HIGH PERFORMANCE SEPARATION PHASES

HPLC | UHPLC | LC/MS | Flash

VDS optilab
Chromatographie
Technik GmbH

Use a better column

VDSpher®

- routine analysis
- polar substance separation
- special ion analysis
- ultra-fast analysis
- 100% water compatibility
- biomacromolecule analysis
- LC/MS compatibility
- substance purification
- cost-effective separation

Use a better column

VDSpher®

- very high degree of purity
- large selection of separating phases
- excellent batch-to-batch reproducibility
- large selection of particle sizes
- high quality bonding and endcapping

Chromatography Columns Made in Berlin

VDS optilab has been producing HPLC columns in all common and special dimensions and with almost all available separation phases since 1987. The high quality of our products have impressed customers from all areas of research & development and quality control, starting from universities and regulatory authorities through small to medium level companies up to large groups in the chemical and pharmaceutical industry. Thanks to the merger with Muder & Wochele GmbH in 2002, our customers as well as we ourselves have profited from the bundled knowledge and expertise of both companies.

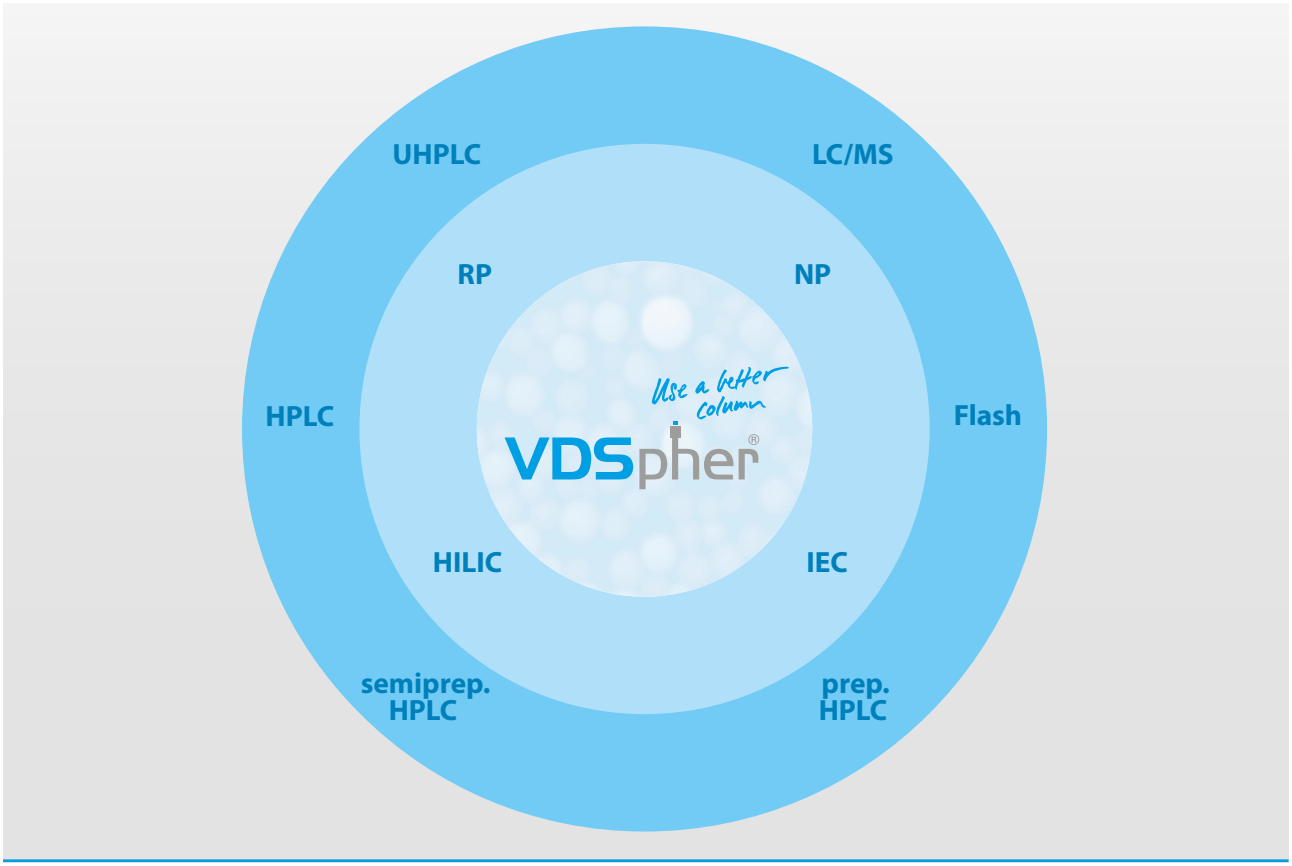
As a result, we were able to develop our own separation phases based on spherical silica gel and present them in 2007 under the VDSpher brand name. Since then we have developed the VDSpher product line further continuously and can offer a wide spectrum of pore sizes and particle diameters as well as modifications, which is used successfully in the normal phase and reversed phase chromatography as well as in the HILIC and ion-exchange chromatography. The scale in which VDSpher is used ranges from UHPLC through analytical HPLC to semi-preparative and preparative chromatography!

VDSpher was developed for users who prefer an uncomplicated and fast LC analysis. Our brand includes the following separation phases:

The available particle diameter range from 1.8 µm to 55 µm. Bigger-sized particles (e.g., 120 µm) can also be delivered upon request. The same selectivity from analytical to preparative chromatography (from UHPLC to preparative HPLC) enables a switch over to a different particle size. For the user, the VDSpher phases permit a target-oriented "upscaling" from analytical HPLC through semi-preparative / preparative HPLC to separation in production scale. Even a “downscaling” of analytical HPLC to UHPLC is simple and possible without performing numerous time-consuming tests. For analytical HPLC the VDSpher phase of choice has a pore size of 100 Å and is suitable for analytes from small to medium molecular weight. In order to analyse larger molecules, VDS optilab offers wide pore silica gels with pore sizes in the range of 150 Å to 1000 Å. For preparative applications through to production scale, even 75 Å separation phases are part of our programme. Benefit from the advantages of our VDSpher phases:

- very good batch-to-batch stability
- wide selectivity spectrum
- very good selectivity
- very high purity of the silica gels
- narrow pore size distribution
- narrow particle size distribution
- high plate numbers

VDSpher separation phases are available for a broad range of applications:



Basic Silica Gels: VDSpher® Classic & VDSpher® PUR

All VDSpher phases are based on two different basic silica gels: VDSpher Classic & PUR, which differ from each other essentially in their purity. The physical specifications of both basic silica gels are summarized in **Table 1.**

The purity of the basic silica gel influences noticeably a chromatographic separation, because the metal content changes the properties of the silica gel and the surface (cf. **Figure 1**). Hence, the interactions between the metal parts and the electron-rich analytes cannot be neglected, as illustrated in **Figure 2**. Due to the higher metal content of the VDSpher Classic 100 C18-E phase the analytes were retarded much stronger as compared to VDSpher PUR 100 C18-E.

All VDSpher Classic & PUR silica gels are suitable for analytical, semi-preparative and preparative scale. To achieve the best possible column packing, we recommend VDSpher PUR phases for analytical issues and VDSpher Classic phases for preparative purposes.

	VDSpher® Classic						VDSpher® PUR			
Pore size [Å]	75	100	150	200	300	1000	100	150	200	300
Surface [m^2/g]	500	320	175	130	90	30	320	175	130	90
Pore volume [ml/g]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Si concentration [%]	99.95	99.97	99.97	99.97	99.97	99.97	99.995	99.995	99.995	99.995
Metal content [ppm]	<125	<100	<100	<100	<100	<100	<20	<20	<20	<20
Density [g/ml]	0.45	0.45	0.45	0.45	0.45	0.42	0.45	0.45	0.45	0.45

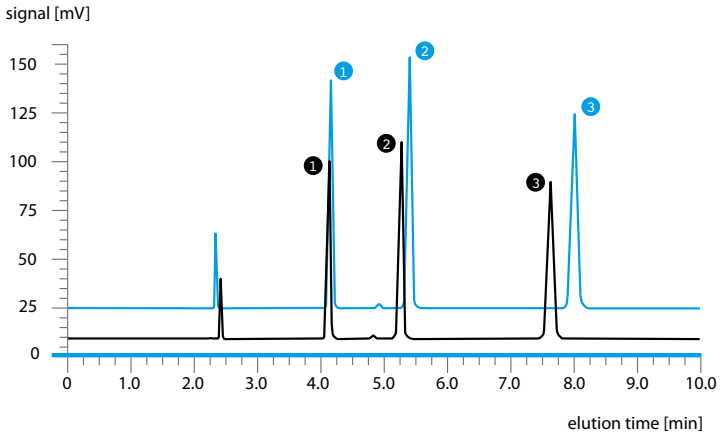
Table 1: *Physical specifications of the basic silica gels VDSpher® Classic & PUR.*

Figure 1: *Influence of metal content on the basic silica gel hydrophobicity.*

VDSpher® Classic vs VDSpher® PUR

Compounds

(1) Benzene
(2) Naphtalene
(3) Anthracene



VDSpher® Use a better column

Columns	5 µm, 250 mm x 4.6 mm
VDSpher®	Classic 100 C18-E
VDSpher®	PUR 100 C18-E

Chromatographic conditions

Solvents:	A: Acetonitrile B: Water
Method:	isocratic 85% A / 15% B
Flow rate:	1.0 mL / min
Pressure:	VDSpher® Classic: 6.1 MPa VDSpher® PUR: 5.0 MPa
Temp.:	30 °C
Detection:	UV 254 nm

For more information, quotation requests and purchase orders please visit our website (www.vdsoptilab.de)
or contact us: info@vdsoptilab.de

Figure 2: *Influence of purity of the basic silica gel on the retention: Comparison of VDSpher® Classic 100 C18-E and VDSpher® PUR 100 C18-E.*

