Tips

## Extraction in Seconds

DPX® stands for Dispersive Pipette EXtraction. HybridSPE® DPX® Tips are pipette tips that incorporate loosely contained HybridSPE® sorbent material that is mixed with the sample solution when aspirated to accomplish solid phase extraction. HybridSPE® technology is a simple and generic sample prep platform designed for the gross level removal of endogenous phospholipid interferences from biological plasma and serum prior to LC-MS or LC-MS/MS analysis (see page 8).

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®-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

To learn more about our HybridSPE DPX Tips, visit

[SigmaAldrich.com/DPX](https://www.sigmaaldrich.com/DPX)

Figure 7. HybridSPE® DPX® Tips



**The unique mixing technique employed provides numerous advantages:**

- Minimal elution solvent volumes
- Rapid extraction times (less than 3 min. per sample/wellplate)
- High extraction efficiencies
- Easy to perform extractions
- Lower costs
- Higher throughput
- Minimal training required
- Environmentally friendly

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



# Go Hands Free with Online SPE

## 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” using the LC instrument. Samples are directly injected onto the SPE cartridge located on the 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 with greater reproducibility
- Clean samples leading to
  - Greater column life
  - Less instrument downtime
  - More accurate and reproducible data

### We currently offer 3 phase chemistries:

- **HybridSPE®** - for complete removal of phospholipids (a leading cause of matrix effects) from biological samples (see previous pages for mechanisms)
- **C8** - for reversed-phase extraction of hydrophobic or nonpolar to moderately polar compounds
- **RP-Amide** - for reversed-phase extraction of nonpolar to polar compounds, compared to pure alkyl phases offers improved retention & performance for polar analytes, especially those that can interact via hydrogen bonding

Figure 8. Supel™ Genie HybridSPE® online starter kit (55324-U)



Figure 9. Supel™ Genie C8 online SPE cartridges, 2 pack (55512-U)



## Supel™ Genie Online SPE Cartridges

HybridSPE® phase offers complete phospholipid removal from biological samples:

Figure 10. Phospholipids in Plasma Sample without Supel™ Genie HybridSPE® Online Cartridge (1<sup>st</sup> injection)

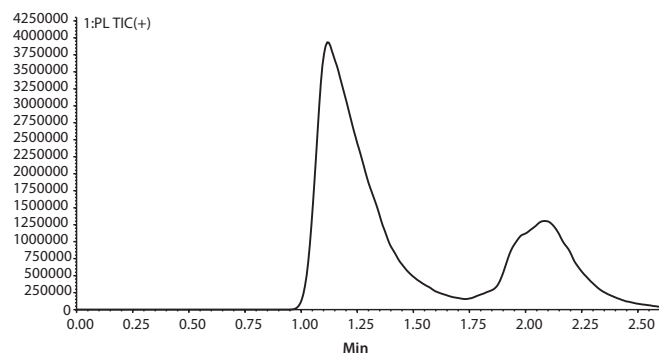
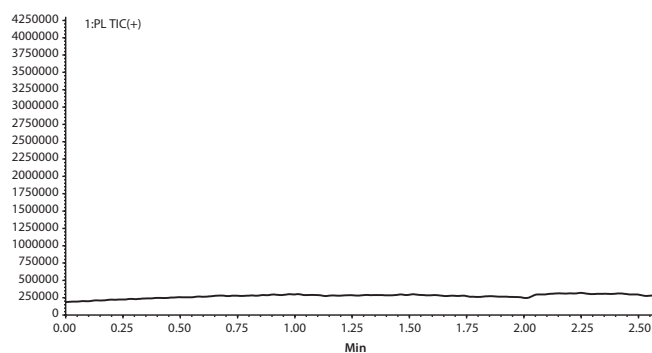


Figure 11. Phospholipids in Plasma Sample with Supel™ Genie HybridSPE® Online Cartridge (120<sup>th</sup> injection)



Check out our other applications at [SigmaAldrich.com/onlinespe](https://SigmaAldrich.com/onlinespe)

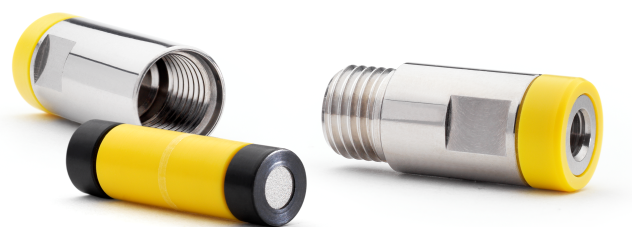
Starter Kits come with reusable hardware that will fit any Supel™Genie cartridge, as well as one cartridge of the selected phase chemistry. Additional cartridge packs include cartridges only.

### HybridSPE® Products

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

### RP-Amide & C8 Products

Description	Cat. No.
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



Need help choosing? Want more information on initial setup?

Our Tech Service Team is happy to help get you started.

Email our experts at: [techserv@sial.com](mailto:techserv@sial.com)

For more information or to order, visit: [SigmaAldrich.com/onlinespe](https://SigmaAldrich.com/onlinespe)

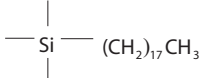
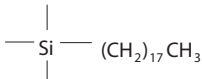
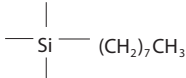
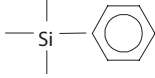
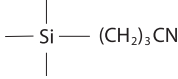
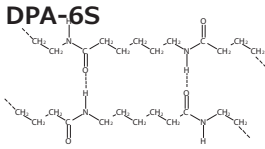
# Discovery® SPE

## Reversed-Phase

Discovery® reversed-phase SPE products are specifically developed, tested and quality controlled for pharmaceutical and clinical applications. Experience greater and more reproducible recoveries for the quick and effective extraction, isolation and concentration

of pharmaceuticals from biological fluids and other aqueous sample matrices.

For Discovery® silica specifications, see page 2. For general guidelines on reversed-phase SPE, see page 49.

<b>DSC-18</b> 	<ul style="list-style-type: none"> <li>• Polymerically bonded, octadecyl (18% C), endcapped</li> <li>• Higher 18% C loading for increased binding capacities and higher recoveries</li> <li>• The least selective phase: retains most organic analytes from aqueous matrices</li> <li>• Beneficial for extracting numerous analytes diverse in structure from the same sample</li> </ul>
<b>DSC-18Lt</b> 	<ul style="list-style-type: none"> <li>• Monomerically bonded, octadecyl (11% C), endcapped</li> <li>• Increased retention for moderately polar hydrophobic molecules</li> <li>• Used to elute very large hydrophobic molecules that are too strongly retained on DSC-18. Use this less retentive phase for the rapid release of hydrophobic compounds using weaker organic solvents at lower volumes</li> </ul>
<b>DSC-8</b> 	<ul style="list-style-type: none"> <li>• Monomerically bonded, octyl (9% C), endcapped; lower carbon content than DSC-18Lt</li> <li>• Used to elute very large hydrophobic molecules too strongly retained on DSC-18 or DSC-18Lt</li> <li>• Use this less retentive phase for the rapid release of hydrophobic molecules using weaker organic solvents at lower volumes</li> </ul>
<b>DSC-Ph</b> 	<ul style="list-style-type: none"> <li>• Monomerically bonded, phenyl (7% C), endcapped</li> <li>• Similar in polarity to DSC-8; however, electron dense aromatic ring offers some unique selectivity and retention</li> </ul>
<b>DSC-CN</b> 	<ul style="list-style-type: none"> <li>• Monomerically bonded, cyanopropyl (7% C), endcapped</li> <li>• Can behave as either reversed-phase or normal-phase</li> <li>• Ideal for very hydrophobic analytes that may be irreversibly retained on more hydrophobic sorbents such as DSC-18</li> <li>• Less retentive than DSC-Si or DSC-Diol when used as normal phase (organic matrices such as hexane or oils)</li> <li>• Allows for the rapid release of very polar molecules irreversibly retained on very polar sorbents</li> </ul>
<b>DPA-6S</b> 	<ul style="list-style-type: none"> <li>• Polyamide Resin: Particle Size: 50-160 µm, Surf pH: 4.5-7.5, Density: 0.2-0.3 cm³/g, Water Content: &lt;5%</li> <li>• Used to adsorb polar compounds (-OH groups, esp. phenolic compounds) from aqueous or methanolic solutions under the reversed-phase mechanism through strong hydrogen bonding between compound hydroxyl groups and amide groups of the resin</li> <li>• Useful for extracting tannins, chlorophyll, humic acid, pharmacologically active terpenoids, flavonoids, gallic acid, catechol A, protocatechuic acid and phloroglucinol</li> <li>• Also useful for extracting aromatic carboxylic acids, nitroaromatic compounds and irreversibly retains quinones</li> </ul>

### Discovery® Reversed-Phase SPE Products

Description	Qty.	DSC-18	DSC-18Lt	DSC-8	DSC-Ph	DSC-CN	DPA-6S
<b>Discovery® SPE Tubes</b>							
50 mg/1 mL	108	52601-U	Custom	52703-U	Custom	Custom	52624-U
100 mg/1 mL	108	52602-U	52611-U	52707-U	Custom	52694-U	Custom
500 mg/3 mL	54	52603-U	52613-U	52713-U	52727-U	52695-U	<sup>1</sup> 52625-U
500 mg/6 mL	30	52604-U	52615-U	52714-U	52728-U	52696-U	<sup>2</sup> 52626-U
1 g/6 mL	30	52606-U	52616-U	52716-U	Custom	52697-U	<sup>3</sup> 52627-U
2 g/12 mL	20	52607-U	52618-U	Custom	Custom	52698-U	<sup>4</sup> 52629-U
5 g/20 mL	20	52608-U	Custom	Custom	Custom	Custom	<sup>5</sup> 52631-U
10 g/60 mL	16	52609-U	Custom	Custom	Custom	Custom	Custom
<b>Discovery® SPE 96-Well Plates</b>							
100 mg/well	1	575603-U	Custom	Custom	Custom	Custom	Custom
50 mg/well	1	Custom	Custom	Custom	Custom	Custom	Custom
25 mg/well	1	575601-U	Custom	Custom	Custom	Custom	Custom
<b>Bulk Packing</b>							
100 g		52600-U					<sup>6</sup> 52633-U

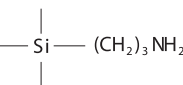
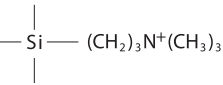

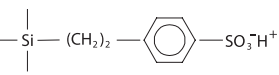
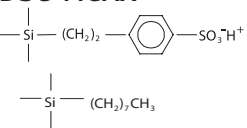
<sup>1</sup> 250 mg/3 mL, <sup>2</sup> 250 mg/6 mL, <sup>3</sup> 500 mg/6 mL, <sup>4</sup> 1 g/12 mL, <sup>5</sup> 2 g/20 mL, <sup>6</sup> 50 g

## Ion-Exchange and Mixed-Mode

Discovery® ion-exchange SPE products are specifically developed, tested and quality controlled for pharmaceutical and clinical applications. The Discovery® ion-exchange product line offers excellent selectivity towards charged molecular species enabling the user to extract, isolate, purify and concentrate charged ionizable pharmaceuticals (basic or acidic) from both polar and non-polar sample matrices.

Use mixed-mode SPE (e.g., Discovery® DSC-MCAX) for superior cleanup and selectivity when extracting basic pharmaceutical compounds from biological matrices such as plasma and urine.

For Discovery® silica specifications, see page 2. For general guidelines on ion-exchange and mixed-mode SPE, see page 50.

<b>DSC-NH<sub>2</sub></b> 	<ul style="list-style-type: none"> <li>• Polymerically bonded aminopropyl phase that is very polar in nature (hydrogen bonding) allowing for both normal-phase and ion-exchange applications</li> <li>• A weak anion exchanger with a pK<sub>a</sub> of 9.8. At pH 7.8 or below, the functional groups are positively charged</li> <li>• Allows the rapid release of very strong anions such as sulfonic acids that may be retained irreversibly on SAX</li> <li>• Can be used in some reversed-phase applications (due to ethyl spacer); however, it is predominately used as an ion-exchanger or normal-phase sorbent due to its polar nature</li> </ul>
<b>DSC-SAX</b> 	<ul style="list-style-type: none"> <li>• A polymerically bonded quarternary amine that remains positively charged at all pH levels</li> <li>• Strong anion ion exchanger, commonly used when extracting weaker cations (e.g., carboxylic acids) that may not bind strongly enough to weaker anion exchangers</li> <li>• Selectivity can be modified by changing the counter ion with the appropriate buffer during conditioning</li> <li>• Counter ion is Cl<sup>-</sup></li> </ul>
<b>DSC-WCX</b> 	<ul style="list-style-type: none"> <li>• A polymerically bonded carboxy propyl phase with a K<sup>+</sup> counter ion and a pK<sub>a</sub> of 4.8</li> <li>• Its weak cation exchange properties carries a negative charge at pH 6.8 or above</li> <li>• A pH of 2.8 or below neutralizes this phase for easier elution of strong cationic analytes that are neutralized only at extreme basic conditions</li> <li>• Typically used when dealing with very strong cationic (high pK<sub>a</sub>) compounds that may be irreversibly retained on strong cation exchangers</li> </ul>
<b>DSC-SCX</b> 	<ul style="list-style-type: none"> <li>• A polymerically bonded, benzene sulfonic acid functional group with a H<sup>+</sup> counter ion that is a strong cation exchanger due to its very low pK<sub>a</sub> (&lt;1.0)</li> <li>• Silica support allows for use with all common organic solvents (no shrinking/swelling)</li> <li>• Excellent capacity (0.8 meq/g) for cleaning up solution phase combinatorial chemistry reactions (removing target molecules from reaction by-products and excess reagents)</li> <li>• The presence of the benzene ring offers some mixed-mode capabilities (hydrophobic interactions) that should be considered when extracting cations from aqueous matrices</li> </ul>
<b>DSC-MCAX</b> 	<ul style="list-style-type: none"> <li>• Packed bed contains both octyl (C8) and benzene sulfonic acid (SCX) bondings. (H<sup>+</sup> as counterion)</li> <li>• Developed for superior selectivity/sample cleanup when isolating basic compounds from biological fluids</li> <li>• Dual retention mechanisms broadens retention for a range of neutral, basic, acidic and zwitterionic compounds</li> <li>• Greater ion-exchange capacity for isolating polar basic and zwitterionic compounds</li> <li>• Can be used to fractionate basic/zwitterionic compounds from acidic and neutral compounds</li> </ul>

### Discovery® Ion-Exchange SPE Products

Description	Qty.	DSC-NH <sub>2</sub>	DSC-SAX	DSC-WCX	DSC-SCX	DSC-MCAX
<b>Discovery® SPE Tubes</b>						
50 mg/1 mL	108	52635-U	52661-U	52737-U	52684-U	52781-U
100 mg/1 mL	108	52636-U	52662-U	52739-U	52685-U	52782-U
500 mg/3 mL	54	52637-U	52664-U	52741-U	52686-U	52783-U <sup>1</sup>
500 mg/6 mL	30	52638-U	52665-U	Custom	52688-U	52784-U <sup>2</sup>
1 g/6 mL	30	52640-U	52666-U	52743-U	52689-U	52788-U, 52786-U <sup>3</sup>
2 g/12 mL	20	52641-U	52667-U	Custom	52690-U	—
5 g/20 mL	20	Custom	Custom	Custom	52691-U	—
10 g/60 mL	16	Custom	Custom	Custom	52692-U	—
<b>Discovery® SPE 96-Well Plates</b>						
100 mg/well	1	575615-U	Custom	Custom	Custom	Custom
50 mg/well	1	Custom	Custom	Custom	Custom	Custom
25 mg/well	1	Custom	Custom	Custom	Custom	Custom
<b>Bulk Packing</b>						
	100 g	57212-U	57214-U	57228-U	57221-U	—

<sup>1</sup> 3 mL/100 mg, pk 54, <sup>2</sup> 300 mg/3 mL, pk 54, <sup>3</sup> 300 mg/6 mL, pk 30

## Normal-Phase

Discovery® normal-phase SPE products are specifically developed, tested and quality controlled for normal phase pharmaceutical applications and other modified flash techniques. The Discovery® normal phase product line enables you to quickly and effectively extract, isolate, purify and concentrate polar compounds from non-polar solutions. Its highly selective properties allow

the user to separate or remove structurally similar molecules through successive wash/elutions with increasingly polar solutions.

