SiliaChrom®

HPLC Columns

SILICYCLE Inc.
UltraPure SILICA GELS

Silia*Chrom* HPLC Columns

Using Silia*Chrom* HPLC Columns in chromatographic applications ensures the following:

- Excellent column efficiency.
- Long lifetime and column-to-column reproducibility.
- Broad pH range from 0.8 to 12.
- Compatibility with 100% aqueous and organic mobile phases.
- High surface coverage presenting no bleeding for LC-MS applications.

Presentation of the Silia*Chrom* HPLC Column Series

SiliCycle manufactures a variety of HPLC columns for reversed and normal phase applications. The Silia*Chrom* series contain more than 40 different phases, and we continue to develop additional, unique and powerful HPLC sorbents. Most of the Silia*Chrom* are silica-based products. You can be assured of the quality, from raw material synthesis through to the packing process.

We pack bonded phases in a wide range of column dimensions, including standard narrow bore and analytical columns in lengths of 20 to 250 mm, internal diameters (*ID*) of 2.0 - 4.6 mm, with particle sizes of 2.5, 3.0, 5.0, 10.0 or 20.0 µm. Also, preparative and semi-preparative HPLC columns are available, in 10, 20, 30 and 50 mm ID with lengths of 50, 100, 150 and 250 mm with particle sizes of up to 20 µm. These columns exhibit superior performances for any type of compound. The Silia*Chrom* series, with its unique sol-gel process technology, offers the total solution for HPLC end-users: broad pH range (*0.5 - 12*), compatibility with 100% aqueous and organic mobile phases, low bleeding for LC-MS, high surface coverage, and excellent column efficiency. All columns are packed using a consistent proprietary packing process to achieve uniform and stable bed for long lifetime and column-to-column reproducibility.

The Silia*Chrom* HPLC portfolio offers a broad variety of separations for various types of chromatography such as biochromatography of large molecules, size exclusion chromatography for large proteins and peptides, chiral chromatography for enantioselective separations and supercritical fluid chromatography for API separations. The following pages will highlight Silia*Chrom* phases that can be used for these applications.

SiliCycle; Experts in HPLC Column Packing

Superior HPLC columns can be produced only with excellent packing materials and excellent packing techniques. Silia*Chrom* columns are made from extremely pure silicas and are well known for their high efficiency and high resolution capacity. Based on spherical, totally porous silica, Silia*Chrom* columns provide enhanced chemical and mechanical stability as well as very high loading capacity and full end-capping.

All Silia*Chrom* columns are packed using a proprietary slurry packing process to achieve uniform and column-to-column reproducibility. Silia*Chrom* columns have good selectivity, good asymmetry and long lifetime for HPLC separation of acidic, neutral and basic organic compounds, polar or non-polar.

Standard HPLC Columns

Silia*Chrom* HPLC columns are available in Narrow Bore, Analytical, Semi-Preparative, and Preparative formats.

Silia*Chrom* HPLC Standard Column Dimensions

		Silia*Chrom* HPLC Column Length (mm)						
		20	30	50	100	150	200	250
Particle Size (µm)		2.5, 3 & 5	2.5, 3 & 5	2.5, 3, 5, 7 & 10	2.5, 3, 5, 7, 10 & 20	2.5, 3, 5, 7, 10 & 20	5 & 10	2.5, 3, 5, 7, 10 & 20
Silia*Chrom* HPLC Column Internal Diameter (mm)	2.0	S	S	S	S	S	C	C
	2.1	S	S	S	S	S	C	C
	3.0	C	S	S	S	S	C	C
	4.6	C	S	S	S	S	S	S
	10	C	C	C	S	S	S	S
	20	C	C	C	C	S	S	S
	30	C	C	C	S	S	S	S
	50	C	C	C	S	S	S	S
	100	C	C	C	C	C	C	C

S = Standard C = Custom

« SiliCycle has been able to repeatedly come through and produce high quality semi-prep HPLC columns (50+ mm ID) for several different projects that we have done. For many of these projects prices is not the driving force, the timing is.»

Jason Blanchard from Ricerca Biosciences, Concord, OH, USA

Column Packing Reproducibility

SiliCycle is recognized for its strong expertise in column packing technology. All Silia*Chrom* columns are packed using a consistent packing methodology to achieve an extremely stable and uniform column packing bed leading to high column lifetime and column-to-column reproducibility. To prove this, we packed and tested several analytical columns 4.6 x 250 mm using the same Silia*Sphere* C18 3 µm, 100 Å for reproducibility and high efficiency evaluation.

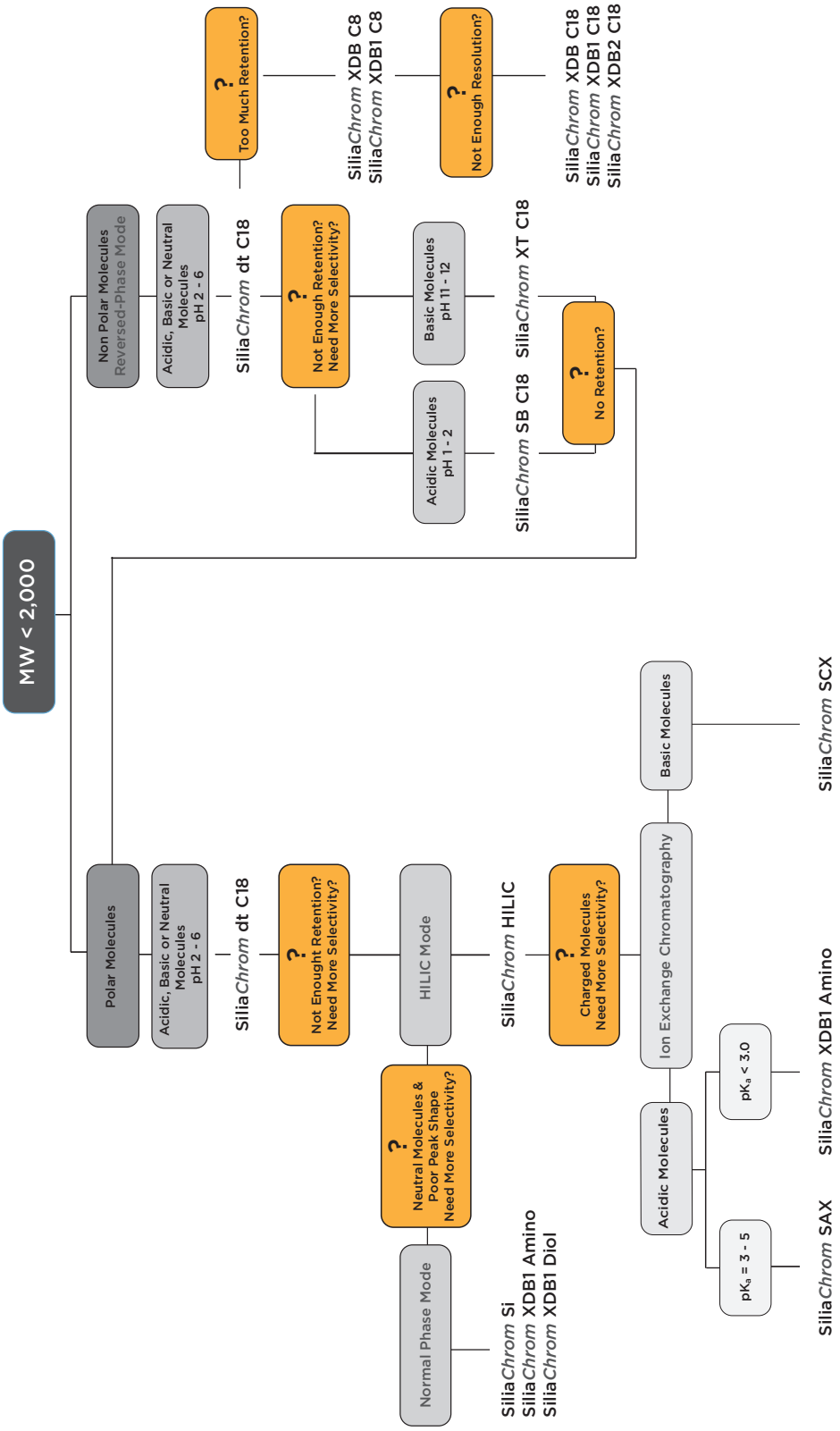
Chromatographic conditions

- **Sample mixture in mobile phase**: Uracil / Phenol / Nitrobenzene / Naphtalene
- **Injection volume**: 2 µL
- **Temperature**: 30°C
- **Flow rate**: 0.8 mL/min
- **Mobile phase**: 15% Water, 85% Methanol

Observed Column Parameters for Napthalene			
Column Number	Retention Time (min)	Theoretical Plates Number / meter	Tailing Factor
1	9.148	28,481	1.01
2	9.382	28,391	1.00
3	9.398	28,712	1.00
4	8.998	28,150	1.01
5	9.307	28,393	1.00
6	9.307	28,267	1.03
7	9.015	28,153	1.04
8	9.373	28,801	1.06
9	9.298	28,357	1.00
10	9.298	28,206	1.04
Average	9.252	28,391	1.02
Standard Deviation	0.147	222	0.02
Relative Standard Deviation	1.589	0.783	2.14

SiliaChrom Selection Guide for Small Molecules

(Molecular Weight < 2,000 Dalton)



Silia*Chrom* HPLC columns Portfolio

How to build your Part Number

Silia*Chrom* HPLC columns are available in Narrow Bore, Analytical, Semi-Preparative, and Preparative sizes.

Below is an example of a Silia*Chrom* product number that shows you the way they are structured;

The product numbers start with the **phase** code, followed by the **particle size**, the **pore size**, the **internal diameter**, and finally the **length** codes.

Note: For Guard Columns, add the letter "G" between the "H" and the phase code.

Example;

Silia*Chrom* dt C18, 3 µm, 100 Å, 4.6 mm x 150 mm = H141803E-N150

Particle Size	
µm	Code
2.5	02
3.0	03
5.0	05
7.0	06
10	07
20	09

Pore Size	
Å	Code
100	E
150	H
300	M

Internal Diameter		
Type of Columns	mm	Code
Narrow Bore	2.0	E
Narrow Bore	2.1	G
Narrow Bore	3.0	H
Analytical	4.6	N
Semi-Preparative	10	Q
Preparative	20	Y
Preparative	30	V
Preparative	50	W
Preparative	100	X

Column Length	
mm	Code
10	010
20	020
30	030
50	050
100	100
150	150
200	200
250	250

Particle Size

Pore Size

Internal Diameter

Column Length

***You may also find and buy your Silia*Chrom* online at www.silicycle.com/products/siliachrom-hplc-columns**

phase code

SiliaChrom HPLC column Characteristics

SiliaChrom	Particle Size (µm)	Pore Size (Å)	Specific Surface Area (m^2/g)	Carbon Load (%)	pH Range	USP Code	T Limit* (°C)	Pressure Limit (psi)	Phase Code
SiliaChrom AQ C18	3, 5, 10	100	380	18	1.5 - 9.0	L1	60	5,000	H1518
SiliaChrom AQ C8	3, 5, 10	100	380	14	1.5 - 9.0	L7	60	5,000	H1508
SiliaChrom dt C18	2.5, 3, 5, 10	100	410 - 440	18	1.5 - 9.0	L1	60	5,000	H1418
SiliaChrom dt Si	3, 5, 10	100	410 - 440	n/a	1.0 - 8.0	L3	45	4,500	H1430
SiliaChrom XT C18	3, 5, 10	150	200	15	1.5 - 12.0	L1	60	5,000	H1718
SiliaChrom XT Fidelity C18	3, 5, 10	100	380	21	1.5 - 12.0	L1	60	5,000	HF1718
SiliaChrom SB C18	3, 5, 10	150	200	12	0.5 - 7.5	L1	60	4,500	H1018
SiliaChrom SB C18-300	5	300	80	5	0.5 - 7.5	L1	60	4,500	H1018
SiliaChrom SB C8	5	150	200	7	1.0 - 7.5	L7	60	4,500	H1008
SiliaChrom SB C8-300	5	300	80	3	1.0 - 7.5	L7	60	4,500	H1008
SiliaChrom XDB C18	5	150	200	15	1.5 - 9.0	L1	60	5,500	H1118
SiliaChrom XDB C8	5	150	200	8	1.5 - 9.0	L7	60	5,500	H1108
SiliaChrom XDB Si	5	150	200	n/a	1.0 - 8.0	L3	45	4,000	H1100
SiliaChrom XDB1 C18	3, 5	100	380 - 400	22	1.5 - 10.0	L1	60	5,500	H1218
SiliaChrom XDB1 C18-300	5, 10	300	80	8	1.5 - 9.0	L1	60	5,500	H1218
SiliaChrom XDB1 C8	5, 10	100	380 - 400	14	1.5 - 8.5	L7	60	5,500	H1208
SiliaChrom XDB1 C8-300	5	300	80	4	1.5 - 8.5	L7	60	5,500	H1208
SiliaChrom XDB1 C4	5	100	380 - 400	7	1.5 - 8.5	L26	60	5,500	H1204
SiliaChrom XDB1 C4-300	3, 5, 10	300	80	3	2.0 - 8.0	L26	60	5,500	H1204
SiliaChrom XDB1 C1	5	100	380 - 400	3	1.5 - 8.5	L13	60	5,500	H1201
SiliaChrom XDB1 C1-300	5	300	80	1	2.0 - 8.0	L13	60	5,500	H1201
SiliaChrom XDB1 CN	5, 10	100	380 - 400	5	2.0 - 8.5	L10	60	5,500	H1220
SiliaChrom XDB1 CN-300	5	300	80	3.5	2.0 - 8.0	L10	60	5,500	H1220
SiliaChrom XDB1 Amino	5, 10	100	380 - 400	7	2.0 - 8.5	L8	45	5,500	H1260
SiliaChrom XDB1 Amino-300	5	300	80	3.5	2.0 - 8.0	L8	45	5,500	H1260
SiliaChrom XDB1 Phenyl	5	100	380 - 400	12	1.5 - 9.0	L11	60	4,000	H1240
SiliaChrom XDB1 Phenyl-300	5	300	80	4.5	2.0 - 8.0	L11	60	4,000	H1240
SiliaChrom XDB1 Diol	5	100	380 - 400	5	2.0 - 8.0	L20	45	4,000	H1250
SiliaChrom XDB1 Diol-300	5	300	80	1	2.0 - 8.0	L20	45	4,000	H1250
SiliaChrom XDB1 Si	3, 5, 10	100	380 - 400	n/a	1.0 - 8.0	L3	45	4,000	H1230
SiliaChrom XDB1 Si-300	3, 5, 10	300	80	n/a	2.0 - 8.0	L3	45	4,000	H1230
SiliaChrom XDB2 C18	3, 5, 10	100	380	18	1.5 - 9.0	L1	60	5,000	H1318
SiliaChrom SCX	3, 5, 10	150	200	10	2.0 - 8.5	L9	45	5,000	H1800
SiliaChrom SCX-300	3, 5	300	80	3.5	2.0 - 8.0	L9	45	5,000	H1800
SiliaChrom SAX	3, 5, 10	100	380	6	2.0 - 8.5	L14	45	5,000	H1900
SiliaChrom SAX-300	3, 5	300	80	1	2.0 - 8.0	L14	45	5,000	H1900
SiliaChrom HILIC	3, 5, 10	100	380	8	2.0 - 8.0	-	60	5,000	H1600
SiliaChrom HILIC-300	5	300	80	2.5	2.0 - 8.0	-	60	5,000	H1600
SiliaChrom RPC	5, 7, 10, 20	n/a	750	polymer	1.0 - 14.0	L21	-	-	H920
SiliaChrom GF	5, 10	100	340	5	2.0 - 8.0	-	45	4,000	H900
SiliaChrom GF-300	5, 10	300	80	1	2.0 - 8.0	-	45	4,000	H900
SiliaChrom GF AMIDE	5, 10	100	340	5	2.0 - 8.0	-	60	4,000	H901
SiliaChrom GF AMIDE-300	5, 10	300	80	1	2.0 - 8.0	-	60	4,000	H901
SiliaChrom Cellulose T-DPC	5, 10	-	-	n/a	2.0 - 7.0	L40	40	< 700	H800
SiliaChrom Cellulose T-MB	5, 10	-	-	n/a	2.0 - 7.0	-	40	< 700	H820
SiliaChrom Amylose T-DPC	5, 10	-	-	n/a	2.0 - 7.0	L51	40	< 700	H810

