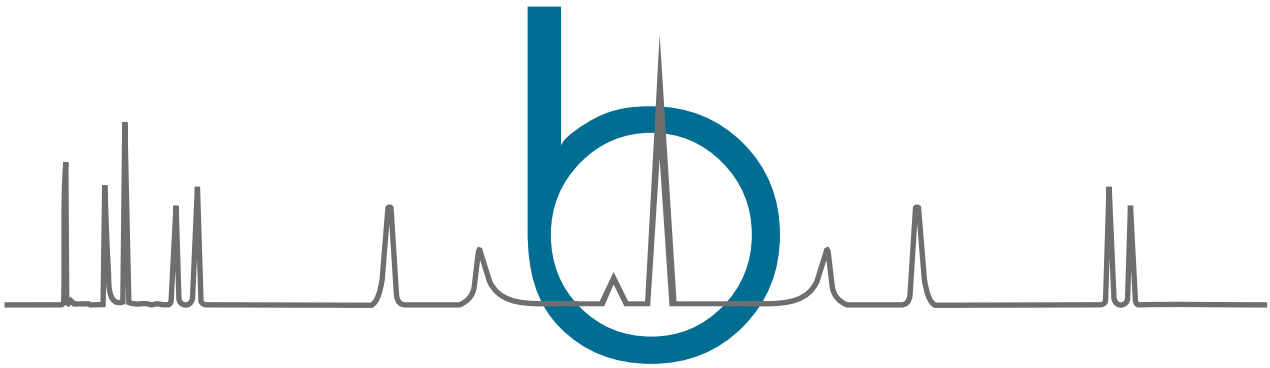
ProntoSIL
HPLC Columns
GERMAN ENGINEERED PERFORMANCE

Thinking Differently



PRODUCT CATALOGUE

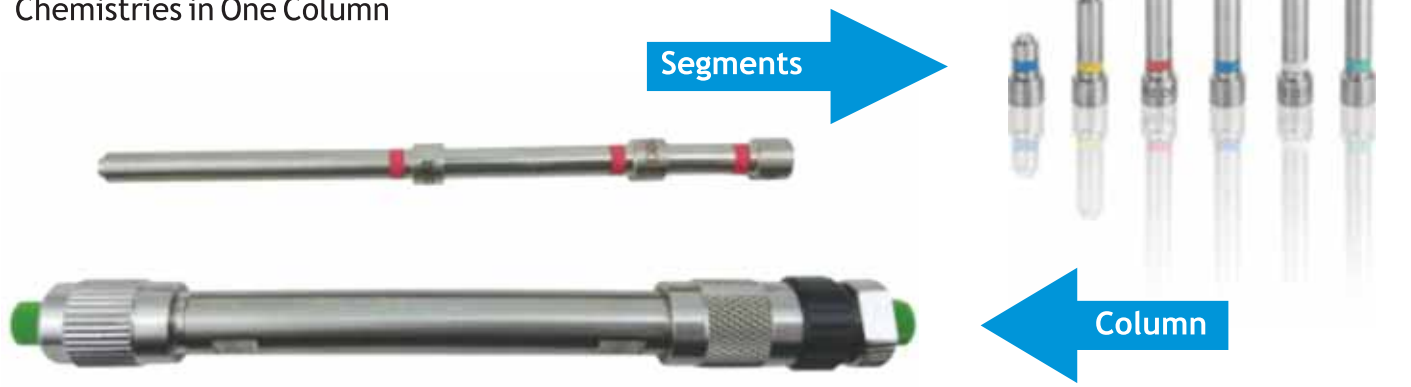
BISCHOFF CHROMATOGRAPHY
Leonberg, Germany

POPLink®

Customize Your HPLC Column with Segments

POPLink Columns

Our patented Segmented Column allows you to couple different Chemistries in One Column



Zero Dead Volume - Same Inner Diameter - Different Chemistries

Different Chemistries - Different segments - ONE COLUMN - POPLink

Introducing :

- Use of two different chemistries in combination for a single run.
- Zero dead volume while using combination of 2 cartridges.
- Reduce the time to figure out the best suitable column for your sample.
- Reduce the retention time drastically due to smaller dimensions of the columns.
- No compromise in resolution with excellent peak shapes.
- Needs lesser solvents and hence conserves environment.

POPLink columns :

- Two cartridges (60mm x 3mm) in a single column.
- The columns are available in both 3um and 5um.
- Customized combination of any two chemistries is possible as per the sample requirements

Standard columns are available in following combinations :

• C8 ACE-EPS + C18 ACE-EPS	• C18 H + C18 SH
• C18 SH + C18 ACE-EPS	• C8 ACE-EPS + C30
• C18 AQ + C18 ACE-EPS	• C8 KromaPlus + C18 KromaPlus
• C18 AQ + C18 SH	• C18 AQ + C18 AQ Plus

Learn more:
bischoff-chrom.com/poplink

BISCHOFF

History

"The most important part of HPLC is the Column"

BISCHOFF, Analysentechnik u-geräte GmbH (internationally known as **BISCHOFF Chromatography**) was founded in 1980 by Klaus Bischoff as a Service provider Company doing Refill of used HPLC-Columns. Since then BISCHOFF chromatography has earned its name as one of the renowned HPLC Manufacturer. Our subsequent involvement in the related areas of development, Applications, Quality Control and Sales along with our long standing relationship with the eminent professors of universities in Germany as well as in other foreign universities have played an influential role in shaping up the company philosophy. We are committed to deliver user and application friendly products of high quality and durability which are manufactured with modern technology to reduce cost and leading to reliable results. As an innovative High-Tech-Company it is also our corporate social responsibility to save on resources and contribute towards environmental protection by optimizing separation processes with miniaturization of instruments.

The unique universal HYPERCHROME column-system which came into existence since 1981, allows dual function of a column which can be used as cartridge as well as conventional separation column.

Within a short time span, through its ability in technique, quality and performance **BISCHOFF Chromatography** has evolved to become one of the leading column manufacturers in Germany and Europe.

Besides the instrument development and the continuous improvement of applied HPLC methods **BISCHOFF Chromatography**, along with the inputs from research groups in the universities has successfully developed new stationary phases or implemented already available phases into marketable products. Our long standing industry experience and continual research has enabled us to develop high quality ultra pure silica gel called ProntoSIL and establish a yardstick in producing other high quality stationary phases in house.

Our state of the art packing technology has resulted into setting a benchmark by producing superior quality HPLC columns.

On behalf of all employees of **BISCHOFF Chromatography** I would like want to assure you the following:

Our main focus / objective is to provide the best high performance separation columns to HPLC users all around the globe. We lay emphasis on QC not only on our products but in our technical advisory service and the "after-sales" service. It is extremely important for us to fulfill and exceed the expectations of our esteemed clientele and to make significant contribution in the fields of "Life Sciences" and other chemical industries.

Oliver Bischoff
Managing Director and Owner

Short Description

- Start measuring your sample on each of the phases you got with your POPLC KIT under isocratic conditions
- Type the retention data you got from your measurements into the POPLC Optimizer Software
- Software will predict a chromatogram and tell you how to combine your segmented column to get your optimal or best possible separation

Your Benefits

- Save time with "Overnight Method Development"
- Combine or couple different column segments packed with different stationary phases
- Save money - You don't have to buy different columns in different dimensions
- Maximum Variety - A POPLC Expert KIT offers you several thousand columns segment combinations

A new way to improve HPLC Separations Phase Optimized Liquid Chromatography - POPLC®

A new way to improve HPLC Separations Phase Optimized Liquid Chromatography - POPLC®The most important tool in HPLC method development is stationary phase selectivity. The number of commercially available RP phases shows the importance of the stationary phase. These are approximately 750 different stationary phases today, and every year new packing materials are introduced. The user now has the task to select the right column for his application from this large number of HPLC phases. This can be very difficult. Very often it involves a costly and time consuming optimization of the mobile phase.

The optimization of a separation becomes much easier once the optimal stationary phase has been found. The Phase Optimized Liquid Chromatography (POPLC®) uses a completely new approach in method development and optimization.

After a rough first choice of mobile phase, only the stationary phase needs to be optimized.

POPLC® is based on the theory of the "PRISMA Model" that has been used before to optimize mobile phases in Liquid Chromatography [Szabolcs Nyiredy, K. Dallenbach-Tölke, O. Sticher in JPC (Journal of Planar Chromatography) 1, (1988), Seite 1241]. (Fig. 4)

The vertical sides of the prism correspond to the retention strength of a given analyte. The optimal retention strength for this analyte lies on a surface that is built by the upper irregular triangle of the prism. It can be realized by combination of the stationary phases A, B and C. Technically this is realized by using a segmented column system.

Method

First retention times are determined with isocratic chromatographic runs of the analytes on different stationary phases using the same mobile phase which is chosen by experience or trial. The stationary phases used for these basic measurements should be of strongly different selectivity. For example C18, C18 with enhanced polar selectivity and Phenyl, C30 or Cyanopropyl (CN) could be used for this purpose. The retention of the analytes on any of these phases is different due to different mechanisms of interaction. The individual retention times are then used for calculations that are performed by optimization software. The software calculates the combination of column segments.

POPLC Optimizer Software for Isocratic Methods

Optimize / Develop HPLC Separations during your Coffee break!

Increasing productivity, confidence in results and cost effectiveness are today's key objectives in HPLC laboratories. POPLC® Optimizer is developed to meet these challenges as a universal software for any type of Liquid Chromatography independent from the brand of instrument or the brand of columns.

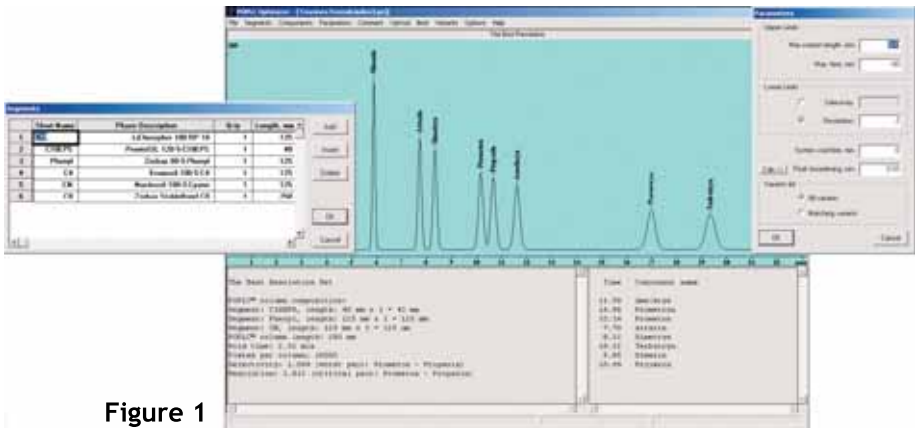


Figure 1

Fig. 1 shows a separation predicted by POPLC® Optimizer using a combination of conventional HPLC columns. None of these columns was able to separate the sample by it's own. The best possible separation showed co-elution, for some compounds, on each column.

To improve the separation the listed columns are virtually divided in twenty five 10 mm segments. The POPLC® Optimizer software will calculate each possible segment combination (in this example 736,286 combinations). The result is an improved isocratic separation using the same parameters. Fig.2 shows the best resolution for this sample using the column combination virtually segmented.

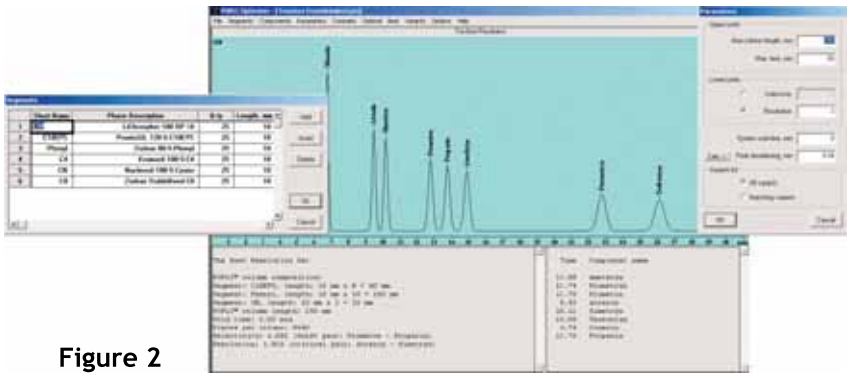
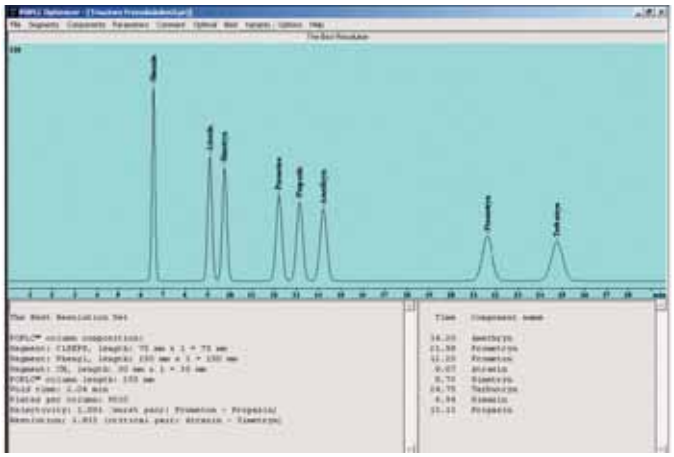


Figure 2



Since only a few column dimensions exist in the market the calculated length for the different stationary phases must be adapted to the availability. In our example (Fig.2) the 60 mm C18 EPS column is translated into 75 mm. The 160 mm Phenyl is translated into 150 mm and the 10 mm CN into 30 mm. With these dimensions the calculation gives a similar chromatogram (Fig.3) compared to the chromatogram in Fig.2.