For Discovery® silica specifications, see page 2.

For general guidelines on normal-phase SPE, see page 51.

<b>DSC-Si</b> $\begin{array}{c}   \\ \text{—Si—OH} \\   \end{array}$	<ul style="list-style-type: none"> <li>Unbonded acid washed silica sorbent ideal for normal-phase SPE and other modified flash techniques</li> <li>Considered the most polar normal-phase sorbent available</li> <li>Excellent capacity for purifying solution phase CombiChem reactions when removing target molecules from reaction by-products and excess reagents</li> </ul>
<b>DSC-Diol</b> $\begin{array}{c} \text{OH} \quad \text{OH} \\   \quad   \\ \text{—Si—(CH}_2\text{)}_3\text{CH—CH}_2 \\   \end{array}$	<ul style="list-style-type: none"> <li>Polymerically bonded, 2,3-Dihydroxypropoxypropyl (7% C)</li> <li>Polar sorbent most commonly used for normal-phase applications (polar extractions from non-polar matrices)</li> <li>The sorbent's dihydroxy groups facilitate strong hydrogen bonding</li> <li>Excellent selectivity when extracting structurally similar molecules</li> </ul>
<b>DSC-CN</b> $\begin{array}{c}   \\ \text{—Si—(CH}_2\text{)}_3\text{CN} \\   \end{array}$	<ul style="list-style-type: none"> <li>Monomerically bonded, cyanopropyl (7% C), endcapped</li> <li>Can behave as either reversed-phase or normal-phase</li> <li>Ideal for very hydrophobic analytes that may be irreversibly retained on more hydrophobic sorbents such as DSC-18</li> <li>Less retentive than DSC-Si or DSC-Diol when used as normal-phase (organic matrices such as hexane or oils)</li> <li>Allows for the rapid release of very polar molecules irreversibly retained on very polar sorbents</li> </ul>
<b>DSC-NH<sub>2</sub></b> $\begin{array}{c}   \\ \text{—Si—(CH}_2\text{)}_3\text{NH}_2 \\   \end{array}$	<ul style="list-style-type: none"> <li>Polymerically bonded, aminopropyl phase that is very polar in nature (hydrogen bonding) allowing for both normal-phase and ion-exchange applications</li> <li>A weak anion exchanger with a pK<sub>a</sub> of 9.8. At pH 7.8 or below, the functional groups are positively charged</li> <li>Allows the rapid release of very strong anions such as sulfonic acids that may be retained irreversibly on SAX (a quarternary amine sorbent that is always positively charged)</li> <li>Can be used in some reversed-phase applications (due to ethyl spacer); however, it is predominately used as an ion-exchanger or normal-phase sorbent due to its polar nature</li> </ul>

### Discovery® Normal-Phase SPE Products

Description	Qty.	DSC-CN	DSC-Si	DSC-Diol	DSC-NH <sub>2</sub>
<b>Discovery® SPE Tubes</b>					
50 mg/1 mL	108	52693-U	52652-U	Custom	52635-U
100 mg/1 mL	108	52694-U	52653-U	52748-U	52636-U
500 mg/3 mL	54	52695-U	52654-U	52751-U	52637-U
500 mg/6 mL	30	52696-U	52655-U	52752-U	52638-U
1 g/6 mL	30	52697-U	52656-U	52753-U	52640-U
2 g/12 mL	20	Custom	52657-U	Custom	52641-U
5 g/20 mL	20	52699-U	52658-U	Custom	52642-U
10 g/60 mL	16	52700-U	52659-U	Custom	52644-U
<b>Discovery® SPE 96-Well Plates</b>					
100 mg/well	1	Custom	Custom	Custom	575615-U
50 mg/well	1	Custom	575608-U	Custom	Custom
25 mg/well	1	Custom	Custom	Custom	Custom
<b>Bulk Packing</b>					
	100 g	Custom	Custom	Custom	57212-U



# Supel™ – Select Polymeric SPE

## For Routine Aqueous Sample Clean Up

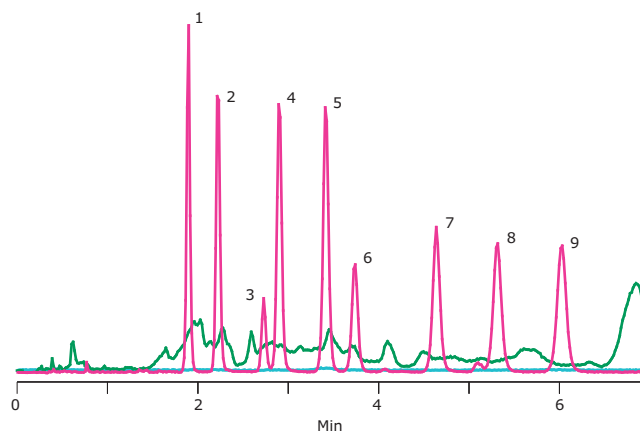
Hydrophilic-lipophilic balance and Ion-Exchange phases for a wide range of applications and pH conditions.

- Ideal for the solid phase extraction of a broad range of compounds from aqueous samples
- HLB Phase Chemistry: Hydrophilic modified styrene polymer
- SAX Phase Chemistry: Quaternary amine functionalized hydrophilic modified styrene polymer

- SCX Phase Chemistry: Sulfonic acid functionalized hydrophilic modified styrene polymer
- pH Compatibility: 0 - 14
- Particle Size: 50 - 70 µm
- MS Suitability: Yes
- Surface Area: 160 - 420 m<sup>2</sup>/g
- Pore Volume: 0.8 - 1.2 mL/g
- Pore Size: 80 - 200 Å

### Urine Sample Clean Up with Supel™ – Select SPE

<b>Sample/matrix</b>	1 mL urine spiked to 100 ng/mL of bath salt mixture
<b>SPE tube</b>	Supel™ – Select SCX, 30 mg/1 mL (54240-U)
<b>Conditioning</b>	1 mL 1% formic acid in acetonitrile, then 1 mL water
<b>Sample addition</b>	1 mL spiked urine
<b>Washing</b>	1 mL water, 1 mL 1% formic acid in acetonitrile, 1 mL water
<b>Elution</b>	2 mL 10% ammonium hydroxide in acetonitrile
<b>Eluate post-treatment</b>	thoroughly mix via vortex agitation, evaporate 1 mL aliquot to dryness, reconstitute in 100 µL water: methanol
<b>Column</b>	Ascentis® Express HILIC (Si), 10 cm x 2.1 mm I.D., 2.7 µm (53939-U)
<b>Mobile phase</b>	(A) 5 mM ammonium formate acetonitrile; (B) 5 mM ammonium formate water; (98:2, A:B)
<b>Flow rate</b>	0.6 mL/min
<b>Pressure</b>	127 bar
<b>Column temp</b>	35 °C
<b>Detector</b>	MS, ESI+, 100-1000 m/z
<b>Injection</b>	1 µL
<b>Sample</b>	200 ng/mL in acetonitrile



- Spiked Urine Sample Monitored Ions After Supel™ – Select SCX Cleanup  
 ■ Diluted Spiked Urine Monitored Ions Without SPE Cleanup  
 ■ Urine Blank Monitored Ions After Supel™ – Select SCX Cleanup
1. 3,4-Methylenedioxypyrovalerone (MDPV)
  2. Buphedrone
  3. 3-Fluoromethcathinone
  4. Butylone
  5. Ethylone
  6. 4-Fluoromethcathinone
  7. Mephedrone
  8. Methylone
  9. Methedrone

## Supel™-Select SPE Products

Description	Qty.	Cat. No.
<b>Supel™ – Select HLB 96-well SPE</b>		
10 mg/ well	1	<b>Inquire</b>
30 mg /well	1	<b>575661-U</b>
60 mg/ well	1	<b>575662-U</b>
<b>Supel™ – Select SAX 96-well SPE</b>		
10 mg/well	1	<b>Inquire</b>
30 mg/well	1	<b>575660-U</b>
60 mg/well	1	<b>575663-U</b>
<b>Supel™ – Select SCX 96-well SPE</b>		
10 mg/well	1	<b>Inquire</b>
30 mg/well	1	<b>575664-U</b>
60 mg/well	1	<b>575665-U</b>
<b>Supel™ – Select HLB SPE</b>		
30 mg/1 mL	100	<b>54181-U</b>
60 mg/3 mL	50	<b>54182-U</b>
200 mg/6 mL	30	<b>54183-U</b>

Description	Qty.	Cat. No.
500 mg/12 mL	20	<b>54184-U</b>
1 g/20 mL	20	<b>54186-U</b>
<b>Supel™ – Select SAX SPE</b>		
30 mg/1 mL	100	<b>54231-U</b>
60 mg/3 mL	50	<b>54233-U</b>
200 mg/6 mL	30	<b>54235-U</b>
500 mg/12 mL	20	<b>54236-U</b>
1 g/20 mL	20	<b>54237-U</b>
<b>Supel™ – Select SCX SPE</b>		
30 mg/1 mL	100	<b>54240-U</b>
60 mg/3 mL	50	<b>54241-U</b>
200 mg/6 mL	30	<b>54242-U</b>
500 mg/12 mL	20	<b>54243-U</b>
1 g/20 mL	20	<b>54245-U</b>

For more information, visit  
[SigmaAldrich.com/supel-select](https://SigmaAldrich.com/supel-select)

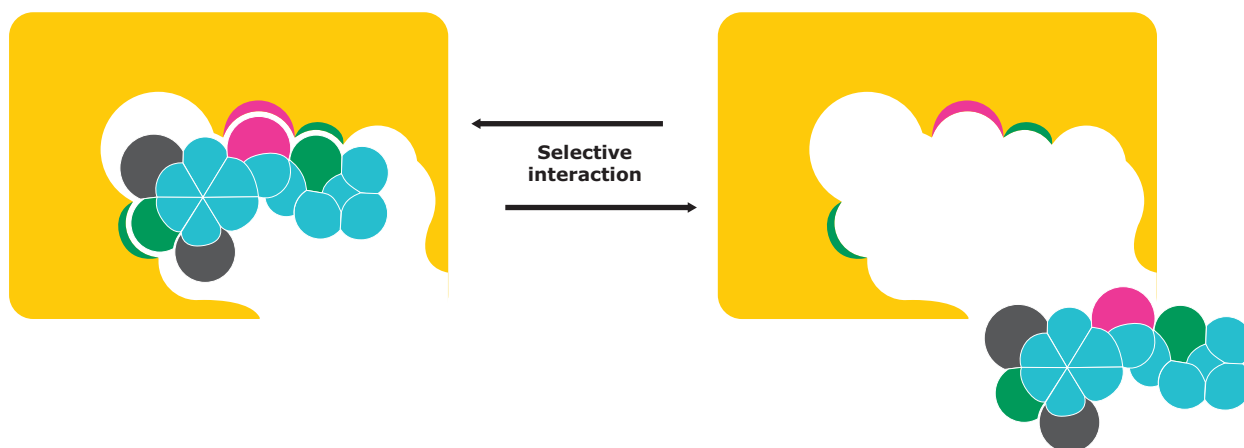
## SupelMIP® SPE: Molecularly Imprinted Polymers

The SupelMIP® line of solid phase extraction (SPE) products comprises highly cross-linked polymers that are engineered to extract a single analyte of interest, or a class of structurally related analytes with

an extremely high degree of selectivity. The use of MIPs in sample preparation may result in decreased ion-suppression as well as the ability to reach lower detection limits.

### SupelMIP® Phases and Methods Available for:

- **PAHs** (polycyclic aromatic hydrocarbons) in edible oils
- **Nitroimidazoles** in milk, eggs, and other food matrices
- **Non-steroidal anti-inflammatory drugs** (NSAIDs) in wastewater and other sample matrices
- **Fluoroquinolones** in bovine kidney, honey, and milk
- **Chloramphenicol** in milk, plasma, honey, urine, and shrimp/prawns
- **NNAL** 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol in urine
- **TSNAs** (Tobacco Specific Nitrosamines) in urine and tobacco
- **β-agonists** in tissue, urine, and wastewater
- **Clenbuterol** in urine
- **Patulin** in fruit matrices
- **Aminoglycosides** in animal tissue, cell culture, and honey
- **Bisphenol A** (BPA) in broth or milk-based matrices

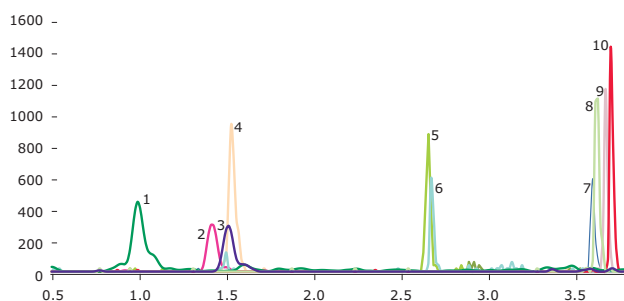


## LC-MS/MS Analysis of Aminoglycosides after SupelMIP® SPE Cleanup

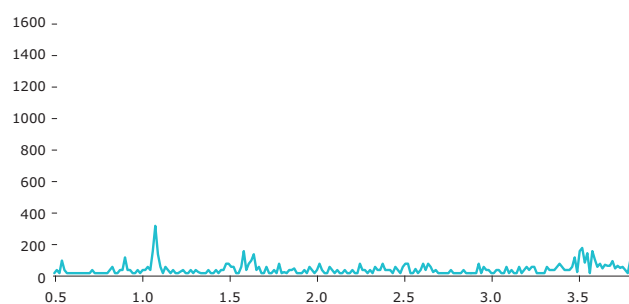
<b>Sample/matrix</b>	3 mL of pork extract (For additional information regarding this application, refer to an article from Supelco® Reporter 32.2 available at <a href="https://www.sigmaaldrich.com/supelmip">SigmaAldrich.com/supelmip</a> )
<b>SPE tube/cartridge</b>	SupelMIP® SPE – Aminoglycosides, 50 mg/3 mL (52777-U)
<b>Conditioning</b>	1 mL of methanol, then 1 mL of 50 mM potassium phosphate in water (pH = 7.8)
<b>Sample addition</b>	3 mL of pork extract
<b>Washing</b>	3 mL of water, followed by drying with slight vacuum for 10 seconds
<b>Washing</b>	1 mL of 50:50 dichloromethane:methanol (v/v), followed by drying with slight vacuum for 10 seconds
<b>Elution</b>	1 mL of 1% formic acid containing 5 mM heptafluorobutyric acid (HFBA) in 80:20 water:acetonitrile (v/v)
<b>Eluate post-treatment</b>	thoroughly mix via vortex agitation, and transfer to polypropylene HPLC vials
<b>Column</b>	Ascentis® Express C18, 10 cm × 2.1 mm I.D., 2.7 µm (53823-U)
<b>Mobile phase</b>	(A) 5mM heptafluorobutyric in water; (B) 5 mM heptafluorobutyric in acetonitrile
<b>Gradient</b>	20 to 90% B in 3.0 min; held at 90% B for 1 min; 90 to 20% B in 0.1 min; held at 20% B for 5.9 min
<b>Flow rate</b>	0.4 mL/min
<b>Column temp.</b>	40°C
<b>Detector</b>	MS/MS, ESI(+), MRM
<b>Injection</b>	10 µL

Analyte	Precursor	Product
Gentamicin C1	478.1	157.2
Streptomycin	582.1	263.2
Neomycin	615.0	161.1
Kanamycin	485.2	163.1
Tobramycin	468.1	163.1
Amikacin	586.2	163.1
Hygromycin B	528.1	177.1
Spectinomycin	351.1	333.1
Dihydrostreptomycin	584.2	263.1
Apramycin	540.2	217.1

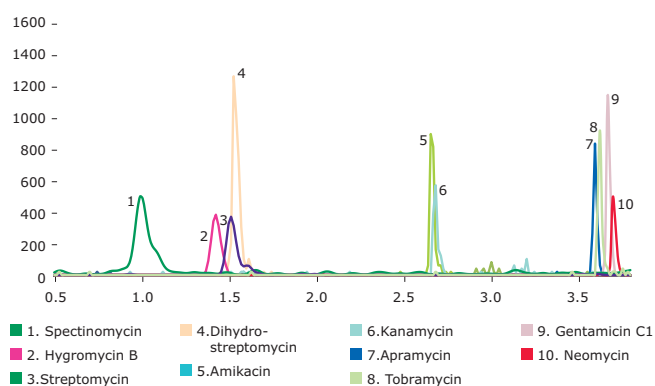
(a) Matrix Matched Standard



(b) Pork Muscle Blank



(c) Pork Muscle Spiked with 100 ng/g of Aminoglycosides



## ZipTip® Pipette Tips

### Concentrating and purifying samples for MALDI MS

The ZipTip® Pipette is a 10 µl pipette tip with a 0.6 or 0.2 µl bed of chromatography media fixed at its end. It is ideal for concentrating and purifying samples for sensitive analyses such as MALDI MS. The ZipTip® pipette tip provides a reproducible, high-recovery method for concentrating and purifying femtomoles to picomoles of peptides, proteins and oligonucleotides for improved analytical data. To simplify your analysis even further, you can fractionate complex peptide mixtures by step elution.