*At pH range 5.0 - 7.5

SiliaChrom HPLC Selection Guide by Manufacturer

SiliaChrom HPLC Selection Guide by Manufacturer				
SiliaChrom HPLC Column	Agilent®	Eka Chemicals® (AksoNobel)	EMD Merck®	Phenomenex®
Reversed-Phases				
SiliaChrom dt C18 **SiliaChrom AQ C18**	Zorbax® SB Aq			Synergy™ Hydro RP Synergy™ Fusion RP
SiliaChrom XT C18	Zorbax® Extend C18	Kromasil® Eternity		Gemini® C18
SiliaChrom XT Fidelity C18	Zorbax® Extend C18	Kromasil® Eternity		Gemini®-NX C18
SiliaChrom SB C18	Zorbax® SB C18			
SiliaChrom SB C8	Zorbax® SB C8			
SiliaChrom XDB C18	Pursuit™ C18 Zorbax® XDB C18			
SiliaChrom XDB C8	Pursuit™ C8 Zorbax® XDB C8			
SiliaChrom XDB1 C18	Pursuit™ XRS C18	Kromasil® C18	LiChrospher® RP18e	Luna® C18
SiliaChrom XDB1 C8	Pursuit™ XRS C8	Kromasil® C8	LiChrospher® RP8	Luna® C8
SiliaChrom XDB1 Phenyl	Zorbax® SB Phenyl	Kromasil® Phenyl		
SiliaChrom XDB1 CN	Zorbax® SB CN			Luna® CN
SiliaChrom XDB2 C18	Zorbax® Eclipse Plus C18 Zorbax® Rx C18			Luna® C18(2)
Normal Phases				
SiliaChrom XDB1 Si	Zorbax® SIL Pursuit® XRS Si	Kromasil® Si	LiChrospher® Si 100	Luna® Silica
SiliaChrom XDB1 Diol		Kromasil® Diol	LiChrospher® Diol	Luna® Diol
SiliaChrom XDB1 Amino	Zorbax® SB NH_2	Kromasil® NH_2		Luna® NH_2
Ion Exchange Phases				
SiliaChrom SCX				Luna® SCX
SiliaChrom SAX	Agilent® SB-AX			Luna® SAX

SiliaChrom HPLC Chiral Selection Guide by Manufacturer				
SiliaChrom Chiral Column	Daicel	Eka Chemicals (AksoNobel)	Phenomenex	Supelco
SiliaChrom Cellulose T-DPC	ChiralCell® OD	Kromasil® CelluCoat	Lux® Cellulose-1	Astec™ Cellulose DMP
SiliaChrom Cellulose T-MB	ChiralCell® OJ		Lux® Cellulose-3	
SiliaChrom Amylose T-DPC	Chiralpak™ AD			

Supelco®	Thermo Fisher Scientific®	YMC®	Waters®	Others
	Acclaim® Polar Advantage Hypersil™ GOLD aQ C18	YMC™-PACK ODS -AQ	Atlantis® T3 Symmetry™ Shiels C18	Inertsil® ODS-3 ACE AQ C18
		YMC™ Triart C18	XTerra® C18	Nucleodur® C18 HTec
		YMC™ Triart C18	XBridge™ C18	
			X-Select™ CSH C18	
Discovery® C18 SUPELCOSIL™ LC-18-DB		YMC™-PACK ODS-A		Pinnacle™ DB C18
		YMC™-PACK C8		Pinnacle™ DB C8
Ascentis® C18		YMC™-PACK Pro RS	Sunfire™ C18 Symmetry™ C18	Ace® C18 HL Alltima™ HP C18 HiLoad
Ascentis® C8	Acclaim® C8	YMC™-PACK Pro C8	Sunfire™ C8 Symmetry™ C8	Ultra™ C8 ProntoSIL™ C8 SH
Ascentis® Phenyl	Hypersil® Phenyl	YMC™-PACK Ph		Ace® Phenyl ProntoSIL™ Phenyl
Ascentis® Cyano	Hypersil® Cyano	YMC™-PACK CN		ACE® CN
	Acclaim® 120 C18	YMC™-PACK Pro C18	SunFire™ C18	Ace® C18 Pinnacle™ II C18
Ascentis® Si		YMC™-PACK SIL	SunFire™ Si	Partasil™ Silica Nucleodur® SiOH
		YMC™-PACK Diol NP		ProntoSIL® Diol
	Acclaim® WAX	YMC™-PACK NH_2	Spherisorb® Amino	Ultra® Amino ProntoSIL® Amino E
	Hypersil® SCX		Spherisorb® SCX	Nucleosil® SCX Partisil® SCX
	Hypersil® SAX		Spherisorb® Amino	Nucleosil® SAX Partisil® SAX

« Your analytical HPLC columns are the best!
I improved my separations with several of my methods and
got an award for my efforts. Thanks for the great products!!! »

Cliff Klimas from Bristol-Myers-Squibb, Pennington, NJ, USA

Silia*Chrom* HPLC Selection Guide by USP Code

Silia*Chrom* HPLC Selection Guide by USP Code			
USP Code	Packing Type	Description	Silia*Chrom* HPLC Columns
L1	Bonding: Octadecyl (C18) Support Type: Silica Particle size: 1.5 – 10 µm	Octadecyl silane chemically bonded to porous or non-porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter, or a monolithic rod	Silia*Chrom* dt C18 Silia*Chrom* AQ C18 Silia*Chrom* XT C18 Silia*Chrom* XT Fidelity C18 Silia*Chrom* SB C18 Silia*Chrom* XDB C18 Silia*Chrom* XDB1 C18 Silia*Chrom* XDB2 C18
L3	Bonding: Silica Support Type: Silica Particle size: 1.5 – 10 µm	Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Silia*Chrom* dt Si Silia*Chrom* XDB Si Silia*Chrom* XDB1 Si
L7	Bonding: Octyl (C8) Support Type: Silica Particle size: 1.5 – 10 µm	Octylsilane chemically bonded to totally or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Silia*Chrom* AQ C8 Silia*Chrom* SB C8 Silia*Chrom* XDB C8 Silia*Chrom* XDB1 C8
L8	Bonding: Amine (NH2) Support Type: Silica Particle size: 1.5 – 10 µm	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter.	Silia*Chrom* XDB1 Amino
L9	Bonding: Strong cation exchange Support Type: Silica Particle size: 3 – 10 µm	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter.	Silia*Chrom* SCX
L10	Bonding: Nitrile (CN) Support Type: Silica Particle size: 1.5 – 10 µm	Nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	Silia*Chrom* XDB1 CN
L11	Bonding: Phenyl Support Type: Silica Particle size: 1.5 – 10 µm	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	Silia*Chrom* XDB1 Phenyl
L13	Bonding: TMS (C1) Support Type: Silica Particle size: 3 – 10 µm	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.	Silia*Chrom* XDB1 C1
L14	Bonding: Strong anion exchange Support Type: Silica Particle size: 5 – 10 µm	Silica gel having a chemicallly bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm in diameter.	Silia*Chrom* SAX
L17	Bonding: Strong cation exchange Support Type: Polymer Particle size: 6 – 12 µm	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 6 to 12 µm in diameter.	Silia*Chrom* IEC SC-H
L21	Bonding: N/A Support Type: Polymer Particle size: 3 – 30 µm	A rigid, spherical styrene-divinylbenzene copolymer, 3 to 30 µm in diameter.	Silia*Chrom* RPC
L22	Bonding: Strong cation exchange Support Type: Polymer Particle size: ~10 µm	A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size.	Silia*Chrom* IEC SC-M
L26	Bonding: Butyl (C4) Support Type: Silica Particle size: 1.5 – 10 µm	Butyl silane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter.	Silia*Chrom* XDB1 C4
L40	Bonding: Chiral Support Type: Silica Particle size: 5 – 20 µm	Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 5 to 20 µm in diameter.	Silia*Chrom* Chiral Cellulose T-DPC
L42	Bonding: Mixed-mode C18/C8 Support Type: Silica Particle size: ~5 µm	Octylsilane and octadecylsilane groups chemically bonded to porous silica particles, 5 µm in diameter.	Silia*Chrom* C18/C8
L51	Bonding: Chiral Support Type: Silica Particle size: 5 – 10 µm	Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica particles, 5 to 10 µm in diameter.	Silia*Chrom* Chiral Amylose T-DPC

How to Choose the Right Silia*Chrom* C18 Phase

C18 reversed-phase is the most used sorbent for HPLC applications. SiliCycle has developed over the years several C18 phases for specific analytes and/or matrices. The table below presents all Silia*Chrom* C18 phases available in the SiliCycle portfolio including a short description and characteristics. This table will help you choose the right Silia*Chrom* C18 phase based on your separation needs.

Silia*Chrom* C18 Reversed-Phase Characteristics

Silia*Chrom* Phases	Description	%C	Pore Size (Å)	Surface Area (m^2/g)	pH Stability Range	Phase Description
Silia*Chrom* dt C18 **Silia*Chrom* AQ C18**	**Universal 100% aqueous compatible C18 column.** Most versatile column of the SiliCycle portfolio. Great retention for hydrophilic compounds. High sensitivity for LC-MS analysis. Same C18 functionalization but the Silia*Chrom* dt C18 is free of metal content.	18	100	410 - 440 380	1.5 - 9.0	Page 101
Silia*Chrom* SB C18	**Column designed for extremely low pH conditions** Compatibility with 100% aqueous mobile phase. Great sensitivity for LC-MS.	12	150	200	0.5 - 7.5	Page 107
Silia*Chrom* XT C18	**High stability under high pH conditions** Ideal for basic compounds.	15	150	380	1.5 - 12.0	Page 109
Silia*Chrom* XT Fidelity C18	**Excellent stability under extreme pH and temperature conditions** Ideal HPLC column for either metabolic or metabolite analysis.	21	150	380	1.5 - 12.0	Page 109
Silia*Chrom* XDB1 C18	**Highest level of hydrophobicity of the SiliCycle C18 phases** Designed for dirty samples. Oldest C18 phase technology.	22	100	380 - 400	1.5 - 10.0	Page 112
Silia*Chrom* XDB2 C18	**Mid-level hydrophobicity and most popular phase for QC analysis** Typical average value of carbon loading.	18	100	380 - 400	1.5 - 9.0	Page 114
Silia*Chrom* XDB C18	**Lowest level of hydrophobocity of the SiliCycle C18 phase** Ideal for separation of highly hydrophobic molecules such as fatty acids, barbiturates, fat-soluble vitamins & steroids.	15	150	200	1.5 - 9.0	Page 111
Silia*Chrom* XDB1 C18-300	**Highest level of hydrophobicity for a C18 with wide pore size** Designed for biochromatography applications (*peptides, proteines or nucleic acids*).	8	300	80	1.5 - 9.0	Page 112

« Needed a set of columns that work with a wide pH range. SiliaChrom XT columns did the trick. »

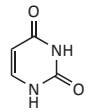
Victor Nicolaev from Sanofi, Oro Valley, AZ, USA

Silia*Chrom* Reversed-Phase HPLC Column Character Evaluation

Our Silia*Chrom* HPLC columns are evaluated by USP and NIST tests for classification purpose and based on the selectivity chart. These tests allow the characterization and the comparison of various HPLC columns in order to determine the following parameters: void volume, retention capacity of hydrophobic compound, selectivity, efficiency and silanol activity. To run this test, we use a mixture of the five organic compounds listed below. Furthermore, we used the same test for side-by-side comparison on various Silia*Chrom* C18 columns against three well-known suppliers[1].

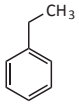
Reaction Mixture

- **Uracil (1)**: void volume marker (T_0)
- **Toluene (2)**: retention capacity of hydrophobic compounds (k'_{Tol})
- **Ethylbenzene (3)**: marker for the calculation of column efficiency for hydrophobic compounds ($k'_{Ethylbenzene}$)
- **Ratio Toluene/Ethylbenzene**: determination of selectivity ($\alpha_{Ethylbenzene/Toluene}$)
- **Amitriptyline (4)**: activity towards bases (*silianol activity evaluation*)
- **Quinizarin (5)**: activity towards chelating reagents (*metal contamination evaluation*)

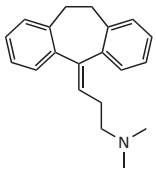


Uracil (1)

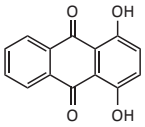
Toluene (2)



Ethylbenzene (3)



Amitriptyline (4)



Quinizarin (5)

Description of the Column Aspects Evaluated

Hydrophobicity is measured by the retention factor of the hydrophobic analyte (*ethylbenzene*) using the following equation:

$$k' = \frac{(T_R - T_0)}{T_0} = \frac{\text{Ethylbenzene retention time – Uracil retention time (}\textit{Void volume}\text{)}}{\text{Uracil retention time (}\textit{Void volume}\text{)}}$$

Selectivity (α) is measured by the retention factor ratio between two similar compounds, ethylbenzene (k_2) and toluene (k_1):

$\alpha = k_2/k_1$

Column Efficiency is usally measured by the plate count (*N*) obtained for the ethylbenzene peak.

Chelating Tailing Factor – Metal Content is measured by the quinizarin peak symmetry. A symmetric peak shape indicates low activity toward chelating agent (*absence of metals*) and an asymmetric peak shape indicates the presence of metals by peak tailing (*high activity toward chelating reagents*).

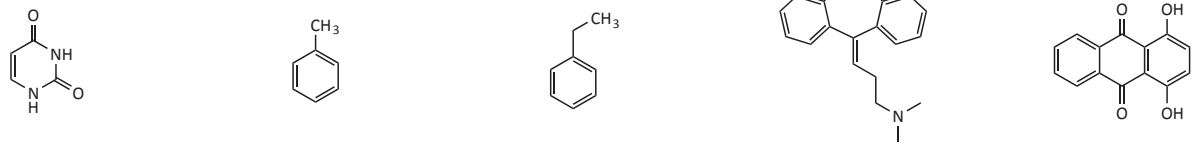
Tailing Factor of Amitriptyline (Amitr.) – Silanol Activity is measured by the peak symmetry of amitriptyline (*basic compound*). Important silanol activity is often associated with peak tailing or an asymmetric peak. In other words, a highly deactivated column will have a lower peak asymmetry.