Figure 3

In Fig.4 a combination of column segments is illustrated which is not virtual. They are available in the unique and patented POPLC® Basic-Kits and can be used instead of regular columns. POPLC® Optimizer software will predict the optimized column and selectivity combination. The Kits allow the immediate proof of prediction. The measured retention times will have a small deviation from the predicted retention times. That means you are able to predict the most selective and fast separation without running an instrument for optimization.

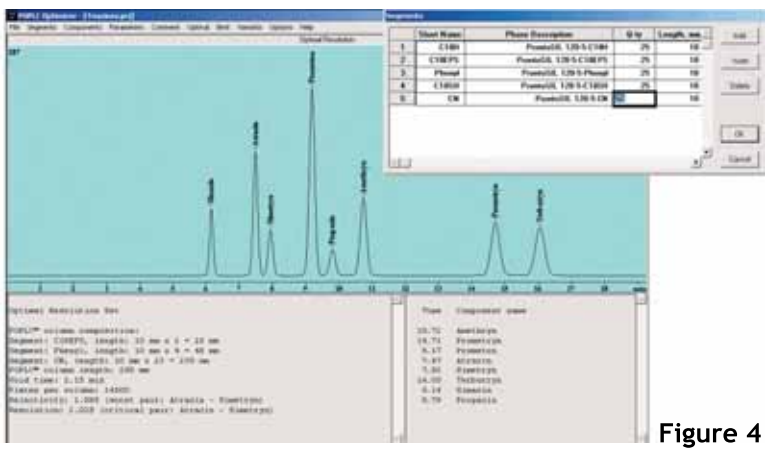


Figure 4

Preparative and Semi Preparative Columns

Bischoff Chromatography is one of the global players in market that meet the challenging demands of preparative chromatography. We aim in providing chromatographic solutions for any compound, right from discovery, scaling up to production and its quality control.

The main aim of preparative chromatography is to produce a quantity of pure compound with ease in the most economical way. The preparative columns by Bischoff chromatography are available in wide range of columns sizes to carry out almost any preparative scale separations. These preparative columns are packed under a very high pressure and in a specialized hardware to increase the bed density as high as possible to obtain sharp peaks and good resolution. This uniform high density packing prevents formation of voids during use. This results in preparative columns with higher efficiency and durability.

Our Preparative Columns are available in all our phases in the following dimensions :

Length (In mm) :
500, 300, 250, 200, 100,
150, 125, 75, 60, 50, 40, 30

Inner diameter (In mm) :
62, 40, 50, 32, 20, 16, 8

Type	Column Dimensions			
	Code	ID	L mm	AD
PREP 1612	1216...	16	125	25
PREP 2012	1220...	20	125	25
PREP 0316	0316...	16	30	25
PREP 2003	0320...	20	30	25
PREP 0332	0332...	32	30	45
SPC	0480...	8	40	12
PREP 2005	0520...	20	50	25
PREP 3205	0532...	32	50	45
PREP 4005	0540...	40	50	60
PREP 5005	0550...	50	50	80
PREP 4006	0640...	40	60	60
PREP 2007	0720...	20	75	25
PREP 5007	0750...	50	75	8
PREP 1610	1016...	16	100	25
PREP 2010	1020...	20	100	25
PREP 5010	1050...	50	100	80
SPSC	1280...	8	125	12

Type	Column Dimensions			
	Code	ID	L mm	AD
PREP 3212	1232...	32	125	45
PREP 4012	1240...	40	125	60
PREP 1015	1510...	10	150	16
PREP 2015	1520...	20	150	25
PREP 3215	1532...	32	150	45
PREP 4020	2040...	40	200	60
PREP 5020	2050...	50	200	80
PREP 6220	2062...	62	200	90
SNC	2580...	8	250	12
PREP 1025	2510...	10	250	16
PREP 1625	2516...	16	250	25
PREP 2025	2520...	20	250	25
PREP 3225	2532...	32	250	45
PREP 4025	2540...	40	250	60
PREP 3050	3050...	50	300	80
PREP 3062	3062...	62	300	90
PREP 2050	5020...	20	500	25
PREP 3250	5032...	32	500	45

ProntoSIL columns for SFC

Bischoff chromatography offers achiral phases for supercritical fluid chromatography (SFC) applications to provide the chemist with a number of options for SFC separations. These columns are available in many chemistries. Columns include stationary phases based on silica that are coated or covalently bonded.

These columns are specifically packed in SFC compatible hardware and tested individually to guarantee performance. The low viscosity of supercritical carbon dioxide allows for separations that are three times faster or more than normal phase HPLC. Speed of SFC Separations, conservation of organic solvents and more concentrated product fractions make SFC a desirable preparative chromatographic technique for purifying chemical mixtures.

ProntoSIL SFC Semi-Prep columns are available with inner diameters from 10mm to 50mm and in lengths from 50mm to 250mm. Individual SFC documentation is included with every column.

Use ProntoSIL SFC columns for following benefits :

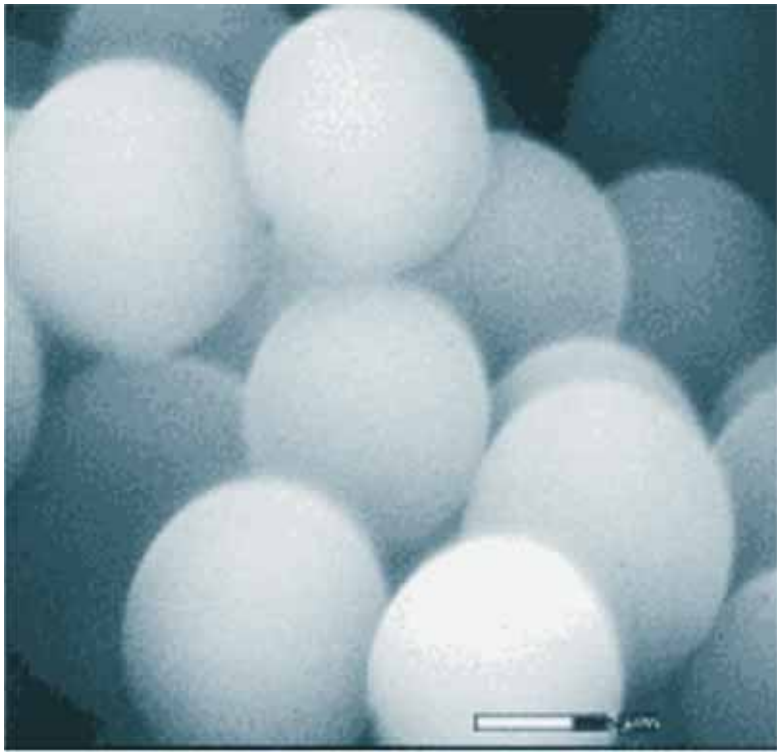
1. Complex samples can be separated with better resolution at a faster rate with shorter run times.
2. Sharp Peak shapes on co-ordination compounds.
3. Extremely fast equilibration.
4. Excellent reproducibility.
5. Used mostly for non polar as well as less polar compounds.
6. 3-5 times faster separation than HPLC.
7. Cost effective purification using SFC.

ProntoSIL SFC Bondings :

- ProntoSIL Cyano SFC
- ProntoSIL Diol SFC
- ProntoSIL Ethylpyridine SFC
- ProntoSIL Silica SFC

Ultra Pure Silica

Spherical, completely Porous Silica



The ultimate ultra-pure stationary phase, is a spherical, completely porous silica gel. It is manufactured under the most stringently controlled conditions, guaranteeing constant particle and pore distribution as well as constant size and volume of the pores. This manufacturing process permits no performance-reducing micropores but yields constant specific surface area. ProntoSIL packing materials have extraordinary purity and are free from metallic contaminants that could hinder optimum peak shape.

In the development of ProntoSIL phases, our many years of experience have led to more than the usual physical parameters such as specific surface area, pore size, pore volume and for RP phases percentage carbon content. Additional parameters provided include silica gel composition, metallic impurities, hydrophobic strength, steric selectivity, peak symmetry for basic analytes, silanol capacity and ion exchange sites. These parameters are determined by procedures such as NMR and ICP in addition to chromatographic techniques. This multiplicity of characteristics yields extremely reproducible carrier materials providing a basis for reliable, constant, batch-independent, high-performance separation columns.

Ultra Pure Silica Gel

The silica gel utilized in the manufacturing of ProntoSIL is 99.999% pure silica gel. A particle size distribution analyzer is used to test each batch for constant particle size. Surface area, pore size and pore volume are determined by a BET analyzer. AAS and ICP are utilized to test for metallic impurities. The extremely low level of metallic impurities guarantees that no sample will be adsorbed and no complexes will be formed during chromatographic analysis.

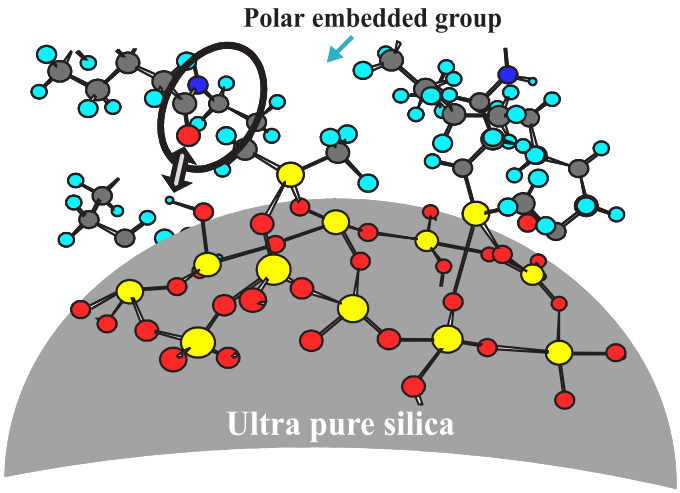
Bonding and End-Capping

The silica gel is chemically modified using the most convenient procedures, in part developed by Bischoff Chromatography. Efficient bonding and the elimination of harmful silanol group residues is monitored by NMR. The absence of silanol group residues is shown by the excellent chromatographic performance with respect to basic and acidic analytes. Both yield equally good chromatograms.

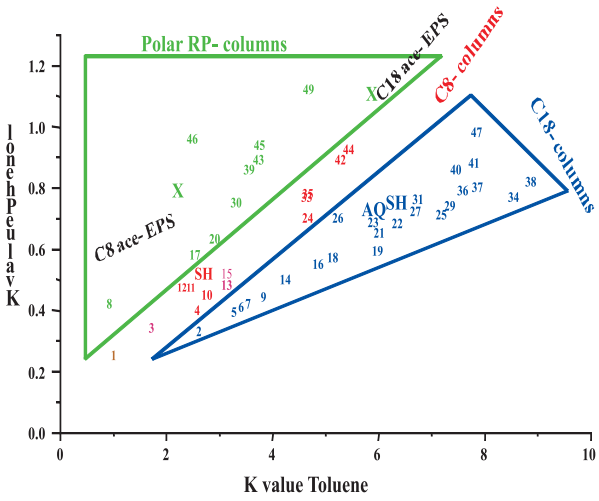
Reversed-phase and Normal-phase packings

Depending upon the relative polarities of the mobile and stationary phases of partition chromatography, it is distinguished into two types. Early work in liquid chromatography was based upon highly polar stationary phases such as water or triethyleneglycol supported on silica or alumina particles, a relatively nonpolar solvent such as hexane or I-propylether then served as the mobile phase. This type of chromatography is now referred to as normal-phase chromatography. In reversed-phase chromatography, the stationary phase is nonpolar, often a hydrocarbon, and the mobile phase is relatively polar (such as water, methanol, or acetonitrile).

In normal-phase chromatography, the least polar component is eluted first, because in a relative sense, it is most soluble in the mobile phase, increasing the polarity of the mobile phase has the effect of decreasing the elution time. In contrast, in the reversed phase method, the most polar component appears first, and increasing the mobile phase polarity increases the elution time.



Surface of ProntoSIL C18 ace-EPS



Classification of RP-Columns

The R group on the siloxane is most commonly C8 chain (n-octyl) or a C18 chain (n-octyldecyl) in reverse phase bonded packings. With such preparations, the long-chain hydrocarbon groups are aligned parallel to one another and perpendicular to the particle surface, giving structure like a brush or bristle. The brush behaves as a liquid hydrocarbon medium similar in nature to an ordinary liquid-liquid stationary phase.