#### Features and Benefits

- Single-step desalting, concentration, and purification
- Fractionate complex samples for more meaningful data
- Ideal for peptides, proteins, nucleic acids, and more
- No dead volume for maximum recovery
- Eliminates time-consuming chromatography

#### Applications

- Mass Spectrometry including MALDI-TOF



Description	Qty/Pk	Cat. No.
<b>ZipTip® Pipette Tips</b>		
ZipTip® with 0.6 mL strong cation resin	8	ZTSCXS008
ZipTip® with 0.6 mL strong cation resin	96	ZTSCXS096
ZipTip® with 0.6 mL C4 resin	8	ZTC04S008
ZipTip® with 0.6 mL C4 resin	96	ZTC04S096
ZipTip® with 0.6 mL C4 resin	960	ZTC04S960
ZipTip® with 0.6 mL C18 resin	8	ZTC18S008
ZipTip® with 0.6 mL C18 resin	96	ZTC18S096
ZipTip® with 0.6 mL C18 resin	960	ZTC18S960
ZipTip® with 0.2 mL C18 resin	8	ZTC18M008
ZipTip® with 0.2 mL C18 resin	96	ZTC18M096
ZipTip® with 0.2 mL C18 resin	960	ZTC18M960

Enjoy the Quality of Millex® Filters

# skip the pain

Introducing the Smplicity® G2 Filtration system:  
**the better way to use Millex® filters.**

Chromatographers trust Millex® filters for their low extractable profile and high recovery. But manual syringe filtration can lead to fatigue.

Now, enjoy the quality of Millex® filters with the ease of vacuum filtration. The new Smplicity® G2 system vacuum-filters 1-8 samples directly into HPLC vials. As it uses Millex® 33 mm filters, it's compatible with all standard protocols specifying 33 mm Millex® filtration.

Sample Type	Job Hazard Score		
	Low (0-9)	Medium (10-29)	High (30-49)
Syringe Filtration of Non-viscous Sample		12.8	
Syringe Filtration of Viscous Sample		16.0	
<b>Smplicity® G2 System Filtration of Viscous Sample</b>	<b>8.0</b>		

According to a Baseline Risk Identification of Ergonomic Factors (BRIEF™) survey, the Smplicity® G2 system lowered the ergonomic impact of filtration compared to manual syringe filtration. For reference, a task involving manipulating a heavy (13 kg) tool was found to have a medium/high job hazard score of 27. Note that the job hazard score for Smplicity® G2 remains at 8 regardless of the viscosity of the sample.

**Skip the pain –**

Learn more about the

Smplicity® G2 Filtration System:

**[SigmaAldrich.com/Smplicity](https://SigmaAldrich.com/Smplicity)**



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Filtration & Monitoring Products



# Lots of different samples? ONE MEMBRANE.

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To learn more about our HPLC-certified Hydrophilic PTFE Millex® filters, visit

[SigmaAldrich.com/OneMillex](https://SigmaAldrich.com/OneMillex)

## We're committed to filtration tools—and the scientists who use them.

Our filtration and analysis tools are the pacesetters in membrane technology with well-established Millex® syringe filters. So, finding ways to improve wasn't easy—but the answer came from thinking of how we could make filtration easier without compromising performance. The result? Implementing our patented over-molded design to Millex® 13 and 33 mm syringe filters. Redesigns that feel good to use—inside and out.

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

- Millex®-LG, LCR filters contain hydrophilic PTFE membranes and are HPLC-certified for low levels of UV-absorbing extractables.
- Hydrophilic PTFE membranes have broad chemical compatibility, enabling filtration of aqueous and organic solvents.
- 33 mm hydrophilic PTFE Millex® filters have faster flow rates than 25 mm filters.
- Easy to read membrane type and pore size printed on every Millex® filter.
- Over-molded design provides excellent seal integrity and burst strength.

## Ordering Information

Description	Pore Size	Diameter	Qty/Pk	Luer-slip Outlet Cat. No.	Tube Outlet Cat. No.
Millex®-LG, hydrophilic PTFE membrane (All items have a light blue overmolded band.)	0.20 µm	13 mm	100	SLLGX13NL	—
			1000	SLLGX13NK	—
		33 mm	50	SLLG033NS	—
			250	SLLG033NB	—
			1000	SLLG033NK	—
Millex®-LCR, hydrophilic PTFE membrane (All items have a light blue overmolded band.)	0.45 µm	13 mm	100	SLCRX13NL	SLCRX13TL
			1000	SLCRX13NK	—
		33 mm	50	SLCR033NS	—
			250	SLCR033NB	—
			1000	SLCR033NK	—

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Millex® Filters

Our 33 mm nonsterile Millex® filters are compatible with the Samplicity® G2 filtration system.

For more information, visit  
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# LC-MS SOLVENTS AND ADDITIVES DESIGNED FOR UHPLC

Quality exceeding your expectations

- Purity for low detection limits
- Suitability tested by UHPLC-MS/TOF
- Lot-to-lot reproducibility
- Microfiltered (0.2 µm), packaged in clear borosilicate glass containers



# LC-MS/UHPLC-MS Solvents and Additives

## LC-MS Solvents for UHPLC-MS

### Key Features and Benefits

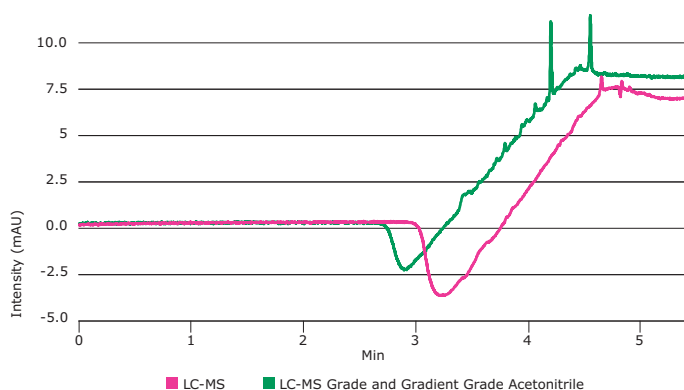
- Prepared and tested specifically for UHPLC
- Suitability tested by (+/- MS modes)
- Bottled in clear, white borosilicate glass for quality
- Filtered through a 0.2  $\mu\text{m}$  filter

### Exclusively Designed for UHPLC

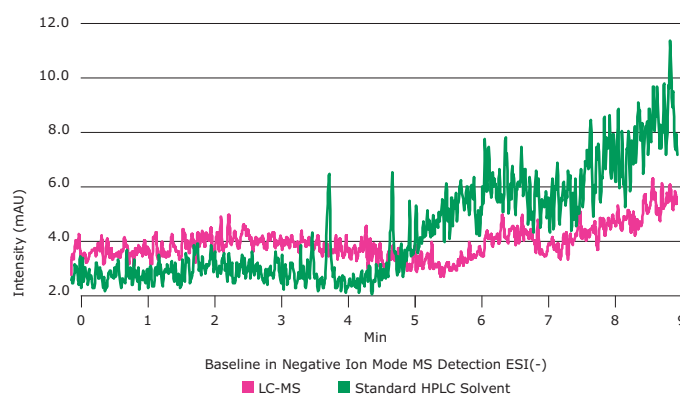
The introduction of UHPLC in bioanalytical assays has created a need for a line of higher purity grade solvents that have passed more demanding quality control procedures. LC-MS solvents and reagents were developed and stability tested to meet the critical needs for purity required to take advantage of the ever-higher UHPLC column efficiencies and lower MS detection limits.

### Performance under Gradient Conditions

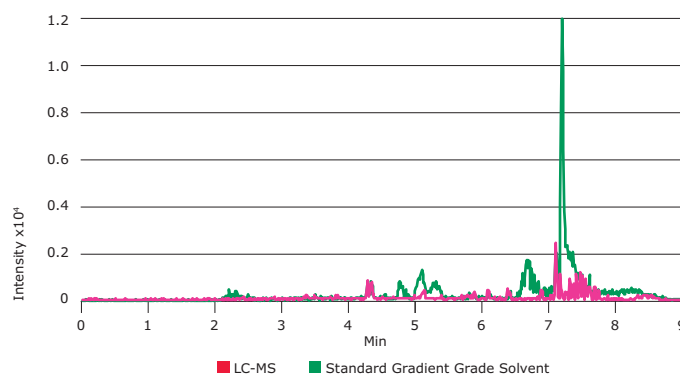
Using a gradient of acetonitrile in water, our new LC-MS grade solvents have extremely low total UV baseline drift compared to classical LC-MS grade and gradient grade acetonitrile. The drift is below 8 mAU at 210 nm for acetonitrile and the new grade has significantly lower baseline and fewer minor peaks.



Blank runs of standard reversed-phase gradients of 30% to 80% acetonitrile in water generally result in low and stable baselines with HPLC columns packed with particles  $>3 \mu\text{m}$ . However, this is not the case with UHPLC separations because of the typically higher flow rates and short columns. Performance tests conducted in ESI +/- ion mode using acetonitrile in water gradient show rising baseline for the blank gradient of standard HPLC grade solvents compared to LC-MS solvents.



Additional tests with water/acetonitrile gradients show that UHPLC gradients using standard solvents have many more impurity peaks.



For a complete list of LC-MS and HPLC solvents, buffers and additives, visit [SigmaAldrich.com/lc-ms](https://www.sigmaaldrich.com/lc-ms)

# High-Purity LC-MS Mobile Phase Additives

## Key Features and Benefits

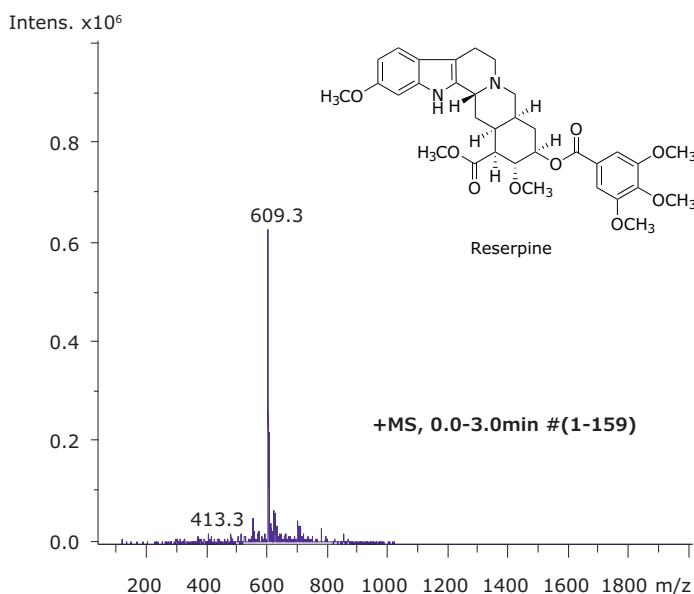
- LC-MS application tested for consistent quality according to the reserpine test
- Optimized to improve ionization and resolution
- Extremely low levels of inorganic and organic impurities
- Manufactured specifically for accurate and fast LC-MS
- Highest quality acids, bases & salts - specified in the certificate of analysis

## Introduction

It is common practice in LC-MS to add certain reagents to the mobile phase, or to introduce them post-column prior to the interface to influence analyte ionization. Most often the goal is for an improvement in the analyte signal. In addition, 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. To help you obtain the highest quality analysis, we offer a wide range of high purity mobile phase additives for LC-MS applications. The LC-MS portfolio includes the most commonly used acids, bases and volatile salts of high purity tested for LC-MS applications. Impurities, such as alkali ions, plasticizers or surfactants, that can be commonly found in lower-grade solvents are particularly problematic as they interfere strongly with LC-MS, resulting in higher background noise and formation of adducts. Only ultrapure reagents enable high signal-to-noise ratios, which results in the highest and most reliable performance for small and large molecule applications.

## Reserpine test

All of our LC-MS solvents and reagents are specified using the standard reserpine test. Reserpine (608.68) is used as the reference substance to quantify possible impurities in the LiChropur® LC-MS reagents. It is performed by diluting 2.5% (v/v) acid, base or 2.5% (w/v) salt in 50/50 (v/v) acetonitrile/water. Every lot produced is analyzed via flow injection analysis mass spectrometry (FIA-MS). The dissolved reagent and the appropriate reserpine reference solutions are introduced into the MS ion source syringe pumps. The total ion chromatogram (TIC) is accumulated during three minutes. The relative intensities of the detected masses are compared with the reserpine signal. For electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive mode, the specified amount of reserpine is 2 ppb for acids and bases, and 20 ppb for salts. In the negative mode, the specified amount of reserpine is 20 ppb for both.



Typical solvent QC mass spectrum, ESI positive (flow injection analysis)

### Acid additives

Volatile, low molecular weight organic acids such as formic and acetic acid such as novel difluoroacetic acid reagent (DFA) improve ionization and resolution of a wide range of molecules. Addition of organic acids to the mobile phase can help to overcome the ionization-suppressing effect of trifluoroacetic acid (TFA) present in the mobile phases used for the analysis of proteins and peptides.

### Neutral salts

Neutral volatile salts, such as ammonium acetate or ammonium formate are typically used as buffer compounds to control the ionization state of the analytes (and phases) which has a strong influence on the LC-MS separation and performance.

### Sodium adduct formation

Alkali adducts diminish instrument sensitivity. When adduct formation tendency is strong, often the addition of defined amounts of sodium ions (mostly pre-column) can help to obtain uniform and stable molecular ions for detection in LC-MS.

## Extensive QC testing ensures highest specification

Residue on ignition (evaporation residue) tests show the low content of insoluble matter in the reagent. This provides confidence that your eluents have the low particle content needed for accurate LC-MS measurement.