Chromatographic conditions:

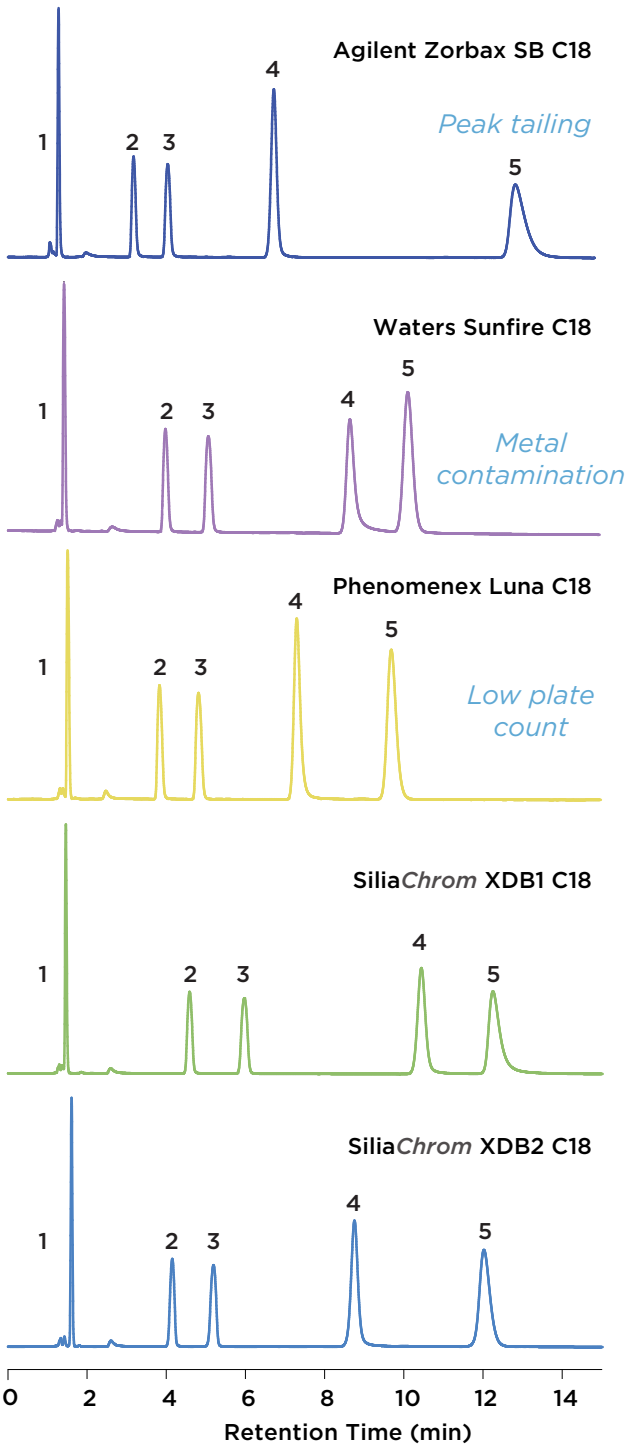
- **HPLC System**: Thermo Surveyor with PDA
- **HPLC Software**: *Xcalibur handling version 2.0*
- **Column Size**: All HPLC columns: 4.6 x 150 mm, 5 µm
- **Mobile Phase**: Methanol/buffer (*80/20, v/v*)
- **Buffer**: 20 mM of phosphate buffer adjusted at pH=7.0
- **Temperature**: 30°C
- **Flow rate**: 1.000 mL/min
- **Temperature**: UV scan (*PDA*), Total scan 200-600 nm

([1]Pharmacopeial Forum, Vol. 31(2) March-Apr. 2005, p.637)

SiliaChrom C18 HPLC Columns Versus the Competition



Uracil (1) Toluene (2) Ethylbenzene (3) Amitriptyline (4) Quinizarin (5)



C18 Column Character Evaluation Comparaison

HPLC Columns	Hydrophobicity		Selectivity
	k' Toluene	k' Ethylbenz.	α Ethylbenz./Tol.
SiliaChrom XDB1 C18	2.14	3.09	1.44
SiliaChrom XDB2 C18	0.61	2.22	1.41
Phenomenex Luna C18	1.50	2.13	1.42
Agilent Zorbax SB C18	1.38	2.01	1.45
Waters Sunfire C18	1.72	2.45	1.43

C18 Column Character Evaluation Comparaison

HPLC Columns	Efficiency		Metal Content	Silanol Activity
	N (/ meter) Ethylbenz.	R	A_s Quinizarin	TF Amitr.
SiliaChrom XDB1 C18	45,000	4.73	1.09	1.65
SiliaChrom XDB2 C18	28,000	3,30	1.10	1.18
Phenomenex Luna C18	22,000	2.90	1.23	1.20
Agilent Zorbax SB C18	25,000	3.20	1.10	1.55
Waters Sunfire C18	35,500	3.90	1.80	1.12

Results interpretation:

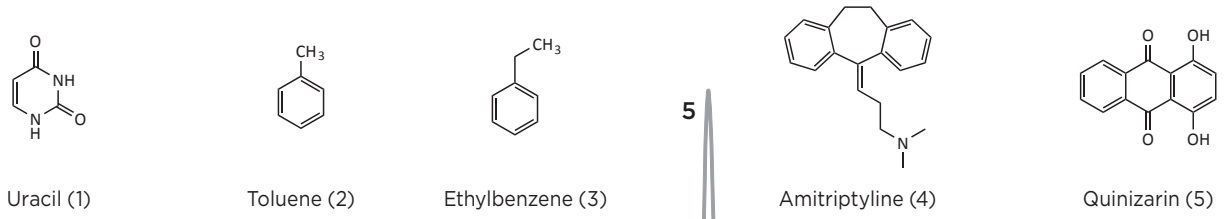
SiliaChrom columns compared advantageously over the competition; they present high column performances and with our wide portfolio, you can select the most suitable phase depending on the compound's nature. For example:

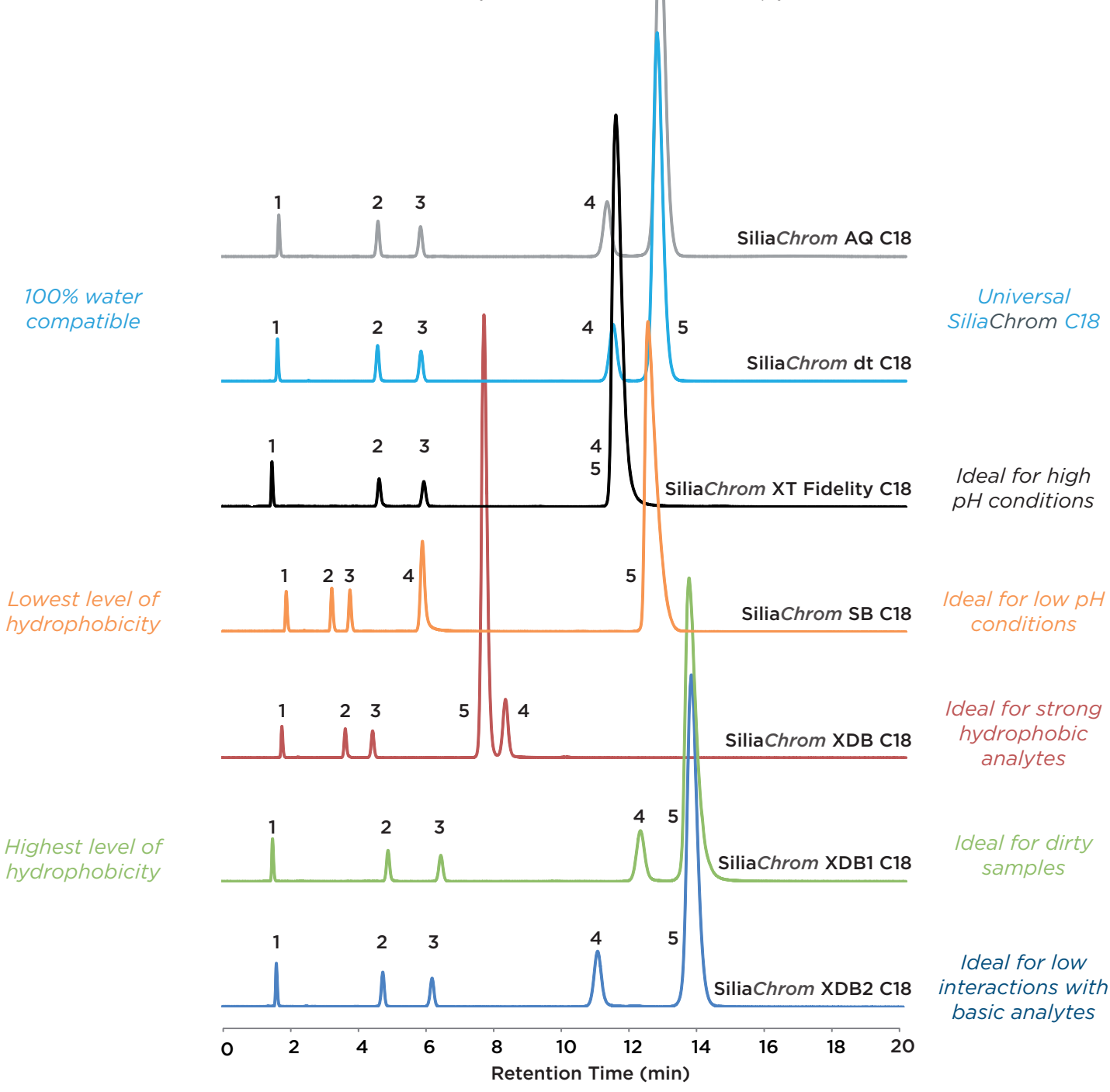
Basic analytes: SiliaChrom XDB2
Less polar analytes: SiliaChrom XDB1

The SiliaChrom columns performed very well compared to the competition. The Phenomenex Luna C18 has a lower efficiency as shown by its plate count (*N*). The Agilent Zorbax SB C18 column, shows peak tailing for amitriptyline which means that there are still some free OH presents on the surface (*activity towards bases*). Finally, the Waters Sunfire C18 seems to have high metal impurities as shown by the peak asymmetry obtained for quinizarin.

Full Range of Selectivity with Silia*Chrom* C18 HPLC Columns

Our most popular Silia*Chrom* reversed-phase C18 HPLC columns were evaluated by USP and NIST tests for classification purpose based on the selectivity chart. Select the most suitable Silia*Chrom* C18 based on your sample's properties.





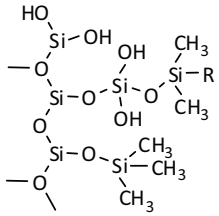
SiliaChrom dt C18
SiliaChrom AQ C8 and C18

Description

Universal 100% aqueous compatible HPLC columns **SiliaChrom dt** adsorbent presents an optimum ratio of C18 short TMS chains and some free silanol groups. This new technology shows good peak shapes for any type of molecule (*acid, neutral and base*). The silica framework is exempt of any metal permitting a high sensivity for LC-MS applications.

SiliaChrom AQ presents the same modified surface chemistry as dt but the silica framework contain low level of metal. C8 and C18 functions are available.

Structure



For C18 R = $(CH_2)_{17}CH_3$

SiliaChrom dt Purity: 99.9999% SiO_2 (*no metal content*)

SiliaChrom AQ Purity: 99.999% SiO_2

Sorbent Characteristics

- **Pore Size: 100 Å**
- **Specific Surface Area: SiliaChrom dt C18 410 - 440 m²/g**
  SiliaChrom AQ C8 & C18 380 m²/g
- **Particle Sizes Available: 2.5, 3, 5 and 10 µm**
- **USP Code: SiliaChrom dt C18 and AQ C18: L1**
  SiliaChrom AQ C8: L7
- **Typical Carbon Loading: SiliaChrom dt & AQ C18: 18%**
  SiliaChrom AQ C8 14%

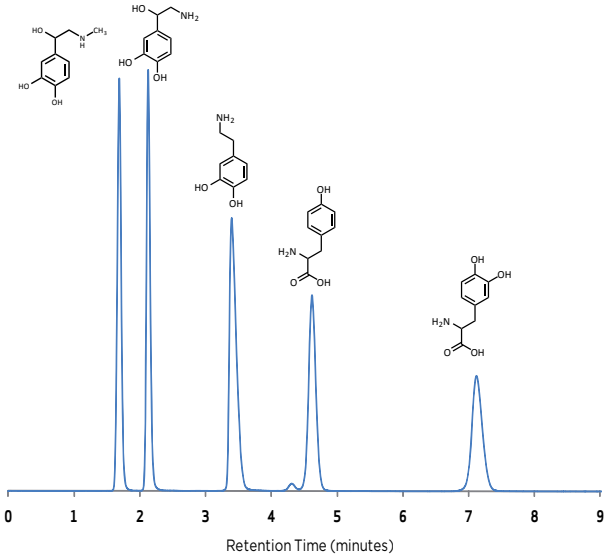
SiliaChrom dt and AQ Main Characteristics

- **Enhanced retention of hydrophilic molecules**
- **Inertness for acidic and basic analytes**
- **Compatible from 100% aqueous mobile phase to 100% organic**
- **Exceptional stability from pH 1.5 to 9.0**
- **Good tolerance to direct injection of biological matrix (*dirty samples*)**
- **Reduces the need for mobile phase modifiers**
- **Low bleeding and high sensitivity for LC-MS**
- **Partially endcapped**

Forensic

Separation of Catecholamines in Acidic Mobile Phase

Catecholamines are hydrophilic compounds with acidic functions. The mobile phase needs to be acidic to have the catecholamines under the molecular configuration and use the sorbent hydrophilic character to drive the separation.



Chromatographic conditions

- **Column**: SiliaChrom dt C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H141805E-N150
- **Mobile phase**: 1% Acetic Acid in water
- **Temperature**: 23°C
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 265 nm
- **Injection volume**: 5 µL

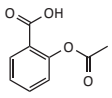
« Polar metabolites separation is very challenging. Using SiliaChrom, dt C18 in normal phase solved the problem. »

Huns Nejad from BASF, Research Triangle Park, NC, USA

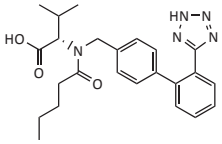
Assay for QC Testing of Blood Pressure and Cholesterol Medication

Pharmaceutical

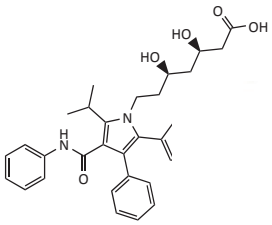
The Silia*Chrom* dt C18 presents a high lot-to-lot reproducibility, which makes it an excellent choice for quality control analysis in phamaceutical laboratories.



A: Aspirine



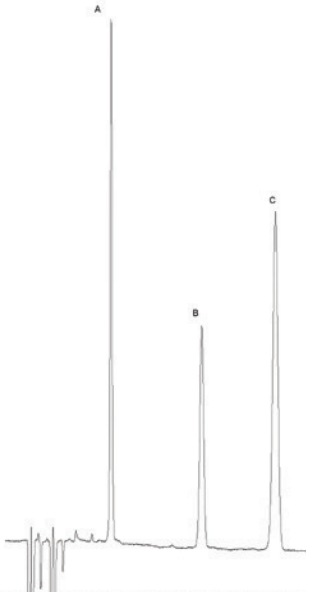
B: Valsartan



C: Atorvastatin

Chromatographic conditions

- **Column**: Silia*Chrom* dt C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H141805E-N150
- **Mobile phase**: Methanol/H_2O (70/30), 0.1% (v/v) formic acid
- **Temperature**: 30°C
- **Flow rate**: 0.800 mL/min
- **Detector**: UV at 280 nm
- **Injection volume**: 10 µL



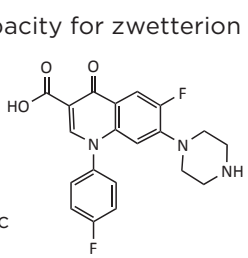
Peak Shape Evaluation for Zwetterion Fluoroquinolones

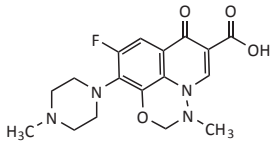
Pharmaceutical

The Silia*Chrom* dt C18 presents a high separation capacity for zwetterion analysis.

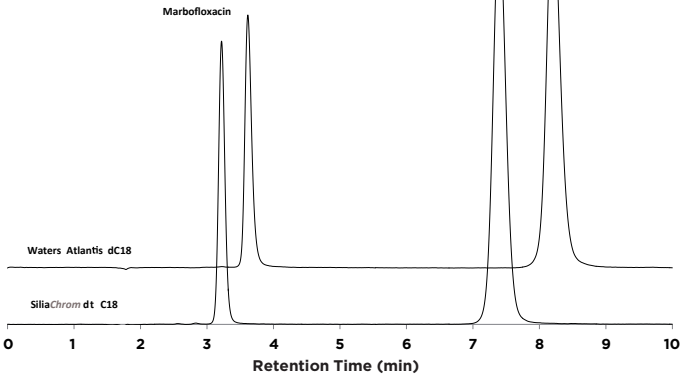
Chromatographic conditions

- **Column**: Silia*Chrom* dt C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H141805E-N150
- **Mobile phase**: 2.5 mM potassium phosphate monobasic (*adjust to pH 2.5 with H_3PO_4*)/ethanol (*68/32*)
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 275 nm
- **Injection volume**: 10 µL





Sarafloxacin



Peak Shape Results		
Product	Asymmetry (USP) Silia*Chrom* dt C18	Asymmetry (USP) Atlantis dC18
Marbofloxacin	1.11	1.29
Sarafloxacin	1.08	1.14

Pharmaceutical

Ropinirole and Amitriptyline Detection in Human Plasma

SiliaChrom dt C18 presents low bleeding and is excellent for dirty samples. Partial endcapping allows for some interactions with free silanol groups. The use of SiliaPrep CleanDRUG prior to injection onto the column will insure a very clean sample which results in very low ionic suppression when using in LC-MS/MS analysis. Another big advantage is the high selectivity of SiliaChrom dt C18 for all concentration levels.