Physical adsorption occurs at the modified surface of the brush coating. The molecules of the mobile phase then compete with the analyte molecules for position on the organic surface. Regardless of the detailed mechanism or retention, a bonded coating can be treated as if it were a conventional, physically retained liquid. In commercial normal-phase bonded packings R group in the siloxane structure is a polar functional group such as the Cyano ($-C_2H_2CN$), Diol ($-C_3H_6OCH_2OH$), Amino ($-C_3H_6NH_2$). The polarities of these packing materials vary over a considerable range. With normal-phase packings, elution is carried out with relatively nonpolar solvents, such as ethyl ether, chloroform, and n-hexane.

Properties of Column

The selection of the right column for your separation problem depends on the properties of your sample. The following properties of an analyte are important for selecting a column, size, functional group, polarity, spatial structure and matrix.

Hydrophobicity

Hydrophobicity refers to the property of a stationary phase to retain a hydrophobic compound as much as possible. The strength of the retention capacity of an individual phase depends substantially on the surface modification and the specific surface of the chromatographic support. C18 phases have the highest hydrophobicity. If the hydrocarbon chains are increased, the loading capacity becomes lower, solely due to steric hinderance. If the hydrocarbon chains are shorter, the analytes will have fewer positions to adsorb, leading to lower hydrophobicity.

The specific surface of the support determines how many functional groups will be bonded. Therefore it is said that the larger the surface, the higher the carbon load and therefore, the hydrophobicity of the phase. The surface of a silica gel is dependent on the pore size and pore volume. As long as the pore volume is kept constant, the larger the pores are, the less surface will be available and the lower the hydrophobicity of the stationary phase will be.

Silanophilic Activity

Silanol groups have a large influence on the selectivity of an RP-HPLC phase. On the one hand, undesired secondary interactions result from the silanol groups whereby basic substances will display pronounced tailing through interactions with the acidic silanol groups. On the other hand, such secondary interactions are actually required in some separations to get the desired selectivity. To minimize the silanophilic activity, many stationary phases are “endcapped".

This is carried out as an additional treatment step on the already modified (C18, C8, ...) silica surface by treating the silica gel with a smaller silane (usually trisilane). Since this silane is considerably less bulky than the respective C8 or C18 groups, many of the non-reacted silanol groups will react with the silane and therefore become deactivated. Modern columns based on silica gel have very good endcapping and display an exceedingly low silanophilic activity.

Shape Selectivity

The term "shape selectivity” refers to the ability of the stationary phase to differentiate between planar and nonplanar molecules (molecular recognition). Highly loaded C18 and C30 phases, in particular, exhibit this property. The effect is based upon the fact that non-planar molecules have a greater space requirement and therefore fewer adsorption positions on the stationary phase as compared to planar analytes. Non-planar molecules are therefore less strongly adsorbed on stationary phases with higher shape selectivity.

Tetrabenzonaphthalene TBN

Benzo[a]pyrene BaP

Shape Selectivity Tests Sander &Wise

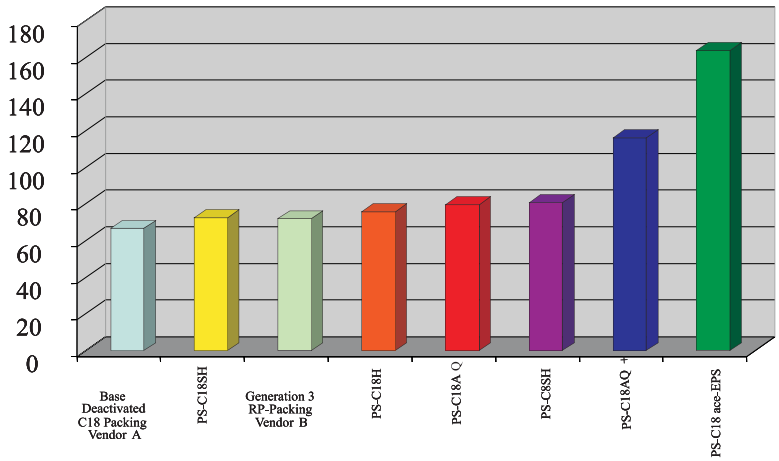
Metal Content

Due to the fact that silica gel was earlier produced from sodium silicate, older generations of silica gel possess a very high metal content (predominantly iron, up to 500 ppm). Metals can lead to problems during analysis of certain analytes. It is known, for instance, that certain proteins cannot be eluted from older HPLC phases. Moreover, the chromatography of complexing agents (e.g. EDTA) with such stationary phases is quite difficult. Since the analytes can only be eluted from the phases as broad asymmetric peaks. Modern silica gels are produced of tetraethoxysilane & consequently possess metal contents < 10 ppm. The separation of bipyridines on two stationary phases of different generations illustrates the difficulties during chromatography. Metals in the silica gel also have a direct effect for the acidity of the silica gel. The higher metal content is, the more acidic the silanol groups on the surface of the silica gel will be. This acidity also substantially influences the reactivity of the silica gel. Since the metal content in older generations of silica gel fluctuates and consequently influences the reactivity, the reproducibility of individual batches of stationary phases is affected. Therefore it is recommended to use as much as possible, stationary phases produced from ultra-pure silica gels.

Polar Selectivity

The polar selectivity of a stationary phase refers to the property of a phase to undergo polar interactions with analytes. During development of modern RP phases, the newer phases have lost their polar selectivity.

Through the synthesis of highly pure silica gels as support materials the surface became more homogeneous, resulting in better coverage by the surface modifications. Modern RP phases with alkyl groups show therefore hardly any polar selectivity. This can be countered however through the targeted introduction of polar functional groups onto the surface of the material or into the alkyl chains.



Polar Selectivity of Different Stationary Phases

pH Stability

In general silica based HPLC columns are stable within pH 2 to pH 8. To measure pH, the measurement has to be done in the aqueous media before mixing with organic solvents. This will give a more accurate and consistent pH measurement than taking the pH in a mixed aqueous/organic media. Some modern HPLC columns can be used outside pH 2 to pH 8. New bonding chemistry allows for operating as low as pH 1 with some stationary phases. However, you should check vendors product information before using a silica based column outside the pH 2 to pH 8. Stationary phases based on ultra pure silica gel can also be used at a pH as high as 11. It depends on the chemical nature of the modifier used in the mobile phase. Large bases (such as pyrolidine) are not able to attack the surface of the silica and, therefore, can be used as mobile phase modifiers when higher pH values are required. If you like to work at pH > 8 by using small bases as the modifier(e.g. ammonia), we recommend the using of stationary phases based on polymers or zirconium dioxide.

Mechanical stability

Stationary phases based on silica are mechanically very stable, and well-packed columns can be used at more than 40 MPa (6000 psi) without any problem. However, pressure shocks should be avoided. Pressure shocks can lead to channeling in the column bed, which may result in peak splitting.

Speciality of column Hardware

Performance of the column is of critical importance. The most important performance criteria for the quality of a packed column are separation efficiency and stability of the packing. These parameters will depend on the packing material as well as on the column hardware. The unique design of HYPERCHROME column hardware is superior to other Hardware in many ways.

Its column nuts are perfectly adapted to the column tube, making it possible to use the hardware as a standard column cartridge. The chromatographic separation, compared to conventional columns, even the packing is protected by a mesh sandwich at the column ends. Direct distortion or mechanical flow distribution of the packing is there by eliminated. The mesh sandwich which replaces typical frits is kept in the column tube by a PTFE seal ring. It also prevents blockages and additional metal contamination which are common for frits.

High performance separation columns are packed using the unique HYPERCHROME column hardware developed in house in own environmental friendly packing process. The spherical, mechanically stable particles of silica gel with extremely narrow size distribution ensure constant column. Every single column undergoes a quality control test to check its chromatographic performance. This test report is attached to the column so you can be sure that the column meets highest quality criteria.

Performance of HPLC system depends on efficiency of column used. Efficiency of column depends on quality of hardware and packing material used.

Zero Dead Volume

Whenever two columns are connected together dead volume efficiency decreases. We have found a solution of this problem: zero dead volume connector (ZDV).

ProntoPEARL Columns / Sub micron size columns

ProntoPEARL sub 2 are new stationary phases with particle size of less than 2µm. The application area is mainly fast HPLC as high efficiencies can be achieved in a short time. You can save up to 90% of analysis time. ProntoPEARL sub 2 packings are available as TPP (Totally Porous Phases) recommended for the separation of small molecules or NPP (Non Porous Phases) used in separation of polymers or biopolymers (proteins and peptides). The narrow particle size distribution leads to extremely high efficiencies in packed columns. Therefore minimum plate column of 200000 plates per meter can be achieved which leads to better efficiency of the column.

ProntoPEARL sub 2 columns are available in following phases:

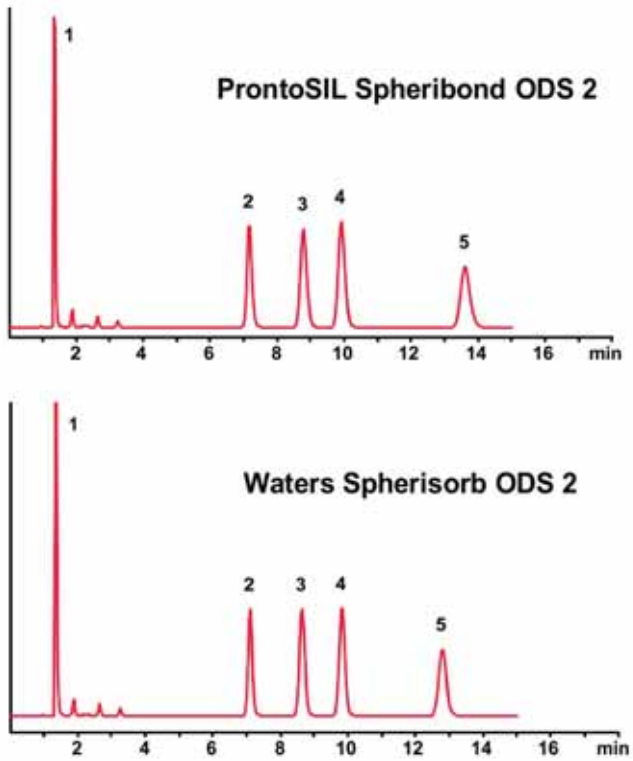
1. ProntoPEARL sub 2 TPP C18 ace-EPS
2. ProntoPEARL sub 2 TPP C18 SH

Smart choice to change Water's Spherisorb ODS1 & ODS2

Introducing

Prontosil Spheribond ODS1 & ODS2

- No revalidation required
- Available in almost any column dimension
- High end packing quality



Tanaka Test

Comparison of ProntoSIL Spheribond ODS 2 and Waters Spherisorb ODS 2

ProntoSIL Spheribond ODS 2 5 µm

Part number:	1546D181SB050
Dimension:	150 x 4.6 mm
Eluent:	H_2O/MeOH 25/75 (w/w)
Flow:	1 ml/min
Detection:	UV 254 nm
Temperature:	40 °C

Sample:
1. Uracil
2. Butylbenzene
3. o-Terphenyl
4. Pentylbenzene
5. Triphenylene

Same Selectivity
Better Price

Bischoff Chromatography offers now alternatives for the Waters-Spherisorb product line.

The new clone packings offer the following benefits:

No revalidation of your existing method is required

The new packings offer the same selectivity under the same chromatographic conditions like the original columns. You can change without any problem.

Available in almost any column dimension

If your chromatography asks for a column outside the typical column dimensions 250 x 4.6 mm we have it. Like all Bischoff columns the new packings are also available in almost any column dimension

High end column packing

Our experience in the packing of the Waters-Spherisorb and Hypersil supports over years in a very high quality and our superior HYPERCHROME column hardware leads to the high end column packing for those supports.

NEW

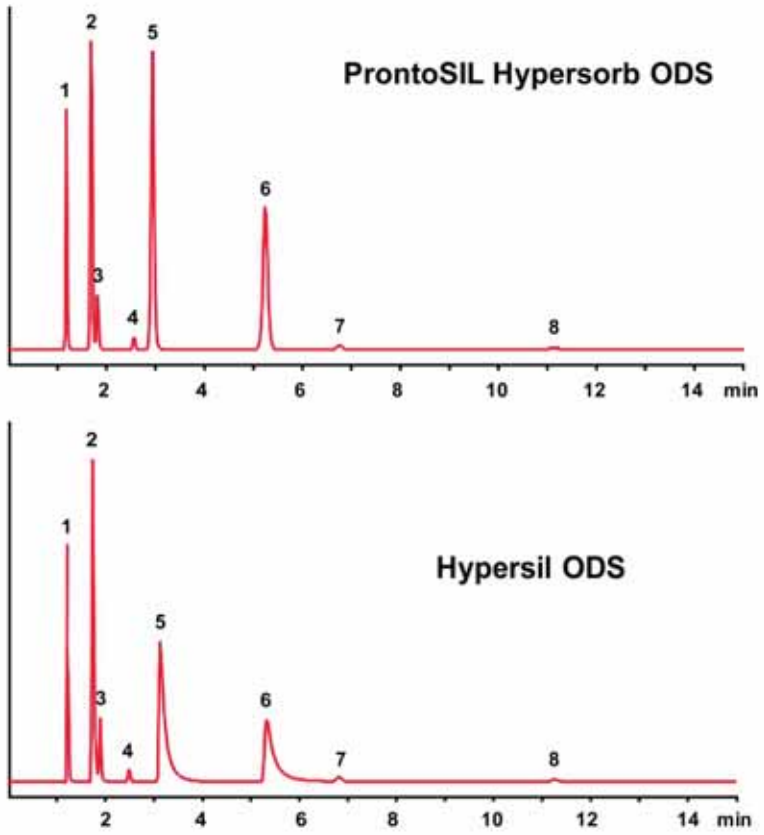
Now Replace Waters µBondapack with ProntoBond

- No revalidation of existing method required.
- Better Packing Technology. Guaranteed Batch to Batch reproducibility
- Each column is tested separately for high quality.
- Also available in any dimensions.