Sodium and Potassium ions are particularly likely to form adducts with the analyte molecules. This leads to complex mass spectra leading to time-consuming data evaluation. The content of trace metals is in the low ppb range for LiChropur® LC-MS reagents to minimizing the risk of adduct formation in the ion source for cleaner results.

Our LiChropur® LC-MS reagents are stored in borosilicate bottles to prevent leaching of alkali ions out of the glass. The content of the potentially complex forming ions aluminum, copper and iron is also specified.

Full specification can be found in the certificate of analysis for each of our LC-MS grade products.

#### Specification (Acids/Bases)

Assay (acidimetric)	≥ 98,0%
Colour	≤ 10 Hazen
Residue on ignition	≤ 2 ppm
Al	≤ 5.0 ppb
Ca	≤ 10.0 ppb
Cu	≤ 1.0 ppb
Fe	≤ 5.0 ppb
K	≤ 5.0 ppb
Mg	≤ 2.0 ppb
Na	≤ 5.0 ppb
NH <sub>4</sub> <sup>+</sup>	≤ 10 ppm
LC-MS Suitability ESI Positive (Reserpine Test)	≤ 2 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine
LC-MS Suitability ESI Negative (Reserpine Test)	≤ 20 ppb (tested with ion trap MS). Intensity of background mass peak based on reserpine

For more information, visit [SigmaAldrich.com/lcms-reagents](https://www.sigmaaldrich.com/lcms-reagents)

## LC-MS Grade Solvents and Additives

Name	Description	Pkg. Size	Cat. No.
<b>Solvents</b>			
Acetonitrile	UHPLC-MS LiChrosolv®	1L, 2L (GL45)	103725
Methanol	UHPLC-MS LiChrosolv®	1L, 2L (GL45)	103726
Water	UHPLC-MS LiChrosolv®	1L, 2L (GL45)	103728
Acetonitrile	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	100029
Methanol	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	106035
Water	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	115333
Ethyl acetate	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	103649
Hexane	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	103701
Heptane	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	103654
2-Propanol	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	102781
<b>Blends</b>			
Acetonitrile + 0.1% Acetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5L, 4L	159004
Acetonitrile + 0.1% Formic acid (v/v)	hypergrade for LC-MS LiChrosolv®	1L, 2.5L, 4L	159002
Acetonitrile + 0.1% Trifluoroacetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L, 4L	159014
Water + 0.1% Acetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L, 4L	159007
Water + 0.1% Formic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5 L, 4L	159013
Water + 0.1% Trifluoroacetic acid (v/v)	hypergrade for LC-MS LiChrosolv®	2.5L, 4L	480112
Acetonitrile with 0.1% formic acid	for UHPLC, for Mass spectrometry	4L	900686
Water with 0.1% formic acid	for UHPLC, for Mass spectrometry	4L	900687
Methanol with 0.1% formic acid	for UHPLC, for Mass spectrometry	4L	632546
<b>Mobile Phase Additives</b>			
Acetic acid 100%	for LC-MS LiChropur™	50 ml	533001
Formic acid 98% – 100%	for LC-MS LiChropur™	50 ml	533002
Ammonia solution 25%	for LC-MS LiChropur™	50 ml	533003
Ammonium acetate	for LC-MS LiChropur™	50 g	533004
Ammonium hydrogen carbonate	for LC-MS LiChropur™	50 g	533005
Ammonium acetate	eluent additive for LC-MS	25g, 100g	73594
Ammonium formate	for Mass Spectrometry	25g, 100g	70221
Sodium formate solution	suitable for LC-MS LiChropur™	100 ml	51197
Difluoroacetic acid	for LC-MS LiChropur™	1 ml, 10x1 ml, 50 ml	00922
Trifluoroacetic acid	eluent additive for LC-MS LiChropur™	10x1 ml, 10 ml, 50 ml	80457
2,2,2-Trifluoroethanol	eluent additive for LC-MS LiChropur™	1 ml, 10x1 ml, 50 ml	18370
1,1,1,3,3,3-Hexafluoro-2-propanol	for LC-MS LiChropur™	10 ml, 50 ml	18127

### LC-MS Tips and Tricks

Mobile phase components should be chosen with key elements in mind:

- Volatility is an essential requirement
- Promotion of ionization
- pH and organic type (methanol and IPA may be useful in MS to promote better ionization response)
- Higher levels of organic solvent can improve ionization
- Suppression of ionization
- Certain solvents and additives can lower response by entering into reactions with solute ions (in liquid or gaseous state) and lowering their gas phase concentration before entering the mass analyzer

For a complete list of LC-MS and HPLC solvents, buffers and additives, visit [SigmaAldrich.com/lc-ms](https://www.sigmaaldrich.com/lc-ms)

## Mobile Phase Buffers

The proper choice of buffer, in terms of buffering species, ionic strength and pH, is the most critical step in reversed-phase chromatography method development for ionic analytes. In sensitive LC-MS separations that depend heavily on the correct choice of acid, base, buffering species and other additives, a buffer must be chosen based on its ability to maintain and not suppress analyte ionization at the MS interface.

The typical pH range for reversed-phase separations on silica-based columns is pH 2 to 8. Your choice of buffer is typically governed by the desired pH. It is important that the buffer has a  $pK_a$  close to the desired pH since buffers control pH best at their  $pK_a$ . A rule of thumb is to choose a buffer with a  $pK_a$  value <2 units of the desired mobile phase pH.

### HPLC Buffers, $pK_a$ Values and Useful pH Range

Buffer	$pK_a$ (25°C)	Useful pH Range
TFA	0.5	<1.5
Sulfonate	1.8	<1–2.8
Phosphate	2.1	1.1–3.1
Chloroacetate	2.9	1.9–3.9
Formate	3.8	2.8–4.8
Acetate	4.8	3.8–5.8
Sulfonate	6.9	5.9–7.9
Phosphate	7.2	6.2–8.2
Ammonia	9.2	8.2–10.2
Phosphate	12.3	11.3–13.3

## Guidelines for Preparing Mobile Phases

It should be understood that slight variations in pH and buffer concentration could have a dramatic effect on the chromatographic process; consistent and specific techniques should be a regular practice in the preparation of mobile phases. A common practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of

buffer. The pH of the solution should be adjusted, if necessary, and then dilute to final volume of water prior to adding or blending of organic solvents. Then, add a volumetrically measured amount of organic solvent to obtain the final mobile phase. Thorough blending, degassing, and filtering prior to use is also recommended.



# Chemical Derivatization Reagents for LC-MS

Modern mass spectrometry techniques such as APCI or ESI are highly successful in providing valuable structural information, and allow the detection of very low analyte concentrations in various sample matrices. For certain samples e.g. non-polar compounds, and in research areas, such as clinical metabolomics and forensics analytics, there are many cases where such methods can be insufficiently sensitive.

Derivatization reactions in mass spectrometry are used to improve ionization efficiency [1-4]. The derivatization reagents have functional groups possessing high proton (cation) affinity that stabilize a positive charge. Of similar importance when derivatizing is the improvement of qualitative analysis by modifying fragmentation behavior to form unique product ions and the mass shift. Finally, derivatization can enhance precise quantitative analysis for profiling of relatively small analyte molecules, particularly in metabolomics.

## References

1. Zaikin V, Halket J, 2009. A handbook of derivatives for mass spectrometry. Chichester: IM Publications LLP,
2. Santa T. 2013. Derivatization in liquid chromatography for mass spectrometric detection Drug Discov. Ther. 7:9-17
3. Santa T. 2011. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry.
4. Biomed. Chromatogr. 25:1-10
5. Santa T, Al-Dirbashi OY, Fukushima T. 2007. Derivatization reagents in liquid chromatography/electrospray ionization tandem mass spectrometry for biomedical analysis. Drug Discov. Ther. 1:108-118.

For more information, visit  
[SigmaAldrich.com/derivatization](https://www.sigmaaldrich.com/derivatization)

Cat. No.	Derivatization Reagent	Analyte Functional Group	Typical Application
05689	Diethyl ethoxymethylenemalonate	Amine	Amino acids
29208	(N-Succinimidylloxycarbonylmethyl) tris(2,4,6-trimethoxyphenyl)phosphonium bromide	Amine	Protein sequence analysis
61224	N-Succinimidyl 4-(dimethylamino)benzoate	Amine	Glycerophosphoethanolamine lipids
73177	1-Fluoro-2,4-dinitrobenzene	Amine	Prim./sec. aliphatic amines
73103	Dibenzyl ethoxymethylenemalonate	Amine	Amino acids
03334	Dansylhydrazine	Carbonyl	—
4465962	Amplifex Keto Reagent Kit	Carbonyl	—
5037804	Amplifex Diene Reagent Kit	Diene	—
65562	2-Picolylamine	Carbonyl	Steroids
89397	Girard's reagent T	Carbonyl	Nucleosides
93742	Pentafluorophenylhydrazine	Carbonyl	Oligosaccharides
79291	4-[2-(N,N-Dimethylamino)ethylaminosulfonyl]-7-(2-aminoethylamino)-2,1,3-benzoxadiazole	Carboxylic acid	Fatty acids
42579	4-Phenyl-1,2,4-triazoline-3,5-dione	Diene	Vitamin D
97622	2-Mercaptoethanol	Double bond	Microcystins
00721	4-(Dimethyl-d <sub>6</sub> -amino)benzoyl chloride	Hydroxy	Deuterium mass shift
03641	Dansyl chloride	Hydroxy	—
05022	N,N-Dimethylglycine	Hydroxy	Cholesterol
06696	3-Amino-9-ethylcarbazole	Hydroxy	Sugars
67954	4-(Dimethylamino)benzoyl chloride	Hydroxy	17β-Estradiol
72702	3,5-Dinitrobenzoyl chloride	Hydroxy	Tetrahydrocorticosterones
41368	p-Toluenesulfonyl isocyanate	Hydroxy	Steroids
49432	Pyridine-3-sulfonyl chloride	Hydroxy	Steroids
55952	Fusaric acid	Hydroxy	Steroids
93535	N-(Propionyloxy)succinimide	Amine	Histones

## MALDI Matrices Selection Table

Matrix-assisted laser desorption/ionization (MALDI) has expanded MS into the analysis of high molecular mass, non-volatile, and thermally labile compounds, such as intact proteins and oligonucleotides. Moreover, it has become an important technique in proteomics research.<sup>1-3</sup> Further significant applications of MALDI-MS include the analysis of polymers, glycans, lipids, and metabolites.

A typical MALDI matrix substance is an aromatic acid with a chromophore that absorbs strongly at the wavelength of the incident laser. The MALDI technique generally involves mixing the sample with a matrix substance, followed by crystallization by different techniques on the MALDI sample plate. The crystallized sample-matrix mixture is irradiated by laser light, usually UV. As the matrix absorbs the light energy, it vaporizes into the gas phase, resulting in an indirect ionization of the sample molecules.<sup>4-6</sup>

Choosing a suitable matrix of high quality is the key to the success of a MALDI-MS experiment. Organic impurities can lead to extraneous peaks, especially in the low mass range. Trace levels of ions, especially Na<sup>+</sup> and K<sup>+</sup>, form adducts with sample molecules. These adducts differ in mass according to the number of positive ions and complicate the MS spectrum. Since the matrix substance is generally applied in large excess to the sample, a very high purity is even more crucial.

The MALDI Matrices Selection Table below facilitates choosing the appropriate matrix for the use in proteomics and metabolomics.

### Features and Benefits

- High chemical purity
- Low trace metal content to minimize adduct formation and simplify the resulting MS spectrum
- Ultra pure grades of the most popular matrix substances with extremely strict specifications concerning purity, trace metal content, appearance, and solubility

### References

1. Karas, M., *et al.*, Matrix-assisted ultraviolet laser desorption of non-volatile compounds. *Int. J. Mass Spectrom. Ion Proc.*, **78**, 53-68 (1987).
2. Hillenkamp, F., and Peter-Katalinic, J. (eds.), *MALDI MS. A Practical Guide to Instrumentation, Methods and Applications*, Wiley-VCH (2007).
3. Aebersold, R., and Mann, M., Mass spectrometry-based proteomics. *Nature*, **422**, 198-207 (2003).
4. Dreisewerd, K., The desorption process in MALDI. *Chem. Rev.*, **103**, 395-425 (2003).
5. Karas, M., and Krüger, R., Ion formation in MALDI. *Chem. Rev.*, **103**, 427-439 (2003).
6. Knochenmuss, R., and Zenobi, R., MALDI ionization: The role of in-plume processes. *Chem. Rev.*, **103**, 441-452 (2003).

Cat. No.	Description	Purity	Abbreviation	Proteins	Peptides	Glycans	Oligonucleotides	Polymers	Lipids	Other Analytes	Pack Sizes
92817	9-Aminoacridine	≥99.5%	9-AA							• Metabolites	1 g
89063	4-Bromo- $\alpha$ -cyanocinnamic acid	≥95%	BrCCA		•					• Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
68914	4-Bromo- $\alpha$ -cyanocinnamic acid - 4-Chloro- $\alpha$ -cyanocinnamic acid mixture	≥95%	BrCCA:ClCCA		•					• Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
05851	4-Aminoquinoline	≥99.0%					•			amino acids	1 g
56229	9-Nitroanthracene	≥98.5%	9-NA					•		fullerenes, humic acids	100 mg 1 g
69028	4-Phenyl- $\alpha$ -cyanocinnamamide	≥98.5%							•	MALDI imaging	100 mg
76884	Anthranilamide	≥99.0%		•	•	•					1 g
78246	Curcumin	≥99.5%							•	pharmaceuticals, drugs, MALDI imaging	100 mg
83788	(2E)-3-(9-Anthryl)-2-cyanoacrylic acid	≥97.0%								low molecular weight compounds	100 mg
87884	trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile	≥99.0%				•		•		Gold nanoparticles, fullerenes, organometallics, macrocycles	250 mg 1 g

Cat. No.	Description	Purity	Abbreviation	Proteins	Peptides	Glycans	Oligonucleotides	Polymers	Lipids	Other Analytes	Pack Sizes
94477	(E)-2-Cyano-3-(2-naphthyl) acrylic acid	≥98.0%								low molecular weight compounds	100 mg
55841	4-Bromo- $\alpha$ -cyanocinnamic acid - $\alpha$ -Cyano-2,4-difluorocinnamic acid mixture	≥95%	BrCCA:DiFCCA		•				•	Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
60018	Caffeic acid	≥99.0%		•	•						1 g, 5 g
94141	4-Chloro- $\alpha$ -cyanocinnamic acid	≥95%	CICCA		•				•	Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
39379	4-Chloro- $\alpha$ -cyanocinnamic acid - $\alpha$ -Cyano-2,4-difluorocinnamic acid mixture	≥95%	CICCA:DiFCCA		•				•		100 mg
77646	$\alpha$ -Cyano-2, 4-difluorocinnamic acid	≥95%	DiFCCA		•					Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
77081	$\alpha$ -Cyano-4-fluorocinnamic acid	≥95%	FCCA		•					Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
70990	$\alpha$ -Cyano-4-hydroxycinnamic acid	≥99.0%	CHCA	•	•	•					250 mg, 1 g
39468	$\alpha$ -Cyano-4-hydroxycinnamic acid	≥99.5%, Ultra pure	CHCA	•	•	•					10x10 mg
03841	$\alpha$ -Cyano-4-hydroxycinnamic acid - $\alpha$ -Cyano-2, 4-difluorocinnamic acid - $\alpha$ -Cyano-2, 3, 4, 5, 6-pentafluorocinnamic acid mixture	≥95%	CHCA:DiFCCA: PentaFCCA		•					Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
38419	$\alpha$ -Cyano-2, 3, 4, 5, 6-pentafluorocinnamic acid	≥95%	PentawFCCA		•					Phosphopeptides, phospholipids, chlorinated lipids, drugs, fragile analytes, ionic liquids (quantification), ME-SIMS, CID-MS/MS	100 mg
56451	1,5-Diamino naphthalene	≥99.0%	1,5-DAN		•		•			In-Source-Decay	250 mg
37468	2',6'-Dihydroxy acetophenone	≥99.5%	2,6-DHAP	•	•	•			•		1 g, 5 g
85707	2, 5-Dihydroxybenzoic acid	≥99.0%	DHB	•	•	•		•	•	Organic molecules	10 mg, 250 mg, 1 g
39319	2, 5-Dihydroxybenzoic acid	≥99.5%, Ultra pure	DHB	•	•	•		•	•	Organic molecules	10x10 mg
46278	trans-Ferulic acid	≥99.0%	FA	•	•						1 g, 5 g
54793	2-(4-Hydroxy phenylazo) benzoic acid	≥99.5%	HABA	•	•	•		•			1 g, 5 g
56197	3-Hydroxypicolinic acid	≥99.0%	3-HPA				•			Oligosaccharides	250 mg, 1 g
73148	3-Nitrobenzyl alcohol	≥99.5%									5 g
80362	3-Nitrobenzonitrile	≥99.0%	3-NBN							Tissues via MAIV	1 g
84228	Salicylamide	≥99.0%					•				1 g
85429	Sinapic acid	≥99.0%	SA	•	•					Dendrimers, Fullerenes	1 g, 5 g
49508	Sinapic acid	≥99.5%	SA	•	•					Dendrimers, Fullerenes	10 x 10 mg
50862	Super-DHB BioReagent		Super-DHB	•	•	•					10 x 10 mg, 1 g, 5 g
91928	2', 4', 6'-Trihydroxy acetophenone monohydrate	≥99.5%	THAP	•	•	•	•				1 g, 5 g

# Standards & Certified Reference Materials for Accurate LC-MS Analyses

We provide a comprehensive and trusted portfolio including our Supelco®, Pestanal®, TraceCERT®, Cerilliant®, Certified Spiking Solutions® and Vetrinal™ brands so you can assure the accuracy and precision of your LC-MS analyses.