Chromatographic conditions

- **Column**: SiliaChrom dt C18, 2.5 µm
- **Column size**: 3.0 x 30 mm
- **SiliCycle PN**: H141802E-H030

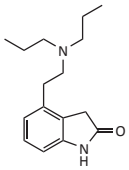
  Sample preparation by SPE
  SiliaPrep CleanDRUG 3 mL/200 mg
  PN: SPEC-R651230B-03G
- **Mobile phase:**
  MPA: 1 mM ammonium formate in (*ACN/water, 10/90*), 0.1% formic acid (*v/v*)
  MPB: 1 mM ammonium formate in (*ACN/water, 90/10*), 0.1% formic acid (*v/v*)

Gradient		
Time (min)	MPA (%)	MPB (%)
0.00 - 0.20	85	15
0.21 - 1.20	50	50
1.21 - 1.60	0	100
1.61 - 3.50	85	15

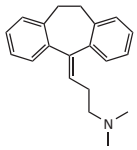
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **MS splitting flow**: 0.30 mL/min
- **Injection volume**: 5 µL

Tandem mass spectroscopy conditions

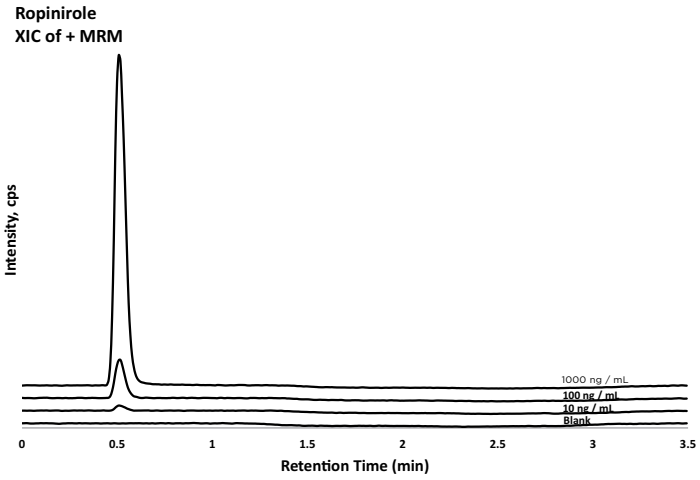
- **Detector**: Sciex API 3000, Applied Biosystem
- **Ion Source**: Positive Electrospray (*ESI+*)
- **Turbolon Ion Spray heater gas flow**: 8000 cc/min
- **Turbolon Ion Spray heater temperature**: 375°C
- **MRM Transition**: Ropinirole: m/z (*261.2 → 114.2*)
  Amitriptyline: m/z (*278.4 → 233.1*)

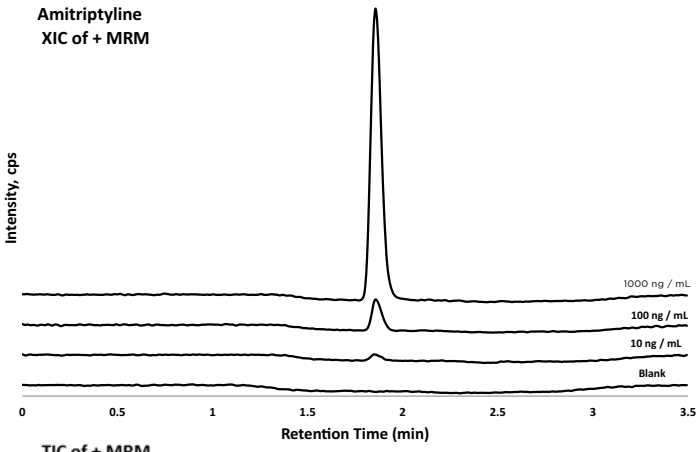


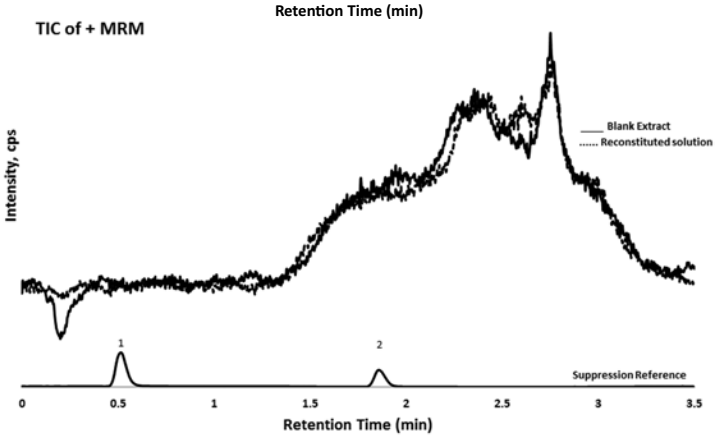
Ropinirole



Amitriptyline

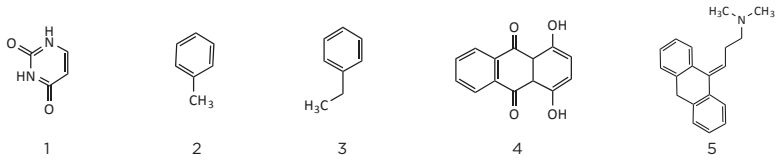






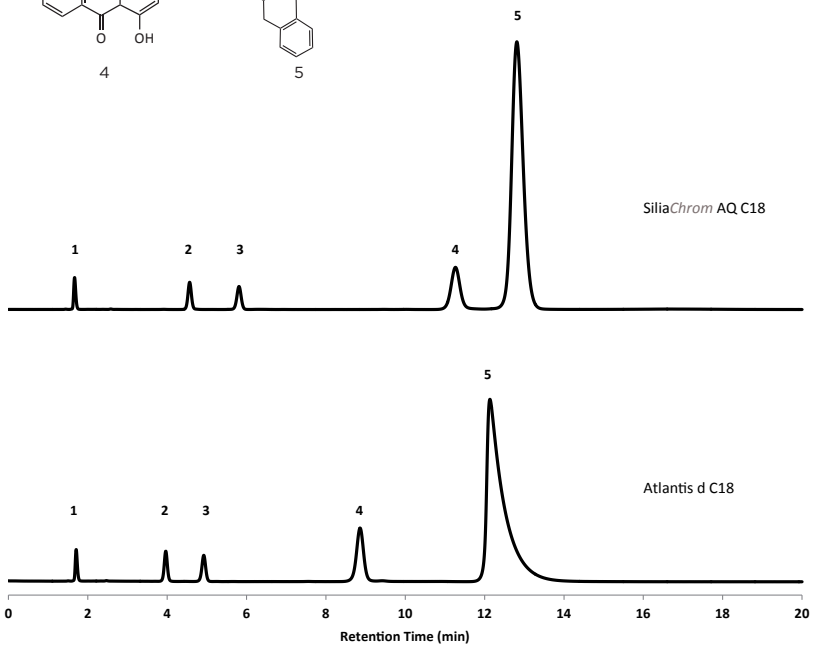
SiliaChrom AQ C18 is Highly Efficient for Basic Compounds

Amitriptyline, a strong basic compound, can be adsorbed on residual silanols on the surface of the packing material. With the traditional endcapping technique, this results in poor peak shapes. SiliCycle has developed a new method of silanol deactivation to eliminate the peak tailing from adsorption of compounds on residual silanol groups. This enables highly qualitative and quantitative analysis of strong basic compounds.



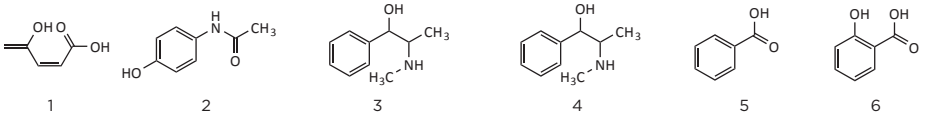
Chromatographic conditions

- **Column**: SiliaChrom AQ C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H151805E-N150
- **Mobile phase**: 80/20 methanol/ 20 mM potassium phosphate pH 7.00
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm
- **Injection volume**: 1 µL



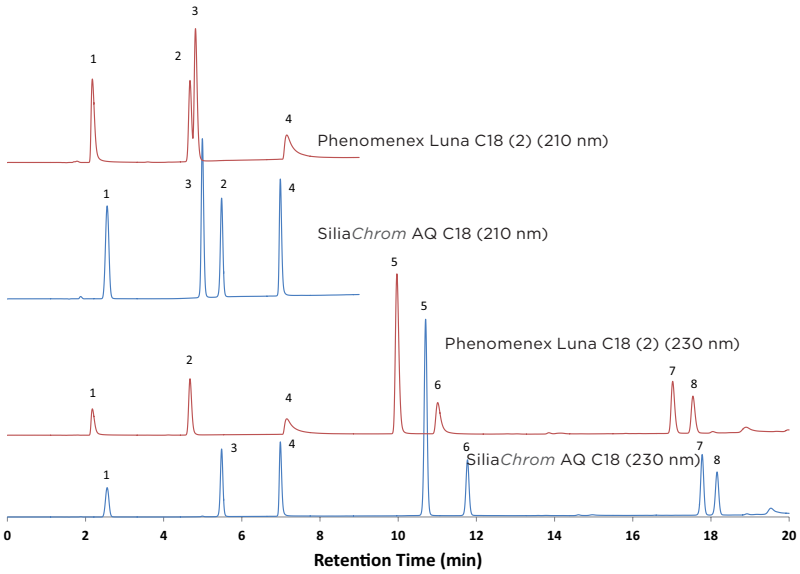
Evaluation of Resolution and Peak Shape

The SiliaChrom AQ C18 column is universal, efficient even for mixtures of basic and acidic compounds.



Chromatographic conditions

- **Column**:
  SiliaChrom AQ C18, 5 µm
  Phenomenex Luna, C18 (2) 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H151805E-N150
- **Mobile phase**:
  MPA: 5 mM potassium phosphate monobasic (*adjust to pH 2.5 with H_3PO_4*)/ACN (*90/10*)
  MPB: 5 mM potassium phosphate monobasic (*adjust to pH 2.5 with H_3PO_4*)/ACN (*10/90*)
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm
- **Injection volume**: 5 µL



Pharmaceutical

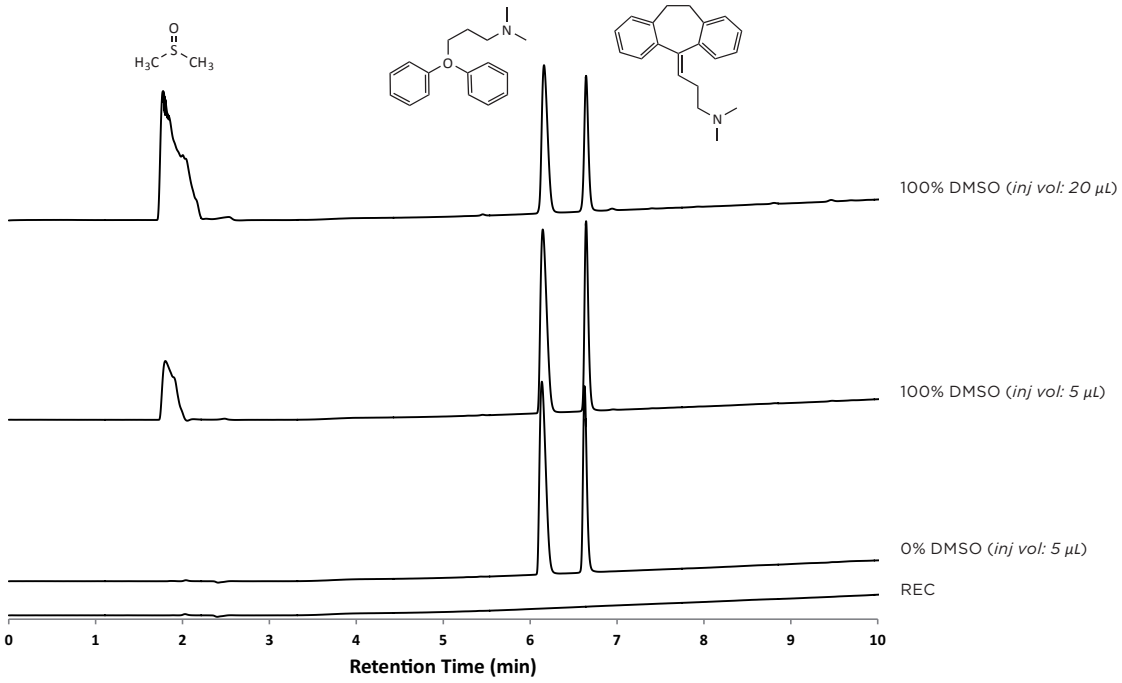
Retention Capacity of DMSO on Silia*Chrom* AQ C18

DMSO (*Dimethylsulfoxide*) is an excellent solvent to solubilize most compounds. Unfortunately, this solvent is not volatile and with some C18 columns, the DMSO can interact with the stationary phase and decrease the selectivity. In this case, the only way to inhibit this effect is to use preparative chromatography. In this study, we show that DMSO does not interact with our Silia*Chrom* AQ C18. A linear gradient has been used from a highly aqueous mobile phase to a highly organic phase.

Chromatographic conditions

- **Column**: Silia*Chrom* AQ C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H151805E-N150
- **Mobile phase**: MPA 0.1% formic acid in water
  MPB 0.1% formic acid in ACN
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm
- **Reconstitution solution (*REC*)**: DMSO

Gradient		
Time (min)	% MPA	% MPB
0	90	10
9	10	90
10	10	90
11	90	10



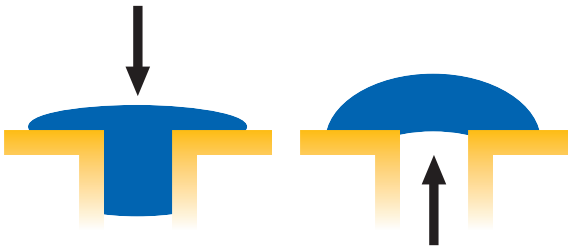
Statistic Analysis Results						
Conditions	As $_{DMSO}$	Tr $_{DMSO}$ (min)	K'$_{DMSO}$	W$_{DMSO}$	Tr $_{diphenhydramine}$ (min)	Tr $_{amitriptyline}$ (min)
0% DMSO 5 µL	-	-	-	-	6.14	6.63
100% DMSO 5 µL	2.29	1.80	0.09	0.3	6.15	6.64
100% DMSO 20 µL	4.10	1.78	0.08	0.5	6.16	6.64

Conclusion: The study shows that DMSO does not interact with the Silia*Chrom* AQ C18. No specific retention is observed. The Silia*Chrom* AQ C18 is an excellent choice to purify components contaminated with DMSO.

Dewetting Phenomena

Pharmaceutical Forensic

The dewetting phenomena is the formation of drops on the solid surface caused by hydrophobic repulsions of highly hydrophobic sorbents. This phenomena is illustrated by the following scheme.

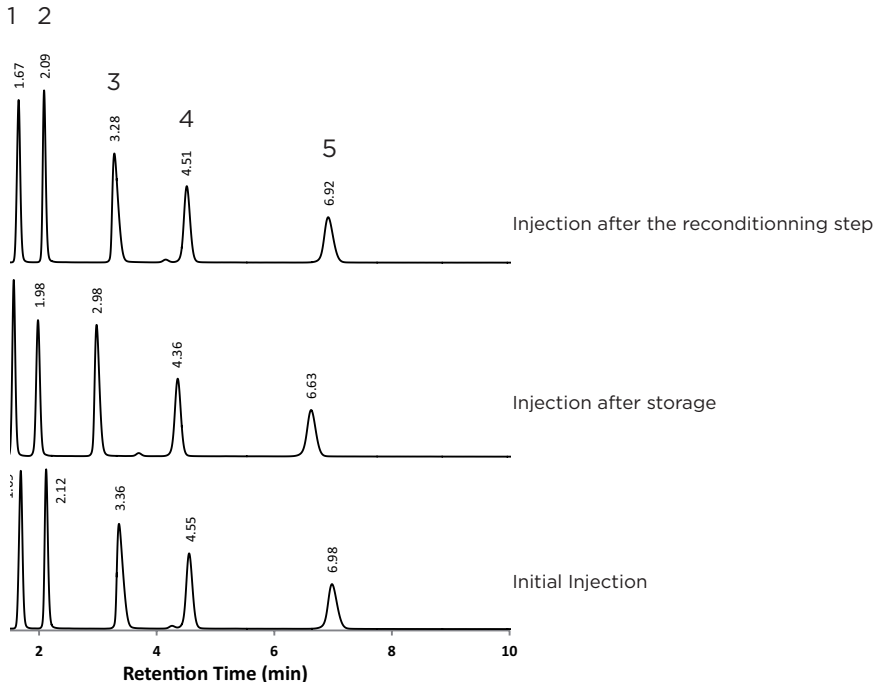


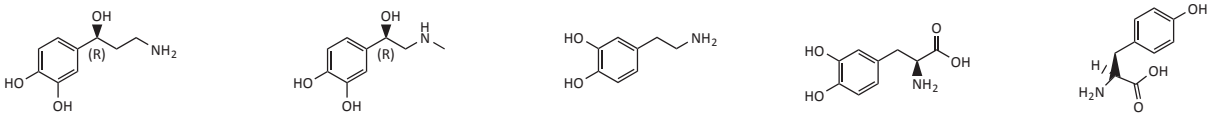
General procedure

- The mixture of catecholamines is eluted on the column
- The flow is then stopped
- The column is stored in this condition during 18 h
- The mixture is then re-injected after a reconditioning step

Chromatographic conditions

- **Column**: Silia*Chrom* AQ C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H151805E-N150
- **Mobile phase**: 1% acetic acid in water
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 265 nm
- **Injection volume**: 5 µL





1.Norepinephrine 2.Epinephrine 3.Dopamine 4.Levodopa 5.Tyrosine

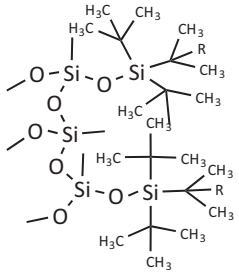
Conclusion: A small decrease in retention time is observed, but is not significant. The displacement has been resolved after the reconditioning step. The Silia*Chrom* AQ C18 does not present the dewetting phenomena.