Particle Size Distribution

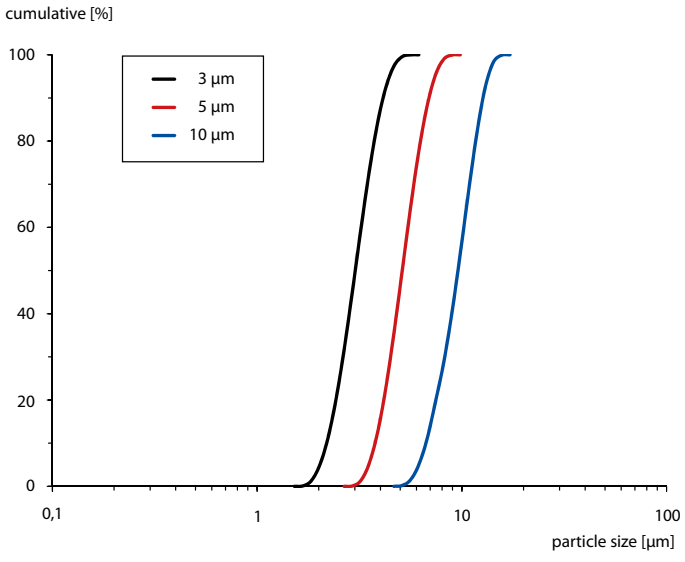
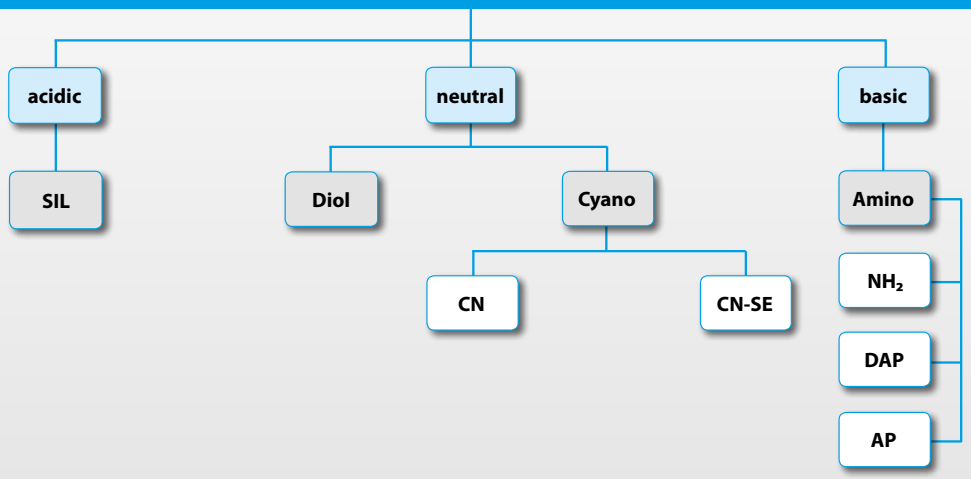


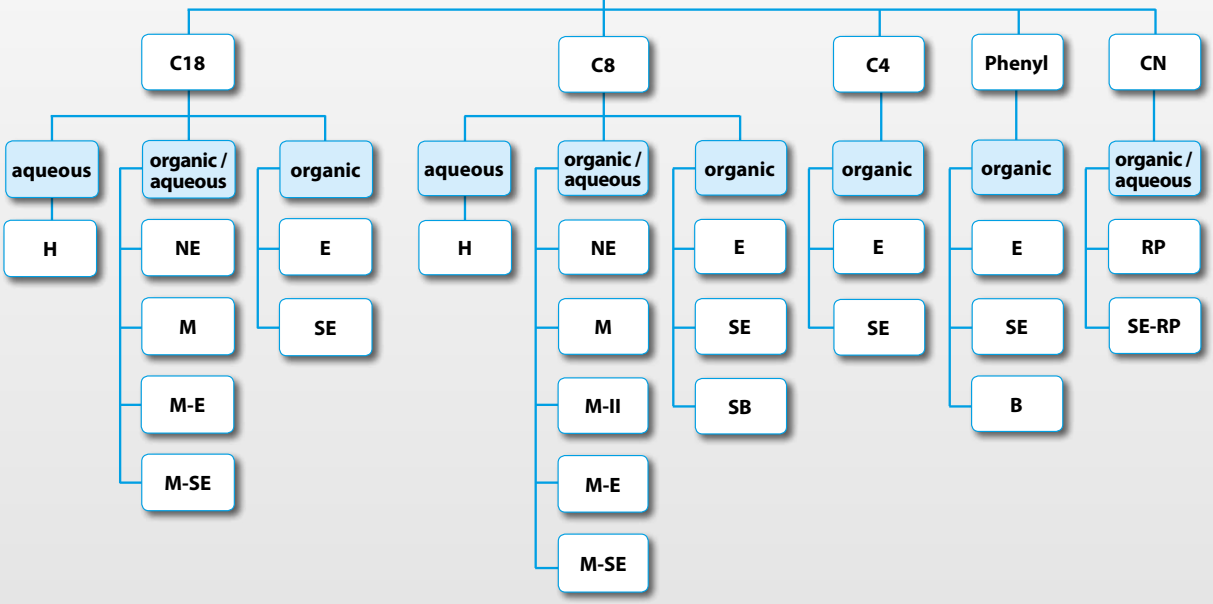
Figure 3: *Cumulative display of the particle size distribution of three VDSpher® PUR silica gels.*

Starting from the basic silica gels VDSpher Classic & PUR, a large number of different modifications offers a wide range of VDSpher separation phases which are displayed schematically in our flow sheets.

VDSpher® Phases for NP Separations



VDSpher® Phases for RP Separations



VDSpher® Normal Phases

For the normal phase (NP) chromatography, unmodified as well as several modified silica gels are available. A multiplicity of normal phase separations can be enabled by them. **Table 2** and **Table 3** (cf. page 8) provide a summary of the available VDSpher Classic & PUR normal phase modifications and their physical specifications. The available particle sizes can be obtained from our current price list.

VDSpher® Classic & PUR Normal Phase Modifications			
Phase	**Modification**	**Endcapping**	**USP Code**
SIL [1], [2]	none	no	L3
Diol [2]	dihydroxy alkyl	no	L20
CN [1]	alkyl nitrile	yes	L10
CN-SE	alkyl nitrile	yes (special)	L10
NH_2 [1]	alkyl amine	no	L8
AP [2]	alkyl amine	no	---
DAP [2]	alkyl amine	no	---

[1] Also available as U-VDSpher for UHPLC (cf. section U-VDSpher® Phases).
[2] Also available as VDSpher Flash for flash chromatography (cf. section VDSpher® Flash Phases).

Table 2: *VDSpher® Classic & PUR normal phase modifications.*

Especially to be highlighted are the advantages of the chemically modified silica gels, which enable a fast equilibration, reproducable analyses despite fluctuating water content in the mobile phase and a problem-free application of the gradient run.

Moreover, it must be considered that the silanol groups at the surface of the silica gel respond slightly acidic, while amino modifications have an alkaline effect. In contrast, Diol and CN are considered as neutral modifications.

Depending on the type of modification, different mechanisms can be used for separation:

- SIL: polar interactions, high silanophilic activity
- Diol: polar interactions, hydrogen bridge connections, silanophilic activity
- CN: polar interactions, π-π interactions, hydrophobic interactions, very low silanophilic activity
- CN-SE: polar interactions, π-π interactions, hydrophobic interactions, no silanophilic activity
- NH_2: polar interactions, hydrophobic interactions, ion exchange, silanophilic activity
- AP: polar interactions, hydrophobic interactions, ion exchange, silanophilic activity
- DAP: polar interactions, hydrophobic interactions, ion exchange, silanophilic activity

Physical Specifications of VDSpher® Classic & PUR Normal Phases				
Phase	**Available Pore Sizes [Å]**	**Carbon Content [%]**	**Nitrogen Content [%]**	**pH Range**
SIL	Classic: 75, 100, 150, 200, 300, 1000 PUR: 100, 150, 200, 300	0	0	2 - 8
Diol	Classic & PUR: 100	4.5	0	2 - 8
CN	Classic & PUR: 100	6.5	1.3	2 - 8
CN-SE	PUR: 100, 150	100 Å: 7.0, 150 Å: 5.0	100 Å: 1.5, 150 Å: 1.2	2 - 9
NH_2	Classic & PUR: 100	4.0	1.5	2 - 7.5
AP	Classic: 100	4.2	1.5	2 - 7.5
DAP	Classic: 100	4.5	2.3	2 - 7.5

Table 3: *Physical specifications of VDSpher® Classic & PUR normal phases.*

VDSpher® Reversed Phases

Reversed phase (RP) chromatography is the unique method of HPLC used most frequently. Therefore, the number of available reversed phases is increasing steadily, so that as far as possible, a matching stationary phase is available for each separation problem. For example, C18 stopped being just C18 long time ago: many different approaches of modification and endcapping result in a large bandwidth of very different C18 phases. Even our brand VDSpher is going through this development and can offer many different modifications and variations. Numerous phases can be selected for C18, C8, C4, Phenyl and CN modifications. A general overview is given in **Table 4**. Further, there are more modifications in our VDSpher product line with VDSpher OptiAqua, VDSpher OptiBio and VDSpher MS, which are covered in subsequent sections.

Below, C18 phases are used to show how the individual modifications and variants of endcapping differ from one another.

– C18-NE	monomeric bonding	no endcapping
– C18-E	monomeric bonding	liquid phase endcapping
– C18-SE	monomeric bonding	gas phase endcapping
– C18-M	polymeric bonding	no endcapping
– C18-M-E	polymeric bonding	liquid phase endcapping
– C18-M-SE	polymeric bonding	gas phase endcapping
– C18-H	single bonding	polar spacer

VDSpher® Classic & PUR Reversed Phase Modifications			
Phase	**Modification**	**Endcapping**	**USP Code**
C18-E [1], [2]	C18	yes	L1
C18-NE	C18	no	L1
C18-SE	C18	yes (special)	L1
C18-M	C18	no	L1
C18-M-E [2]	C18	yes	L1
C18-M-SE [1]	C18	yes (special)	L1
C18-H [1], [2]	C18	yes (polar)	L1
C8-E [1]	C8	yes	L7
C8-SB	C8	yes	L7
C8-NE	C8	no	L7
C8-SE	C8	yes (special)	L7
C8-M	C8	no	L7
C8-M-II	C8	no	L7
C8-M-E	C8	yes	L7
C8-M-SE	C8	yes (special)	L7
C8-H	C8	yes (polar)	L7
C4-E	C4	yes	L26
C4-SE	C4	yes (special)	L26
Phenyl-E [1]	alkyl phenyl	yes	L11
Phenyl-SE	alkyl phenyl	yes (special)	L11
Phenyl-B	alkyl phenyl	yes	L11
CN-RP [1]	alkyl nitrile	yes	L10
CN-SE-RP	alkyl nitrile	yes (special)	L10

[1] Also available as U-VDSpher for UHPLC (cf. section U-VDSpher® Phases).
[2] Also available as VDSpher Flash for flash chromatography (cf. section VDSpher® Flash Phases).