Our manufacturing sites are accredited to ISO/IEC 17025 and ISO 17034 at a minimum. Our portfolio of over 20,000 products includes standards for environmental, petrochemical, pharmaceutical, clinical diagnostic, toxicology, forensic, food, beverage, cosmetic and veterinary applications and much more

## Discover our comprehensive range of reference materials:

- Amino Acids, Peptides & Proteins
- Biomarkers & Metabolomics
- Carbohydrates
- Food & Cosmetic Components
- Lipids & Fatty Acids
- Matrix Materials
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- Pesticides
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- Proficiency Testing
- Vitamins

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## LC-MS/MS Analysis of Fentanyl and Fentanyl Analogs

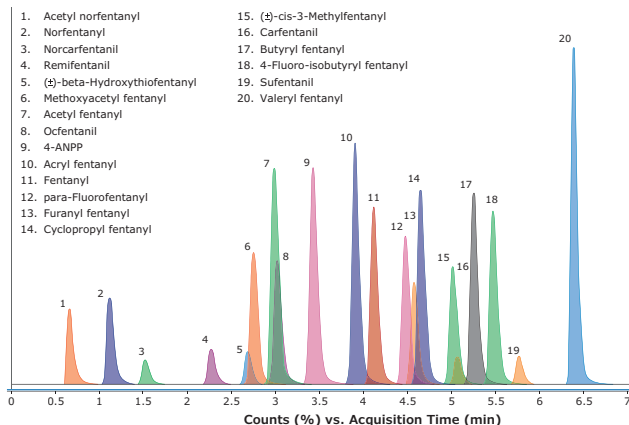
The emergence of fentanyls as drugs of abuse represents a great challenge to current forensic toxicology. Recently, a huge number of new fentanyl structural variants, also known as designer fentanyls, have appeared on the illicit drug market. Often mixed with traditional opioid drugs, these highly potent fentanyl analogs have caused harmful intoxication and dramatically increased opioid related mortality in the United States, Europe and Asia. Due to the very high potency of some fentanyl analogs, forensic testing labs face the challenge of detecting minimal trace amounts of these drugs in biological samples while ensuring laboratory safety.

To help address these safety challenges, we provide Certified Spiking Solutions® of the most potent fentanyl analogues, such as Carfentanyl, in an ampule additionally protected in a “safety can”, ensuring safe transport and handling in the lab. Since all fentanyls are classified as controlled substances, we offer Cerilliant® fentanyl reference standards as DEA-exempt solutions for added convenience.

Visit:

[SigmaAldrich.com/standards](https://SigmaAldrich.com/standards)

### LC/MS/MS Analysis of Fentanyl and Fentanyl Analogs



Column	Supelco Titan™ C18, 5 cm × 2.1 mm I.D., 1.9 µm particles (577122-U)	
Column temp.	40 °C	
Sample	1.0 ng/mL in 90:10 methanol:water	
Injection volume	1.0 µL	
Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in acetonitrile	
Flow rate	0.4 mL/min	
Gradient	<b>Time (min)</b>	<b>% B</b>
	0	15
	0.3	15
	5.5	30
	7.0	95
	9.0	95
Instrument	Agilent 1290 Infinity II UHPLC	
	Agilent 6495 Triple Quad LC/MS	
MS/MS mode	MRM	
Ion mode	Positive	

## Fentanyls

Product	Description
M-194-0.5ML	(±)-cis-3-Methylfentanyl HCl, 100 µg/mL (as free base) in methanol
H-130-0.5ML	(±)-β-Hydroxythiofentanyl HCl, 100 µg/mL (as free base) in methanol
A-139-0.5ML	4-ANPP, 100 µg/mL in methanol
A-157-0.5ML	4-ANPP-D5, 100 µg/mL in methanol
F-050-0.5ML	4-Fluoroisobutyrylfentanyl, 100 µg/mL in methanol
A-109-1ML	Acetyl fentanyl, 1.0 mg/mL in methanol
A-129-1ML	Acetyl fentanyl, 50 µg/mL in methanol
A-110-1ML	Acetyl fentanyl-13C6, 100 µg/mL in methanol
A-130-1ML	Acetyl fentanyl-13C6, 50 µg/mL in methanol
A-115-1ML	Acetyl norfentanyl oxalate 1.0 mg/mL (as free base) in methanol
A-116-1ML	Acetyl norfentanyl-13C6 oxalate, 100 µg/mL (as free base) in methanol
A-140-0.5ML	Acryl fentanyl HCl, 100 µg/mL (as free base) in methanol
A-071-1ML	Alfentanil HCl, 1.0 mg/mL (as free base) in methanol
B-066-0.5ML	Butyryl fentanyl, 100 µg/mL in methanol
C-162-1EA	Carfentanil Oxalate, 100 µg/mL in methanol
C-163-1EA	Carfentanyl-D5 Oxalate, 100 µg/mL (as free base) in methanol
C-177-0.5ML	Cyclopropyl fentanyl HCl, 100 µg/mL (as free base) in methanol
F-013-1ML	Fentanyl, 1.0 mg/mL in methanol
F-002-1ML	Fentanyl, 100 µg/mL in methanol
F-001-1ML	Fentanyl-D5, 100 µg/mL in methanol
F-046-0.5ML	Furanyl fentanyl HCl, 100 µg/mL (as free base) in methanol

Product	Description
F-053-0.5ML	Furanyl fentanyl-D5 HCl, 100 µg/mL (as free base) in methanol
I-038-0.5ML	Isobutyryl fentanyl HCl, 100 µg/mL (as free base) in methanol
M-200-0.5ML	Methoxyacetyl fentanyl HCl, 100 µg/mL (as free base) in methanol
N-114-1EA	Norcarfentanil Oxalate, 100 µg/mL (as free base) in methanol
N-031-1ML	Norfentanyl oxalate, 1.0 mg/mL (as free base) in methanol
N-055-1ML	Norfentanyl-D5 oxalate, 1.0 mg/mL (as free base) in methanol
N-030-1ML	Norfentanyl-D5 oxalate, 100 µg/mL (as free base) in methanol
O-047-0.5ML	Ocfentanil, 100 µg/mL in methanol
F-054-0.5ML	ortho-Fluorofentanyl HCl, 100 µg/mL (as free base) in methanol
F-048-0.5ML	para-Fluorobutyryl fentanyl (PFBF), 100 µg/mL in methanol
F-049-0.5ML	para-Fluorofentanyl, 100 µg/mL in methanol
R-026-1ML	Remifentanil acid, 100 µg/mL in acetonitrile
R-024-1ML	Remifentanil HCl, 100 µg/mL (as free base) in methanol
S-008-1ML	Sufentanil Citrate, 100 µg/mL (as free base) in methanol
S-018-1ML	Sufentanil-D5, 100 µg/mL in methanol
V-048-0.5ML	Valeryl fentanyl HCl, 100 µg/mL (as free base) in methanol
V-068-0.5ML	Valeryl fentanyl-D5 HCl, 100 µg/mL (as free base) in methanol



# LC-MS Accessories

## Vials and Syringes for LC-MS Samples

### Key Features and Benefits

- Low Adsorption (LA) vials designed to enhance MS detection
- Low volume (center drain) vials designed to reduce sample volume needed to run your assay
- Large selection of manual and autosampler syringes to meet any purpose or sample type
- New digital syringe to enhance accuracy and meet validation concerns for sample handling
- Support and delivery you expect and deserve

### Vast Selection of Vials for MS Applications

Clear or amber? Glass or plastic? Choose from a wide variety of vials for any LC-MS application or your every-day usage. We offer the newest and most up-to-date selection of vials for your analytical needs including our Low Adsorption (LA) or CD™ vials (Center Draining). The MRQ30 CD™ vials allow maximum sample recovery down to 2 µL of sample remaining in the vial after sample withdrawal, an improvement over standard vials. We know some vials can be instrument specific, so if you have trouble choosing, visit **SigmaAldrich.com/vials**, or call your local Technical Service representative for assistance in choosing the right product for your application.

### High-Quality Syringes for any Sample Matrix

We carries the best syringe selection and the top brands you use every day. Whether you need a liquid or gastight, manual or autosampler syringe, we have the appropriate quality and size you need for your application and sample volume. To see our Syringe Selection Guide and our entire syringe line, visit **SigmaAldrich.com/syringes**. To order, call your local Technical Service representative at 1-800-325-5832.

### Low Adsorption Sample Vials and Caps

Description	Cat. No.
<b>CD (Center Draining) Vial Kits*</b>	
Clear glass vial, 1.5 mL, PTFE/silicone septa	29655-U
Clear glass vial, 1.5 mL, PTFE/silicone septa with slit	29656-U
<b>MRQ30 Vial Kits*</b>	
Clear glass vial, 1.2 mL, PTFE/silicone septa	29658-U
Clear glass vial, 1.2 mL, PTFE/silicone septa with slit	29659-U
<b>QSertVial™ (0.3 mL) Vial Kits*</b>	
Clear glass, natural PTFE/silicone septa	29661-U
Clear glass, natural PTFE/silicone septa with slit	29662-U
Amber glass vial, natural PTFE/silicone septa	29663-U
Amber glass vial, natural PTFE/silicone septa with slit	29664-U
<b>Low Adsorption 2 mL (12 × 32 mm) Vial Kits*</b>	
Clear glass vial with marking spot, natural PTFE/silicone septa	29651-U
Clear glass vial with marking spot, natural PTFE/silicone septa with slit	29652-U
Amber glass vial with marking spot, natural PTFE/silicone septa	29653-U
Amber glass vial with marking spot, natural PTFE/silicone septa with slit	29654-U
<b>Replacement Mass Spec Quality (MSQ) Caps with Septa</b>	
Caps with septa, 9 mm, natural PTFE/silicone, pack of 100	29665-U
Caps with septa, 9 mm, natural PTFE/silicone with slit, pack of 100	29666-U

\*Kits include 100 each of vial, cap and septa



### Popular Hamilton® Syringes for Rheodyne®, Valco® VISC-2, Altex™ and SSI™ Injection Valves

Description	Needle Type	Volume	Mfr Model	Pkg. Size	Cat. No.
<b>Hamilton® 700 Series Syringes</b>					
10 µL Syringe with Cemented Needle	22s ga, blunt tip	10 µL	701 SNR	1	58380-U
25 µL Syringe with Cemented Needle	22s ga, blunt tip	25 µL	702 SNR	1	58381
50 µL Syringe with Cemented Needle	22s ga, blunt tip	50 µL	705 SNR	1	58382
100 µL Syringe with Cemented Needle	22s ga, blunt tip	100 µL	710 SNR	1	58383
250 µL Syringe with Cemented Needle	22 ga, blunt tip*	250 µL	725 SNR	1	58384
500 µL Syringe with Cemented Needle	22 ga, blunt tip	500 µL	750 SNR	1	26222-U
<b>Hamilton® 1700 Series Syringes</b>					
100 µL Syringe with Removable Needle	22s ga, blunt tip	100 µL	1710 RNR	1	20888
250 µL Syringe with Removable Needle	22 ga, blunt tip	250 µL	1725 RNR	1	20889
500 µL Syringe with Removable Needle	22 ga, blunt tip	500 µL	1750 RNR	1	20890-U

\*The nominal I.D. of the needle is 0.413 mm for a 22 gauge needle and 0.168 mm for 22s gauge.



# UHPLC Fittings

## Upchurch Scientific® UHPLC Fingertight Fittings

Manufactured from a proprietary PEEK blend, the Upchurch Scientific® UHPLC Fingertight fittings can be used at temperatures up to 200 °C and higher pressure (to 23,000 psi/1,585 bar in some cases)

than more traditional HPLC fittings. These fittings are available in one piece 10–32 and the traditional two-piece design.

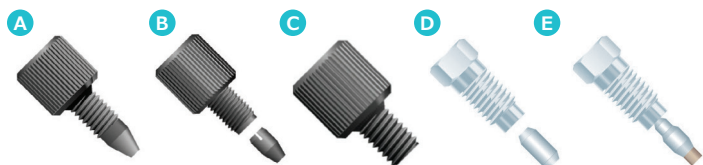
Description	Qty.	Cat. No.
Ultra-High Pressure Fitting, PEEK		
<b>A</b> Fingertight I Nut (Black)	Pk/10	<b>51262-U</b>
<b>B</b> SealTight™ Short Fitting (Black)	Pk/10	<b>51263-U</b>
<b>C</b> LiteTouch® Nut (Black) (51256-U requires 51258-U)	Pk/10	<b>51256-U</b>

## Upchurch Scientific® UHPLC Stainless Steel Fittings

With new column technology pushing the pressure limits of standard HPLC fittings, Upchurch Scientific® has specially designed UHPLC stainless steel that

can withstand much higher pressures than standard HPLC analysis.

Description	Qty.	Cat. No.
Ultra-High Pressure Fitting, Stainless Steel		
<b>D</b> With Ferrule for 1/16" Tubing	Pk/10	<b>51264-U</b>
<b>E</b> With Ferrule for 1/32" Tubing	Pk/10	<b>51265-U</b>



## Upchurch Scientific® UHPLC Unions and Adapters

The design of the UHPLC unions, 51274-U and 51277-U, allows a convenient connection between 1/32" O.D. tubing and 360 µm tubing. The union contains a stainless steel body and capsule, both with excellent chemical compatibility. It is coupled with a direct-connect ferrule made from a proprietary PEEK polymer blend allowing for tubing connections up to 15,000 psi and temperatures of 100 °C or below.