SiliaChrom SB C18 and C8

Description

SiliaChrom SB C18 and **C8** surfaces are treated with an organic form of silicon to increase the number of silanol groups on the surface. After this step, the surface is bonded with a silane containing two functions. One function is a protecting group that shields the area and protects the surface from an acid attack from the mobile phase. The H_3O^+ ion does not have access to the surface to break the O-Si bond (*steric effect*). The other function is the linear hydrophobic chain with 18 or 8 carbons.

Structure



For C18 R = $(CH_2)_{17}CH_3$
For C8 R = $(CH_2)_7CH_3$

SiliaChrom SB C18

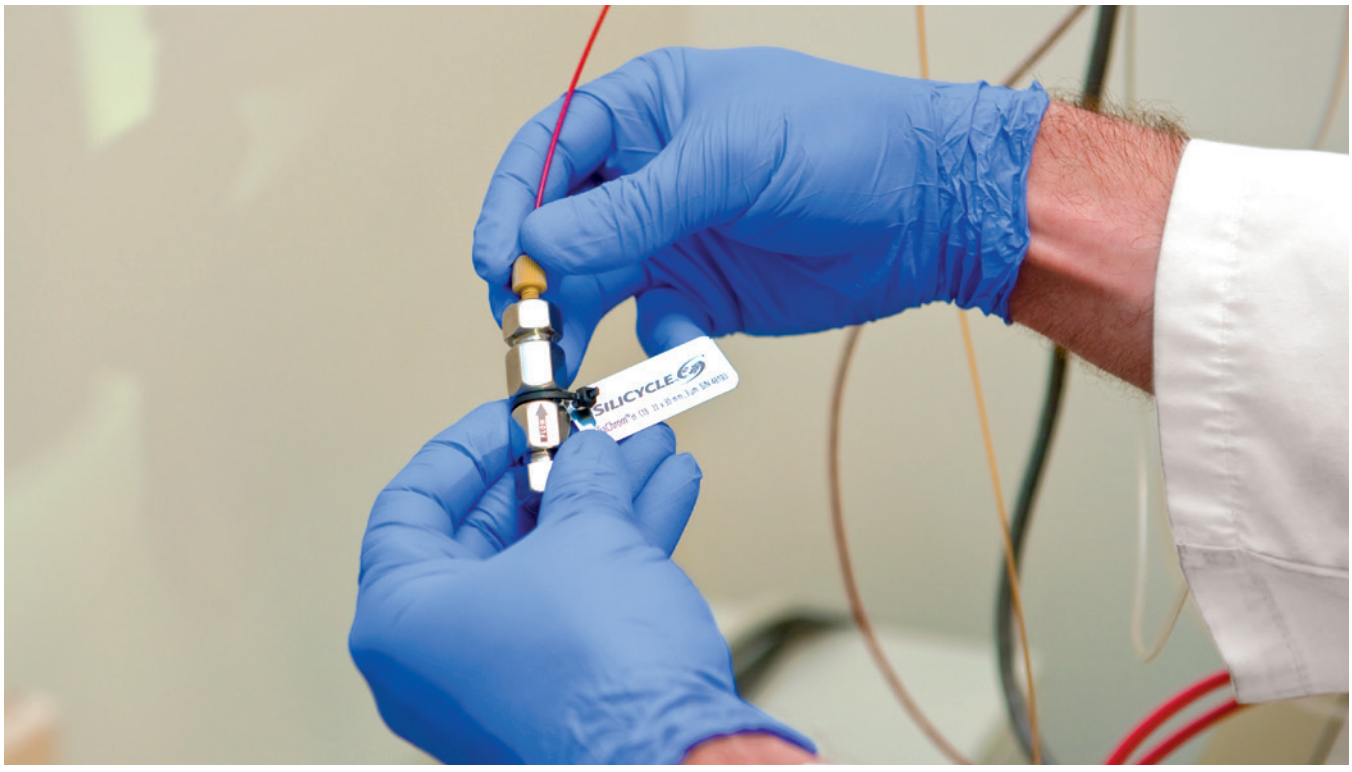
SiliaChrom SB C8

Sorbent Characteristics

- **Pore Size: 150 Å**
- **Specific Surface Area: 200 - 220 m^2/g**
- **Particle Sizes Available: 3, 5 and 10 µm**
- **USP Code: SiliaChrom SB C18: L1**
  SiliaChrom SB C8: L7
- **Typical Carbon Loading: SiliaChrom SB C18: 12%**
  SiliaChrom SB C8: 7%

SiliaChrom SB Main Characteristics

- **Extremely low pH limits (*0.5 - 7.5*)**
- **Extremely low bleeding for LC-MS applications under acidic conditions**
- **Compatible with mobile phases ranging 100% water to 100% organic**
- **Non endcapped**

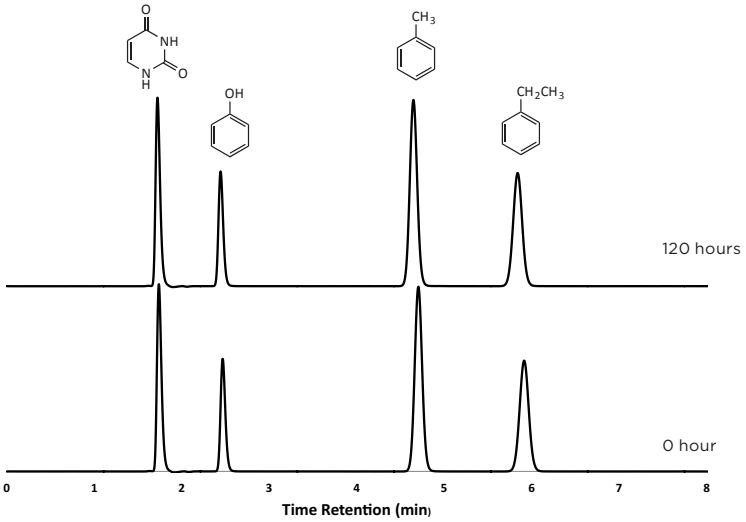


Stability of Silia*Chrom* SB C18 at Low pH Conditions

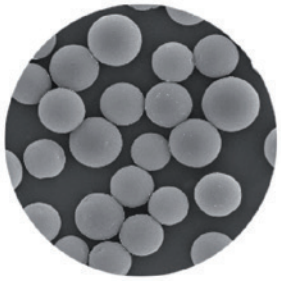
Acidic mobile phases have widespread applications in the reversed phase HPLC separation of many important pharmaceutical and environmental compounds. Analytes such as pharmaceuticals and biomolecules often show peak shape, retention and selectivity changes when the mobile phase pH is changed from neutral to acidic pH (*pH 1.0*). In fact, lowering the pH helps to suppress silanol interactions between basic compounds and the residual surface silanols, thus resulting in less tailing and better retention of acidic compounds (*pKa lower than 2*).

Chromatographic conditions

- **Column**: Silia*Chrom* SB C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H101805H-N150
- **Mobile phase**: 2% TFA in ACN/water (*60/40*)
  Solution pH: 1.00
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 270 nm
- **Injection volume**: 10 µL

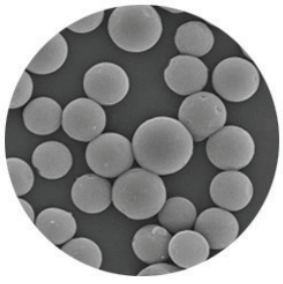


Silia*Chrom* SB C18 (*Ethylbenzene*)			
Time (hour)	RT (min)	TF (USP)	N (USP)
0	5.91	1.01	14,014
24	5.89	1.02	14,085
48	5.77	1.02	14,023
72	5.83	1.02	14,076
96	5.85	1.01	14,087
120	5.84	1.02	14,050
Mean	5.85	1.02	14,056
RSD (%)	**0.84**	**0.51**	**0.23**



Silia*Chrom* SB C18 before

No column degradation under extreme pH conditions



Silia*Chrom* SB C18 after

The HPLC column was used under extreme pH conditions and, even after 5 days of continuous injections, the number of theoretical plates (*N*), the tailing factor (*TF*) and the retention time (*RT*) are comparable. The sorbent kept its chemical and structural integrity, which we have proven with similar chromatograms and scanning electron microscope pictures (*SEM*) before and after 120 hours of use.

In conclusion, our Silia*Chrom* SB C18 and SB C8 columns are stable at low pH conditions.

SiliaChrom XT C18 and XT C18 Fidelity

Description

SiliaChrom XT C18 and **XT C18 Fidelity** are suitable with low or high pH conditions. The key is to have a hybrid surface to reduce the solubility of silica at high pH. In fact, the SiliaChrom XT C18 and the XT C18 Fidelity silica are coated with a monomeric methyltriethoxysilane/tetraethoxysilane prepolymer, followed by a special thermic treatment to get a rigid surface that is less soluble than untreated silica itself at high pH.

The SiliaChrom XT C18 column is designed for applications at very high pH (*up to 12*) at room temperature but is also suitable for low pH (*down to 1.5*).

The SiliaChrom XT C18 Fidelity is used at high pH conditions and offers a higher thermal stability. The only difference between SiliaChrom XT C18 and the XT C18 Fidelity is the carbon loading. The SiliaChrom XT C18 Fidelity (21% C) presents a higher hydrophibic capacity than the SiliaChrom XT C18 (15% C).

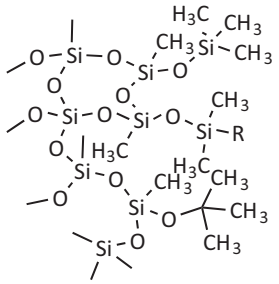
Sorbent Characteristics

- **Pore Size: SiliaChrom XT C18: 150 Å**
  SiliaChrom XT C18 Fidelity: 100 Å
- **Specific Surface Area: 380 m²/g**
- **Particle Sizes Available: 3, 5 and 10 µm**
- **USP Code: L1**
- **Typical Carbon Loading: SiliaChrom XT C18: 15%**
  SiliaChrom XT C18 Fidelity: 21%

SiliaChrom XT Main Characteristics

- **Excellent durability at high pH (*up to 12*)**
- **Ideal for basic compounds**
- **High thermal stability**
- **Ideal for auto-purification (*Prep. LC-MS*)**
- **Double endcapped**
- **Best HPLC columns for either metabolic or metabolite studies**

Structure



R = $(CH_2)_{17}CH_3$

SiliaChrom XT C18 and XT C18 Fidelity

« The high quality nature of the HPLC columns and plates from SiliCycle has allowed us to achieve a level of reproducibility with our compound libraries that would be unheard of with any other production line. »

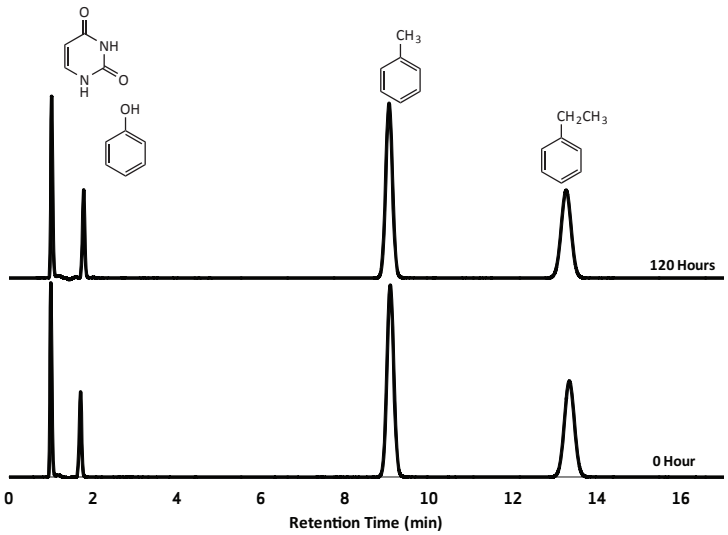
Steven Marois from Boston University CMLD, Boston, MA, USA

Stability of Silia*Chrom* XT C18 Fidelity at High pH Conditions

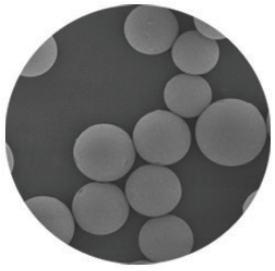
For some applications, it is necessary to work at high pH to increase the selectivity or to optimize peak shape. This is the case with basic organic compounds (pK_a > 9.0). It is the reason why it is important to have chromatographic phases stable at alkaline pH. This study demonstrates the stability of the Silia*Chrom* XT C18 Fidelity at high pH.

Chromatographic conditions

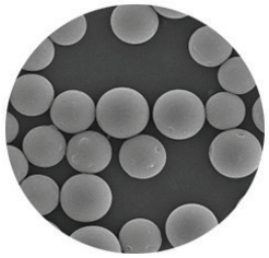
- **Column**: Silia*Chrom* XT C18 Fidelity, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: HF171805H-N150
- **Mobile phase**: 0.2% TEA in ACN/water (*55/45*) (*v/v*) Solution pH: 11.5
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 270 nm



Silia*Chrom* XT C18 Fidelity (*Ethylbenzene*)			
Time (hour)	RT (min)	TF (USP)	N (USP)
0	13.35	1.01	13,623
24	13.29	1.01	13,648
48	13.27	1.01	13,689
72	13.25	1.00	13,604
96	13.24	1.00	13,649
120	13.28	1.00	13,582
Mean	13.28	1.01	13,633
RSD (%)	**0.29**	**0.54**	**0.28**



Silia*Chrom* XT C18 Fidelity before



Silia*Chrom* XT C18 Fidelity after

The HPLC column was used under extreme pH conditions, and even after 5 days of continuous injections, the number of theoretical plates (*N*), the tailing factor (*TF*) and the retention times (*RT*) remain constant. The sorbent kept its chemical and structural integrity, which we have proven with similar chromatograms and scanning electron microscope (*SEM*) pictures before and after 120 hours of use.

In conclusion, our Silia*Chrom* XT C18 Fidelity column is stable at high pH conditions.

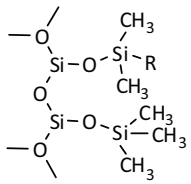
SiliaChrom XDB C18 and C8

Description

SiliaChrom XDB C18 and **C8** are made of a special silica with a larger pore size and lower surface area for the separation of large hydrophobic molecules. The relatively low surface area allows a shorter retention time for such compounds.

SiliaChrom XDB phases are ideal for separation of barbiturates, fat-soluble vitamins, fatty acids and steroids.

Structure



For C18 R = $(CH_2)_{17}CH_3$
For C8 R = $(CH_2)_7CH_3$

SiliaChrom XDB C18

SiliaChrom XDB C8

Sorbent Characteristics

- **Pore Size: 150 Å**
- **Specific Surface Area: 200 m²/g**
- **Particle Sizes Available: 3, 5 and 10 µm**
- **USP Code: SiliaChrom SB C18 L1**
  SiliaChrom SB C8 L7
- **Typical Carbon Loading: SiliaChrom XDB C18: 15%**
  SiliaChrom XDB C8: 8%

SiliaChrom XDB C18 Main Characteristics

- **Better choice for molecules > 500 Dalton**
- **High Loading capacity**
- **Wide pH range: 1.5 to 9.0**
- **Double endcapped**

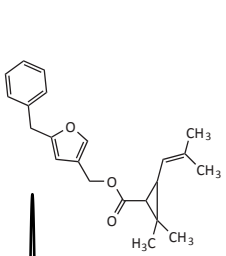
Resolution and Peak Shape of a Highly Hydrophobic Domestic Insecticide

Environment

This application illustrates the high separation efficiency of the SiliaChrom XDB C18 for very hydrophobic compounds.