ProntoSIL Hypersorb ODS

Your Alternative for Hypersil ODS

- No revalidation required
- Available in almost any column dimension
- High end packing quality



Engelhardt Test

Comparison of ProntoSIL Hypersorb ODS and Hypersil ODS

ProntoSIL Hypersorb ODS 5 µm

Part number:	1546F180PY050
Dimension:	150 x 4.6 mm
Eluent:	H_2O/MeOH 51/49 (w/w)
Flow:	1 ml/min
Detection:	UV 254 nm
Temperature:	40 °C
Injection:	5 µl

Sample:

1. Uracil
2. Aniline
3. Phenol
4. Impurity
5. p-Ethylaniline
6. N,N-Dimethylaniline
7. Toluene
8. Ethylbenzene

Same Selectivity
Better Peakshape

Bischoff Chromatography now offers alternatives for Hypersil ODS.

The new clone packings offer the following benefits:

No revalidation of your existing method is required

The new packings offer the same selectivity under the same chromatographic conditions like the original columns. You can change without any problem.

Available in almost any column dimension

If your chromatography asks for a column outside the typical column dimensions 250 x 4.6 mm we have it. Like all Bischoff columns the new packings are also available in almost any column dimension

High end column packing

Our experience in the packing of the Waters-Spherisorb and Hypersil supports over years in a very high quality and our superior HYPERCHROME column hardware leads to the high end column packing for those supports.

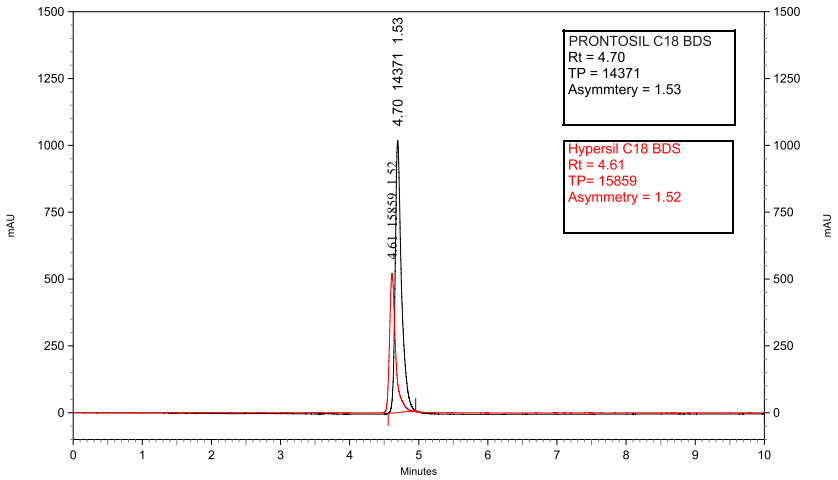
ProntoSIL C18 BDS

Corresponding to USP "L" listing ProntoSIL C18 BDS phase falls under L1 category.

"L1" - Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10µm in diameter.

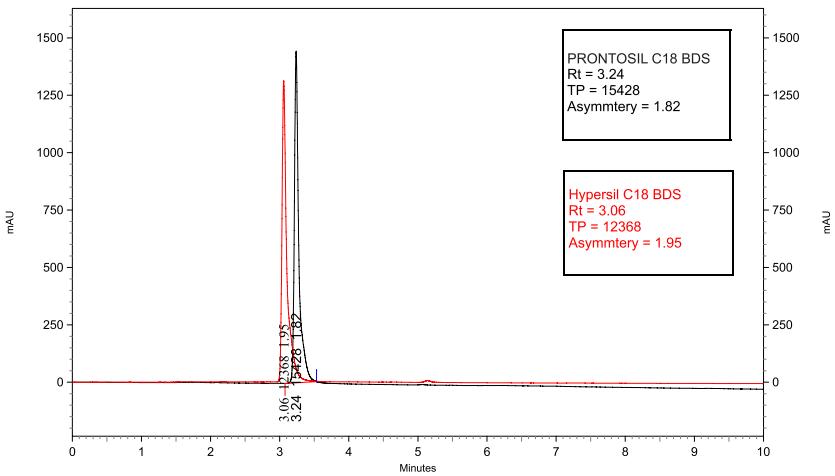
PRONTOSIL C18 BDS is the stationary phase in the PRONTOSIL line which is similar to Hypersil C18 BDS & HyperClone BDS. It is fully end-capped. Following table compares the specifications for two phases:

Specifications	PRONTOSIL C18 BDS	Hypersil C18 BDS
L Category	L1	L1
Carbon Load %	11	11
End-capping	Yes	Yes
Temperature ^{0}C	60	60
Pore Size	130Å	130Å
pH	2-8	2-8
Surface Area	170 m2/gm	170 m2/gm



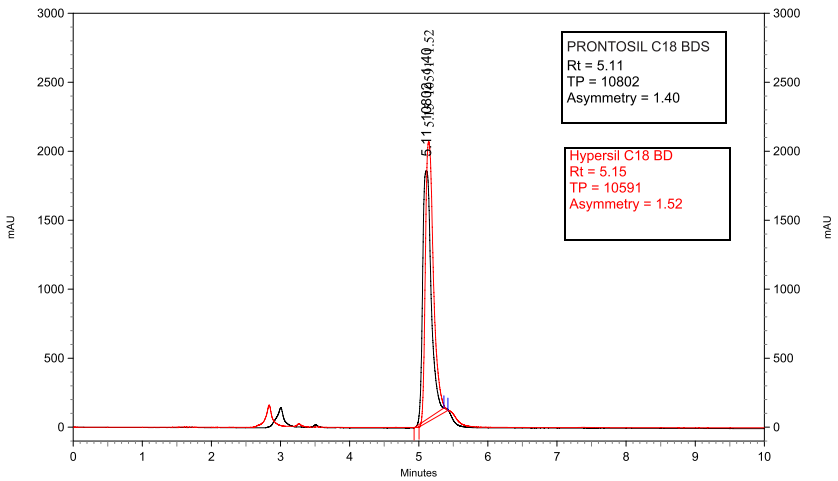
Basic Component Dimethyl aniline on Hypersil C18 BDS and PRONTOSIL C18 BDS

- **No revalidation of your existing method**



Acidic Component Phenol on Hypersil C18 BDS and PRONTOSIL C18 BDS

- **Better resolution & peak shape**



Neutral Component Toluene on Hypersil C18 BDS and PRONTOSIL C18 BDS

- **Each column tested individually**

NOW Replace Kromasil by Kromaplus

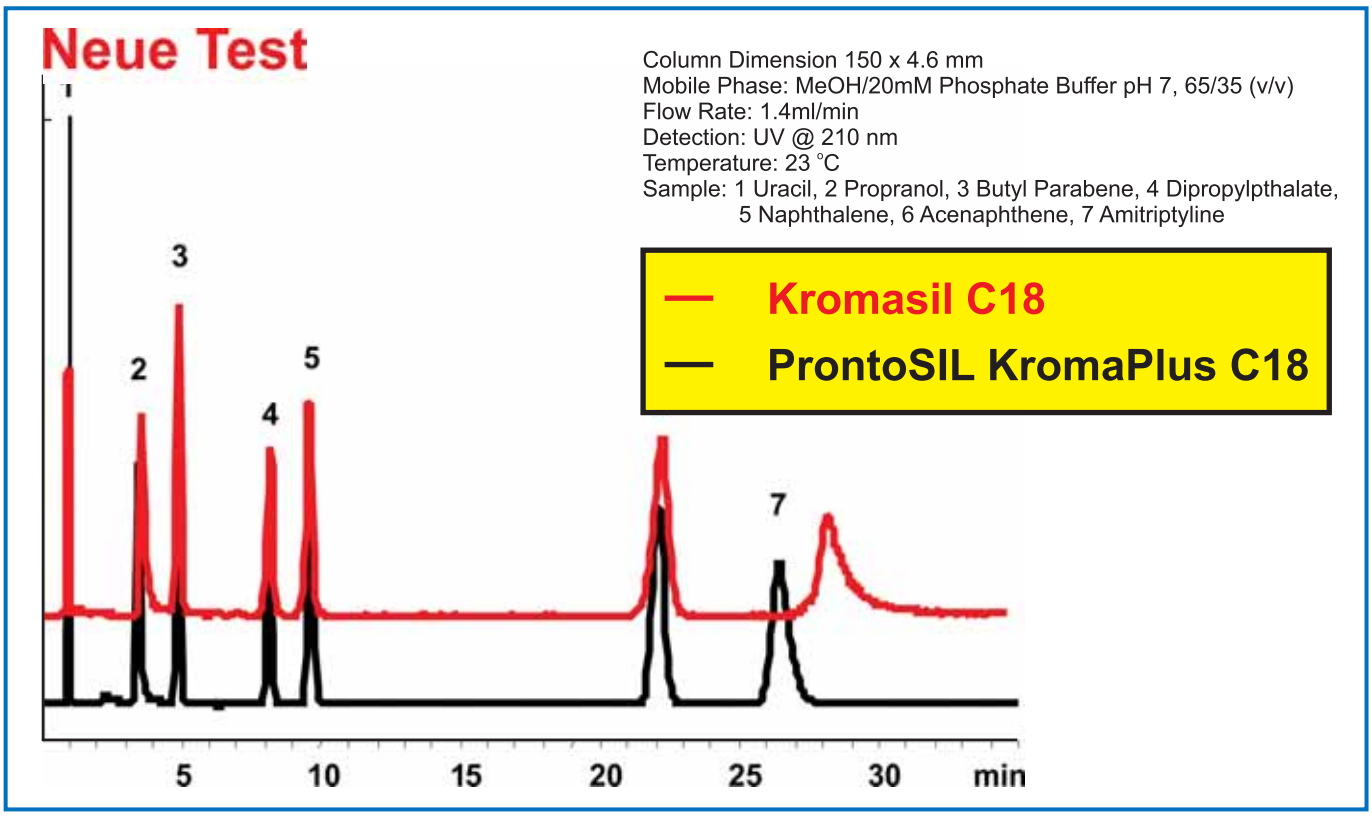
ProntoSIL KromaPlus C18

ProntoSIL KromaPlus C18 is based on the well known high performance spherical silica ProntoSIL for analytical and prep scale liquid chromatography. Our long experience and knowledge about silica gels for HPLC in combination with our outstanding bonding technology led to ProntoSIL KromaPlus C18. It is ultra pure and gives high reproducibility and chemical stability by using monofunctional silanes and full end-capping. ProntoSIL KromaPlus C18 is stable from pH 1 to 10. Without changing the existing method you can replace KromaSIL C18 by ProntoSIL KromaPlus C18. To guarantee the batch reproducibility each Batch has to undergo specific tests.

Parameter	Spec.	Parameter	Spec.
Mean Pore Diameter (Å)	110 +/- 10	Specific Surface Area (m^2/g)	300 +/- 30
Pore Volume (ml/g)	0.8 +/- 0.1		
Mean Particle Size (µm)	5.5 +/- 0.5	Particle Size Distribution (D10/D90)	< 1.6
Carbon Content (%)	20		

- Better peak shape
- Much Attractive price for all sizes.
- Available in any dimensions.
- Also Available in 3.5 microns

Also available in Kromaplus C8



Also

KromaPlus Bulk material now @ Economical Price.

BISCHOFF

MERCK Material packed by Bischoff Chromatography.

High performance HPLC columns are packed using the ORIGINAL MERCK packing along with unique HYPERCHROME column hardware and packed with our own environment friendly packing process. Every single column undergoes a quality control test to check its chromatographic performance. This test report is provided with each column so you can be sure that the column meets highest quality criteria.