Table 4: *VDSpher® Classic & PUR reversed phase modifications.*

Depending on the type of reagents used, a single ("monomeric") or a multiple ("polymeric") bonding at the surface silanol groups of the basic silica gel is achieved in the modification step, which then results in a brush-like or a branched structure. For steric reasons however, all the silanol groups cannot be modified in both cases, so that the phases still show a noticeable silanophilic activity. To reduce this or to avoid it altogether, an endcapping with trimethylchlorosilane is carried out in a subsequent step. The reaction guidance of the endcapping also plays a role here: in the liquid phase, only approx. 40% of the remaining silanoles can react with trimethylchlorosilane, while in the gas phase, it is up to 99%. In another variant, a reagent is used in addition to the monomeric modification which carries a polar group and therefore serves as "polar spacer" between the C18 chains. The special properties of this phase are described in detail further down. The different VDSpher C18 phases arise from the combination of the modification and endcapping methods described above.

Bonding and endcapping have a decisive influence on the hydrophobicity of a phase. The higher carbon load in a multiple bonded phase ensures a more hydrophobic behaviour than for a single bonded phase. On the other hand, free silanol groups have a hydrophilic effect. The behaviour of the VDSpher C18 phases can therefore be displayed as in **Figure 4**. For orientation, two VDSpher OptiAqua and OptiBio phases are even considered.

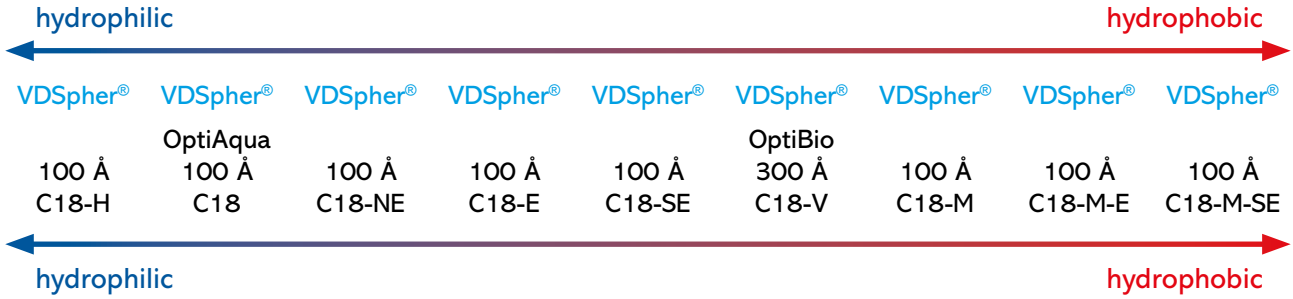


Figure 4: *Hydrophobicity of the VDSpher® C18 modifications.*

Two examples are given in **Figure 5** and **Figure 6**, which illustrate the different separation behaviour of VDSpher reversed phases. The standard separation of benzene, naphthalene and anthracene (cf. **Figure 5**) differ according to the carbon content in each column. The most hydrophobic phase VDSpher Classic 100 C18-M-SE retards anthracene for the longest duration as compared to the phase VDSpher Classic 100 C18-E with medium hydrophobicity, while the most hydrophilic phase VDSpher Classic 100 C18-H enables a significantly less strong retention of hydrophobic analytes.

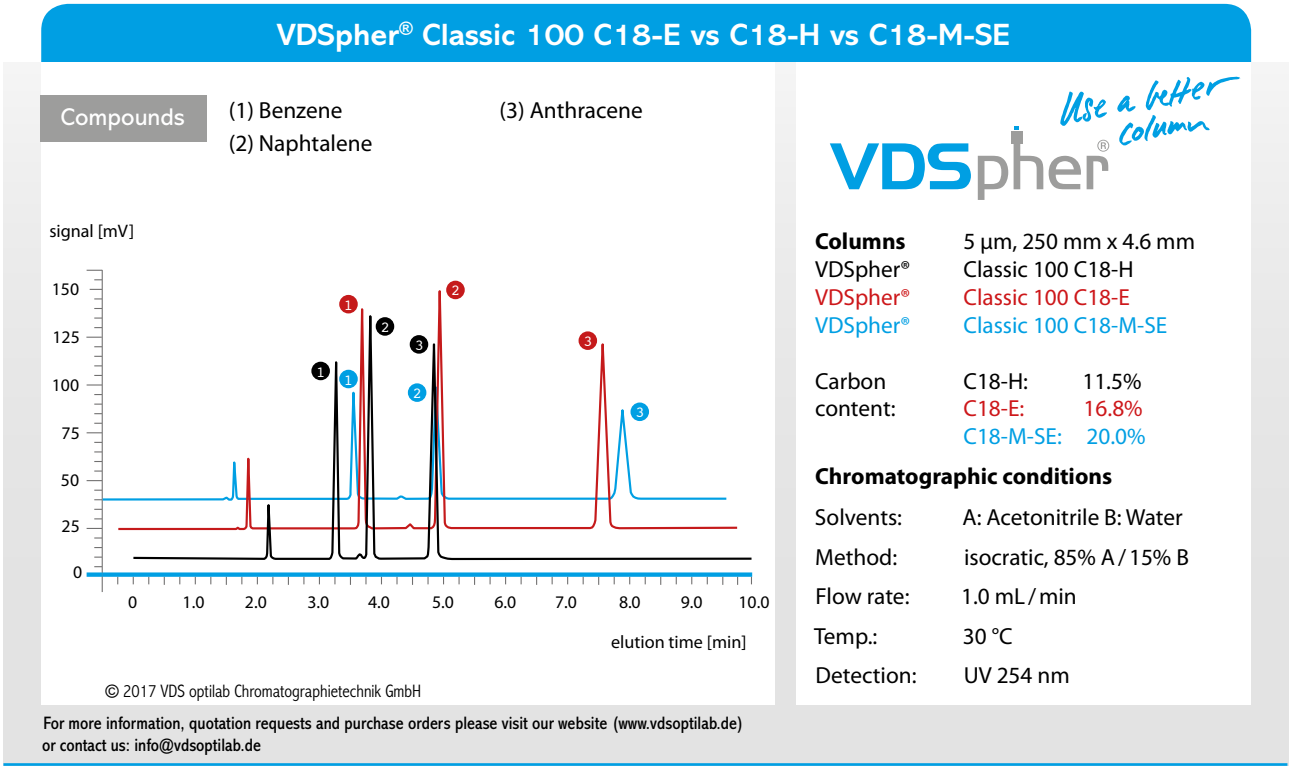


Figure 5: *Influence of hydrophobicity of different VDSpher® C18 phases on the retention: Comparison of VDSpher® Classic 100 C18-E, VDSpher® Classic 100 C18-H and VDSpher® Classic 100 C18-M-SE.Comparison of VDSpher® Classic 100 C18-E, VDSpher® Classic 100 C18-H and VDSpher® Classic 100 C18-M-SE.*

Besides, the polar group of the phase VDSpherClassic 100 C18-H can also be used to achieve a different selectivity: **Figure 6** shows the purification of insulin (Peak 2) with the phases VDSpher Classic 100 C8-E and VDSpher Classic 100 C18-H. For this example, the phase VDSpher Classic 100 C8-E was selected, because it has a carbon load that is comparable to that of VDSpher Classic 100 C18-H. It is to be noted that on using VDSpher Classic 100 C8-E, insulin elutes between its impurities, while in the "polar" phase VDSpher Classic 100 C18-H, insulin retardation is longer than for the impurities.

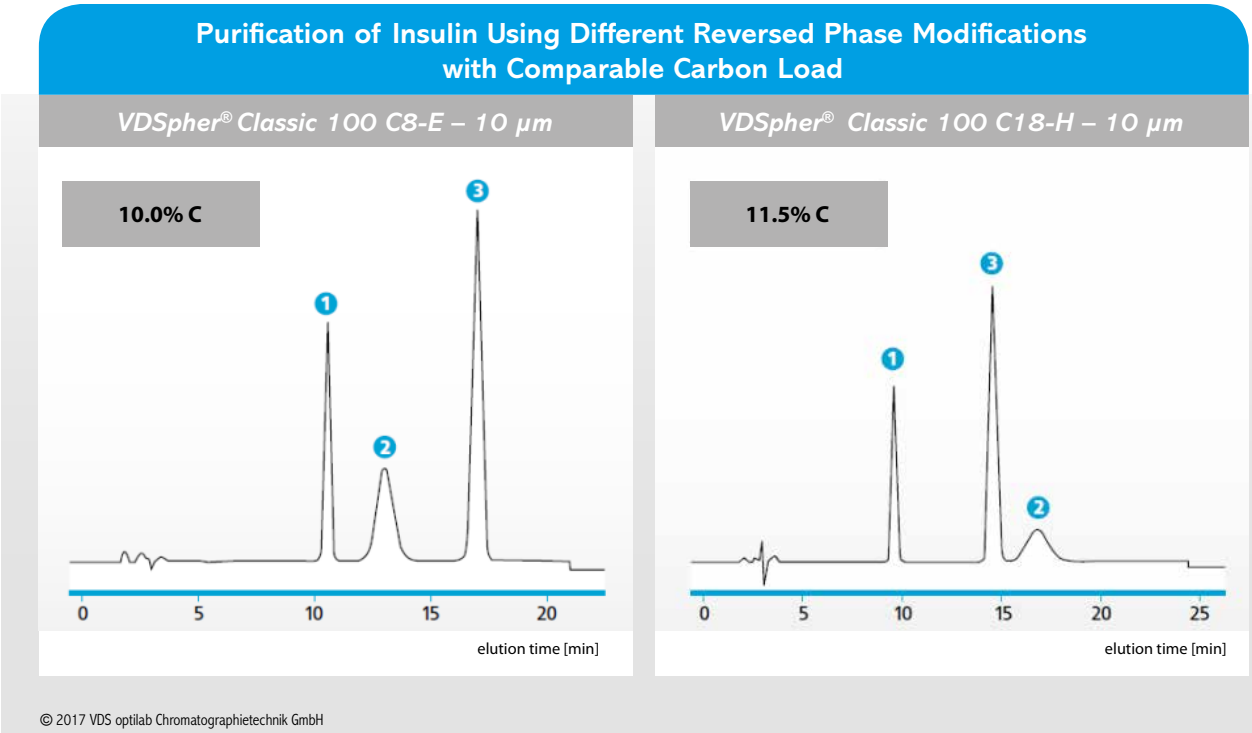


Figure 6: *Different selectivity of insulin purification using different VDSpher® reversed phases: Comparison of VDSpher® Classic 100 C8-E and VDSpher® Classic 100 C18-H.*

Normally, the standard phase VDSpher PUR 100 C18-E is a very good starting point for many separation problems. It is of medium hydrophobicity and shows a low silanophilic activity.
VDSpher PUR 100 C18-E has therefore proved its worth in many applications e.g., for the isolation of natural substances, for the determination of caffeine in coffee, tea and other caffeine containing drinks (cf. **Figure 7** page 12) and even for the determination of amino acids.
The phase VDSpher C18-SE, which does not show any silanophilic activity is slightly more hydrophobic. Hydrophobic substances are therefore retarded more by this phase. Besides, due to the complete endcapping and the resulting inertness, the analysis of alkaline substances is implemented better and an increased stability is achieved in strong acidic and slightly alkaline media.
On the other side, the not endcapped phase VDSpher PUR 100 C18-NE is available, if importance is given to silanophilic activity during separation. The free silanol groups also increase the water mobility as compared to the endcapped phases. For working with 100% water as mobile phase, we recommend the very hydrophilic phase VDSpher PUR 100 C18-H. The polar spacer ensures that the C18 chains do not collapse despite the high water content. VDSpher PUR 100 C18-H is therefore ideal for the analysis of polar analytes and smaller water-soluble biomolecules.