Chromatographic conditions

- **Column**: SiliaChrom XDB C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H111805H-N150
- **Mobile phase**: ACN/water (*90/10*)
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 235 nm
- **Injection Volume**: 1 µL



Bioresmethrin

Column Performance Results

Compounds	Retention Time (min)	Peak Asymmetry Factor (USP)	Theoretical Plates (USP)
Uracil	1.72	1.26	5,936
Bioresmethrin	4.34	1.03	14,090

Silia*Chrom* XDB1 Family

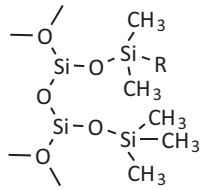
Description

Silia*Chrom* XDB1 phases have a wider range of polarity than other SiliCycle HPLC phases (*C18 to normal phase*). These phases have the maximum bonding density regardless of the compound's polarity. This allows for the least amount of interaction between the analytes and the surface OH's. These phases are not recommended for samples containing highly hydrophobic compounds.

All Silia*Chrom* XDB1 are available in 3, 5 and 10 µm except the Diol-300 which is not available in 3 µm.

The Silia*Chrom* XDB1 C18 is designed for maximum hydrophobicity and efficiency for dirty samples.

Structure



R = $(CH_2)_{17}CH_3$

Silia*Chrom* XDB1 C18

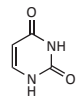
Sorbent Characteristics

See table next page.

Silia*Chrom* XDB1 Family Main Characteristics

- **Better choice for molecules > 500 Dalton**
- **High loading capacity**
- **Double endcapped**

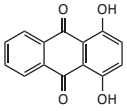
Highly Base Deactivated C18



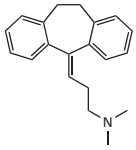
1. Uracil

2. Toluene

3. Ethylbenzene



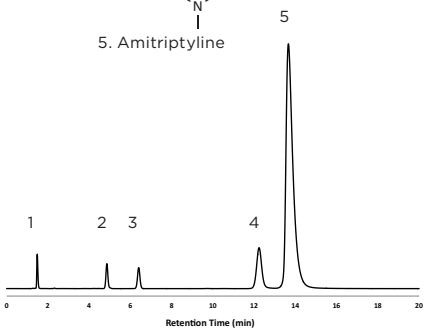
4. Quinizarin



5. Amitriptyline

Chromatographic conditions

- **Column**: Silia*Chrom* XDB1 C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H121805E-N150
- **Mobile phase**: MeOH/20 mM potassium phosphate monobasic pH = 7.00 (*80/20*)
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm
- **Injection Volume**: 1 µL



Column Performance Results

Compounds	Retention Time (min)	Peak Asymmetry Factor (USP)	Theoretical Plates (USP)
Uracil	1.49	1.27	3,778
Toluene	4.86	1.09	12,144
Ethylbenzene	6.40	1.02	13,026
Quinizarin	12.24	1.07	11,525
Amitriptyline	13.66	1.76	8,190

SiliaChrom XDB1 Sorbent Characteristics

SiliaChrom XDB1 Sorbent Characteristics						
SiliaChrom Phases	Description	USP Code	%C	Pore Size (Å)	Surface Area (m^2/g)	pH Stability Range
Reversed-Phases						
SiliaChrom XDB1 C18	Designed for maximum hydrophobicity and efficiency for dirty samples.	L1	22	100	380 - 400	1.5 - 10.0
SiliaChrom XDB1 C18-300		L1	8	300	80	1.5 - 9.0
SiliaChrom XDB1 C8	Exceptionally stable with high bonding coverage and low silanol activity.	L7	14	100	380 - 400	1.5 - 8.5
SiliaChrom XDB1 C8-300		L7	4	300	80	1.5 - 8.5
SiliaChrom XDB1 C4		L26	7	100	380 - 400	1.5 - 8.5
SiliaChrom XDB1 C4-300		L26	3	300	80	2.0 - 8.0
SiliaChrom XDB1 C1		L13	3	100	380 - 400	1.5 - 8.5
SiliaChrom XDB1 C1-300		L13	1	300	80	2.0 - 8.0
SiliaChrom XDB1 CN	Maximum hydrophobicity and works in normal and reversed-phase conditions.	L10	5	100	380 - 400	2.0 - 8.5
SiliaChrom XDB1 CN-300		L10	3.5	300	80	2.0 - 8.0
SiliaChrom XDB1 Phenyl	Highly retentive phase for aromatic and unsaturated compounds.	L11	12	100	380 - 400	1.5 - 9.0
SiliaChrom XDB1 Phenyl-300		L11	4.5	300	80	2.0 - 8.0
Normal Phases						
SiliaChrom XDB1 Si	Designed for normal phase conditions, presents a high surface area and a low metal content.	L3	n/a	100	380 - 400	1.0 - 8.0
SiliaChrom XDB1 Si-300		L3	n/a	300	80	2.0 - 8.0
SiliaChrom XDB1 Diol	Excellent for normal phase applications with the highest hydrophobic activity.	n/a	5	100	380 - 400	2.0 - 8.0
SiliaChrom XDB1 Diol-300		n/a	1	300	80	2.0 - 8.0
SiliaChrom XDB1 Amino	Superior general purpose amino phase. Recommended for normal phase analysis and excellent for sugar analysis.	L8	6	100	380 - 400	2.0 - 8.5
SiliaChrom XDB1 Amino-300		L8	2.5	300	80	2.0 - 8.0

« I have successfully used regular HPLC Analytical Columns for some analytical purpose, it works perfectly and accomdate good separation. »

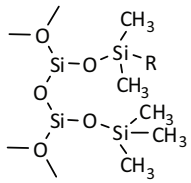
Xiaohai Li from Scripps Reshearch Institute, Jupiter, FL, USA

Silia*Chrom* XDB2 C18

Description

Silia*Chrom* XDB2 C18 is designed to be a mid-hydrophobic C18 phase with 18% of carbon loading, like most of the popular reversed-phase HPLC columns on the market. This phase demonstrates a balanced hydrophobic adsorption in order to avoid excessive retention of hydrophobic compounds.

Structure



R = $(CH_2)_{17}CH_3$

Silia*Chrom* XDB2 C18

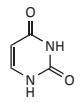
Sorbent Characteristics

- **Pore Size: 100 Å**
- **Specific Surface Area: 380 - 400 m²/g**
- **Particle Sizes Available: 3, 5 and 10 µm**
- **USP Code: L1**
- **Typical Carbon Loading: 18%**
- **pH Stability: 1.5 - 9.0**

Silia*Chrom* XDB2 C18 Main Characteristics

- **Great column-to-column and batch-to-batch reproducibility (*popular for QC/QA laboratory*)**
- **Typical average value for carbon loading (*18%*)**
- **Good peak shape for basic, acidic and neutral analytes**
- **Stronger separation power for isomers**
- **Double endcapped**

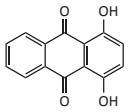
Highly Base Deactivated C18



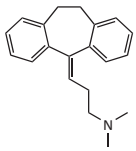
1. Uracil

2. Toluene

3. Ethylbenzene



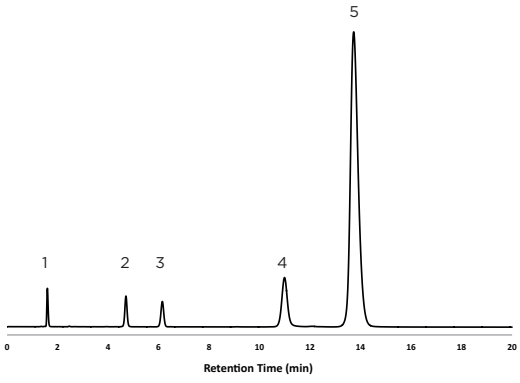
4. Quinizarin



5. Amitriptyline

Chromatographic conditions

- **Column**: Silia*Chrom* XDB2 C18, 5 µm
- **Column size**: 4.6 x 150 mm
- **SiliCycle PN**: H131805E-N150
- **Mobile phase**: MeOH/20 mM potassium phosphate monobasic pH = 7.00 (*80/20*)
- **Temperature**: 23°C
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm
- **Injection Volume**: 1 µL



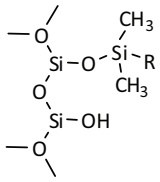
Column Performance Results			
Compounds	Retention Time (min)	Peak Asymmetry Factor (USP)	Theoretical Plates (USP)
Uracil	1.61	1.24	4 618
Toluene	4.73	1.04	12 858
Ethylbenzene	6.19	1.00	13 633
Quinizarin	11.18	1.03	12 277
Amitriptyline	13.53	1.29	9 451

SiliaChrom HILIC

Description

SiliaChrom HILIC (*hydrophilic interaction chromatography*) HPLC columns are designed to retain highly polar analytes. SiliaChrom HILIC has a selectivity that is complementary to reversed-phase columns. In fact, it has a higher retention for hydrophilic compounds in HILIC mode. HILIC sorbent is more stable and offers higher reproducibility than normal phase silica or amino columns. This phase is ideal for MedChem laboratories and is approved for SFC applications.

Structure



R = $(CH_2)_3NH\text{-}CO\text{-}NH_2$

Sorbent Characteristics

- **Pore Size: 100 Å**
- **Specific Surface Area: 380 m²/g**
- **Particle Sizes Available: 3, 5 and 10 µm**
- **Typical Carbon Loading: 8%**

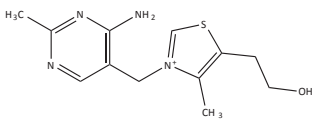
SiliaChrom HILIC Main Characteristics

- **Unique chemistry (*urea*)**
- **Accepts normal and reversed-phase applications**
- **Best replacement for amino HPLC column**
- **Provides high efficiency and rapid equilibration**
- **Enhanced sensitivity in mass spectrometry**
- **Non endcapped**

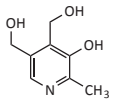
SiliaChrom HILIC

Food

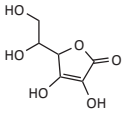
SiliaChrom HILIC: Separation of Vitamin B Complex and Vitamin C

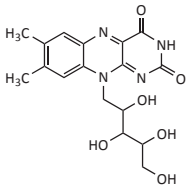


A. Thiamine (B1)



B. Pyridoxine (B6)

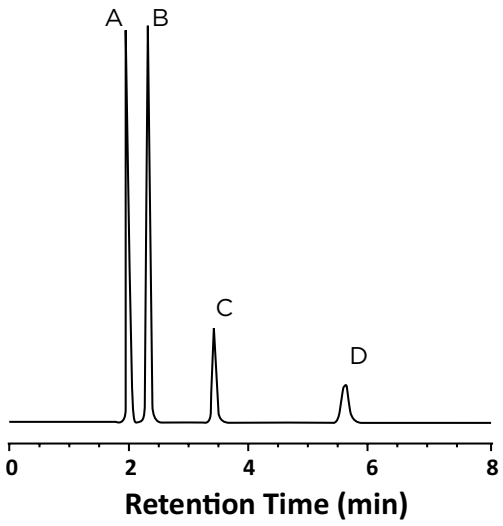




D. Riboflavine (B2)

Chromatographic conditions

- **Column**: SiliaChrom HILIC, 5 µm
- **Column size**: 4.6 x 200 mm
- **SiliCycle PN**: H131805E-N150
- **Mobile phase**: 0.1% TFA in water/0.1% in ACN (*90/10*)
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 280 nm

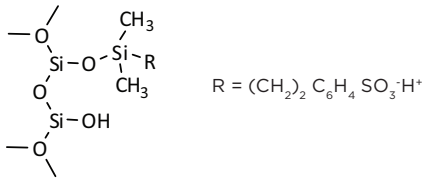


Silia*Chrom* SCX-SAX

Description

Silia*Chrom* SCX provides excellent resolution and peak shape for cationic analytes. The benzene sulfonic acid function of the Silia*Chrom* SCX is providing the cationic phase and also the π - π (*aromatic*) interaction. The Silia*Chrom* SCX is used for specific analysis of amino acids, anilines, drug salts, inorganic cations, and nucleosides.

Structure



Silia*Chrom* SCX

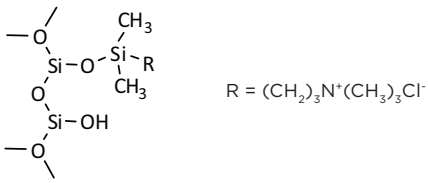
Sorbent Characteristics

- **Pore Size: Silia*Chrom* SCX: 150 Å**
  Silia*Chrom* SAX: 100 Å
- **Specific Surface Area: Silia*Chrom* SCX: 200 m²/g**
  Silia*Chrom* SAX: 380 m²/g
- **Particle Sizes Available: 3, 5 and 10 µm**
- **USP Code: Silia*Chrom* SCX L9**
  Silia*Chrom* SAX L14
- **Typical Carbon Loading: Silia*Chrom* SCX: 10%**
  Silia*Chrom* SAX: 6%

Description

Silia*Chrom* SAX provides excellent resolution and peak shape for anionic analytes. Silia*Chrom* SAX presents propyltrimethyl ammonium chloride functions allowing ion exchange interactions to acheive effective ionin chromatography. Silia*Chrom* SAX is used for specific analysis of pesticides, herbicides, inorganic anions and biological species such as nucleotides and glucosinolates

Structure



Silia*Chrom* SAX

Silia*Chrom* SCX and SAX Main Characteristics

- **Narrow peak shape**
- **Rapid equilibration**
- **Compatible with organic modifiers**
- **Provides high efficiency and rapid separations**
- **Endcapped**

Other Silia*Chrom* Products

Apart from the classic stationary phases, SiliCycle has also developped specific HPLC columns based on a silica matrix like our mixed-mode HPLC columns.

Mixed-Mode Silia*Chrom* HPLC Columns

Conjugate two surface function chemistries to optimize your separation in a single experiment. SiliCycle offers the following Silia*Chrom* Mixed-Mode HPLC columns:

- Silia*Chrom* C18/C8
- Silia*Chrom* C18/Amide
- Silia*Chrom* C18/Phenyl
- Silia*Chrom* C18/CN
- Silia*Chrom* C18/SCX
- Silia*Chrom* C18/SAX
- Silia*Chrom* C18/Nitrophenyl

SiliaChrom HPLC Columns for Biochromatography

Bio-Pharma

The rapid progress in the areas of genomics, proteomics, metabolomics and other biotechnology sectors has pushed scientists to develop innovative and efficient chromatographic methods. These methods have opened the way to better understanding of biomolecules and now offer impactful solutions effective at each level of the development of new commercial biopharmaceutical ingredients. Sorbent materials used in biochromatography and small molecule chromatography are similar but they require specific characteristics such as wide pore sizes and/or precise chemical resistance.

Separation and determination of peptides, proteins and nucleic acids can be done via different chromatography techniques. This section will highlight the SiliaChrom HPLC columns used in each following technique:

- Reversed-phase biochromatography for molecular weights (*MW*) < 5,000 Da
- Reversed-phase biochromatography for MW between 5,000 and 100,000 Da
- Ion exchange chromatography (*IEC*)
- Size exclusion chromatography (*SEC*)

SiliaChrom Reversed-Phases for Biochromatography (*MW < 5,000 Da*)

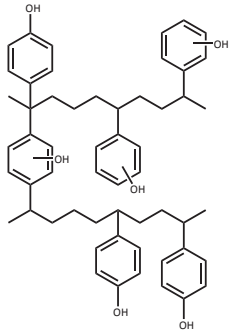
SiliaChrom Reversed-Phases for Biochromatography (*MW < 5,000 Da*)					
SiliaChrom Phases	Pore Size (Å)	%C	pH Stability Range	Characteristics	Phase Description
XT C18	150	21	1.5 - 12.0	Superior separation of basic & hydrophobic compounds Excellent peak shape in every condition. Excellent durability.	Page 109
XT C18 Fidelity	150	15	1.5 - 12.0		Page 109
dt C18	100	18	1.5 - 9.0	Superior separation of hydrophilic compounds. Mobile phase compatibility 100% aqueous to 100% organic. Inert and stable for acidic & basic analytes.	Page 101
RPC	n/a	Polymer	1.0 - 14.0	Guarantees chemical stability between pH 1.0 to 14.0 Basic compounds are well separated without peak tailling.	Page 117

Polymeric-Based SiliaChrom RPC

Description

SiliaChrom RPC phase is a hydrophobic copolymer based on polystyrene and divinylbenzene. The macroporous RPC reversed-phase resins are available in different particle sizes within a very narrow size distribution. The chemically inert polymer matrix of the SiliaChrom RPC guaranted chemical stability and allows for use with applications in the pH range from 1 to 14. The capacity factor (*K'*) values measured for aromatic and conjugated molecules on RPC columns are high due to the very pure uniform hydrophobic surface. The high efficiency and high selectivity of SiliaChrom RPC columns allow the separation of analytes in minutes. Even basic substances are separated efficiently without any peak tailing.