Lichrospher and Lichrospher Select B Columns

USP Listing	Description	Code	Particle Size	Pore Size	End capping
L1	Lichrospher 100 RP 18e	...E181LS050	5 µm	100 Å	fully
L1	Lichrospher 100 RP 18e	...E181LS100	10 µm	100 Å	fully
L1	Lichrospher 100 RP 18	...E180LS050	5 µm	100 Å	-
L1	Lichrospher 100 RP 18	...E180LS100	10 µm	100 Å	-
L7	Lichrospher 100 RP 8e	...E081LS050	5 µm	100 Å	fully
L7	Lichrospher 100 RP 8e	...E081LS100	10 µm	100Å	fully
L7	Lichrospher 100 RP 8	...E080LS050	5 µm	100 Å	-
L7	Lichrospher 100 RP 8	...E080LS100	10 µm	100 Å	-
L10	Lichrospher 100 CN	...E200LS050	5 µm	100 Å	-
L10	Lichrospher 100 CN	...E200LS100	10 µm	100 Å	-
L8	Lichrospher 100 NH2	...E190LS050	5 µm	100 Å	-
L8	Lichrospher 100 NH2	...E190LS100	10 µm	100 Å	-
L20	Lichrospher 100 Diol	...E410LS050	5 µm	100 Å	-
L20	Lichrospher 100 Diol	...E410LS100	10 µm	100 Å	-
L3	Lichrospher 60 Si	...C000LS050	5 µm	100 Å	-
L3	Lichrospher 60 Si	...C000LS100	10 µm	100 Å	-
L3	Lichrospher 100 Si	...E000LS100	10 µm	100 Å	-
L7	Lichrospher 60 RP Select B	...C081LS050	5 µm	60 Å	-
L7	Lichrospher 60 RP Select B	...C081LS100	10 µm	60 Å	-

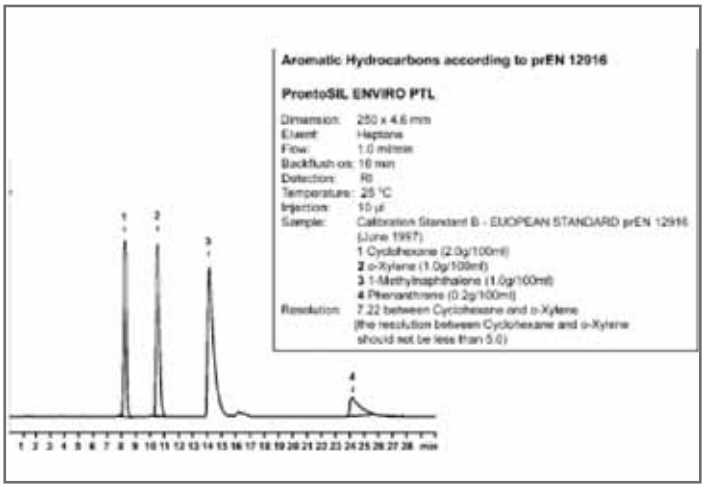
Lichrosorb and Lichrosorb Select B Columns

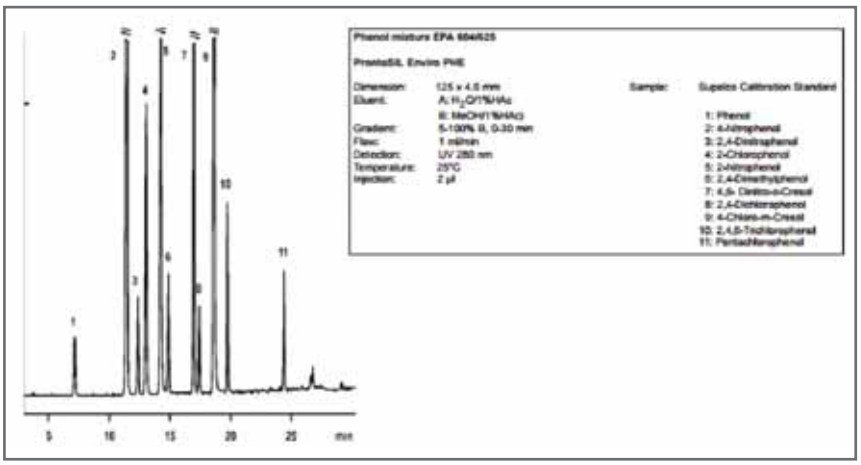
USP Listing	Description	Code	Particle Size	Pore Size	End capping
L1	Lichrosorb RP 18	...E680LB050	5 µm	100 A	-
L1	Lichrosorb RP 18	...E680LB100	10 µm	100 A	-
L7	Lichrosorb RP 8	...E580LB050	5 µm	100 A	-
L7	Lichrosorb RP 8	...E580LB070	7 µm	100 A	-
L7	Lichrosorb RP 8	...E580LB100	10 µm	100 A	-
L16	Lichrosorb RP 2	...E510LB070	7 µm	100 A	-
L10	Lichrosorb CN	...E700LB050	5 µm	100 A	-
L8	Lichrosorb NH2	...E690LB050	5 µm	100 A	-
L8	Lichrosorb NH2	...E690LB070	7 µm	100 A	-
L8	Lichrosorb NH2	...E690LB100	10 µm	100 A	-
L20	Lichrosorb Diol	...E910LB050	5 µm	100 A	-
L20	Lichrosorb Diol	...E910LB100	7 µm	100 A	-
L3	Lichrosorb 60 Si	...C500LB050	5 µm	60 A	-
L3	Lichrosorb 60 Si	...C500LB070	7 µm	60 A	-
L3	Lichrosorb 60 Si	...C500LB100	10 µm	60 A	-
L3	Lichrosorb 100 Si	...E000LS050	5 µm	100 A	-
L3	Lichrosorb 100 Si	...E000LB100	10 µm	100 A	-
L7	Lichrosorb RP Select B	...E581LB100	10 µm	100 A	-

ProntoSIL Enviro Special Columns

ProntoSIL Enviro PTL

The Enviro PTL phase is a special stationary phase for the group determination of non-aromatic, monoaromatic, diaromatic and polyaromatic hydrocarbons in the petroleum industry. This stationary phase was tailored exactly to the requirements of European standard prEN 12916 (June 1997). In this method, a minimum resolution between cyclohexane (non-aromatic hydrocarbon) and o-xylene (monoaromatic hydrocarbon) of 7 is required. Therefore, this phase is offered only in a column length of 250 mm. Before delivery, the columns are tested with the standard of the European standard and this test certificate is attached to the columns.



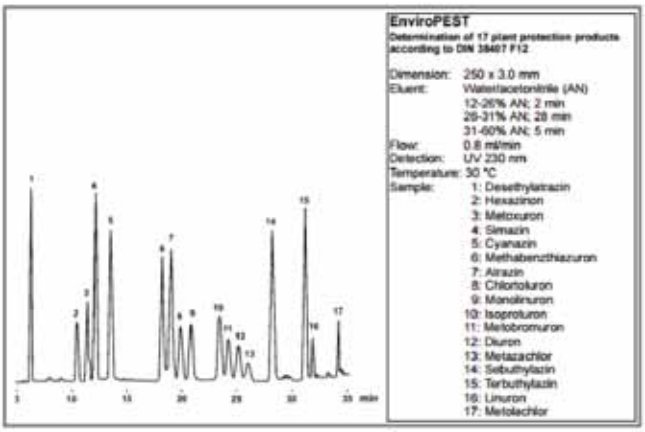


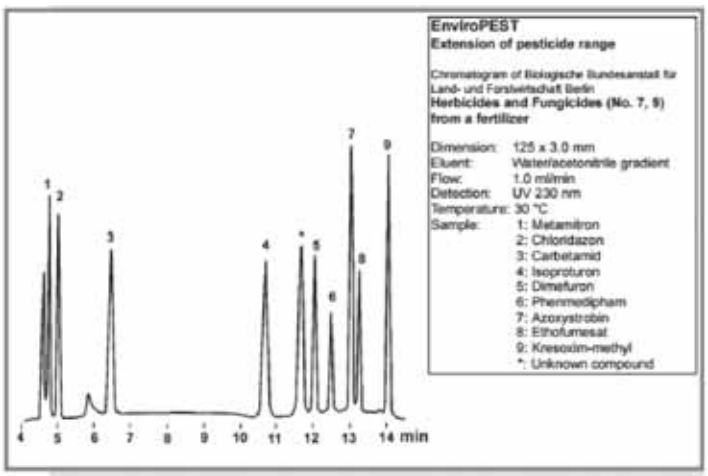
ProntoSIL Enviro-PHE

The Enviro PHE phase is a special stationary phase for the determination of phenols in environmental analysis. This stationary phase was tailored exactly to the requirements of EPA Method 604/625. In this method 11 phenols must be baseline separated and determined. Prior to shipment, the columns are tested with a standard EPA method and this test certificate is attached to the columns.

ProntoSIL Enviro-PES

The Enviro-PES phase is a special stationary phase for the determination of pesticides in environmental analysis. This stationary phase was tailored exactly to the requirements of DIN regulation 38407 F12. In this method, 17 pesticides must be determined. With this column, this complex mixture can be separated in a cycle time of 45 minutes. Prior to shipment, the columns are tested with the appropriate pesticide standard and this test certificate is attached to the columns.





ProntoSIL Enviro-PAH

The Enviro PAH phase is a special stationary phase for the determination of polyaromatic hydrocarbons (PAHs) in environmental analysis. This stationary phase was tailored exactly to the requirements of EPA Method 610. In this method 16 polyaromatic hydrocarbons must be baseline separated and determined. Prior to shipment, the columns are tested with a standard EPA method and this test certificate is attached to the columns.

Phases

ProntoSIL C18 H : Classic C18

ProntoSIL C18H is the standard C18 support of the alliance for chromatography. ProntoSIL C18 H is applicable in a wide range of RP- chromatography. The packing is fully endcapped and possesses all of the excellent properties a new generation stationary phase can offer. Keeping in line with all ProntoSIL products, this support is based on an ultra pure silica. The wide pore supports show excellent properties for the separation of biomolecules such as proteins and peptides.

ProntoSIL C18 SH : Enhanced methylene group selectivity

ProntoSIL C18 SH is applicable in a wide range of RP- chromatography. The packing is fully end-capped. Due to carbon load it shows an excellent shape selectivity and stability even at pH 2. Longer end-capped chains produce packing that are more retentive. In addition, longer chain lengths permit the use of larger samples.

ProntoSIL C18 AQ : Unique bonding technology

ProntoSIL C18 AQ with its unique bonding technology has been especially developed for the use in aqueous mobile phases with an organic content below 10%. Standard stationary phases with conventional bonding give very poor peak shapes under these chromatographic conditions due to the collapse of C18 brushes in aqueous eluents. ProntoSIL C18 AQ gives excellent peak shapes in these mobile phases resulting in enhanced selectivities. The advantages of the AQ packings can be demonstrated in applications of polar analytes. It is a special reversed phase material for separating a broad spectrum of hydrophilic analytes that show no retention on other reversed phase materials. Strongly polar samples soluble only in water can be separated using ProntoSIL C18 AQ. The eluent can even be water with no added organic solvent. ProntoSIL C18 AQ can also be used to separate hydrophobic compounds like other C18 or ODS phases. In ProntoSIL C18 AQ the primary separation mechanism is hydrophobic interaction.

ProntoSIL C18 AQ Plus : Excellent peak in pure aqueous eluents

ProntoSIL AQ Plus can also be used in aqueous mobile phase with an organic content below 10%. In comparison to ProntoSIL C18 AQ, ProntoSIL C18 AQ Plus shows an enhanced stability at low pH's down to pH 2. Also the packing shows excellent peak shapes in pure aqueous eluents but differs in shape selectivity compared to ProntoSIL C18 AQ. The application field for this support is mainly in combinatorial chemistry where the standard separation conditions are fast gradients from 0-100% organic and where the mobile phases include 0.1% TFA.

ProntoSIL C18 Eurobond : Classical bonding technology

Optimized synthesis procedure for this classical bonded C18 phase leads to this best high quality product ProntoSIL Eurobond C18. The selectivity of the ProntoSIL Eurobond C18 phase is in between the selectivity offered by the two stationary phases. ProntoSIL C18 H Phases and ProntoSIL C18 SH. The ProntoSIL Eurobond C18 is fully end-capped and can be used in the broad range of RP- application.

ProntoSIL Kromaplus C18 : Widely used in pharmaceutical industries

ProntoSIL Kromaplus C18 is based on the well known high performance spherical silica for analytical and preparative liquid chromatography. It is ultra pure and gives high reproducibility and chemical stability by using monofunctional silanes and full end-capping. ProntoSIL Kromaplus C18 is stable from pH 2 to 9.

ProntoSIL C18 ace-EPS : C18 with embedded amide group

The ProntoSIL C18 ace-EPS belongs to the new group of stationary RP-supports with embedded groups. The packing is very stable over a wide pH range (pH 2-9). In addition, it offers a maximum of hydrophobicity combined with a maximum of polar selectivity. The silanophilic activity of the support is very low. Ultra strong basic compounds such as amitriptyline can be eluted from the column at neutral pH values with excellent symmetrical peak shapes.

The main application area of these packings is the pharmaceutical industry, where analytes often have basic or acidic groups. For the separation of these compounds these supports exhibit an enhanced polar selectivity. In comparison to a classical bonded C18 column acidic compounds show a higher retention where as basic compounds show a slight decrease of retention on an embedded polar column. The C18 ace-EPS bonding type is available in several particle in several particle and pore sizes.