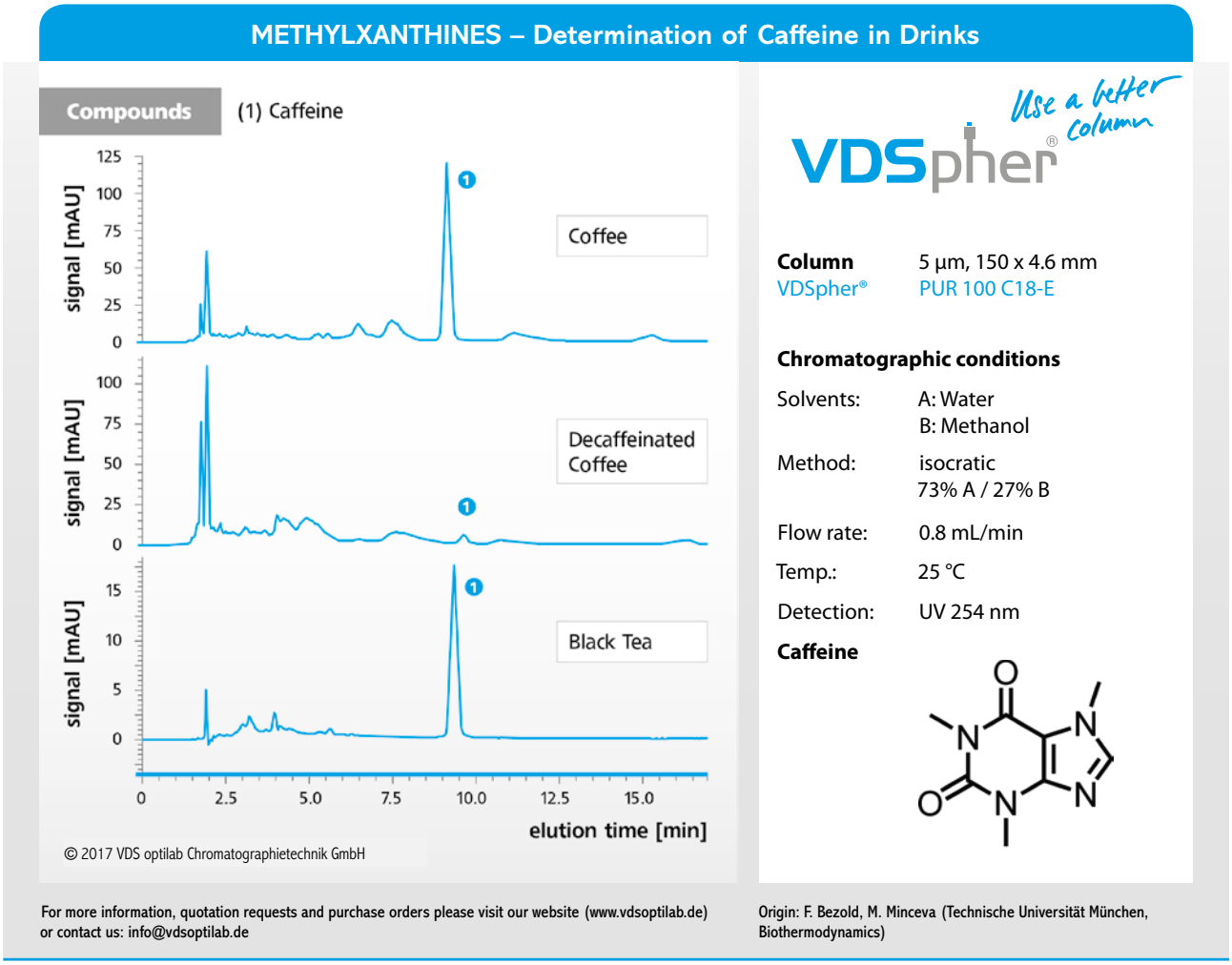


Figure 7: *Determination of caffeine in drinks using VDSpher® PUR 100 C18-E.*

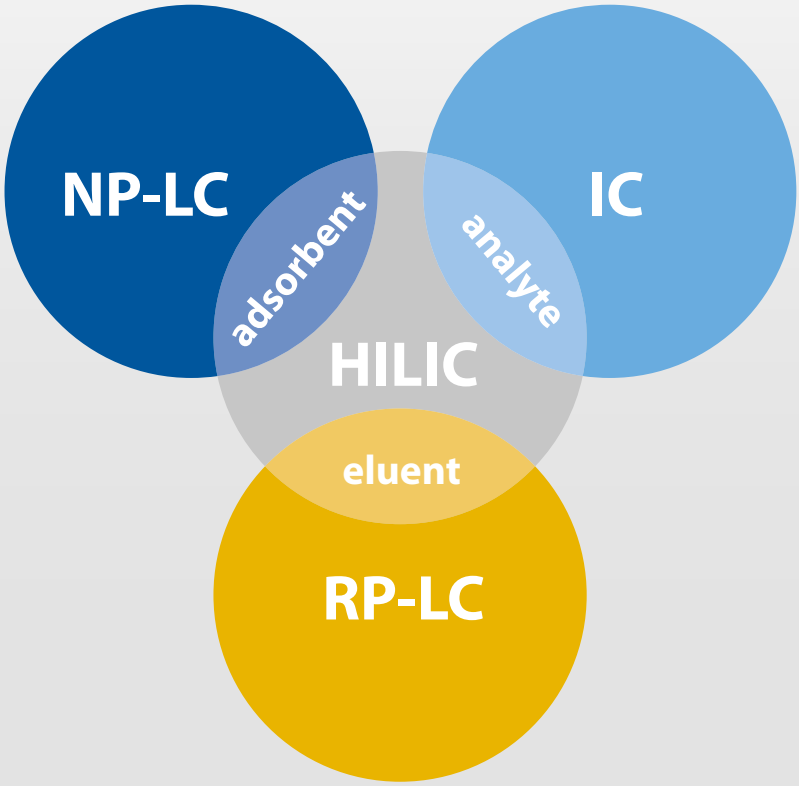
The phases VDSpher C18-M, VDSpher C18-M-E and VDSpher C18-M-SE can be selected for very hydrophobic applications. These phases have a very high carbon load due to their multiple bonding. The branched surface structure shields the surface of the silica gel effectively, so that despite high hydrophobicity, working with 100% water as eluent is possible. As in the single bonding phases, not endcapped VDSpher C18-M is available in the liquid phase endcapped VDSpher C18-M-E and in the gas phase encapped VDSpher C18-M-SE modifications, so that the influence of silanol groups and carbon content can be considered for the desired application.The described modifications and their effects are not limited to just C18: a large number of different VDSpher phases are available for C8 and C4 modifications as well. Because of the low carbon load as compared to VDSpher C18 phases, VDSpher C8 and especially VDSpher C4 phases are less hydrophobic. Moreover, the silanophilic activity is more pronounced due to the better accessibility of the silica gel surface. VDSpher Phenyl-E with liquid phase endcapping and VDSpher Phenyl-SE with gas phase endcapping are alternatives of the aliphatically modified reversed phases. π-π interactions influence the separation through due to the alkylphenyl modification and other selectivities are enabled e.g., for polar and non-polar aromatic hydrocarbons or fatty acids.

Physical Specifications of VDSpher® Classic & PUR Reversed Phases				
Phase	**Available Pore Sizes [Å]**	**Carbon Content [%]**	**Nitrogen Content [%]**	**pH Range**
C18-E	Classic: 75, 100, 150 PUR: 100, 150	75 Å: 21.0, 100 Å: 16.8 150 Å: 10.5	0	2 - 7.5
C18-NE	Classic & PUR: 100	16.3	0	2 - 7.5
C18-SE	Classic: 75, 100 PUR: 100	75 Å: 21.3 100 Å: 17.0	0	2 - 9
C18-M	Classic & PUR: 100	17.5	0	2 - 8
C18-M-E	Classic & PUR: 100	19.0	0	2 - 8
C18-M-SE	Classic & PUR: 100	20.0	0	1 - 10
C18-H	Classic & PUR: 100	11.5	0	2 - 7.5
C8-E	Classic: 75, 100, 150 PUR: 100, 150	75 Å: 13.8, 100 Å: 10.0 150 Å: 5.9	0	2 - 7.5
C8-SB	Classic: 75, 100 PUR: 100	75 Å: 13.0 100 Å: 11.3	0	2 - 8.5
C8-NE	PUR: 100	9.9	0	2 - 7.5
C8-SE	Classic: 75, 100, 150 PUR: 100	75 Å: 13.9, 100 Å: 10.4 150 Å: 6.1	0	2 - 9
C8-M	Classic: 100	7.0	0	2 - 8
C8-M-II	PUR: 100	8.2	0	2 - 8
C8-M-E	PUR: 100	10.7	0	2 - 8
C8-M-SE	PUR: 100	11.0	0	2 - 10
C8-H	Classic & PUR: 100	8.5	0	2 - 7.5
C4-E	Classic & PUR: 100	7.0	0	2 - 7.5
C4-SE	Classic & PUR: 100	7.1	0	2 - 9
Phenyl-E	Classic & PUR: 100	10.5	0	2 - 7.5
Phenyl-SE	PUR: 100	10.7	0	2 - 9
Phenyl-B	PUR: 100	12.0	0	2 - 7.5
CN-RP	Classic & PUR: 100	6.5	1.3	2 - 8
CN-SE-RP	PUR: 100, 150	100 Å: 7.0, 150 Å: 5.0	100 Å: 1.5, 150 Å: 1.2	2 - 9

Table 5: *Physical specifications of VDSpher® Classic & PUR reversed phases.*

VDSpher PUR Phenyl-B is a special phase, which enables aliphatic as well as aromatic interactions and therefore represents an interesting alternative to many phenyl-hexyl phases on the market. The product portfolio is rounded off with the VDSpher reversed phases by the alkyl nitrile-modified separation phases VDSpher CN-RP and CN-SE-RP, which are e.g., ideal for the separation of alkaline molecules. The available particle sizes can be obtained from our current price list.