Structure



Phase Code: H920

SiliaChrom Reversed-Phases for Biochromatography (*MW 5,000 - 100,000 Da*)

SiliaChrom Reversed Phases for Biochromatography (*MW 5,000 - 100,000*)					
SiliaChrom Phases	Pore Size (Å)	%C	pH Stability Range	Characteristics	Phase Description
XDB1 C18-300	300	8	1.5 - 9.0	SiliaChrom C18 phase with wide pore size specialy designed for peptide & protein separation	Page 112
XDB1 C8-300	300	4.5	1.5 - 8.0	SiliaChrom C8 phase with wide pore diameter presenting lower hydrophobicity than C18	Page 112
XDB1 C4-300	300	3	2.0 - 8.0	SiliaChrom C4 phase with wide pore diameter presenting lower hydrophobicity than C8 ideal for protein separation	Page 113
XDB1 CN-300	300	3.5	2.0 - 8.0	This Cyano phase provides the maximum hydrophobicy for normal phase analysis conditions	Page 113
XDB1 Phenyl-300	300	4.5	2.0 - 8.0	Reversed-phase permitting π - π interactions Excellent for aromatic and unsaturated compounds	Page 113

SiliaChrom GF Phases for Size Exclusion Chromatography

Size exclusion chromatography (*SEC*) also known as gel permeation chromatography (*GPC*) or gel filtration chromatography, separates molecules according to their size (*or, more accurately, according to their hydrodynamic diameter or hydrodynamic volume*). Smaller molecules are able to enter the pores of the media and, are therefore trapped and removed from the flow of the mobile phase. The average residence time in the pores depends upon the effective size of the analyte and the pore size itself. Larger molecules are excluded with essentially no retention. SiliaChrom GF column series are an appropriate set of phases to be used for size exclusion chromatography with silica-based material in normal phase conditions.

SiliaChrom GF Phases for Size Exclusion Chromatography					
SiliaChrom Phases	Functional Group	Pore Size (Å)	pH Stability Range	Separation of molecules with molecular weights between:	Phase Code
GF	Diol	100	2.0 - 8.0	5,000 and 100,000 Da	H900
GF-300	Diol	300	2.0 - 8.0	50,000 and 1,000,000 Da	H900
GF AMIDE	Amide	100	2.0 - 8.0	5,000 and 100,000 Da	H901
GF AMIDE-300	Amide	300	2.0 - 8.0	50,000 and 1,000,000 Da	H901

SiliaChrom IEC Phases for Ion Exchange Chromatography

Bio-Pharma Pharmaceutical

SiliaChrom IEC series are composed of polystyrene polymer-based packing bearing different functionalities such as weak or strong cationic and anionic functions. SiliaChrom IEC phases are compatible with most mobile phases and samples with a pH range from 1 to 14. Polymer-based columns tend to have lower efficiencies for small molecules compared to silica-based columns due to their smaller surface area.

Nevertheless, SiliaChrom IEC packings are a good alternative for samples that require a mobile phase pH outside the normal operating range of standard silica-based columns. SiliaChrom IEC columns are generally used for ion exchange separations, and are also useful for non-aqueous gel permeation chromatography size exclusion analysis and ion exclusion analysis of organic acids and carbohydrates.

SiliaChrom IEC Phases for Ion Exchange Chromatography

SiliaChrom Phases	Functional Group	%C	pH Stability Range	Characteristics	Phase Code
SiliaChrom IEC SA	Dimethylammonium Chloride	8	2.0 - 8.0	Strong anion exchanger	H950
SiliaChrom IEC SC	Sulfonic Acid	4.5	2.0 - 8.0	Strong cation exchanger	H930
SiliaChrom IEC WA	Amino	3	2.0 - 8.0	Weak anion exchanger	H960
SiliaChrom IEC WC	Carboxylic Acid	4.5	2.0 - 8.0	Weak cation exchanger	H940

Each SiliaChrom IEC phases is available in particule size 5, 7, 10 and 20 µm

Silia*Chrom* Chiral Phases for Chiral Chromatography

Pharmaceutical Bio-Pharma

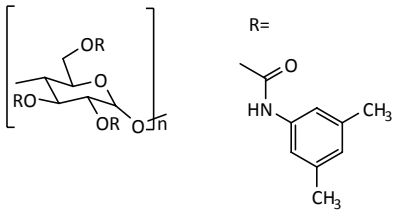
Silia*Chrom* Chiral Phases

Silia*Chrom* Chiral coated polysaccharide stationary phases are made with a spherical high quality silica support physically coated with a polymeric chiral selector such as amylose or cellulose derivatives. Due to the coated nature of these supports, solvents should be carefully selected for normal phase conditions.

Description

Silia*Chrom* Chiral Amylose T-DPC
Amylose tris-(3,5-dimethylphenylcarbamate) coated on a spherical silica support (*USP L51*). Silia*Chrom* Chiral Amylose T-DPC is used for chiral separation of alkaloids, tropines, amines, and beta blockers.

Structure

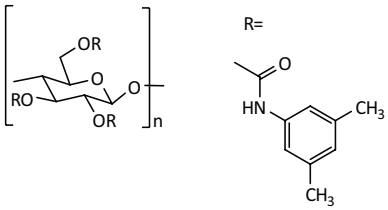


Silia*Chrom* Chiral Amylose T-DPC Phase Code: H810

Description

Silia*Chrom* Chiral Cellulose T-DPC
Cellulose tris-(3,5-dimethylphenylcarbamate) coated on a spherical silica support (*USP L40*). Silia*Chrom* Chiral Cellulose T-DPC is the most popular phase for chiral separation of alkaloids, tropines, amines, and beta blockers.

Structure

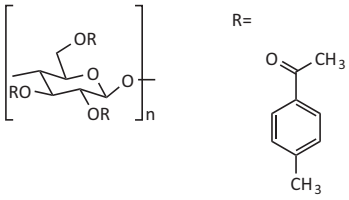


Silia*Chrom* Chiral Cellulose T-DPC Phase Code: H800

Description

Silia*Chrom* Chiral Cellulose T-MB
Cellulose tris-(4-methylbenzoate) coated on a spherical silica support. Silia*Chrom* Chiral Cellulose T-MB is used for chiral separation of aryl methyl esters and aryl methoxy esters.

Structure

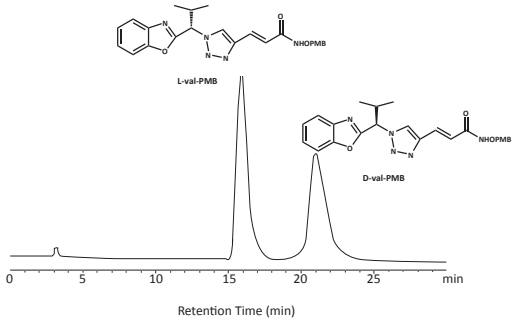


Silia*Chrom* Chiral Cellulose T-MB Phase Code: H820

Silia*Chrom* Chiral Amylose T-DPC Enantiomeric Separation of L and D-val PMB

Chromatographic conditions

- **Column**: Silia*Chrom* Chiral Amylose T-DPC, 5 µm
- **Column size**: 4.6 x 250 mm
- **SiliCycle PN**: H81005T-N250
- **Mobile phase**: Hexane/Isopropanol (*80/20*)
- **Flow rate**: 1.000 mL/min
- **Detector**: UV at 254 nm



SiliaChrom Phases for Supercritical Fluid Chromatography (SFC)

Supercritical Fluid Chromatography (*SFC*) is a globally accepted powerful «green» chromatographic technique for separation of enantiomeric compounds and complex mixtures. For decades, it has been the preferred technique for preparative chromatography. The recent advances in preparative and analytical equipment for SFC coupled with the industry demand for reliable rapid analysis chromatography has created the need for a dependable source for SFC columns and necessary technical support. SFC is a chromatographic technique where the main component of the mobile phase is Carbone Dioxide (*CO_2*). A CO_2 based mobile phase composition is a «green» alternative to conventional HPLC mobile phases. The use of CO_2 based mobile phases enables the use of high performance preparative columns (*10 to 50 mm ID*) with a variety of particle sizes from 3 to 10 µm. Many SFC separations have successfully utilized stationary phases from normal phase HPLC such as unmodified silica, Diol, Amino and Cyano without the need for special packing techniques or hardware. The low viscosity of supercritical CO_2 allows separations to occur 3 to 5 times faster with 70 to 90% less in solvent usage than those for normal phase HPLC. Speed of the SFC separations, conservation of organic solvents and more concentrated product fractions make SFC a desirable preparative chromatographic technique for purifying chemical mixtures.

- Hydrophobicity +

SiliaChrom Phases for Supercritical Fluid Chromatography				
SiliaChrom Phases	Pore Size (Å)	Carbon Loading %	Particle Size (µm)	Phase Description
SiliaChrom XDB1 Si	100	-	3, 5, 10	Page 113
SiliaChrom XDB1 Diol	100	5	3, 5, 10	Page 113
SiliaChrom XDB1 Amino	100	6	3, 5, 10	Page 113
SiliaChrom XDB1 CN	100	5	3, 5, 10	Page 113
SiliaChrom Hilic	100	8	3, 5, 10	Page 115

Silia*Chrom* Guard Columns and Holders

Silia*Chrom* HPLC Guard Columns are designed to effectively protect both analytical and preparative HPLC columns. The usage of this shorter column is highly recommended to prolong column lifetime and does not alter the chromatography. Silia*Chrom* Guard Columns are cost effective and easy to use as a pre-filter to remove contaminants prior to injection. In liquid chromatography, contaminants introduced into the column can cause:

- Higher backpressure
- Resolution loss
- Baseline noise or drift
- Peak shape changes
- Irreversible damages (*column + system*)

Silia*Chrom* Guard Columns Packing and Dimensions

For optimal results and maximal protection, it is recommanded to always use a guard column packed with the same packing material than the HPLC column. However, only the same chemistry is really needed. Particle size can be different but it is highly recommended to match the characteristics of the HPLC column used.

Silia*Chrom* Guard Columns are available in two different lengths (*10 and 20 mm*) and four internal diameters (*ID: 2.1, 4.6, 10 and 20 mm*). In most cases, a 10 mm length would be enough but if the sample contains important quantity of impurities, the 20 mm would then be more suitable.

The Guard Column internal diameter should be the same as the HPLC column or one size smaller. Never use a guard column with a larger ID than to the HPLC column (*risk of efficiency loss*).

Silia*Chrom* Guard Columns and HPLC Column Combinations

		Silia*Chrom* Guard Cartridges Internal Diameter (mm)			
		2.1	4.6	10	20
Silia*Chrom* HPLC Column Internal Diameter (mm)	2.0	r			
	2.1	r			
	3.0	r			
	4.6		r		
	10		O	r	
	20			O	r
	30				r
	50				r

X = Preferred O = Possible

SiliaChrom Guard Holders

To use a SiliaChrom Guard Column you need to purchase the appropriate holder:

SiliaChrom Guard Holders					
Product Number		SiliaChrom Guard Cartridges Internal Diameter (mm)			
		2.1	4.6	10	20
Holders	HDW-000	r			
	HDW-001		r		
	HDW-002			r	
	HDW-003				r

Installation Procedure

1. If a new capillary tubing has been installed or if the LC system has not been operated for some time, flush the lines free of particulate before attaching the SiliaChrom Guard Column.
2. Insert the stainless column fitting (*B*) into the metal housing male connector (*A*) of the SiliaChrom Guard Holder.
3. Insert the SiliaChrom Guard Column (*C*) into the metal housing male connector (*A*) of the SiliaChrom Guard Holder. Make sure that the flat side of the stainless column fitting (*B*) is placed in front the SiliaChrom Guard Column frit (*C*).
4. Insert the stainless column fitting (*D*) into the metal housing female connector (*E*) of the SiliaChrom Guard Holder.
5. Finger tight both parts of the assembled SiliaChrom Guard Holder until leak free.
6. Connect the assembled SiliaChrom Guard Holder into the male fitting of the LC system tubing.
7. Once you have connected the SiliaChrom Guard Holder to the system and the LC column, connect the LC column to the detector and start pumping the working mobile phase at a low flow rate to equilibrate both the Guard Column and the LC column.
8. Gradually increase the flow rate to working condition and check for leaks. If leaking still occurs after persistent hand tightening, replace the leaking fitting.

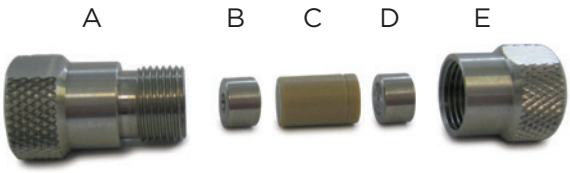
A Metal housing male connector

B Stainless column fitting

C SiliaChrom Guard Column

D Stainless column fitting

E Metal housing female connector



Silia*Chrom* Cleaning and Regeneration Procedures

If adequate care is taken, it is possible to maintain column efficiency and reliability over an extended period of time. This section is intended to give information on the different procedures to help extend HPLC column lifetime.

Difference between cleaning and regeneration

We usually make the assumption that, after a separation, all the material initially present in the column or cartridge has been eluted. After a run, the column is simply washed with 2-3 column volumes of the initial solvent mixture before starting a new separation. However, some impurities that are strongly retained on the column will accumulate at the intlet, if the mobile-phase composition is not strong enough to elute them during a regular run. Some non-negligible problems can arise when this happen: loss of performance, back-pressure build up, peak tailing, retention time shift or baseline drift.

To avoid this, it is highly recommended to perform regular cleaning of the column before any of these symptoms occurs. This process is simple and does not require modification of the usual chromatographic set up. When cleaning is not sufficient, a more thorough treatment, i.e. regeneration, may be necessary to avoid discarding the column.

Suggested Cleaning Procedure

The more you use a cleaning procedure, the less rigorous conditions be necessary. Cleaning should be performed after running a known “dirty” sample and prior to column storage using lower flow rate than usual (*typically from 20% to 50%*).

Column volume estimation is done using the following equation:

Column Volume (*packing’s volume included*) in mL = π * [Column Radius in cm]^2 * [Column Length in cm]

Silia*Chrom* Suggested Cleaning Procedure

Silia*Chrom* HPLC Column	Suggested Procedure
Reversed-Phase Columns (*C18, C8, C4, Amine, Cyano, Phenyl, etc.*)	- Water/ACN (*95/5*) to remove buffer - Water/ACN (*5/95*) - Mobile phase used during the separation
Normal Phase Columns (*Amine, Cyano, Diol, etc.*)	- MeOH/$CHCl_3$ (*50/50*) - Ethyl Acetate - Mobile phase used during the separation Note: Never use water.
Unbonded Silica Columns (*Silica*)	- Hexane - Isopropanol - Methylene Chloride - Mobile phase used during the separation
Ion Exchange Columns (*SCX, SAX, etc.*)	- 5 mM Phosphate Buffer pH 7.00 - Acetic Acid/Water (*10/90*) - Water - Methanol - Water

SiliaChrom Suggested Storage Conditions

When SiliaChrom HPLC Columns are not used for an extended period of time, do not allow high aqueous or high salt mobile phases to remain in the column. Remove aqueous buffers remaining in the column by washing with 20-30 column volumes of a 50% methanol or acetonitrile aqueous solution, followed by 20 column volumes of organic solvent such as methanol or acetonitrile.