ProntoSIL C8 ace-EPS : Enhanced polar selctivity

The ProntoSIL C8 ace-EPS belongs to the new group of stationary RP support with polar embeded groups. The packing is stable at pH range 2-9. In comparison to the corresponding C18 packing the ProntoSIL C8 ace-EPS shows higher polar selectivity. Due to the shorter alkyl chain the influence of the polar group in contribution to the retention mechanism of the stationary phase is increased. The silanophilic activity of the support is very low. Ultra strong basic compounds with pka values higher than 9 (like amitriptyline) can be eluted from the column in neutral pH values with excellent symmetrical peak shapes. The main application area of these packings is in the pharmaceutical industry where the analytes often have basic or acidic groups. For the separation of these compounds, these supports are showing an enhanced polar selectivity.

ProntoSIL C8 SH : Excellent shape selectivity

ProntoSIL C8 SH is a classical C8-type stationary phase. It is fully end-capped. Due to the bonding technology it shows an excellent shape selectivity and stability even at pH 2. The 300 Å packing show excellent properties for the separation of large bio molecules like proteins and peptides.

ProntoSIL C30 : High Carbon load

ProntoSIL C30 is a stationary phase with a high carbon load. The high coverage of the support results in a very dense packing and in an excellent shape selectivity and stability even at pH 2. The C30 bonding type is available with several pore sizes and in several particle sizes. Especially the wide pore supports are showing an enhanced shape selectivity. The application field of the C30 packing is the separation of isomers of carotenoids and other long alkyl chain solutes, like that can not be separated on classical C18 columns. Longer chains produce packings that are more retentive. Longer chain lengths permit the use of larger samples.

ProntoSIL C1 : Lowest Retention

The C1 packing shows the lowest retention of the complete product line. The application area is mainly the separation of non polar solutes. It can also be used for the separation of proteins in the HIC (Hydrophobic interaction chromatography). Due to the bonding technology the C1 bonding type is stable down to pH 2.

ProntoSIL C4 : Enhanced stability even at pH 2

Due to the bonding technology it shows an enhanced stability even at pH 2. The C4 packings show excellent properties for the separation of large bio molecules like proteins and peptides not only in the RP-mode but also in the HIC- mode (Hydrophobic Interaction Chromatography).

ProntoSIL Si : Purity of 99.999%

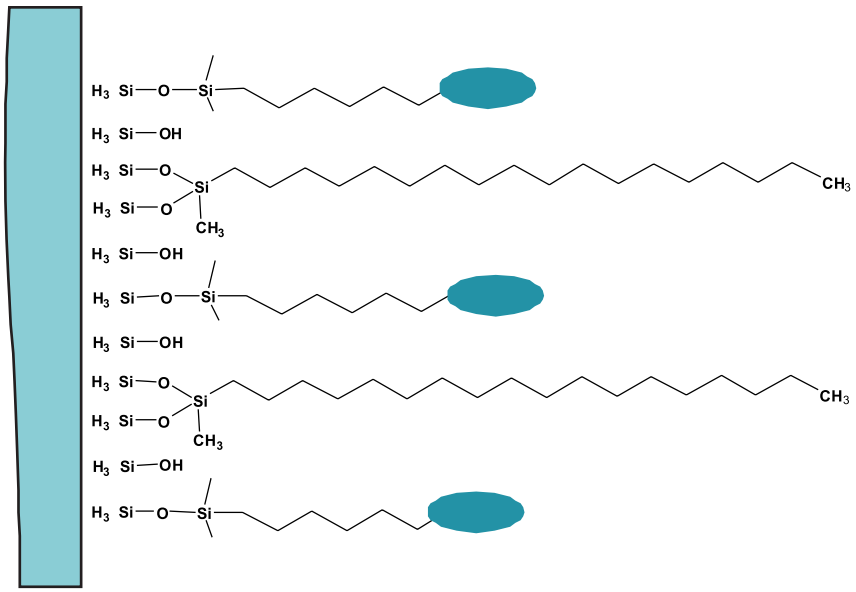
The ProntoSIL Si is the silica support of the Alliance for chromatography. Due to the fact that it is manufactured under very stringent conditions the resulting silica has a purity of 99.999%. The optimum manufacturing process guarantees an excellent batch to batch reproducibility. It has wide range of different applications i.e, In SEC (size exclusion chromatography) but also for the NP HPLC of large molecules is given.

ProntoSIL Amino : Amino Propyl bonded phase

ProntoSIL Amino is an Amino Propyl bonded phase. It can be used in NP mode as alternative to silica but offers different selectivity. In RP-mode it is mainly used for carbohydrate analysis. In IC mode the bonded phase can be used as a weak anion exchanger (WAX) for the analysis of anions and organic acids.

The analyte which do not differ enough in terms of polarity and spatial structure, other interactions must be encouraged in order to achieve the desired selectivity. The use of stationary phases with attached amino propyl polar group, stimulate the hydrophobic interaction as well as π-π interaction. These modifications achieve completely different selectivities for many separations.

Polar Group: Amino, Cyano, Diol



ProntoSIL CN : Cyano Propyl bonded phase

ProntoSIL is a cyano-propyl bonded phase. It can be used in normal phase mode and reversed phase mode. In RP- mode, the application area is the separation of strong basic solutes. In NP mode it offers a complementary selectivity to the other NP phases Silica, Amino and Diol. Due to quick equilibration time of the CN bonded phase it is the best choice for gradient elution in NP mode.

ProntoSIL Phenyl : Different selectivities than C8 & C18

ProntoSIL Phenyl is a RP packing that offers different selectivities in comparison to brush type stationary phases like C8 or C18. It is fully end-capped. Due to the bonding technology it shows excellent stability even at pH 2. The packing shows an enhanced selectivity and hydrophobicity.

ProntoSIL Diol : Shorter equilibriation time

ProntoSIL OH is a diol bonded phase. The Diol packing is an alternative to the silica packings. The equilibration times of the support is shorter. In comparison to the corresponding silica support. The selectivities are comparable. Due to the lower activity of these packings they can also be used for SEC-applications.

Selection by Application

The selection of the right column for your separation problem depends on the properties for your sample. The following properties of an analyte are important for selecting a column, size, functional groups, polarity, spatial structure and matrix.

No Special Properties

The analyte is either too large or too small (MW between 100 and 5000g/mol). It possesses neither extremely acidic or extremely basic functional groups. The analytes do not have any significant steric structure and no isomers (e.g. cis / trans isomers) are present. In such a case, a conventional C18 column can be normally used, If possible, a modern C18 phase manufactured from highly pure silica gel should be used. These have, in comparison to older materials, the advantage of better batch to batch reproducibility. In addition, newer materials also have a better particle size distribution, allowing for higher plate numbers to be achieved at lower back pressure.

Polar Compounds

In order to retard very polar analytes in reversed phase chromatography, very polar mobile phases are required. Generally it is necessary to work with pure aqueous eluents or even to add ion pair reagents to the mobile phase. Conventional reversed phases cannot be used with pure aqueous eluents. Since the density of the C18 “brushes” on the surface of the silica gel is too high in these phases, the brushes will collapse as soon as the organic portion of the mobile phase is below 5%. In chromatograms, the retention of these analytes fall apart or very broad and asymmetric peaks will be eluted. For aqueous media special stationary phases have been developed. ProntoSIL C18 AQ phase exhibit the same selectivity as conventional C18 phases. ProntoSIL C18 AQ Plus phase possesses a polar hydrophilic end-capping. The polar groups on the surface attract the polar water and therefore prevent the collapsing of the alkyl chains. ProntoSIL C18 ace-EPS phases are 100% wettable by aqueous eluents. A polar group is attached to the alkyl chains of this phase and therefore produces an analogous behavior as ProntoSIL AQ Plus. In comparison to ProntoSIL AQ, the two other possess a polar selectivity imparted by the introduction of a polar functionality, leading to a completely different separation behavior in comparison to conventional C18 phases.

Non-Polar Compounds

Non-polar compounds display a very high retention on conventional reversed phases and can only be eluted from the column with extremely strong eluents. The solution here is to use short chain reversed phases (C8, C4,C1) which produce substantially weaker interactions with the analytes. Particularly suited to the separation of these compounds are materials with large pores (200Å and 300Å) and reversed phases from non-porous silica gel. Since they have less specific surface available they show weaker interactions with the analytes. An additional possibility is to switch to normal phase mode. Non-polar compounds are often very easy to separate by normal phase chromatography since even the slightest change in the water content of the eluents result in an alteration of the retention times. Water is the strongest eluents result in an alteration of the retention times. Water is the strongest eluent in normal phase chromatography. Water is also present in all non-polar solvents in trace amounts, making retention time shifts nearly unavoidable.

High Molecular Weight

Analytes with high molecular weights have considerably lower diffusion coefficients compared to smaller molecules. Such an analytes path in and out of the pores is clearly slower in comparison to smaller molecules. This results in broad and asymmetric peaks if the normal pore diameter of 100Å is used. The use of conventional materials with very large molecular weights can even lead to size exclusion. In such cases, a streak of the compound over the complete separation column is observed in the chromatogram. For

this reason, such compounds are separated on “wide-pore” materials. The typical pore sizes of these supports are about 200Å and 300Å. All bonding which are available in smaller pore sizes, are also available in “wide-pore”. As an alternative, non-porous materials can also be used. The interaction between the analytes through the pores becomes thereby completely invalidated. Separation columns which have been filled with nonporous support materials offer the most efficient separations for macromolecules.

Isomers and anlytes which differ from each other primarily through their spatial structure

Isomers and analytes which differ from each other primarily through their spatial structure are very difficult to separate. Their hydrophobicity is very similar. Consequently, such separations are regularly carried out by normal phase chromatography on silica phases. The basic problem in normal phase chromatography however is that retention times are shifting. Highly loaded C18 and C30 phases are an alternative and offer an elegant solution to work in reversed phase mode. The distance between the individual alkyl chains on these phases is so minimal, that the separation takes place through the shape selectivity of the phases.

No separation of the analytes on conventional columns

If separation of the analytes is not possible on traditional phases, which is due to the fact that the analytes do not differ enough in terms of polarity and spatial structure, other interactions must be encouraged in order to achieve the desired selectivity. The use of stationary phases with attached polar groups, such as phenyl, cyanopropyl, or aminopropyl groups stimulate the hydrophobic interaction as well as π-π interaction. These modifications achieve completely different selectivities for many separations. Such kind of stationary phases should only be considered as alternatives than as primary choice.

Application	Column suggestion
Ascorbic acid	ProntoSIL Eurobond C18
Amitriptyline	ProntoSIL CN
Aniline	ProntoSIL Eurobond C18
Acetic acid	ProntoSIL C18 AQ
Angiotensin I	ProntoSIL C18 H
Angiotensin II	ProntoSIL C18 H
5-Aminolevulinic acid	ProntoSIL C18 H
n-plus Adhumulon	ProntoSIL C18 SH
Acetaldehyde	ProntoSIL C18 H
Acetone	ProntoSIL C18 H
Acrolein	ProntoSIL C18 H
Acetylsalicylic acid	ProntoSIL C18 AQ
Adrenalin	ProntoSIL C18 AQ
Arenalin	ProntoSIL C18 AQ
Benzoic acid	ProntoSIL C18 AQ
Bradykinin	ProntoSIL C18 H
BSA	ProntoSIL C18 H
Butylparaben	ProntoSIL Phenyl
2-Butanal	ProntoSIL C18 H
Benzaldehyde	ProntoSIL C18 H
Bicalutamide	ProntoSIL C18 AQ
Coffeine	ProntoSIL Eurobond C18
Chlorophenamine	ProntoSIL Eurobond C18
Camphor	ProntoSIL Eurobond C18
Citric acid	ProntoSIL C18 AQ
Cyanocobalmine	ProntoSIL C18 AQ
Cresol	ProntoSIL C18 H

Application	Column suggestion
Cholecalciferol	ProntoSIL C18 H
Cholesterol	ProntoSIL C18 H
ß-Carotine	ProntoSIL C30
Cytochrome C	ProntoSIL C4
Chymotrypsinogen	ProntoSIL C4
Cohumulon	ProntoSIL C18 SH
Colupulon	ProntoSIL C18 SH
n-plus Colupulon	ProntoSIL C18 SH
α-Carotine	ProntoSIL C18 SH
Crotonic aldehyde	ProntoSIL C18 H
Cisplatin	ProntoSIL C8 SH
Carvedilol	ProntoSIL C8 SH
Doxepin	ProntoSIL CN
Desipramine	ProntoSIL CN
Dihydroxybenzylamine	ProntoSIL C18 AQ
Dopamine	ProntoSIL C18 AQ
Doxepin	ProntoSIL C18 AQ
2,3-Dihydroxybenzoic acid	ProntoSIL CN
2,5-Dihydroxybenzoic acid	ProntoSIL CN
Deoxyhumulon	ProntoSIL C18 SH
DNPH	ProntoSIL C18 H
Decanone	ProntoSIL C18 AQ
2-Dodecanone	ProntoSIL C18 AQ
2,4-Dinitrophenylhydrazine	ProntoSIL C18 H
2,4-Dimethylbenzaldehyde	ProntoSIL C18 H
2,4-Dimethylpentanone	ProntoSIL C18 H
3,3-Dimethylbutanone	ProntoSIL C18 H
DHBA	ProntoSIL C18 AQ