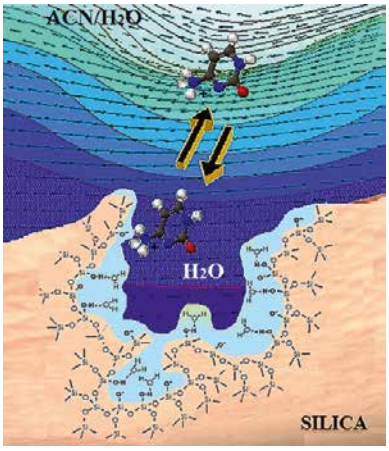
VDSpher® PUR HILIC Phases



HILIC (Hydrophilic Interaction Liquid Chromatography) is a special variant of HPLC in which hydrophilic normal phase modifications are handled with mobile phases normally used in reversed phase chromatography. The separation of very polar substances is promoted this way, which is otherwise extremely difficult to analyse by the proven HPLC methods.

In the HILIC mode, an aqueous layer is formed on the surface of the stationary phase. The separation of analytes is based on a complex combination of different effects, because in addition to the usual interactions, the distribution of the analytes between mobile phase and water layer adds a crucial influence.

The ability to retard polar substances is one of the special strengths of HILIC. Thus for example, Uracil, which is used frequently as dead time marker in HPLC, retards strongly in the HILIC mode, as illustrated in **Figure 8**.



B. Buszewski *et al.*, *Anal. Chem.* **2012**, *402,* 231-247. The figure was kindly provided by Bogusław Buszewski (Environmental Chemistry and Bioanalytics, Nicolaus Copernicus University, Toruń, Poland).

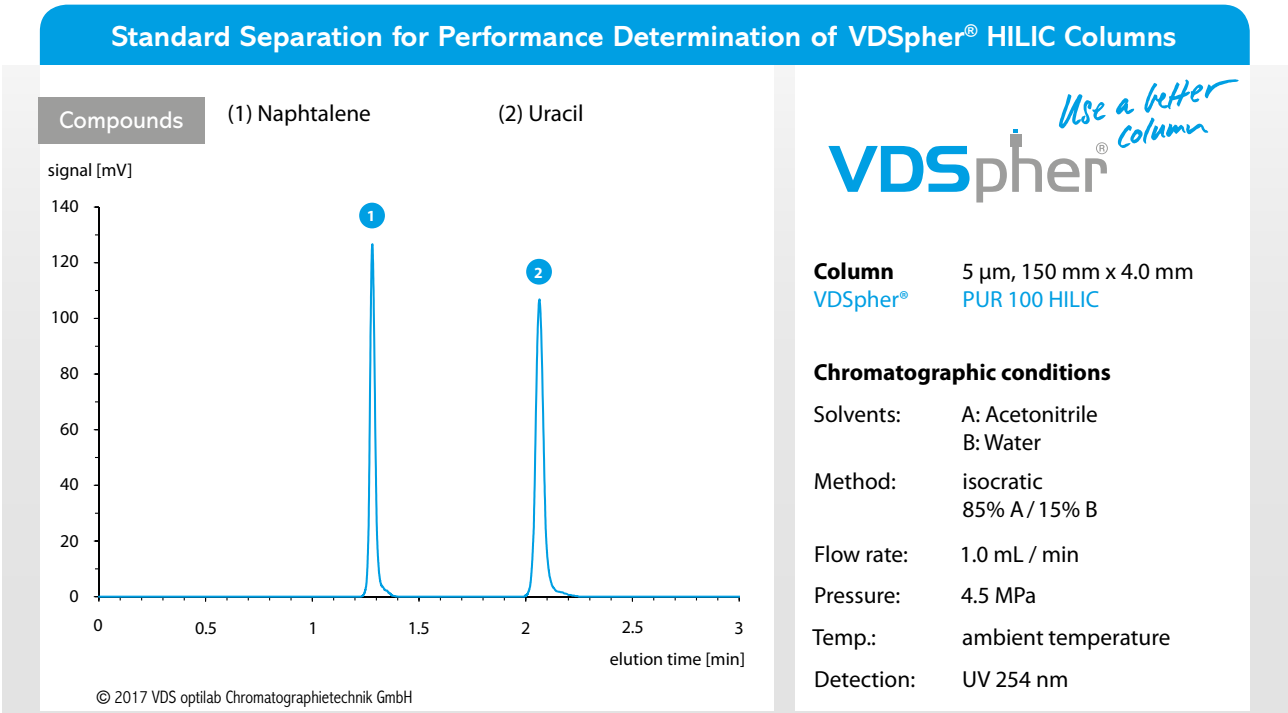


Figure 8: *Separation of naphthalene and uracil with VDSpher® PUR 100 HILIC.*

The available VDSpher PUR 100 HILIC phases are shown in **Table 6** and normally have a pore size of 100 Å and a medium particle diameter of 5 µm. The optimum phase for each HILIC application can be selected via the different available modifications. Thus VDSpher PUR 100 HILIC-AM and especially VDSpher PUR 100 HILIC-SAC are ideal for sugar separations, while the analysis of nucleobases can be performed ideally with VDSpher PUR 100 HILIC-Z.

VDSpher® PUR 100 HILIC Modifications		
Phase	**Modification**	**USP Code**
HILIC	none	L3
HILIC-OH	dihydroxy alkyl	L20
HILIC-AM	alkyl amine	L8
HILIC-SAC	alkyl amine	L8
HILIC-Z	zwitterionic	-

Table 6: *VDSpher® PUR 100 HILIC modifications.*

VDSpher® Ion-Exchange Phases

The product line VDSpher is completed with ion-exchange phases based on silica gel.
The phase VDSpher PUR 100 NH_2, known as a normal phase material, can be applied in the ion-exchange mode equally. Additionally, the amino phases VDSpher Classic 100 AP and VDSpher Classic 100 DAP are available for ion-exchange chromatography (IEX). Another option offered as weak anion-exchanger is the phase VDSpher PUR 100 PEI. For this, VDSpher PUR basic silica gel is modified with polyethylenimine via a spacer. VDSpher PUR 100 PEI achieves the optimum anion-exchange capacity in the range of pH = 4 - 7. For the chromatographic separation, ionic strength and changes in pH value of the mobile phase are responsible for the adsorption and desorption of the analytes. The ion-exchange capacities of the available VDSpher 100 anion-exchange phases are shown in **Table 7**.

Ion-Exchange Capacities of the VDSpher® 100 Anion-Exchanger	
Phase	**Ion-Exchange Capacity [mmol/g]**
NH_2 Classic & PUR	0.95
AP Classic	0.85
DAP Classic	1.4
PEI PUR	2.1

Table 7: *Ion-exchange capacities of the VDSpher® 100 anion-exchanger.*

A phase for cation-exchange is also offered with VDSpher PUR 100 OA-1. H^+ serves as the counterion of the functional group and the exchange capacity is 0.5 mmol/g. This phase also has silanophilic activity. The separation of organic acids without buffer addition in particular, is enabled by VDSpher PUR 100 OA-1. Ion-exchange phases are available with a pore size of 100 Å and a particle diameter of 5 µm. VDSpher Classic 100 AP is only available with particle diameter of 15 µm, 30 µm or 55 µm.

U-VDSpher® PUR Phases

More and more users are using UHPLC (Ultra High Performance Liquid Chromatography) for their analysis, due to short analysis times, high efficiency and accordingly, a high economy of the analysis together with the high commercial availability of UHPLC systems and columns.
For UHPLC, suitable packaging materials and columns must satisfy certain claims. Short analysis times are achieved with small column dimensions, the desired high chromatographic resolution however, cannot be achieved with the particle sizes used normally in HPLC. For the UHPLC therefore, silica gel with particle diameters less than 2 µm have come up ("sub-2-µm particles"). Very high plate numbers are achieved with these bulk materials, leading to a very high resolution.
Due to the small particle size, pressures of up to 1200 bar may be expected in UHPLC applications. To guarantee stability of the column packing at high pressures, special processes of column production are required. VDS optilab works continuously on the further development and optimization of production processes for the manufacture of UHPLC columns. Nevertheless, it must be mentioned that at pressures of over 800 bar, one should expect a shortening of column life time.
With U-VDSpher columns, VDS optilab offers the ideal solution for UHPLC applications. The different

U-VDSpher separation phases have the required small particle size (1.8 µm) and are packed in the columns using a special production process. These are therefore, ideal for the requirements of UHPLC.
The available modifications on the basis of silica gel U-VDSpher PUR 100 are shown in **Table 8.** These can be used to perform a large number of normal and reversed phase separations.

Physical Specifications of U-VDSpher® PUR 100 Normal and Reversed Phase Modifications

Phase	Modification	Endcapping	Carbon Content [%]	Nitrogen Content [%]	USP Code
SIL	none	no	0	0	L3
CN	alkyl nitrile	yes	6.5	1.3	L10
CN-RP	alkyl nitrile	yes	6.5	1.3	L10
NH_2	alkyl amine	no	4.0	1.5	L8
C18-E	C18	yes	16.8	0	L1
C18-M-SE	C18	yes (special)	20.0	0	L1
C18-H	C18	yes (polar)	11.5	0	L1
C8-E	C8	yes	10.0	0	L7
Phenyl-E	alkyl phenyl	yes	10.5	0	L11

Table 8: *Physical specifications of U-VDSpher® PUR 100 normal and reversed phase modifications.*