The adapters, 51279-U and 51281-U, have internal threaded ports that feature a zero-dead volume (ZDV) connection between both ends of the tube. The one-piece Ultra High Performance Fingertight fitting allows them to be used in temperature applications up to 200 °C and pressures to 6,000 psi and at room temperatures up to 15,000 psi.

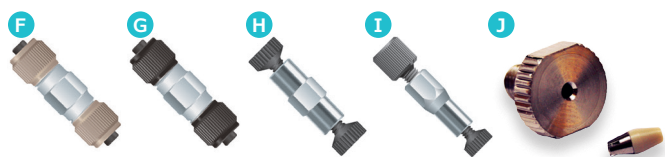
Description	Qty.	Cat. No.
<b>UHPLC Micro Union</b>		
<b>F</b> For 1/32" Tubing	1 ea.	<b>51274-U</b>
<b>G</b> For 360 µm Tubing	1 ea.	<b>51277-U</b>
<b>H</b> For 1/32" Tubing (Fingertight Fittings)	1 ea.	<b>51279-U</b>
<b>UHPLC Adapter</b>		
<b>I</b> For 1/32" and 1/16" Tubing (Fingertight Fittings)	1 ea.	<b>51281-U</b>

## Optimize Technologies® EXP® Fitting System

The EXP® Fitting System is the premier adjustable nut and ferrule compression fitting for extreme high-pressure connections between 1/16" tubing and any 10–32 port. The Titanium Hybrid ferrule

provides a perfect seal with every connection, yet can be released without tools to adjust to the different port depths of various hardware. There is no longer the need to clip off and replace ferrules from tubing.

Description	Qty.	Cat. No.
<b>UHPLC Micro Union</b>		
<b>J</b> EXP® Hand Tight Nut and Titanium Ferrule 10–32	1 ea.	<b>51384-U</b>
EXP® Hand Tight Nut and Titanium Ferrule 10–32	Pk/10	<b>51385-U</b>
Titanium Hybrid Ferrule	Pk/10	<b>51391-U</b>



# OPTI-SOLV EXP Pre-Column Filter

## Key Features and Benefits

- Tested to 30,000 psi
- Easily interchangeable with hand tight EXP® guard column

The OPTI-SOLV® EXP® Hand Tight Pre-Column Filter for extreme high-pressure applications is ideal for protecting HPLC columns with small particles employing ultra high pressure techniques. Such techniques analyze samples in the most demanding applications, which can decrease the life of these expensive columns. EXP® Pre-Column Filters help extend that life and protect your column investment, without sacrificing

- Auto adjusting ZDV column connection
- Hand tight filter replacement – NO TOOLS
- Low volume, low dispersion cartridges

performance. EXP® Pre-Column Filters are available with Titanium Hybrid ferrules for easy direct connection to any UHPLC column. The filter comes as a complete package including fittings to provide repeated tube stop and zero-dead volume column connections. Use EXP® Pre-Column Filters to protect any HPLC column of 2 mm I.D. to 4.6 mm I.D. It can be used up to at least 15,000 psi (1,000 bar).

## OPTI-SOLV® EXP® Pre-Column Filter Holder with EXP® Titanium Hybrid Ferrule

Description	Qty.	Cat. No.
<b>K</b> 2 Ferrules, 1 Nut in pack	1 ea.	<b>51163-U</b>
Replacement Titanium Hybrid Ferrule	Pk/10	<b>51391-U</b>

Order cartridges separately

## OPTI-SOLV® EXP® Pre-Column Filter Cartridge

I.D.	Qty.	Cat. No.
0.5 µm	Pk/5	<b>51164-U</b>
0.5 µm	Pk/10	<b>51165-U</b>
0.2 µm	Pk/5	<b>51166-U</b>
0.2 µm	Pk/10	<b>51167-U</b>

## OPTI-GUARD® 1 mm Guard Columns

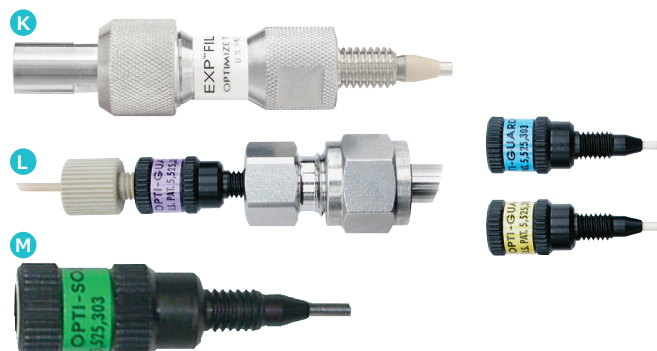
Manufactured from a proprietary PEEK blend, Upchurch Scientific® UHPLC Fingertight fittings can be used at temperatures up to 200 °C and higher pressure (to 23,000 psi/1,585 bar in some cases) than more traditional HPLC fittings. These fittings are available in one piece 10–32 as well as the traditional two-piece design.

Description	Cat. No.
<b>L</b> OPTI-GUARD® C18 (Violet Label)	51177-U
OPTI-GUARD® Silica (Orange Label)	51178-U
OPTI-GUARD® CN (Blue Label)	51179-U
OPTI-GUARD® Anion Exchange (Black Label)	51180-U
OPTI-GUARD® Cation Exchange (White Label)	51181-U
OPTI-GUARD® C18, Biocompatible	51183-U
OPTI-GUARD® C8	51184-U
OPTI-GUARD® Phenyl	51185-U
OPTI-GUARD® Amino, NH2	51187-U

## OPTI-SOLV® Filter

The OPTI-SOLV® filter provides low-impact filtering with a zero dead volume connection. Use the OPTI-SOLV® filter to prolong the life of your analytical column or before the mass spectrometer as a last defense against debris

Description	Qty.	Cat. No.
<b>M</b> OPTI-SOLV® Mini Filter 2 µm	5 ea.	51170-U
OPTI-SOLV® Mini Filter 5 µm	5 ea.	51171-U
OPTI-SOLV® Nano Filter 0.5 µm	5 ea.	51176-U



## LC-MS Post Column Flow Splitters

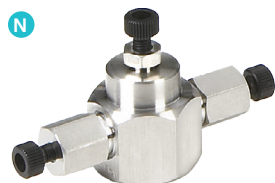
### Key Features and Benefits

- Eliminates laborious adjustments to capillary tubing for split ratio optimization
- Ultra low dead volume design
- Easy-to-use interchangeable fluid resistors
- Rugged stainless steel construction

The design of the ASI QuickSplit™ Flow Splitter with fixed or adjustable split ratio allows split flow ratios to remain stable and reproducible, and unaffected by changes in viscosity or pressure. Conventional splitters use long lengths of capillary tubing but the QuickSplit™ Flow Splitter uses two compact fluid resistor elements which are designed as cartridges for easy replacement. The QuickSplit™ is available in a variety of configurations including fixed split ratios from 3:1 to 20:1 or an adjustable flow splitter with a range of 1:1 to 20:1. The fluid resistors can be purchased separately and are interchangeable, so changes in split ratios can be made quickly and accurately.

Description	Cat. No.
HPLC Post Column Flow Splitters – Fixed	
<b>N</b> Split Ratio = 20:1	<b>56624-U</b>
Split Ratio = 10:1	<b>56625-U</b>
Split Ratio = 5:1	<b>56626-U</b>
Split Ratio = 3:1	<b>56627-U</b>
Mounting Bracket for HPLC Post Column Flow Splitters – Fixed	<b>56630-U</b>

Description	Cat. No.
HPLC Post Column Flow Splitter – Adjustable	
Split Ratio = 1:1 to 20:1	<b>56629-U</b>
HPLC Post Column Resistor Sets – Binary	
Split Ratio = 20:1	<b>56631-U</b>
Split Ratio = 10:1	<b>56632-U</b>
Split Ratio = 5:1	<b>56633-U</b>
Split Ratio = 3:1	<b>56634-U</b>



# All-Glass Filter Holder

## Filter Holder for 47 mm and 90 mm disc filters

The Millipore® All-Glass Filter Holder was designed for HPLC solvent preparation. The vacuum connection is integrated into the filter holder base and flask cap, above the filtrate exit level. This design prevents filtrate from entering the vacuum tubing and allows simple transfer of filtrate (i.e., HPLC solvents) by pouring from the receiver flask.

### Features & Benefits

- All-glass construction has broad chemical compatibility
- Borosilicate glass parts contact liquid, with ground-glass sealing surfaces
- Vacuum connection to flask cap simplifies transfer of filtrate

### Specifications

	47 mm Holder	90 mm Holder
Materials of Construction	Borosilicate glass funnel and base and cap, anodized aluminum spring clamp	
Filter Support Material	Fritted glass	Stainless steel or fritted glass
Filter Size	47 mm	90 mm
Filtration Area	9.6 cm <sup>2</sup>	40 cm <sup>2</sup>
Funnel Volume	300 mL or 500 mL	1 L
Flask Volume	1 L	Sold Separately

### All-Glass Filter Holders (For 47 mm and 90 mm)

Product	Cat. No.
Millipore® All-Glass Filter Holder – Kit 47 mm, Glass frit membrane support, 300 mL funnel	XX1514700
Millipore® All-Glass Filter Holder – Kit 47 mm, Glass frit membrane support, 500 mL funnel	XX5514700
Millipore® All-Glass Filter Holder – Kit 90 mm, Stainless steel screen membrane support, 1L Funnel	XX1019020
Millipore® All-Glass Filter Holder – Kit 90 mm, Glass frit membrane support, 1L Funnel	XX1019022



XX1514700



XX5514700



XX1019020

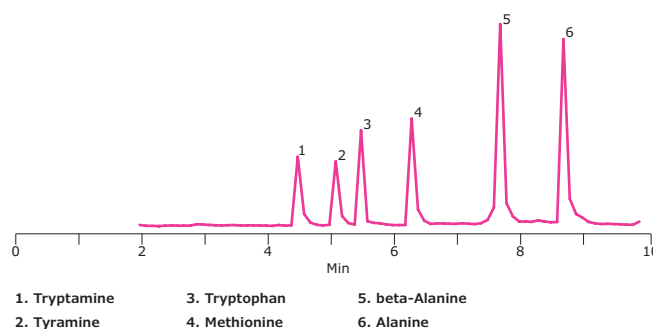
# LC-MS Applications

## Practical Recommendations for Maximum System Performance

- Use 0.005 in. I.D. inlet and outlet tubes. Broadening is much less sensitive to the tube length than to the I.D. Minimize lengths of the inlet and outlet tubes for best performance, but do not worry about having a few extra centimeters of length if it makes maintenance or column installation easier.
- If high pressure becomes a problem, then use acetonitrile as modifier and elevate the column temperature whenever possible. If methanol, THF, or another more viscous modifier is required, then elevating the temperature becomes even more beneficial. Even a modest temperature increase will greatly reduce the mobile phase viscosity and the required pressure while improving mass transfer.
- Keep the sample volumes small – 5 µL or less if the peaks of interest elute early ( $k = 1$ ). Up to 20 µL is acceptable if  $k$  exceeds 10.
- Avoid sample solvents that are stronger than the mobile phase.

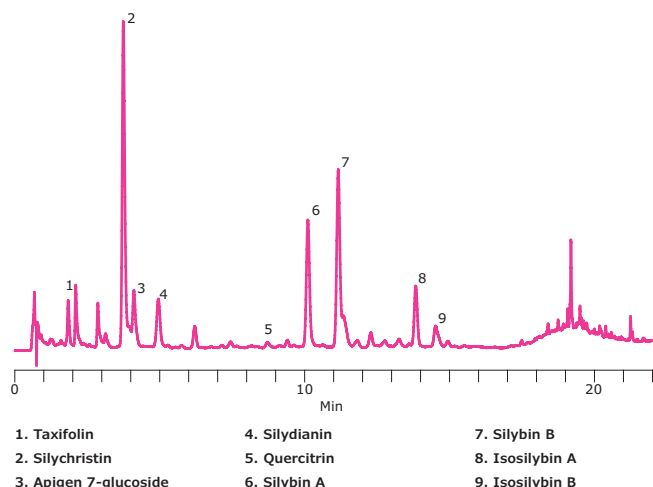
### Amino Acids

<b>Column</b>	Ascentis® Express HILIC, 15 cm × 4.6 mm I.D., 2.7 µm (53981-U)
<b>Mobile phase A</b>	13 mM ammonium acetate in 10:90 (v/v) water: acetonitrile
<b>Mobile phase B</b>	13 mM ammonium acetate in water
<b>Gradient</b>	held at 0% B for 1 min; 0 to 90% B in 19 min
<b>Flow rate</b>	1.0 mL/min
<b>Column temp</b>	35 °C
<b>Detector</b>	ESI(+)
<b>Injection</b>	10 µL
<b>Sample</b>	10 mg/L in Mobile Phase A



### Herbal Supplement Containing Milk Thistle

<b>Column</b>	Ascentis® Express C18, 10 cm × 3.0 mm I.D., 2.7 µm (53814-U)
<b>Mobile phase A</b>	water with 0.1% formic acid
<b>Mobile phase B</b>	methanol
<b>Gradient</b>	held at 35% B for 3 min; 35 to 45% B in 10 min; held at 45% B for 2 min; 45 to 100% B in 5 min
<b>Flow rate</b>	0.6 mL/min
<b>Column temp</b>	35 °C
<b>Detector</b>	UV, 254 nm
<b>Injection</b>	20 µL
<b>Sample</b>	20 mg/mL in water:ethanol (5:95); sonicate 15 minutes; filter 0.45 µm; dilute to water: ethanol (80:20)

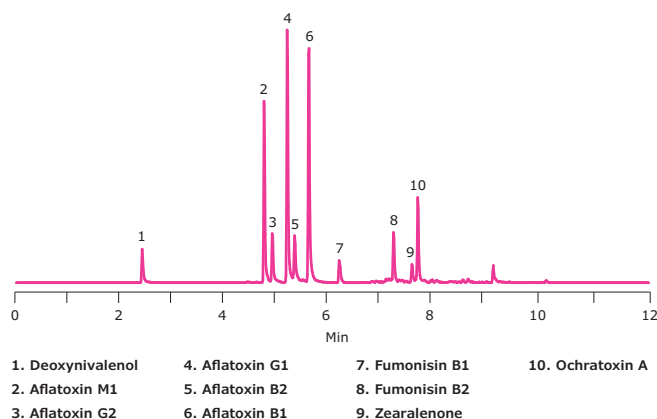


For more information on these columns, see pages 4-5.