Application	Column suggestion
DOPAK	ProntoSIL C18 AQ
Domperidone	ProntoSIL C18 H
Donepezil HCl	ProntoSIL C18 SH
Ethylbenzene	ProntoSIL Eurobond C18
Ethyleneglycolmonosalicylate	ProntoSIL Eurobond C18
Epinephrine	ProntoSIL C18 AQ
Eledoicin	ProntoSIL C18 H
Ergocalciferol	ProntoSIL C18 HErgosterol
ProntoSIL C18 H	
Ethylparaben	ProntoSIL Phenyl
Ethanol	ProntoSIL C18 AQ
Estrone	ProntoSIL C18 H
Fumaric acid	ProntoSIL C18 AQ
Formic acid	ProntoSIL C18 AQ
Folic acid	ProntoSIL C18 AQ
Fructose	ProntoSIL Amino
Fibrinogen (Human)	ProntoSIL C4
Formaldehyde	ProntoSIL C18 AQ
fluconazole	ProntoSIL C18 H
Glutamic acid	ProntoSIL C18 AQ
Guifenesin, phenazopyridine HCl	ProntoSIL C18 H
Hydrocortisone	ProntoSIL C18 H
2-Hydroxybenzoic acid	ProntoSIL C18 AQ
3-Hydroxybenzoic acid	ProntoSIL C18 AQ
4-Hydroxybenzoic acid	ProntoSIL C18 AQ
2-Hexanone	ProntoSIL C18 AQ
2-Heptanone	ProntoSIL C18 AQ
2-Hexadecanone	ProntoSIL C18 AQ
Hexanal	ProntoSIL C18 H
Heptanal	ProntoSIL C18 H
HIAA	ProntoSIL C18 AQ
HVA	ProntoSIL C18 AQ
Imipramine	ProntoSIL CN
Insuline(bovine)	ProntoSIL C18 H
Insuline (human)	ProntoSIL C18 H
Insuline chain A, oxidized	ProntoSIL C18 H
Insuline chain B, oxidized	ProntoSIL C18 H
Iodate	ProntoSIL C8 SH
Isopropanol	ProntoSIL C18 AQ
Lysozyme	ProntoSIL C18 H
Lactose	ProntoSIL Amino
Lactic acid	ProntoSIL C8 SH
ß-Lactoglobuline	ProntoSIL C18 AQ
Levothyroxin	ProntoSIL CN
Levamisole HCL	ProntoSIL C8SH
Malic acid	ProntoSIL C18 AQ
Maleic acid	ProntoSIL C18 AQ
Myoglobin	ProntoSIL C18 H
Methylparaben	ProntoSIL Phenyl
Mannose	ProntoSIL Amino
Maltose	ProntoSIL Amino
Melatonin	ProntoSIL C18 H
Molenoic acid	ProntoSIL C18 AQ

Application	Column suggestion
2-Methylbenzaldehyde	ProntoSIL C18 H
4-Methylbenzaldehyde	ProntoSIL C18 H
Methacrylic Acid Copolymer	ProntoSIL C18 AQ
Mebendazole	ProntoSIL Kromaplus C8
Methocarbamol	ProntoSIL C8 SH
Nortriptyline	ProntoSIL CN
N,N-Dimethylamine	ProntoSIL Eurobond C18
Nicotic acid with methyl ester	ProntoSIL Eurobond C18
Nicotic acid with benzyl ester	ProntoSIL Eurobond C18
Norepinephrine	ProntoSIL C18 AQ
Nicotinic acid	ProntoSIL C18 AQ
Nocotynamide	ProntoSIL C18 AQ
Neurotensin	ProntoSIL C18 H
2-Nonanone	ProntoSIL C18 AQ
Nonanal	ProntoSIL C18 H
Noradrenalin	ProntoSIL C18 AQ
4-Nitrophenylchloroformate	ProntoSIL CN EC
Oxalic acid	ProntoSIL C18 AQ
Oxytocin	ProntoSIL C18 H
Ovalbumin	ProntoSIL C18 H
2-Octanone	ProntoSIL C18 AQ
Octanal	ProntoSIL C18 H
Oxycloxyclozanife BP VET	ProntoSIL C8SH
Paracetamol	ProntoSIL Eurobond C18
Protriptyline	ProntoSIL CN
Phenol	ProntoSIL Eurobond C18
p-Ethylaniline	ProntoSIL Eurobond C18
Pyridine-4-carboxylic acid	ProntoSIL C18 AQ
Pyridine-3-carboxylic acid	ProntoSIL C18 AQ
Pyridine-2,6-dicarboxylic acid	ProntoSIL C18 AQ
Pyridoximine	ProntoSIL C18 AQ
Pyridoxal	ProntoSIL C18 AQ
Pyridoxine	ProntoSIL C18 AQ
Protamine Sulfate	ProntoSIL C18 H
Protamine insuline	ProntoSIL C18 H
Progesterone	ProntoSIL C18 H
Propylparaben	ProntoSIL Phenyl
Perbenzoic acid t-butyl ester	ProntoSIL C18 H
n-Propanol	ProntoSIL C18 AQ
Propanal	ProntoSIL C18 H
Pentanal	ProntoSIL C18 H
2-Pentanone	ProntoSIL C18 AQ
Propylphenanzone	ProntoSIL C18 AQ
Propylthiouracil	ProntoSIl Kromaplus C18
Pantoprazole	ProntoSILC18 H
Perindopril Erbumin	ProntoSIL Kromaplus C8
Progesterone	ProntoSILC18 H
Quinic acid	ProntoSIL C18 AQ
Q9-OH	ProntoSIL C18 SH
Q10-OH	ProntoSIL C18 SH
Q9	ProntoSIL C18 SH
Q10	ProntoSIL C18 SH
Riboflavin	ProntoSIL C18 AQ
Ribonuclease A	ProntoSIL C18 H
Saccharose	ProntoSIL Amino

Application	Column suggestion
Salicylic acid	ProntoSIL CN
Succinic acid	ProntoSIL C18 AQ
Sulfanilamide	ProntoSIL C18 ace-EPS
Sulfadiazine	ProntoSIL C18 ace-EPS
Sulfathiazol	ProntoSIL C18 ace-EPS
Sulfamerazine	ProntoSIL C18 ace-EPS
Sulfamethazine	ProntoSIL C18 ace-EPS
Sildenafil Citrate	ProntoSil C18 H
Seratonine	ProntoSIL C18 AQ
Trimipramine	ProntoSIL CN
Tartaric acid	ProntoSIL C18 AQ
Thiamine	ProntoSIL C18 AQ
α-Tocopherol	ProntoSIL C18 H
ß-Tocopherol	ProntoSIL C30
γ-Tocopherol	ProntoSIL C30
σ-Tocopherol	ProntoSIL C30
Theobromine	ProntoSIL C18 AQ
Theophyline	ProntoSIL C18 AQ
Thyroglobulin	ProntoSIL C4

Application	Column suggestion
2-Tridecanone	ProntoSIL C18 AQ
Toluene	ProntoSIL Eurobond C18
Tetramethylketone	ProntoSIL C18 AQ
2-Undedeconane	ProntoSIL C18 AQ
Vitamin A	ProntoSIL C18 SH
Vitamin A acetate	ProntoSIL C18 SH
Vitamin D 2	ProntoSIL C18 SH
Vitamin D 3	ProntoSIL C18 SH
Vitamin E	ProntoSIL C18 SH
Vitamin K1	ProntoSIL C18 SH
Venalafaxine	ProntoSIL C18SH
Venlafaxine HCl	ProntoSIL C18H
Vitamin B 1	ProntoSIL C18 AQ
Vitamin B 2	ProntoSIL C18 AQ
Vitamin B 3	ProntoSIL C18 AQ
Vitamin B 6	ProntoSIL C18 AQ
Xylose	ProntoSIL Amino

Phase Selection Guide

Name	Normal / Reverse Phase	Application
ProntoSIL Silica	NP	Non-polar, & moderately polar non-ionic organic compounds.
ProntoSIL C1	RP	Least retentive of all alkyl group bonded phases for non-polar solutes. Typically used for moderately polar & multi-functional compounds.
ProntoSIL C4	RP	Separation of peptides and proteins. Shorter retention than C8, C18
ProntoSIL C8 (Octyl silane)	RP	Moderately to highly polar compounds, small peptides and proteins, polar pharmaceuticals, steroids, environmental samples.
ProntoSIL C18 (Octadecyl silane)	RP	Most retentive of alkyl-bonded phases for non-polar to moderately polar compounds. Used widely for pharmaceuticals, steroids, fatty acids, phthalates, environmental etc.
ProntoSIL CN, Cyano (Propyl Nitrile)	NP/RP	Unique selectivity for polar compounds, more suitable than base silica for normal-phase gradient separations. When used in reversed-phase the selectivity is different to that of the C8, C18 phases. Useful for a wide range of pharmaceutical applications. Useful for mixtures of very different solutes.
ProntoSIL NH_2 (Amino Propyl)	NP/RP	In RP, Carbohydrate analysis and other polar compounds. Weak anion exchanger, anions & organic acids using buffers and organic modifiers. In NP, Alternative selectivity to silica. Good for aromatics.
ProntoSIL Phenyl	RP	Aromatic compounds.
ProntoSIL Diol (OH)	NP/RP	In RP, Proteins, peptides. In NP, similar selectivity to silica, but less polar.

Selection by USP Notification

Phase	Particle size	Form	Pore Size	Surface area m^2/g	Carbon Load	Endcapping	pH
L1 Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter, or a monolithic rod.							
ProntoSIL C18 ace-EPS	3µm	Spherical	120 Å	300	18.5	Yes	2 to 10
			200 Å	200	12.5	Yes	
			300 Å	100	8.5	Yes	
	5µm	Spherical	120 Å	300	18.5	Yes	
			200 Å	200	12.5	Yes	
			300 Å	100	8.5	Yes	
	10µm	Spherical	120 Å	300	18.5	Yes	
			200 Å	200	12.5	Yes	
			300 Å	100	8.5	Yes	
ProntoSIL C18 H	3µm	Spherical	60 Å	450	18.5	Yes	2 to 9
			120 Å	300	17.5	Yes	
			200 Å	200	11	Yes	
			300 Å	100	7	Yes	
	5µm	Spherical	60 Å	450	18.5	Yes	
			120 Å	300	17.5	Yes	
			200 Å	200	11	Yes	
			300 Å	100	7	Yes	
	10µm	Spherical	120 Å	300	17.5	Yes	
ProntoSIL C18 SH	3µm	Spherical	120 Å	300	17	Yes	2 to 7
	5µm	Spherical	120 Å	300	17	Yes	
	10µm	Spherical	120 Å	300	17	Yes	
ProntoSIL C18 AQ	3µm	Spherical	120 Å	300	14	Yes	2 to 9
			200 Å	200	9	Yes	
	5µm	Spherical	120 Å	300	14	Yes	
			200 Å	200	9	Yes	
	10µm	Spherical	120 Å	350	14	Yes	
ProntoSIL C18 AQ Plus	5µm	Spherical	120 Å	300	17	Yes	2 to 9
ProntoSIL Eurobond C18	5µm	Spherical	120 Å	300	17	Yes	2 to 9
			120 Å	300	17	Yes	
	10µm	Spherical	120 Å	300	17	Yes	
			120 Å	300	17	Yes	
ProntoSIL Hyersorb ODS	3µm	Spherical	120 Å	170	9.5	Yes	2 to 9
	5µm	Spherical	120 Å	170	9.5	Yes	
ProntoSIL Spheribond ODS 1	3µm	Spherical	80 Å	220	7	No	2 to 9
	5µm	Spherical	80 Å	220	7	No	
ProntoSIL Spheribond ODS 2	3µm	Spherical	80 Å	220	12	Yes	2 to 9
	5µm	Spherical	80 Å	220	12	Yes	
Hipak ODS AB	3µm	Spherical	130 Å	170	11	Yes	2 to 9
	5µm	Spherical	130 Å	170	11	Yes	
ProntoSIL KromaPlus C18	5µm	Spherical	100 Å	300	20	Yes	2 to 9
ProntoSIL C18 BD	5µm	Spherical	130 Å	170	11	Yes	2 to 9
ProntoBond	10µm	Irregular	125 Å	330	10	Yes	2 to 9
Eurobond C18	5µm	Spherical	120 Å	300	17	Yes	2 to 7
	10µm	Spherical	120 Å	300	17	Yes	2 to 7
Crombudget C18	5µm	Spherical	100 Å	320	17	Yes	2 to 8
	10µm	Spherical	100 Å	320	17	Yes	2 to 8
L3 Porous silica particles, 3 to 10 µm in diameter							
ProntoSILSilica	3µm	Spherical	60 Å	450		No	
			120 Å	300		No	
			200 Å	200		No	
			300 Å	100		No	
	5µm	Spherical	60 Å	450		No	
			120 Å	300		No	
			200 Å	200		No	
			300 Å	100		No	
	10µm	Spherical	60 Å	450		No	
			120 Å	300		No	
			200 Å	200		No	
			300 Å	100		No	
L7 Octysilane chemically bonded to totally porous silica particles , 3 to 10 µm in diameter.							
ProntoSIL C8 ace-EPS	3µm	Spherical	120 Å	300	12	Yes	2 to 9
	5µm	Spherical	120 Å	300	12	Yes	
ProntoSILC8 SH	3µm	Spherical	120 Å	300	10	Yes	
			200 Å	200	7	Yes	
			300 Å	100	4	Yes	
Crombudget C8	5µm	Spherical	100 Å	330	12	Yes	2 to 8
	10µm	Spherical	100 Å	330	12	Yes	2 to 8

Phase	Particle size	Form	Pore Size	Surface area m^2/g	Carbon Load	Endcapping	pH
ProntoSIL C8 SH	5µm	Spherical	60 Å	450	12	Yes	
			120 Å	300	10	Yes	
			200 Å	200	7	Yes	
			300 Å	100	4	Yes	
	10µm	Spherical	120 Å	300	10	Yes	
Hipak C8 AB	3µm	Spherical	130 Å	170	7	Yes	2 to 9
	5µm	Spherical	130 Å	170	7	Yes	2 to 9
ProntoSIL KromaPlus C8	5µm	Spherical	100 Å	300	12	Yes	2 to 9
ProntoSIL C8 BD	5µm	Spherical	130 Å	170	7	Yes	2 to 9

L8

An essentially monomolecular layer of aminopropylsilane. Chemically bonded to totally porous silica gel support, 3 to 10µµm in diameter.