Standard Separation for Performance Determination of U-VDSpher® RP Columns

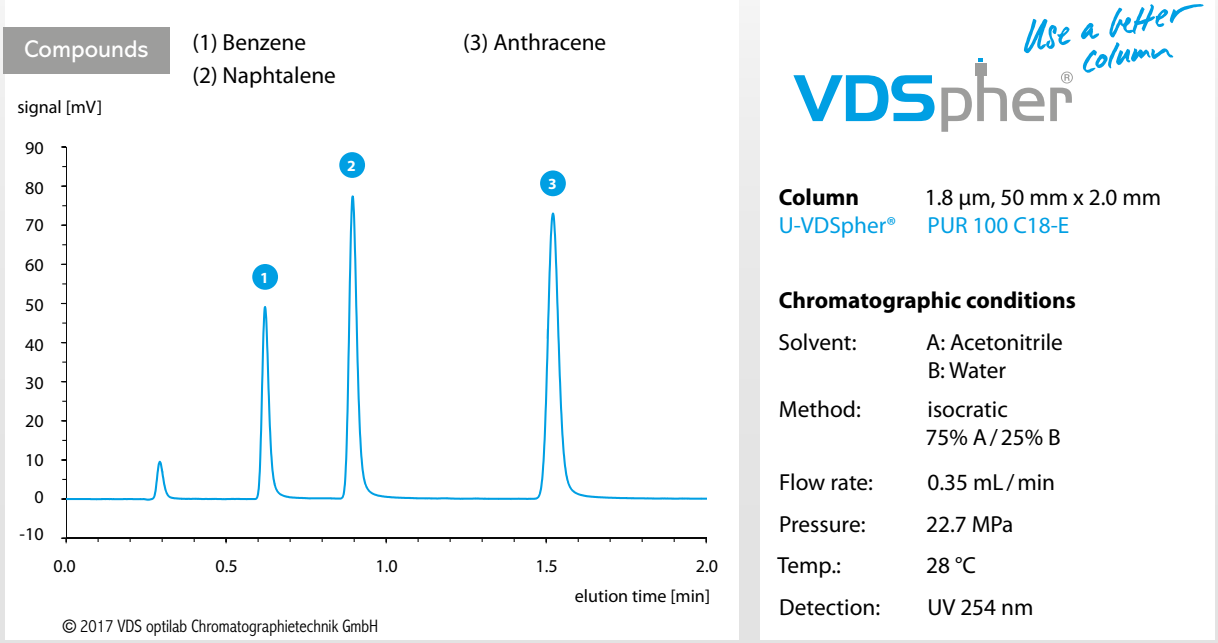


Figure 9: *Standard separation with U-VDSpher® PUR 100 C18-E.*

VDSpher® OptiAqua Phases

VDSpher OptiAqua phases have been developed especially for very hydrophilic applications. A special polar endcapping makes it possible to use 100% water as solvent while still maintaining reversed phase properties.

Six VDSpher OptiAqua phases with different modifications as well as pore sizes and particle diameters have been developed on the basis of VDSpher Classic & PUR, with which a large number of applications can be performed.
Ideal preconditions therefore exist for preparative as well as analytical HPLC.

Analytes of different sizes can also be handled optimally. Thus, different molar mass ranges can be covered, depending on the pore size of the modified silica gel. The available VDSpher OptiAqua & OptiAqua PUR reversed phase modifications are shown in **Table 9**.

VDSpher OptiAqua phases can among other things, also be used to separate polar substanes.

Typical analysts are:

- antibiotics
- biomolecules
- drugs
- nucleobases
- organic acids
- parabens
- pesticides
- sulfonamides
- water-soluble vitamins
- xanthines

VDSpher® OptiAqua & OptiAqua PUR Reversed Phase Modifications

Phase	Available Pore Sizes [Å]	Carbon Content [%]	Analyte Size [g/mol]	USP Code
OptiAqua C18	100	13.0	30 - 800	L1
OptiAqua C8	100	8.1	30 - 800	L7
OptiAqua PUR C18	100, 150	100 Å: 13.0 150 Å: 7.0	100 Å: 30 - 800 150 Å: 120 - 3200	L1
OptiAqua PUR C18-LL	100	100 Å: 10.5	30 - 800	L1
OptiAqua PUR C8	100	8.1	30 - 800	L7
OptiAqua PUR C4	100	6.5	30 - 800	L26

Table 9: *VDSpher® OptiAqua & OptiAqua PUR reversed phase modifications.*

All VDSpher OptiAqua phases are pH stable in a range of 2 to 7.5 and can be used without any problem up to a temperature of 60 °C. The available particle sizes can be obtained from our current price list.

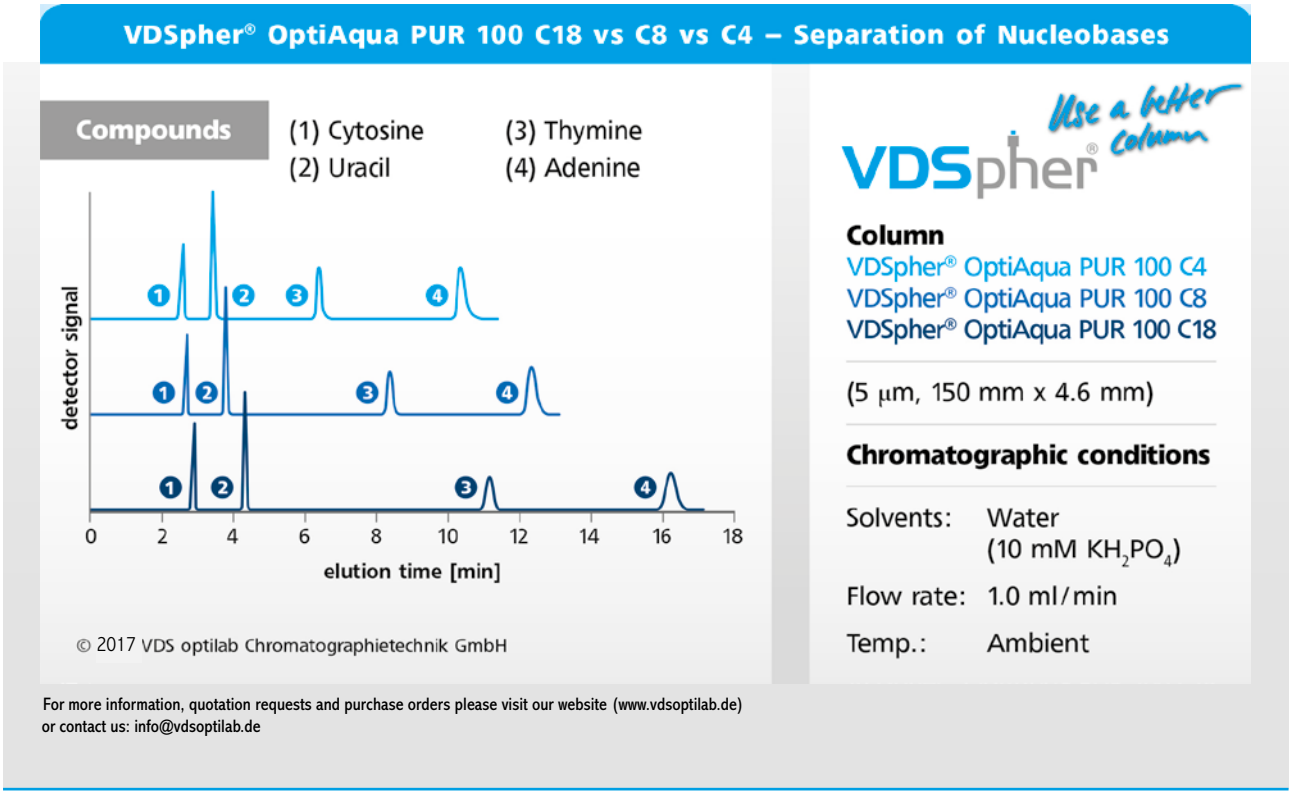


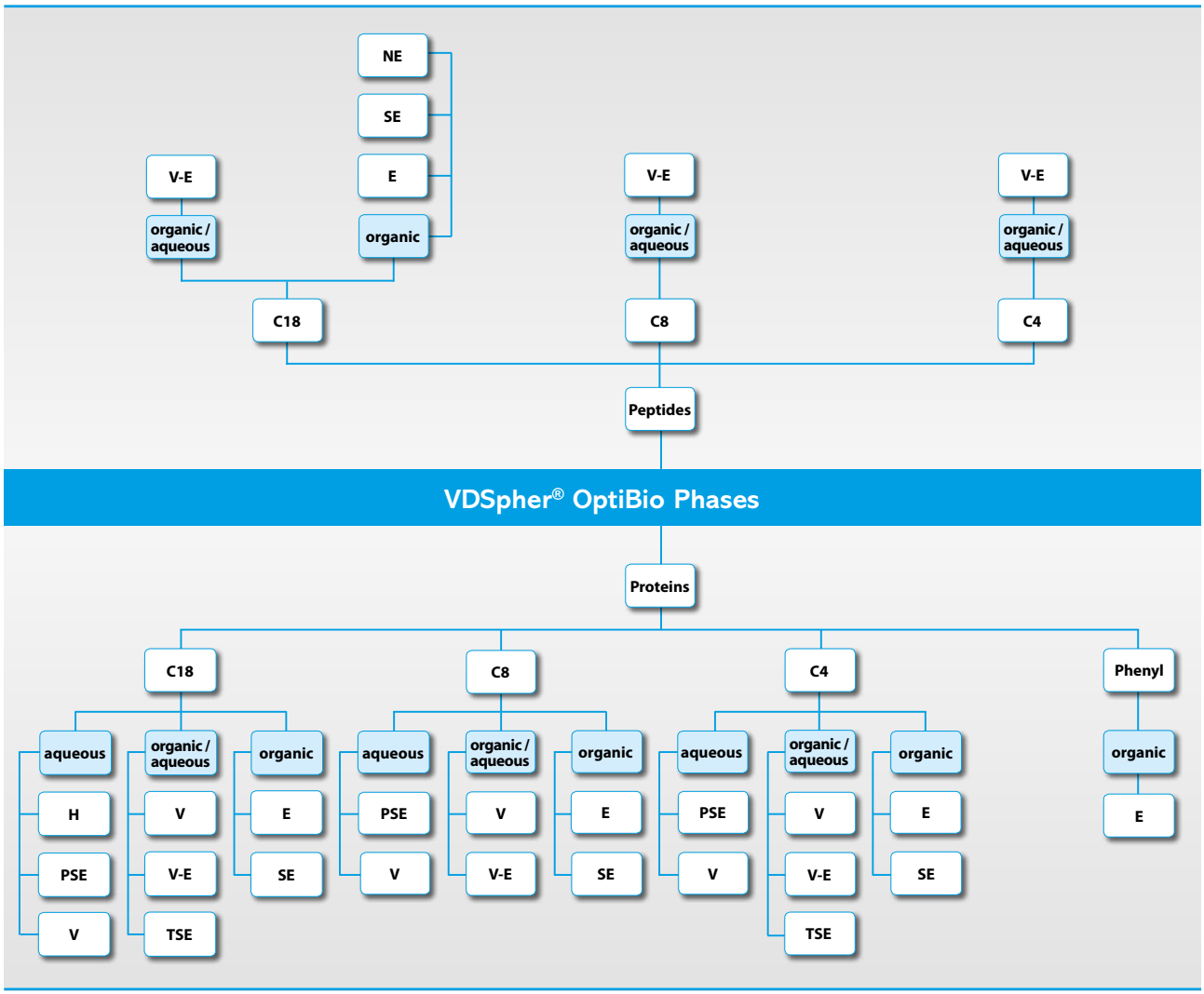
Figure 10: *Separation of nucleobases with different VDSpher® OptiAqua PUR phases.*

VDSpher® OptiBio Phases

VDSpher OptiBio phases have been developed specially for biochemical analysis such as, for example, protein and peptide separation. A total of 28 separation phases with different modifications as well as pore sizes and particle diameters are available, suitable for a broad range of applications. **Table 10** provides an overview of the VDSpher OptiBio & OptiBio PUR reversed phase modifications and their physical specifications. Analytes that can be examined with VDSpher OptiBio columns are e.g., antibodies, oligo-nucleotides, peptides and proteins as well as other biomolecules. Depending on the pore diameter of the VDSpher OptiBio phase, analytes of different sizes and structures can be handled (cf. **Table 11** page 21).