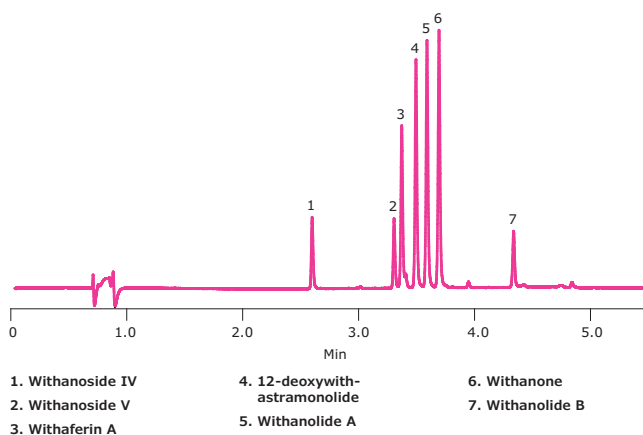
## Mycotoxins

Column	Ascentis® Express C18, 10 cm × 2.1 mm I.D., 2.7 µm (53823-U)
Mobile phase A	5 mM ammonium formate, pH 2.5 (titrated with formic acid)
Mobile phase B	methanol
Gradient	5 to 100% B in 10 min
Flow rate	0.4 mL/min
Pressure	325 bar
Column temp	35 °C
Detector	APCI (+)
Injection	5 µL
Sample	0.15 - 30 mg/L in 90:10, water:acetonitrile



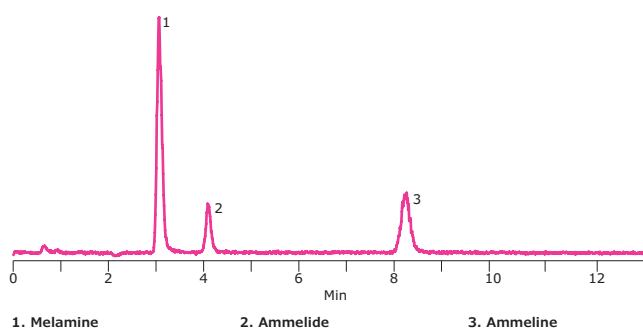
## Withania (Aswagandha)

Column	Ascentis® Express F5, 10 cm × 2.1 mm I.D., 2.7 µm (53569-U)
Mobile phase A	water
Mobile phase B	acetonitrile
Gradient	20 to 100% B in 10 min: held at 100% B for 0.5 min
Flow rate	0.3 mL/min
Pressure	190 bar
Column temp	35 °C
Detector	UV at 227 nm
Injection	5 µL
Sample	20 µg/mL in 80:20, water:methanol



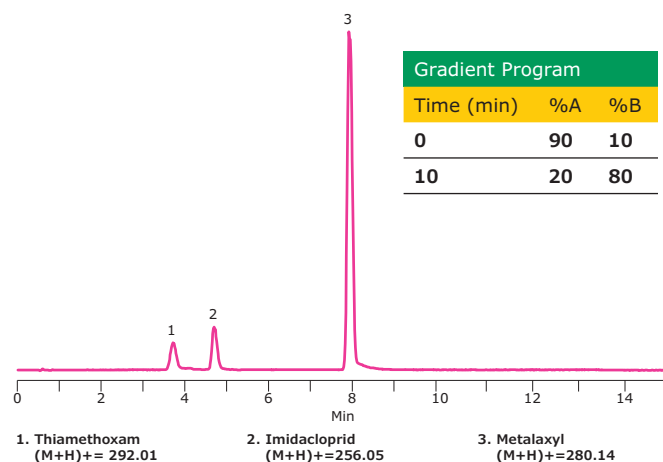
## Melamine and Hydrolysis

Column	Ascentis® Express HILIC, 5 cm × 2.1 mm I.D., 2.7 µm (53934-U)
Mobile phase	5 mM ammonium formate in 95:5 (v/v) acetonitrile:water
Flow rate	0.2 mL/min
Column temp	35 °C
Detector	MS, ESI(+), full scan
Injection	2 µL
Sample	1 mg/L in mobile phase



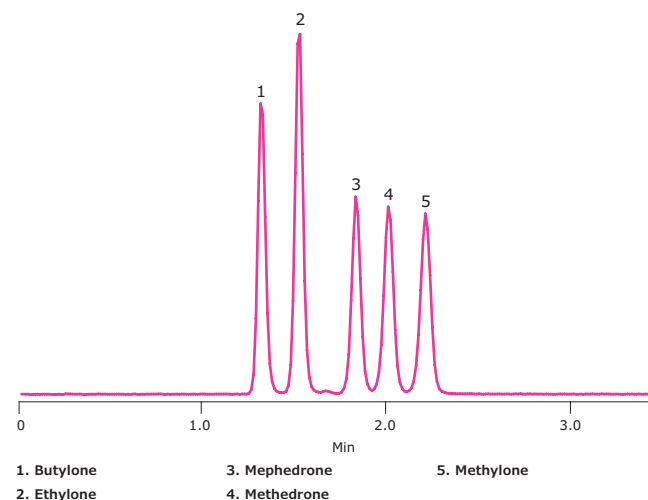
## Pesticides

Column	Ascentis® C18, 5 cm x 2.1 mm I.D., 3 µm particles (581300-U)
Mobile phase A	0.1% ammonium acetate, pH unadjusted
Mobile phase B	acetonitrile
Flow rate	0.2 mL/min.
Column temp	35 °C
Detector	MS, (+) ESI, Selected Ion Recording Mode injection
Injection	5 µL
Sample	1 µg/mL each in 90:10 water:acetonitrile
Sample	0.15 - 30 mg/L in 90:10, water:acetonitrile



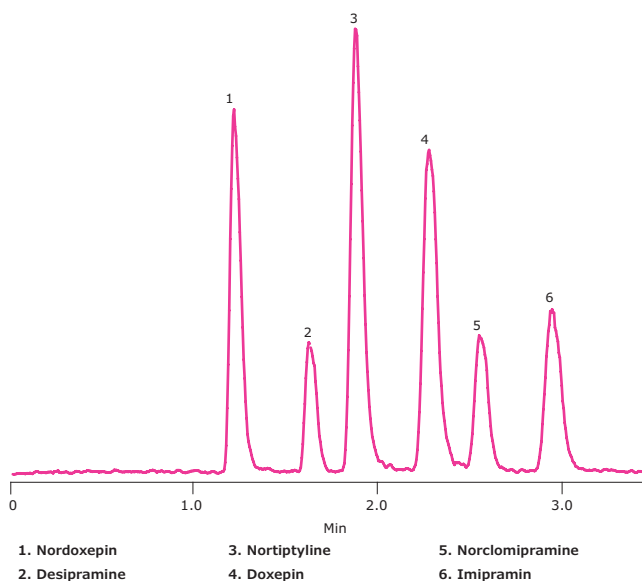
## Illicit Bath Salts

Column	Ascentis® Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm (53934-U)
Mobile phase A	5 mM ammonium formate (95:5 acetonitrile:water) (A025)
Flow rate	0.6 mL/min
Column temp	35 °C
Detector	ESI(+), TIC 100 - 1,000 m/z
Sample	1 µL



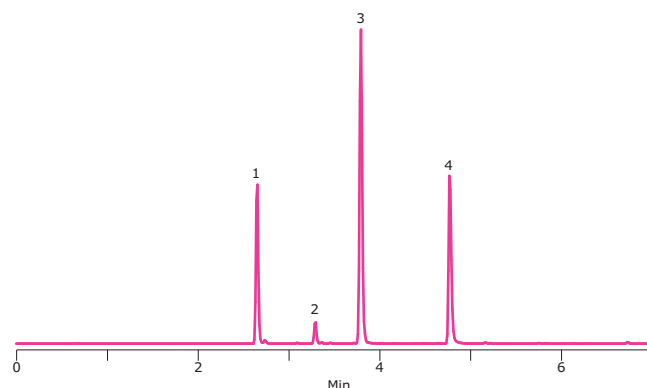
## Tricyclic Antidepressants

Column	Ascentis® Express C18, 10 cm x 2.1 mm ID (53823-U)
Mobile phase A	100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide)
Mobile phase B	water
Mobile phase C	methanol
Flow rate	0.3 mL/min
Column temp	55 °C
Detector	Thermo LCQ Advantage; ESI(+), m/z 250-320
Sample	1 µL
Instrument	Jasco X-LC



## Basic Peptides

Column	BIOshell™, A160 Peptide C18, 10 cm × 2.1 mm I.D., 2.7 µm (66904-U)
Mobile phase A	0.1% (v/v) additive in water
Mobile phase B	25:75, 0.4% (v/v) additive in water:acetonitrile
Additive	formic acid, pH 3.5 (adjusted with ammonium hydroxide)
Gradient	initial = 15% B, slope = 2% MeCN / column volume
Flow rate	0.3 mL/min
Temp.	35 °C
Detector	ESI(+)-TOF
Injection	1 µL
Sample	5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

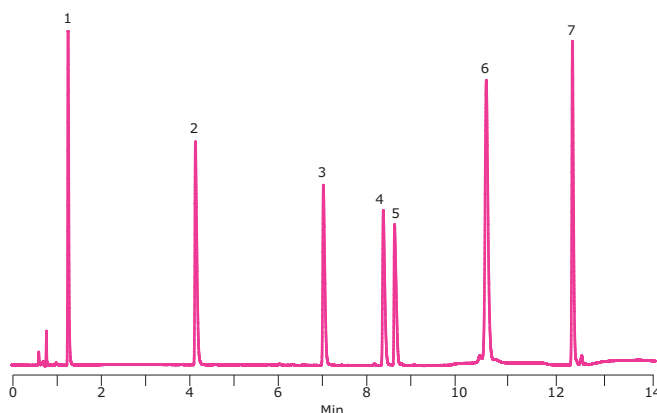


Peptide probes (listed in order of elution):

- |   |   |
|---|---|
| 1. ac-GGGLGGAGGLKG<br>monoisotopic mass: 941.5  | 3. ac-GGAVKALKGLKG<br>monoisotopic mass: 1139.7 |
| 2. ac-KYGLGGAGGLKG<br>monoisotopic mass: 1118.6 | 4. ac-KYALKALKGLKG<br>monoisotopic mass: 1330.8 |

## Peptide Test Mix

Column	BIOshell™ A160 Peptide C18, 10 cm × 4.6 mm I.D., 2.7 µm (66915-U)
Mobile phase A	0.1% (w/v) TFA in 90:10 water:acetonitrile
Mobile phase B	0.095% (w/v) TFA in 25:75 water:acetonitrile
Gradient	initial = 0% B to 50% B in 15 min.
Flow rate	1.5 mL/min
Temp.	30 °C
Detector	UV at 220 nm
Injection	5 µL

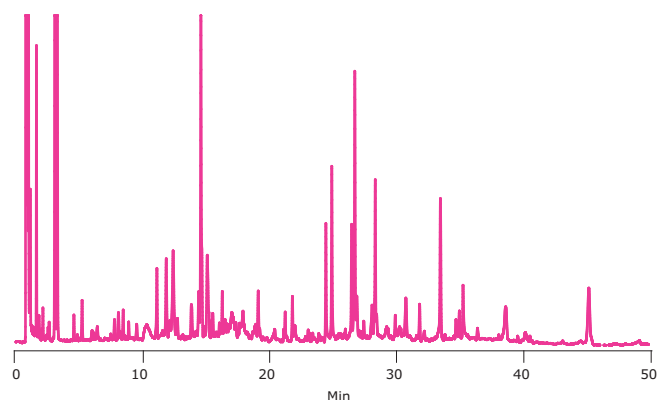


The test mix employed contains the following peptides:

- |                             |                             |
|-----------------------------|-----------------------------|
| 1. Gly-Tyr MW = 252         | 5. Leu-Enkephalin MW = 555  |
| 2. Val-Tyr-Val MW = 379     | 6. Ribonuclease MW = 13,700 |
| 3. Met Enkephalin MW = 574  | 7. Bovine Insulin MW = 5733 |
| 4. Angiotensin II MW = 1032 |                             |

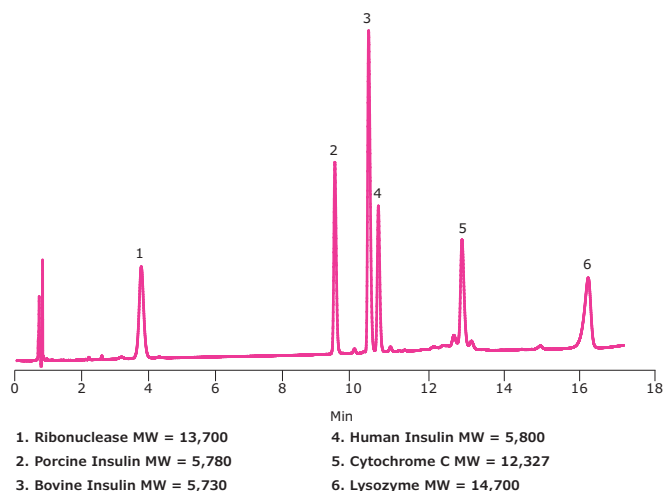
## Carbonic Anhydrase Tryptic Digest

Column	BIOshell™ A160 Peptide C18, 10 cm × 4.6 mm I.D., 2.7 µm (66915-U)
Mobile phase A	0.1% (w/v) TFA in water
Mobile phase B	0.1% TFA (w/v) in 40:60 water:acetonitrile
Gradient	initial = 3% B to 100% B in 53 min.
Flow rate	1.0 mL/min
Temp.	30 °C
Detector	UV at 215 nm
Injection	20 µL



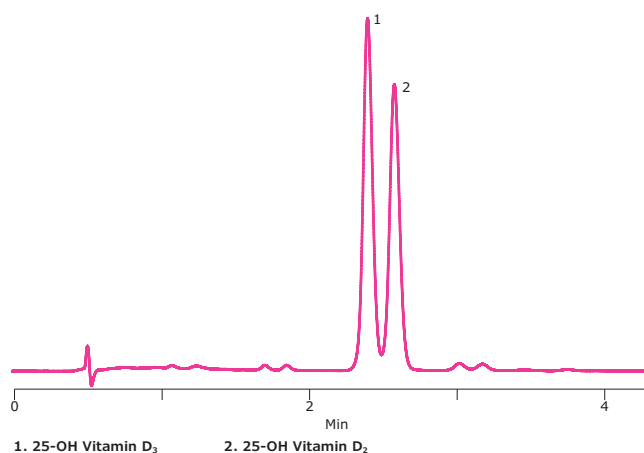
## Small Proteins

Column	BIOshell™ A160 Peptide C18, 10 cm × 4.6 mm I.D., 2.7 μm (66915-U)
Mobile phase A	0.1% (w/v) TFA in 90:10 water:acetonitrile
Mobile phase B	0.095% (w/v) TFA in 25:75 water:acetonitrile
Gradient	initial = 25% B to 40% B in 15 min.; then to 60% B at 20 min.
Flow rate	1.5 mL/min
Temp.	30 °C
Detector	UV at 220 nm
Injection	4 μL



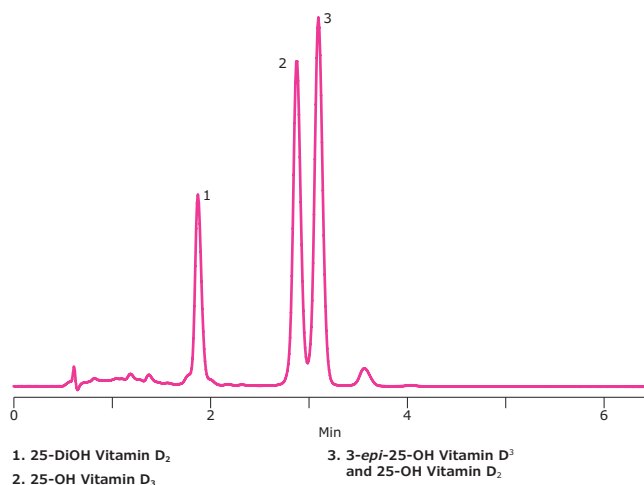
## HPLC Separation of 25-Hydroxyvitamin D<sub>2</sub> and 25-Hydroxyvitamin D<sub>3</sub> on Ascentis® Express F5

Column	Ascentis® Express F5, 10 cm × 2.1 mm I.D., 2.7 μm (53569-U)
Mobile phase	(A) 5 mM ammonium formate; (B) 5 mM ammonium formate in methanol; (25:75, A:B)
Flow rate	0.4 mL/min
Column temp.	40 °C
Detector	ESI(+), m/z 100–1,000
Injection	1 μL, each compound 20 μg/mL in methanol