ProntoSIL Amino	3µm	Spherical	120 Å	300	4	No	2 to 9
	5µm	Spherical	120 Å	300	4	No	
	10µm	Spherical	120 Å	300	4	No	
ProntoSIL Amino E	5µm	Spherical	120 Å	300	5	Yes	2 to 9
ProntoSIL Amino H	5µm	Spherical	120 Å	300	4.5	No	2 to 9

L10

Nitrile groups chemically bonded to porous silica particles, 5 to 10 µm in diameter.

ProntoSIL CN	3µm	Spherical	120 Å	300	5	No	2 to 9
	5µm	Spherical	120 Å	300	5	No	
			120 Å	300	5	Yes	
	10µm	Spherical	120 Å	300	5	No	

L11

Phenyl groups chemically bounded to porous silica particles, 5 to 10 µm in diameter.

ProntoSIL Phenyl	3µm	Spherical	120 Å	300	10	Yes	2 to 9
	5µm	Spherical	60 Å	450	12	Yes	
			120 Å	300	9.5	Yes	
	10µm	Spherical	120 Å	300	9.5	Yes	

L16

Dimethylsilane chemically bonded to porous silica particles, 5 to 10 µm in diameter.

ProntoSIL C1	3µm	Spherical	120 Å	300	3	No	2 to 9
	5µm	Spherical	120 Å	300	3	No	

L20

Butyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter.

ProntoSIL C4	3µm	Spherical	120 Å	300	5.5	No	2 to 9
			300 Å	100	2.5	No	
	5µm	Spherical	60 Å	450	7.5	No	
			120 Å	300	5.5	No	
			200 Å	200	3.5	No	
			300 Å	100	2.5	No	
	10µm	Spherical	120 Å	300	5.5	No	

L26

Dihydroxypropane groups chemically bonded to porous silica particles, 5 to 10 µm in diameter.

ProntoSIL Diol	3µm	Spherical	120 Å	300	4	No	2 to 9
	5µm	Spherical	120 Å	300	4	No	
	10µm	Spherical	120 Å	300	4	No	

L62

C30 Silane bonded phase on a fully porous spherical silica, 3 to 15 µm in diameter.

ProntoSIL C30	3µm	Spherical	120 Å	300	25	No	2 to 9
			200 Å	200	20	No	
			300 Å	100	13	No	
			300 Å	100	13	Yes	
	5µm	Spherical	200 Å	200	20	No	
			300 Å	100	13	No	
			300 Å	100	13	Yes	
	10µm	Spherical	200 Å	200	20	No	

Regeneration of a Column

You can reduce the equilibration time by simply increasing the flow rate to the double. However make sure to purge the column with at least 20 column volumes of eluent.

Regeneration of a column

Impurities from the sample or mobile phase can be absorbed at the column inlet and may cause changes in selectivity or peak splitting. Often these “dirty columns”can be regenerated by applying the following procedures:

Regeneration of RP-packings

C18,C8,C4,C1,C30,CN and Phenyl are RP-packings. To regenerate RP-columns please run the following procedure with solvents of different polarity :

1) Wash with 20 column volumes Water (To elute out salts).
2) Wash with 20 column volumes Acetonitrile (To elute hydophilic contaminants).
3) Wash with 5 column volumes Isopropanol(To elute mildly hydrophobic contaminants).
4) Wash with 20 column volumes Heptane or Hexane or Methylenchloride. (To elute strongly hydrophobic contaminants).
5) Wash with 5 column volumes Isopropanol.
6) Wash with 20 column volumes Acetonitrile.
7) Return the column to its original Reverse- Phase condition.

Regeneration of NP-packings

Silica, Diol, Nitro and Amino are NP packings :

1) Wash column with 20 column volumes of Heptane or Hexane.
2) Wash with 5 column volumes Isopropanol.
3) Wash with 4 column volumes Acetonitrile.
4) Wash with 5 column volumes Isopropanol.
5) Wash with 20 column volumes Heptane or Hexane.
6) Return the column to its original Normal-Phase condition.

Attention : Do not use Water!

Proper storage for Silica based HPLC columns

Proper storage of column is very important for column life and reproducibility .

1) Short-term storage, e.g. overnight, columns can be stored in the eluent.
2) Mid-term storage, e.g. 2-7 days, purge column with pure water and store in a 10% aqueous solution of isopropanol (IPA) to prevent bacterial growth.
3) Long-term storage, e.g. > 7 days, silica based columns should be converted to 100% Methanol or Acetonitrile to minimize hydrolyses.

Caution! Make sure that all buffer salts are remove for the column before exchanging aqueous mobile phase to organic solvents. Buffer salts are not soluble in organic solvents e.g. acetonitrile and may block capillary tubing and the column.

Troubleshooting for HPLC Column

The most common problems that occur during the use of an HPLC column are summarized below

Increase in back pressure

It is most likely that the capillary tubing, the column or the detector cell is blocked. To solve that, open all connections starting from the detector and then back to the injector while the pump is running. Blockage can be located by observing where the pressure drop occurs. Replace the blocked parts. If frits or meshes are causing a blockage clean or exchange them.

Decrease of Resolution or Low Efficiency

1) It can be because the column is contaminated with strongly retained components. This comes from either mobile phase or the sample. Regeneration of column as per protocol can help to solve this problem.
2) If channeling inside the column has occurred. This may have been caused by a mechanical shock to the column bed. Column replacement is the only option.
3) The column has a void at the inlet. Contact column manufacturer. He might be able control the damage.

Increasing back pressure in combination with low efficiency

It may be because of high pH. Silica based column dissolve at pH 8. To resolve that install a saturation column between the pump and the injector valve.

Varying retention times

1) In NP mode: Water is the strongest solvent in NP mode. Absorption of moisture in the air changes retention time. To fix this problem, add 1% polar modifier like methanol it helps to achieve stable retention times.
2) In RP mode: If pH is below 2, RP packing gets hydrolyzed and alkyl chain gets washed out.

Peak fronting

1) In most of the cases the origin of this phenomenon is a solvent strength effect. The solvent that dissolve the sample has a higher solvent strength in comparison to the mobile phase used in chromatography.
2) Channeling in the column.

Precautions to be taken while running any HPLC

1) Filter all mobile phases through a 0.45 um filter before using in HPLC.
2) Always check the solubility of the buffer salts in the mobile phase you are using.
3) If you have particulate material in your sample, filter it.
4) Use pre or guard column filters. It keeps column bed safe.
5) Avoid shocks to column, do gradual changes in pressure and protect column from mechanical shocks.
6) Maintain proper pH range. (2-8)
7) Use solvent weaker than mobile phase to dissolve the sample.

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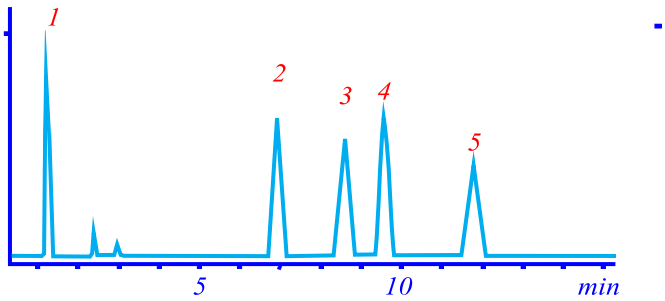
Analysis of Silica Gel

Average Particle Size D50	[µm]	5.0
Mean Pore Diameter	Å	100
Pore Volume	[ml/g]	0.8
Specific Surface Area	[m^2/g]	320
Metal Impurity (ICP)		
Na	[ppm]	<10
Mg	[ppm]	<10
Al	[ppm]	<10
Ca	[ppm]	<10
Fe	[ppm]	<10
Zr	[ppm]	<10
Ti	[ppm]	<10
Particle Shape	Spherical	
Carbon Content	17%	
Endcapping	Yes	
pH	2 - 8	

Tanaka-Test 1
Shape Selectivity/Methylene Group Selectivity

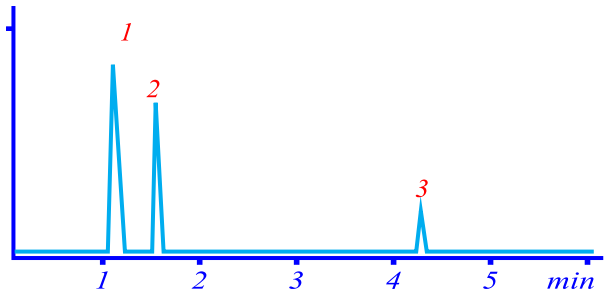


Column Size	125 x 4.0mm	1. Uracil
Eluent	Methanol / Water 75/25 (w/w)	2. Pentyl Benzene
Flow Rate	1.0 ml/min	3. Triphenylene
Column Temperature	40 C	4. Butyl Benzene
Sample Size	10 µl	5. o-Terphenyl
Detection	UV @ 254nm	

Tanaka test 1 : Shape selectivity :

Shape selectivity is ability of the stationery phase to differentiate between planar and non-planer molecules. Molecular recognition is important property exhibited by Chrombudget column. This depends on space requirement of the molecule.

Tanaka-Test 2
Silanophilic Activity at pH 7



Column Size	125 x 4.0mm	
Eluent	Methanol / TRIS Buffer pH 7 30/70 (w/w)	
Flow Rate	1.0 ml/min	
Column Temperature	40 °C	1. Uracil
Sample Size	10 µl	2. Benzyl Amine
Detection	UV @ 254nm	3. Phenol

Tanaka test 2 : Silanophilic activity :

Silanol group have large influence on the selectivity of reverse phase. Undesired secondary interactions results from the silanol group. To reduce this effect Chrombudget has been endapped and it has been deactivated. So column possess exceedingly low silanophilic activity.

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The HPD (High Precision Dosing) pump 3351 is the ideal dosing pump. Compact dimensions, high accuracy and a low-maintenance concept make this pump a reliable partner in the process industry. Both heatable and coolable, the 3351 offers a wide range of application possibilities. All important data and facts are summarized below.

Technical data :

The new High Precision Dosing pump – Multitherm 200 model 3351 for ambient and / or high temperature metering of fluids. The HPD Pump Multitherm 200 3351 covers a wide area of applications with its possibilities of variable flow ranges from micro to large flow rates (depending on the pump head). As a high precision dosing pump it is a unique dosing unit for use at temperatures from – 5°C to 200° C in micro-process technology and mini-plants where high pressure and and / or high temperature is needed.

The new HPLC / DOSING Compact Pump model 3350 for HPLC and High Precision Dosing covers the full area of modern LC and Dosing applications. The new designed electronic with the control of a high resolution stepper motor allows accurate flow rates at high pressure up to 60 MPa from 1µl up to 40.00 ml/min. With interchangeable pump heads the pump is able to cover a flow range from 1 µl to 40.0 ml/min at high and low backpressure. As a high precision compact pump it is the heart for any modular HPLC instrument or Micro- or Miniplant. The pump can be used in isocratic or gradient mode (for gradient a second pump is required).

Available Pump Heads in Stainless Steel 1.4401

- Micro Pump head
Flow: rate1 µl - 1000 µl/min
Pressure range: 0 - 60 Mpa
P/N 2200 0100

- Analytical Pump Head
Flow: rate 0.01 - 5.00 ml/min
Pressure range 0 - 60 Mpa
P/N 2200 0200

- Semi Prep Pump head
Flow: rate 0.1 - 20.00 ml/min
Pressure range 0 - 15 Mpa
P/N 2200 0300

- Prep Pump head
Flow : rate 0.1 - 40.00 ml/min
Pressure range 0 - 5 Mpa
P/N 2200 0400

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Model - 3350

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