Selection of Pore Size According to Molar Mass Range of the Analytes		
Pore Size [Å]	**Spherical Analytes [g/mol]**	**Cylindrical Analytes [g/mol]**
200	250 - 6500	100 - 2800
300	800 - 21500	350 - 9500

Table 10: *Selection of pore size according to molar mass range of the analytes.*



The various modifications differ in terms of hydrophobicity as shown in **Figure 11** using the example of C18 phases. The modifications C18-V, C18-V-E and C18-TSE are very hydrophobic, C18-SE and C18-E have a medium hydrophobicity. As against this, C18-PSE and C18-H have hydrophilic properties.

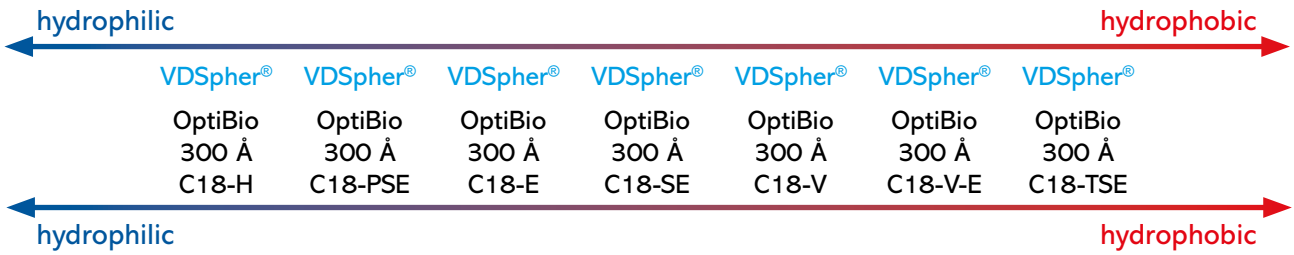


Figure 11: *Hydrophobicity of VDSpher® OptiBio C18 modifications.*

VDSpher® OptiBio & OptiBio PUR Reversed Phase Modifications					
Phase	Available Pore Size [Å]	Endcapping	pH Range	Max. H_2O Content Eluent [%]	USP Code
OptiBio C18-E	200, 300	yes	2 - 7.5	90	L1
OptiBio C18-NE	200	no	2 - 7.5	95	L1
OptiBio C18-SE	200	yes (special)	2 - 9	95	L1
OptiBio C18-V	300	no	2 - 7.5	100	L1
OptiBio C8-E	300	yes	2 - 7.5	90	L7
OptiBio C8-V	300	no	2 - 7.5	100	L7
OptiBio C4-E	300	yes	2 - 7.5	90	L26
OptiBio C4-V	300	no	2 - 7.5	100	L26
OptiBio PUR C18-E	300	yes	2 - 7.5	90	L1
OptiBio PUR C18-SE	300	yes (special)	2 - 9	95	L1
OptiBio PUR C18-V-E	200, 300	yes	2 - 7.5	100	L1
OptiBio PUR C18-TSE	300	yes (special)	2 - 10	100	L1
OptiBio PUR C18-H	300	yes (polar)	2 - 7.5	100	L1
OptiBio PUR C18-PSE	300	yes (hydrophilic)	2 - 8	100	L1
OptiBio PUR C8-E	300	yes	2 - 7.5	90	L7
OptiBio PUR C8-SE	300	yes (special)	2 - 9	95	L7
OptiBio PUR C8-V-E	200, 300	yes	2 - 7.5	100	L7
OptiBio PUR C8-PSE	300	yes (hydrophilic)	2 - 8	100	L7
OptiBio PUR C4-E	300	yes	2 - 7.5	90	L26
OptiBio PUR C4-SE	300	yes (special)	2 - 9	95	L26
OptiBio PUR C4-V-E	200, 300	yes	2 - 7.5	100	L26
OptiBio PUR C4-TSE	300	yes (special)	2 - 9	100	L26
OptiBio PUR C4-PSE	300	yes (hydrophilic)	2 - 8	100	L26
OptiBio PUR Phenyl-E	300	yes	2 - 7.5	90	L11

Table 11: *VDSpher® OptiBio & OptiBio PUR reversed phase modifications.*

All VDSpher OptiBio modifications are pH-stable over a large range and can be used without any problem up to a temperature of 65 °C. Eluents with high water content are applicable for all VDSpher OptiBio phases. The available particle sizes can be obtained from our current price list.
VDSpher OptiBio phases can also be filled in biocompatible PEEK columns or in PLS columns (PEEK-lined stainless steel) on request.

VDSpher® MS Phases

Mass spectrometry is becoming more and more crucial as an online detection method. The requirements of the LC/MS analysis are reflected in the conception of HPLC columns. To reduce the solvent quantity and so as not to endanger the vacuum created in the mass spectrometer, columns of smaller dimensions are normally preferred. To profit from a high resolution further, silica gel with a small particle size is recommended for the column. VDSpher MS satisfies this requirement with 2.5 µm separation phases. If the resolution is not crucial, VDSpher MS columns with 4 µm silica gels are also available.

In principle, any VDSpher column can be used for LC/MS. Because as a sensitive detection method, mass spectrometry is in a position to display very low impurities, depending on the application e.g., carry-overs from previous runs ("carry-over effect"). VDSpher MS phases are selected specially to avoid these effects.

In all, ten different modifications are available. The C18 phases enable a large bandwidth of hydrophilic (C18-H, C18-LC-H) and hydrophobic (C18-DE, C18-B-DE) applications.

The not endcapped phase C18-B has medium hydrophobicity. Additionally, there are hydrophobic VDSpher MS phases with C8, C4, Phenyl and CN modifications.

VDSpher MS phases are available with a pore size of 100 Å. The VDSpher MS 100 normal and reversed phase modifications are shown in **Table 12**.

VDSpher® MS 100 Normal and Reversed Phase Modifications

Phase	Endcapping	Carbon Content [%]	pH Range	USP Code
VDSpher® MS 100 C18-E	yes (special)	17.1	2 - 7.5	L1
VDSpher® MS 100 C18-DE	yes (special)	17.2	2 - 9	L1
VDSpher® MS 100 C18-B	no	17.7	2 - 7.5	L1
VDSpher® MS 100 C18-B-DE	yes (special)	20.1	1 - 11	L1
VDSpher® MS 100 C18-H	yes (polar)	11.5	2 - 7.5	L1
VDSpher® MS 100 C18-LC-H	yes (hydrophilic)	13.5	2 - 8	L1
VDSpher® MS 100 C8-B-DE	yes (special)	11.2	2 - 10	L7
VDSpher® MS 100 C4-B-DE	yes (special)	7.3	2 - 9	L26
VDSpher® MS 100 Phenyl-DE	yes	10.9	2 - 9	L11
VDSpher® MS 100 CN-DE	yes	7.2	2 - 9	L10
VDSpher® MS 100 CN-DE-RP	yes	7.2	2 - 9	L10

Table 12: *VDSpher® MS 100 normal and reversed phase modifications.*

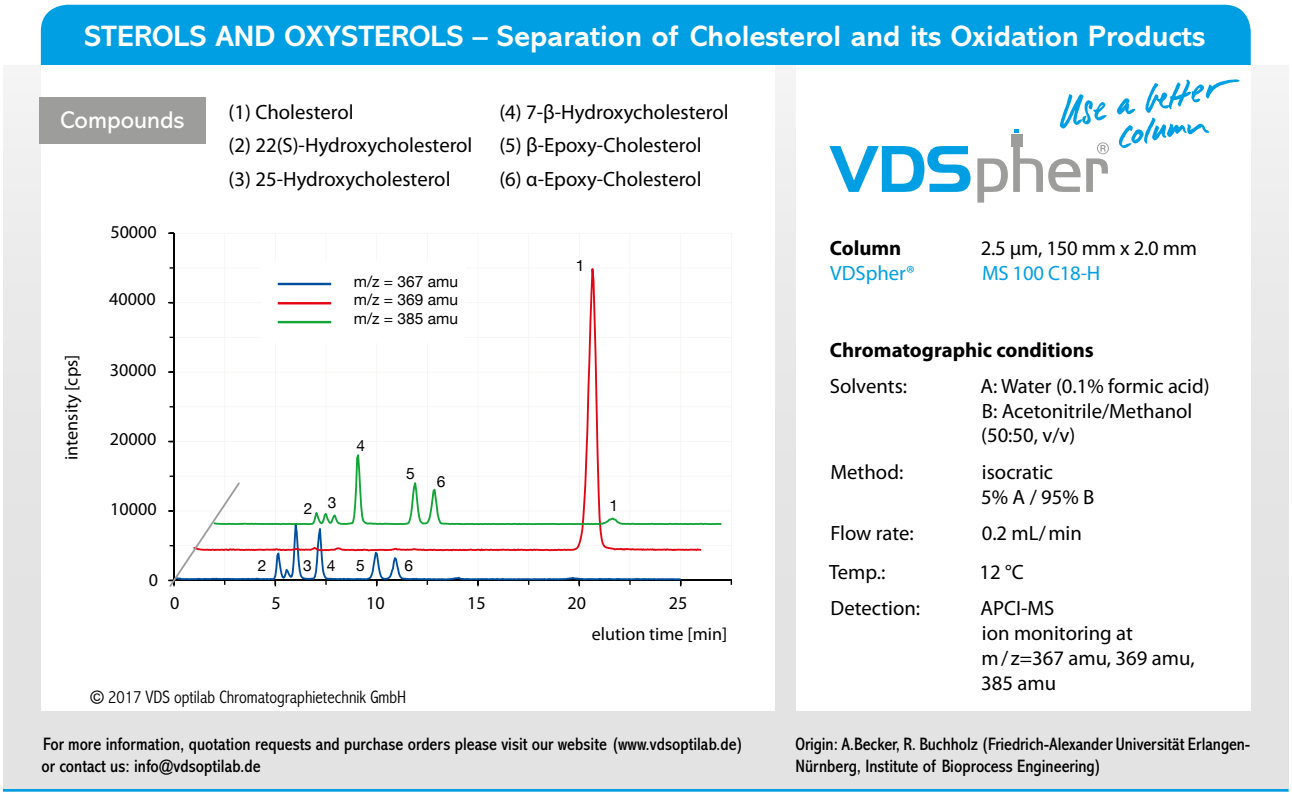


Figure 12: *Separation of cholesterol and its oxidation products with VDSpher® MS 100 C18-H.*