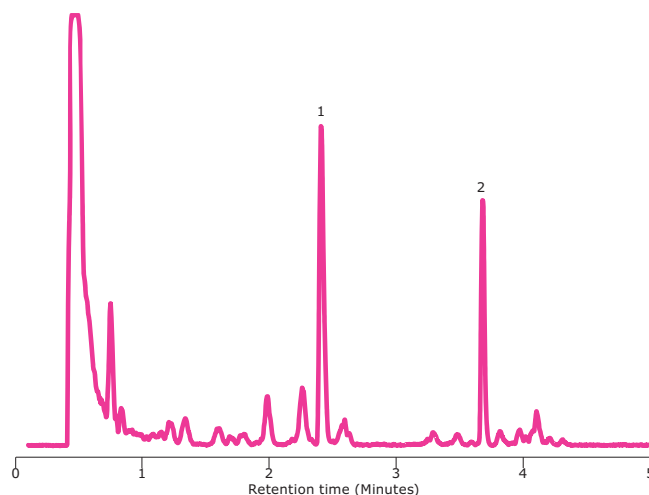
## HPLC Separation of 25-Dihydroxyvitamin D<sub>2</sub>, 25-Hydroxyvitamin D<sub>3</sub>, and 3-*epi*-25-Hydroxyvitamin D<sub>3</sub> on Ascentis® Express F5

Column	Ascentis® Express F5, 10 cm × 2.1 mm I.D., 2.7 μm (53569-U)
Mobile phase	(A) 5 mM ammonium formate; (B) 5 mM ammonium formate in methanol; (25:75, A:B)
Flow rate	0.4 mL/min
Column temp.	40 °C
Detector	ESI(+), m/z 100–1,000
Injection	1 μL, each compound 20 μg/mL in methanol



## Cochineal Red A and Azorubine

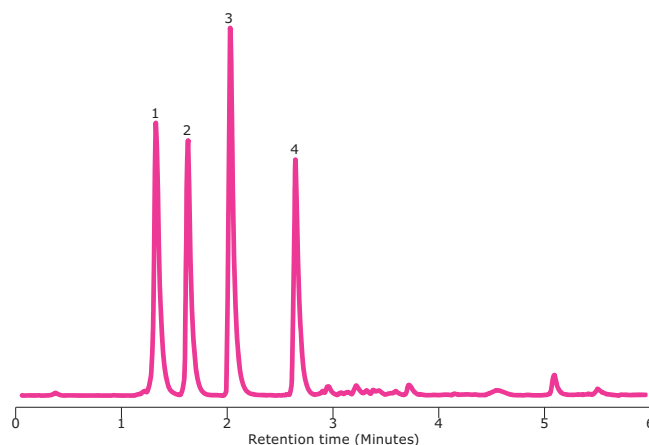
<b>Column</b>	Chromolith® FastGradient RP-18, 5 cm x 2.0 mm I.D.
<b>Mobile phase A</b>	acetonitrile
<b>Mobile phase B</b>	20 mM ammonium acetate, pH 4.7 in water
<b>Gradient</b>	2.5 to 50% A in 6 min; held at 50% A for 2 min
<b>Flow rate</b>	0.4 mL/min
<b>Pressure</b>	856-899 psi (59-62 bar)
<b>Column Temp.</b>	25 °C
<b>Detector</b>	ESI-MS(-) (m/z range 100-550), BPC
<b>Injection</b>	1 µL



1. E-124 (Cochineal Red A, Ponceau 4R) 2. E-122 (Azorubine, Carmosine)

## Flavonoids

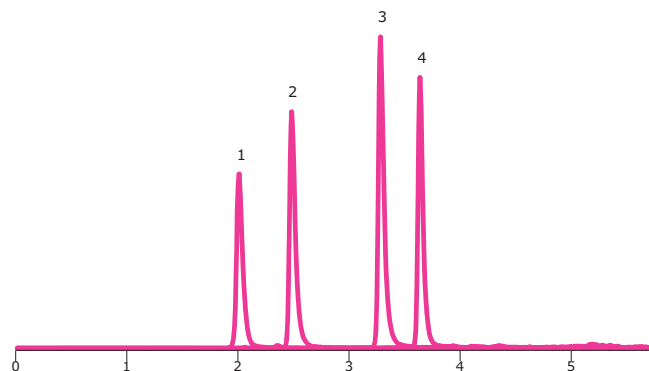
<b>Column</b>	Chromolith® FastGradient RP-18, 5 cm x 2.0 mm I.D.
<b>Mobile phase A</b>	0.1% formic acid in water
<b>Mobile phase B</b>	0.1% formic acid in acetonitrile
<b>Gradient</b>	5 to 95% B in 4 min, held at 95% B for 0.5 min, to 5% B in 0.5 min, held at 5% for 2 min
<b>Flow rate</b>	0.4 mL/min
<b>Pressure</b>	677-245 psi (47-17 bar)
<b>Column Temp.</b>	25 °C
<b>Detector</b>	ESI-MS(+) (m/z range 100-455)
<b>Injection</b>	1 µL
<b>Sample</b>	catechin 317 ppb, epicatechin 270 ppb, quercetin 198 ppb, hesperetin 177 ppb in water



1. (+)-Catechin 2. (-)-Epicatechin 3. Quercitrin 4. Hesperetin

## Isoflavones

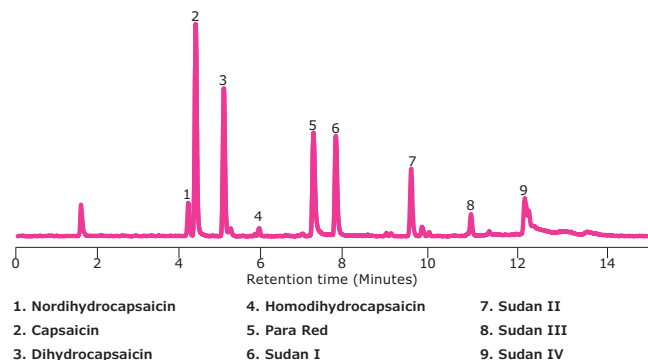
<b>Column</b>	Chromolith® FastGradient RP-18, 5 cm x 2.0 mm I.D.
<b>Mobile phase A</b>	0.1% formic acid in water
<b>Mobile phase B</b>	0.1% formic acid in acetonitrile
<b>Gradient</b>	5 to 15% B in 1.5 min, to 95% B in 4 min, held at 95% B for 1 min, to 5% B in 1 min, held at 5% for 1.5 min
<b>Flow rate</b>	0.5 mL/min
<b>Pressure</b>	821-317 psi (57-22 bar)
<b>Column Temp.</b>	25 °C
<b>Detector</b>	ESI-MS(+) (m/z range 100-600), overlay of four EICs (m/z 271.0, 255.0, 447.1, 417.1)
<b>Injection</b>	1 µL
<b>Sample</b>	genistein 10 ppb, daidzein 10 ppb, glycitin 10 ppb, puerarin 20 ppb in methanol and water (50:50 v/v)



1. Puerarin 2. Glycitin 3. Daidzein 4. Genistein

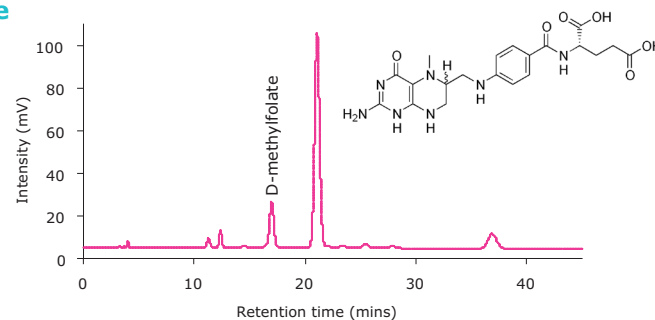
## Sudan Dyes and Capsaicinoids

<b>Column</b>	Chromolith® CapRod™ RP-18, 15 cm x 0.1 mm I.D.
<b>Mobile phase A</b>	0.1% formic acid in water
<b>Mobile phase B</b>	0.1% formic acid in acetonitrile
<b>Gradient</b>	35 to 95% B in 12 min
<b>Flow rate</b>	1.24 µL/min
<b>Pressure</b>	1160 psi (80 bar)
<b>Column Temp.</b>	ambient
<b>Detector</b>	nano-ESI(+) 100-600 m/z
<b>Injection</b>	2.5 nL



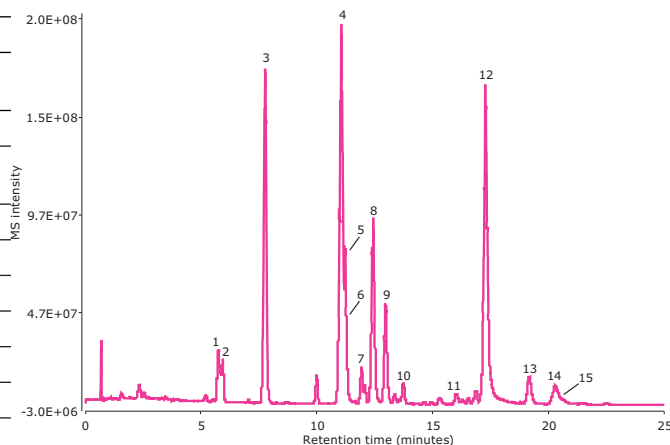
## Separation of L-methyl folate and D-methyl folate

<b>Column</b>	SeQuant® ZIC®-CHILIC 100 Å, 15 cm x 4.6 mm, 3 µm particles
<b>Injection</b>	10 µL
<b>Detection</b>	Shimadzu Prominence, U.V. 280 nm
<b>Cell</b>	
<b>Flow Rate</b>	1.0 mL/min
<b>Mobile Phase</b>	Dissolve 3.54 g of ammonium acetate in 1000 mL Milli-Q® water. Mix buffer and acetonitrile 23:77 (v/v)
<b>Temperature</b>	30 °C
<b>Standard</b>	Take 25 mg of L-methyl folate standard in 100 mL volumetric flask and dissolve in 30 mL of buffer. Sonicate it and make up volume with acetonitrile.
<b>Sample</b>	Take 25 mg of sample in 100 mL volumetric flask and dissolve in 30 mL of buffer. Sonicate it and make up volume with acetonitrile.
<b>Pressure Drop</b>	70 Bar(1015 psi)



## Analysis of a tryptic digest of bovine cytochrome C

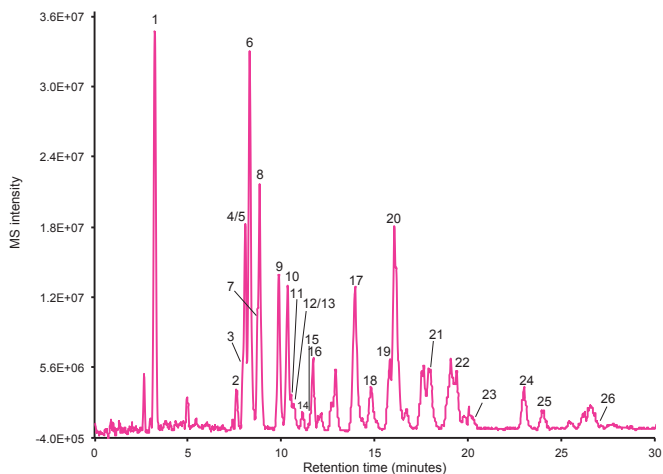
<b>Chromatographic Conditions</b>	
<b>Column</b>	SeQuant® ZIC®-HILIC 200 Å, 5 cm x 2.1 mm, 3.5 µm particles
<b>Injection</b>	0.2 µL
<b>Detection</b>	pos. ESI-MS (m/z range 310-730), BPC; MS/MS (m/z range 100-800)
<b>Cell</b>	0
<b>Flow Rate</b>	0.25 mL/min
<b>Mobile Phase (v/v)</b>	A: acetonitrile + 0.1 % formic acid B: water + 0.1 % formic acid
<b>Temperature</b>	25 °C
<b>Diluent</b>	water
<b>Sample:</b>	A lyophilized tryptic digest of bovine cytochrome C (13 nMol protein) was resuspended utilizing 0.5 mL ACN/water + 0.1% FA 10/90
<b>Pressure Drop</b>	28 - 64 bar





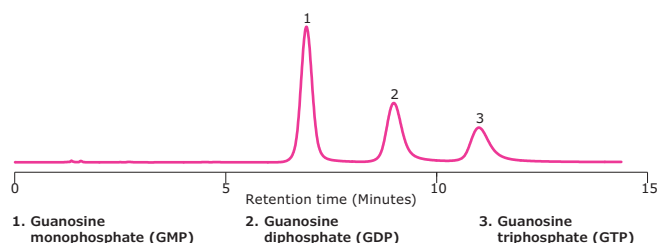
## Analysis of a tryptic digest of bovine serum albumine

Chromatographic Conditions	
Column	SeQuant® ZIC®-HILIC 200 Å, 10 cm x 2.1 mm, 3.5 µm particles
Injection	0.2 µL
Detection	pos. ESI-MS, m/z range 100-2200 (BPC); m/z range 200-1200 (MS/MS)
Cell	0
Flow Rate	0.2 mL/min
Mobile Phase (v/v)	A: acetonitrile + 0.1 % formic acid B: water + 0.1 % formic acid
Temperature	25 °C
Diluent	water
Sample	A lyophilized tryptic digest of bovine serum albumine (1 nMol protein) was resuspended utilizing 0.4 mL ACN/water 50/50 + 0.1% FA
Pressure Drop	78 - 120 bar



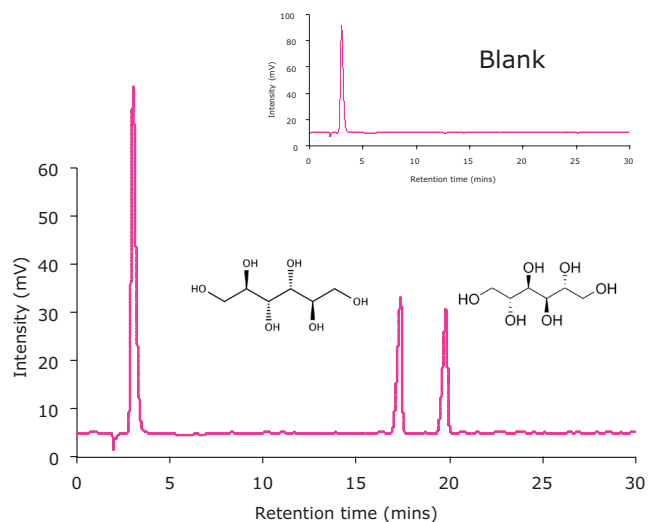
## GMP, GDP and GTP

Column	SeQuant® ZIC®-HILIC 200 Å, 10 cm x 4.6 mm, 5 µm particles
Mobile phase A	acetonitrile
Mobile phase B	ammonium acetate; (70:30, A,B)
Flow rate	0.7 mL/min
Detector	UV (259 nm)
Injection	5 µL



## Assay of Sorbitol & Mannitol ZIC®-cHILIC

Chromatographic Conditions	
Column	SeQuant® ZIC®-cHILIC 100 Å, 25 cm x 4.6 mm, 3 µm particles
Injection	
Detection	Shimadzu Prominence, R.I.
Cell	10 µL
Flow Rate	1.5 mL/min
Mobile Phase (v/v)	Acetonitrile : 0.1% orthophosphoric acid in Milli-Q® water 90/10 (v/v)
Temperature	45 °C (oven), 40 °C (detector cell)
Diluent	Acetonitrile : 0.1% orthophosphoric acid in Milli-Q® water 75/25 (v/v)
System suitability solution	5.0 mg/mL each of sorbitol and Mannitol in diluent
Pressure Drop	116 Bar(1682 psi)



## Notes

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## Notes

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## Notes

[illegible]

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# Supelco®

Analytical Products

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Frankfurter Strasse 250  
64293 Darmstadt, Germany

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