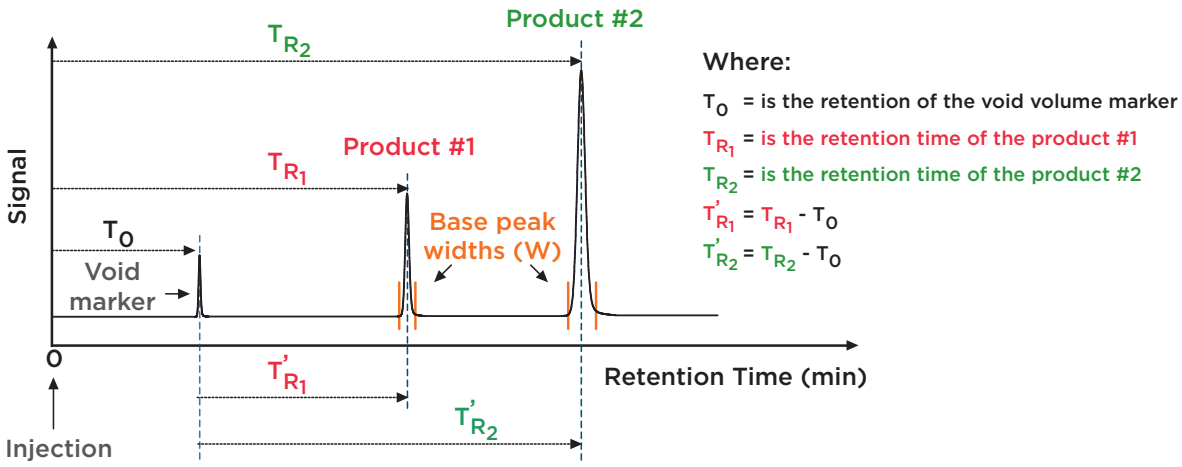
Each column is shipped with two removable column end plugs to prevent the drying of the column bed. Always put these plugs back on tightly before column storage or when column is not being used.

SiliaChrom Suggested Storage Conditions

SiliaChrom HPLC Columns		Recommended Storage Solvent
SiliaChrom AQ C18 SiliaChrom AQ C8 SiliaChrom dt C18 SiliaChrom SB C18 SiliaChrom SB C18-300 SiliaChrom SB C8 SiliaChrom SB C8-300 SiliaChrom XDB C18 SiliaChrom XDB C8 SiliaChrom XDB1 C18 SiliaChrom XDB1 C18-300 SiliaChrom XDB1 C8 SiliaChrom XDB1 C8-300 SiliaChrom XDB1 C4 SiliaChrom XDB1 C4-300 SiliaChrom XDB1 C1 SiliaChrom XDB1 C1-300 SiliaChrom XDB1 CN SiliaChrom XDB1 CN-300 SiliaChrom XDB1 Phenyl SiliaChrom XDB1 Phenyl-300 SiliaChrom XDB2 C18	SiliaChrom XT C18 SiliaChrom XT Fidelity C18 SiliaChrom C18/C8 SiliaChrom C18/Amide SiliaChrom C8/Amide SiliaChrom C18/Phenyl SiliaChrom C18/CN SiliaChrom C18/SCX SiliaChrom C18/SAX SiliaChrom C18/Nitrophenyl SiliaChrom Hilic SiliaChrom Hilic-300 SiliaChrom SCX SiliaChrom SCX-300 SiliaChrom SAX SiliaChrom SAX-300 SiliaChrom GF-300 SiliaChrom GF Amide SiliaChrom GF Amide-300	Methanol or Acetonitrile
SiliaChrom XDB1 Amino SiliaChrom XDB1 Amino-300		Butyl Chloride/Methanol
SiliaChrom Chiral Cellulose T-DPC SiliaChrom Chiral Cellulose T-MB	SiliaChrom Chiral Amylose T-DPC	Hexane/Isopropyl Alcohol (*90/10*)
SiliaChrom dt Si SiliaChrom XDB Si SiliaChrom XDB1 Si SiliaChrom XDB1 Si-300	SiliaChrom XDB1 Diol SiliaChrom XDB1 Diol-300	Isooctane/Ethanol

Important HPLC Definitions and Equations

Typical Chromatogram in liquid chromatography



Capacity Factor or Retention Factor (*k'*) is measured by the retention factor of the analyte compared to an unretained peak (*void volume marker*) using the following equation:

$$k' = \frac{(T_R - T_0)}{T_0}$$

Where:

T_R: is the retention time of the analyte

T_0: is the retention time of the unretained product

Efficiency (*N*) is usually measured by the plate count (*N or also called theoretical plate number*) using various equations. The most popular ones are:

By USP (*United States Pharmacopeia*)

$$N = 16 \times \left[\frac{t}{W}\right]^2$$

Where:

N: is the number of theoretical plates

t: is the retention time of the analyte

W: is the width at the base of the analyte

By DAB (*German Pharmacopeia*)

$$N = 5.54 \times \left[\frac{t}{W_{0.5}}\right]^2$$

Where:

N: is the number of theoretical plates

t: is the retention time of the analyte

$W_{0.5}$: is the width-at-half-height of the analyte

Selectivity (α) is measured by the retention factor ratio between two similar compounds.

$$\alpha = \frac{k'_2}{k'_1}$$

Where:

K_1': is the retention factor of product #1

K_2': is the retention factor of product #2

Separation's difficulty based on the selectivity value. If the selectivity is:

≥ 2:	Easy separation
1.5 - 2:	Possible separation*
1.2 - 1.5:	Difficult separation
≤ 1.2:	Very difficult separation**

* Method adjustment could be required

** Selectivity's optimization may be required

Important HPLC Definitions and Equations *(con't)*

Resolution (R) can be expressed using the two following equations

$$R = \frac{\sqrt{N}}{4} \times \left(\frac{\alpha - 1}{\alpha}\right) \times \left(\frac{1 + k'_2}{k'_2}\right)$$

Where:

N: is the number of theoretical plates

α: is the selectivity

K_2': is the retention factor of product #2

$$R = \frac{2(t_2 - t_1)}{W_2 + W_1}$$

Where:

T_1: is the retention time of the product #1

T_2: is the retention time of the product #2

W_1: is the width at the base of the product #1

W_2: is the width at the base of the product #2

Summary of Influencing Factors in HPLC

To choose the most suitable HPLC column, various parameters need to be taken into account: the desired selectivity and the sample load as well as the efficiency and the resolution. All these parameters are influenced by different factors in HPLC summarized in the table below.

Liquid Chromatography Influencing Factors

Properties	Typical Parameters	Affected Influencing Factors	Limitations
Chromatographic Conditions	Solvent	Retention, Efficiency	Back-pressure & phase stability
	pH	Selectivity, Resolution & Retention	Phase stability
	Flow Rate	Analysis Time, Efficiency & Resolution	Back-pressure & phase stability
Packing Characteristics	Chemistry (*SiO_2, C18, etc.*)	Selectivity, Resolution & Retention	Solvent used
	Pore Size (Å)	Sample Load & Selectivity	Size of the molecule
	Particle Size (µm)	Back-pressure, Efficiency & Resolution	Back-pressure & flow rate
HPLC Column Dimensions	Internal Diameter	Sample Load & Sensitivity	Back-pressure & flow rate
	Length	Analysis Time & Resolution	Back-pressure & analysis time too long

HPLC Method Scaling Up or Scaling Down Theory

When your experimental conditions are well optimized to get the most suitable purification, it is possible to scale up/down your method by keeping the same particle size and sorbent using these two equations:

Adjustment of the Sample Load

$$x_2 = \frac{x_1 \times r_2^2 \times C_L}{r_1^2} \quad \text{where} \quad \left[C_L = \frac{L_2}{L_1}\right]$$

Where:

x_1: is the maximum sample load in initial column

x_2: is the maximum sample load in final column

r_1: is the radius of the initial column

r_2: is the radius of the final column

L_1: is the length of the initial column

L_2: is the length of the final column

Adjustment of the Flow Rate

$$V_2 = \frac{V_1 \times r_2^2}{r_1^2}$$

Where:

V_1: is the flow rate use with the initial column

V_2: is the flow rate use with the final column

r_1: is the radius of the initial column

r_2: is the radius of the final column

How to Select the Right Silia*Chrom* HPLC Column

To select the right HPLC Column to use in your method development, read the section below to select the most appropriate Silia*Chrom* HPLC column to try first. However, before going forward in the selection, you need to have an idea of the sample quantity you need to purify as well as the liquid chromatography equipment available.

Remember: Resolution $R = \frac{\sqrt{N}}{4} \times \left(\frac{\alpha - 1}{\alpha}\right) \times \left(\frac{1 + k'_2}{k'_2}\right)$

Step 1. Find the Desired Selectivity by Selecting the Chemistry

When selecting an HPLC column, the most important factor is the selectivity in order to achieve an optimal resolution. A good knowledge of the composition of the sample mixture is crucial to select the most suitable chromatography mode to use in order to have good interactions between the sorbent and the compounds.

In liquid chromatography, there are various modes of operation possible based on the interaction mechanism of the solute with the stationary phase. Please refer you to previous sections to choose the most suitable phases to get optimal separation results.

Step 2. Select the Pore Diameter

To select the right pore diameter to use, find out the molecular weight of the solute. Typically, for small molecules, 100 - 150 Å pore size is recommended (*molecular weights below 5,000 Da*). For large molecules, such as peptides and proteins, 300 Å or higher is recommended.

Step 3. Find the Desired Efficiency & Resolution

Once you found the right selectivity, the second step is;

Be able to separate your sample with the shortest possible analysis time WITH optimal efficiency.

Two factors can influence the efficiency of a chromatography:

1. The particle size: influence on the resolution and back-pressure
2. The column dimensions (*internal diameter & length*): influence on the resolution and the sample load

Step 3.1. Select the Particle Size

For analytical applications, different particle sizes are available. The most common one being the 5 µm due to a good price/performance ratio. However, if you require a better separation and want to decrease analysis time, then 3 µm would be a better choice. Keep in mind that with a smaller particle size the backpressure will be higher.

For preparative applications, a larger particle size is usually used (*most frequently used is 10 µm*) with a larger column diameter (≥ *20 mm*).

How to Select the Right SiliaChrom HPLC Column (con't)

Step 3.2 Select the Column Dimensions (*Influence on the Resolution*)

For analytical applications, the most often recommended format for initial trial is the 4.6 x 150 mm. Then, if you need more resolution, look at: decreasing the internal diameter or increasing the column length.

3.2.1 Select the Internal Diameter (*Influence on the Sample Load*)

With smaller internal diameters, you reduce solvent consumption due to lower flow rate required but increase analysis time. Furthermore, loading capacity is decreased as the diameter decreases. The table below identifies typical applications associated with typical internal diameters used in HPLC.

Select the Internal Diameter (*ID*)				
Type of columns	ID (mm)	Typical Sample Load	Typical Flow Rate	Typical Applications
Narrow Bore	2.1	0.04 - 1.5 mg	0.1 - 0.3 mL/min	Used with low sample volumes or when more sensitivity and selectivity are needed over 3 mm ID.
	3.0	0.08 - 3.0 mg	0.2 - 0.6 mL/min	Used to reduce flow rate and solvent consumption over 4.6 mm ID. It is gaining popularity.
Analytical	4.6	0.2 - 7.0 mg	0.5 - 1.5 mL/min	This is the most common ID used for traditional quantitative analysis.
Semi-Preparative	10	0.95 - 33.0 mg	2.5 - 7.0 mL/min	Used for small-scale (*mg*) preparative purifications.
Preparative	20	4.0 - 132.0 mg	9.0 - 28.0 mL/min	Used for large-scale (*hundreds of mg to gram*) purifications. The higher the diameter, the greater the loading capacity.
	30	8.5 - 297.0 mg	20.0 - 60.0 mL/min	
	50	24.0 - 800.0 mg	60.0 - 175.0 mL/min	
	100	96.0 - 3,200.0 mg	240.0 - 700.0 mL/min	

3.2.2 Select the Column Length (*Influence on the Resolution*)

The rule of thumb is that in presence of the same packing, longer columns provide better resolution and efficiency over shorter ones but with longer retention times and higher pressure. In general, it is preferable to try using the shortest column length possible. If the resolution is not good enough, increase the column length or use a smaller particle size with the same length. The table below presents the most suitable length/particle combinations.

Select the Column Length		
Length (mm)	Most Suitable Particle Size (µm)	Typical Applications
30 & 50	3 µm or smaller	Used to reduce flow rate and solvent consumption over 100 & 150 mm lengths.
100 & 150	3 or 5 µm	These are the most common lengths used for traditional quantitative analysis.
200 & 250	5 µm or larger	For difficult separations or for higher resolution.

Acceptable Modifications to an HPLC Validated Method

Even if you are using an FDA validated or a USP recommanded method, some operating conditions can be adjusted if the modifications respect the acceptable specifications proposed by Pharmacopeias[1-3] and the FDA[4]. A side-by-side comparison of both the original and the adjusted method needs to be performed to demonstrate that the method's accuracy and precision is not affected by these modifications.

Acceptable Modifications to an HPLC Validated Method		
Parameters	Allowable modification	Examples of possible modifications
Mobile phase pH	± 0.2 units	Validated pH: 7.0 Allowed pH range: 6.8 – 7.2
Concentration of salts in buffer	± 10%	Validated concentration: 20 mM Allowed concentration range: 18 - 22 mM
Ratio of components in mobile phase	Only the minor components can be adjusted by ± 30% or ± 2% absolute (*i.e.: in regards to the total mobile phase*), whichever is the larger but should never exceed ± 10% absolute or removed totally.	**Binary mixtures:** Validated ratio: 50/50 Allowed ratio: 40/60 to 60/40 Validated ratio: 95/5 Allowed ratio: 93.5/6.5 to 96.5/3.5 **Ternary mixtures:** Validated ratio: 60/35/5 Allowed % of the 1st component: 60% Allowed % of the 2nd component: 25 – 45% Allowed % of the 3rd component: 3.5 – 6.5% The total of the three components together need to be 100%.
Wavelength of UV detector	No modification allowed.	n/a
Column length	± 70%	Validated length: 150 mm Allowed length range: 45 - 255 mm
Column inner diameter	± 50%	Validated inner diameter: 4.6 mm Allowed inner diameter range: 2.3 – 10.6 mm
Flow rate	± 50%	Validated flow rate: 1.00 mL/min Allowed flow rate range: 0.5 – 1.5 mL/min
Injection volume	May be increased to as much as 2 times if no adverse effects on LOD and repeatability.	n/a
Particle size	No increase permitted. May be decreased by as much as 50%.	Validated particle size: 5 µm Allowed particle size range: 2.5 - 5 µm
Column temperature	± 20%	Validated temperature: 23°C Allowed length range: 18.4 – 27.6°C

1. USP. USP 32–NF 27, Chromatography <621>. Rockville, MD: USP; 2009:227.
2. USP. Second Supplement to USP 32–NF 27. Rockville, MD: USP; 2009:4147.
3. USP. USP 32–NF 27, Verification of Compendial Procedures <1226>. Rockville, MD: USP; 2009:736.
4. ORA Laboratory Procedure, Food and Drug Administration, modification criteria.

NEW

SiliaChrom HPLC Column Storage Cabinet

Protect your HPLC Column Inventory with the SiliaChrom Column Storage Cabinet

The SiliaChrom Column Storage Cabinet has been designed to safely store your HPLC column investment. Poor column storage can lead to reduced column performance and decreased product life.

The SiliaChrom Column Storage Cabinet is a bench top storage unit of solid steel construction with chrome-plated D-ring handles for added resistance. Up to 30 columns of 300 mm long (*or shorter*) can be stored in 5 separate drawers. Each drawer has a 6 position secure molded foam insert providing several storage possibilities. The foam insert can easily be customized to accommodate shorter columns, guard cartridges or HPLC tools and fittings. Each storage cabinet is stackable and supplied with rubber mounts. With the addition of a mounting bracket (*sold separately*), your SiliaChrom Column Storage Cabinet can be expanded to a multi-unit storage device.

The SiliaChrom Column Storage Cabinet is a cost-effective, expandable solution to conveniently index and store your HPLC column inventory.

Using the SiliaChrom HPLC Column Storage Cabinet ensures the following benefits

- Easy column identification.
- No more misplaced or lost columns.
- Increases column lifetime.
- Saves time and storage space.

SiliaChrom Column Storage Cabinet Characteristics	
Column Storage Cabinet PN	AUT-0167
Mounting Bracket PN	AUT-0168
Dimensions (*W x H x D*)	279 x 325 x 408 mm
Drawer Height	51 mm
Column Formats	From 20 to 300 mm lenght
Column Storage Cabinet Weight	12 Kg