VDSpher® for Preparative Applications

VDSpher has proven itself not only in the analytical scale, but also for the semi-preparative and preparative HPLC. Customers from all areas of research & development as well as from quality control of research facilities to large enterprises of the chemical and pharmaceutical industry use different VDSpher modifications in order to purify and analyse their substances. The excellent scalability enables the simple switching from small particle diameters (1.8 µm to 5 µm) to larger particle sizes (7 µm, 10 µm, 15 µm, 30 µm and beyond) preferred in semi-preparative and preparative applications.
Some VDSpher Classic modifications are available with a pore diameter of 75 Å. The high surface of these materials of 500 m^2/g offers the advantage of very high sample loading, so that time and money can be saved during purification. All VDSpher Classic & PUR silica gels can be used in the semi-preparative and preparative scale. To achieve the best possible column packing we recommend VDSpher Classic phases especially for columns with 20 mm or higher inner diameter. **Table 13** (cf. page 24) provides information on the normally deliverable column dimensions. Needless to mention, column lengths other than the ones specified.

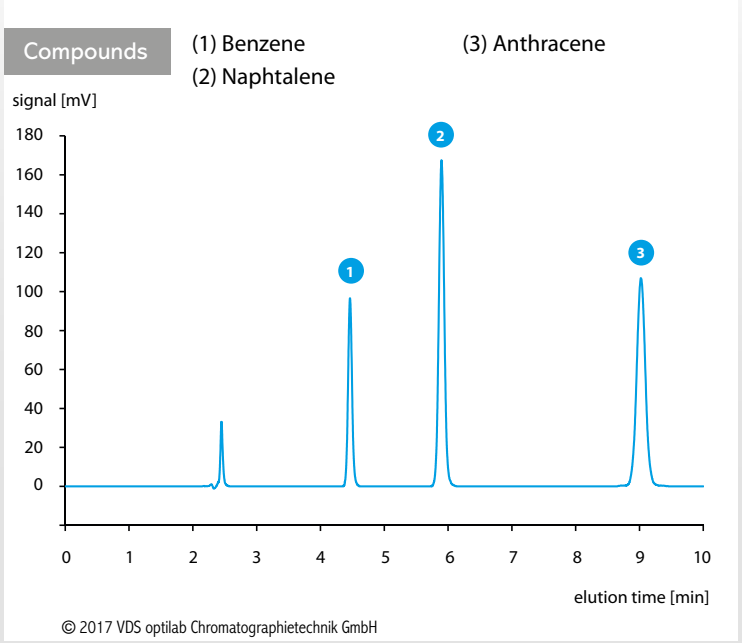
We would also be glad to offer you preparative HPLC columns with inner diameters of 25 mm, 32 mm, 40 mm, 50 mm and 63 mm. We naturally offer a refill service for all our preparative and semi-preparative VDSpher columns. In this way the hardware costs can be reduced.

Normally Available Column Dimensions for Semi-Preparative and Preparative Applications	
Inner Diameter [mm]	**Column Lengths [mm]**
8.0	30 / 40 / 125 / 250
10.0	50 / 150 / 250
16.0	30 / 60 / 125 / 250
20.0	30 / 50 / 100 / 125 / 150 / 250

Table 13: *Normally available column dimensions for semi-preparative and preparative applications.*

We would also like to focus on some VDSpher PUR modifications which are available for selection in a particle size of 7 µm. This particle size represents a valuable compromise between 5 µm and 10 µm: Less pressure than with 5 µm – more plates numbers than 10 µm.
This fact is illustrated in **Table 14**, in which three columns of the phase VDSpher PUR 100 C18-E are compared, having different particle sizes: 5 µm, 7 µm, and 10 µm. The analytical column dimension 250 × 4.6 mm was chosen for demonstration purposes. The pressure relations of the columns show: 7 µm creates hardly double the pressure created by 10 µm, but only about half the pressure caused by a particle size of 5 µm. Hence, the plates numbers of the phase with 7 µm provide a clear gain as compared to a column with 10 µm bulk material.
In brief: Anyone who still has scope in the pressure range for 10 µm particle diameter, can profit essentially from 7 µm bulk material. But anyone who have to work in the upper pressure range of with 5 µm phases, can switch to 7 µm particle diameter without any major compromise in terms of separation.
VDSpher phases with a particle size of 7 µm are available upon request.

Standard Separation for Performance Determination of VDSpher® RP 7 µm Phases



Use a better column
VDSpher®

Column 7 µm, 250 mm x 4.6 mm
VDSpher® PUR 100 C18-E

Chromatographic conditions

Solvents:	A: Acetonitrile B: Water
Method:	isocratic 85% A / 15% B
Flow rate:	1.0 mL/ min
Pressure:	2.5 MPa
Temp.:	ambient temperature
Detection:	UV 254 nm

Figure 13: *Standard separation with VDSpher® PUR 100 C18-E with a particle size of 7 µm.*

Comparison of Pressure and Number of Theoretical Plates per Meter for VDSpher® PUR 100 C18-E		
Particle Size [µm]	**Pressure [bar]**	**N/m (Anthracene Peak)**
5	48	111000
7	25	83000
10	14	54000

Table 14: *Comparison of pressure and number of theroretical plates per meter for VDSpher® PUR 100 C18-E with particle sizes of 5 µm, 7 µm and 10 µm.*

VDSpher® Flash Phases

Flash chromatography is a cost-effective separation method which is used for cleaning and extracting raw materials in synthesis chemistry, combinatory chemistry and also in pharmacokinetics. The normally used irregular silica gels often display limited efficiency. Very high and strong fluctuating metal contents of the silica matrix in different batches can influence a separation considerably and have a negative effect on the reproducibility.

Through an improvement in selectivity and by using spherical silica gels like VDSpher Flash phases, the separation efficiency and therefore the capacity can be increased significantly. The VDSpher Flash phases are characterised by low back pressure, controlled low metal content, narrow particle size distributions and low costs, i.e., a very good price/performance ratio: A narrow particle size distribution with low fine-particle proportion provides a good resolution and therefore a high capacity of the filled Flash Chromatography column at a low back pressure.
In the context of selectivity, the phases listed in **Table 15** (cf. page 26) correspond to the comparable phase modifications of VDSpher phases for the analytical HPLC (reference columns with smaller particle sizes are available on request). This enables a simple upscaling from HPLC to flash chromatography. VDSpher Flash AP is ideally suited in the normal phase mode as special amino phase for the purification of alkaline compounds, in the RP mode (HILIC) for sugar separations and they can also be used as adsorbents (metal scavengers).
A primary and a secondary amine are linked in the phase VDSpher Flash DAP. This results in a higher ion-exchange capacity. When working in the normal phase mode, there are shorter retention times as compared to the VDSpher Flash AP phase.
In addition to the particle sizes listed in **Table 15**, VDSpher Flash bulk materials with a particle diameter of 120 µm (70 – 170 µm particle size distribution) are also available.
The VDSpher Flash bulk materials can be used in the following separation technologies:

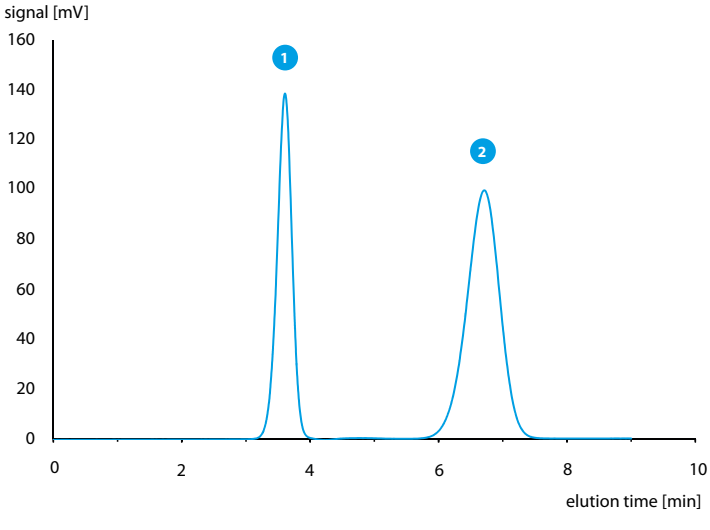
– Flash 30 µm:	HPLC separations / flash chromatography
– Flash 55 µm:	flash chromatography
– Flash 120 µm:	column chromatography (open column)

VDSpher® Flash Phase Modifications			
Phase	**Particle Size [µm]**	**Endcapping**	**pH Range**
Flash 60 SIL	55	no	2 - 8
Flash 75 SIL	30, 55	no	2 - 8
Flash 100 SIL	30, 55	no	2 - 8
Flash 100 C18-E	30	yes	3 - 7.5
Flash 100 C18-H	30, 55	yes (polar)	3 - 7.5
Flash 100 C18-M-E	30, 55	yes	2 - 7.5
Flash 100 C4-H	30, 55	yes (polar)	3 - 7.5
Flash 100 C4-M-E	30, 55	yes	2 - 7.5
Flash 100 Diol	30, 55	no	2 - 8
Flash 100 AP	30, 55	no	2 - 8
Flash 100 DAP	30, 55	no	2 - 8

Table 15: *VDSpher® Flash phase modifications.*

Standard Separation for Performance Determination of VDSpher® Flash Columns

Compounds (1) Benzene (2) Nitrobenzene



Use a better column

VDSpher®

Column 55 µm, 250 mm x 4.6 mm
VDSpher® Flash 75 SIL

Chromatographic conditions

Solvents: A: *n*-Heptane
B: 1,4-Dioxane

Method: isocratic
90% A / 10% B

Flow rate: 1.0 mL / min

Pressure: 0.1 MPa

Temp.: ambient temperature

Detection: UV 254 nm

For more information, quotation requests and purchase orders please visit our website (www.vdsoptilab.de) or contact us: info@vdsoptilab.de

Figure 14: *Standard separation with VDSpher® Flash 75 SIL.*