Chemistry of Reversed Phase HPLC Columns

Introduction:

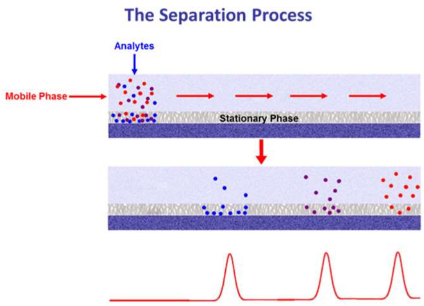
Do You Know Your Stationary Phase Chemistry?

- When the question is asked, which stationary phase is used in an HPLC method, the answer is almost always:
  - The **manufacturers** name (**Agilent**, **Phenomenex**, **Thermo**, **Waters**, **YMC**, etc.) and
  - The phase **brand** name (**Zorbax**, **Luna**, **Hypersil**, **Inertsil**, **Discovery, Symmetry**, etc.) and sometimes
  - The phase chemistry (**C18**, **C8**, **CN**, etc.), but usually only when the bonded phase is an alkyl silica
- When the phase is anything other than a conventional alkyl silica, the fashion is to know the phase by its **trade name** — for example, Agilent Zorbax **Bonus-RP**

Introduction:

Do You Know Your Stationary Phase Chemistry?

- But what is the chemistry of this phase?
- What are the mechanisms of interaction with the analyte and hence how is retention and selectivity gained from this phase?
- How can we troubleshoot separation problems or develop suitable methods without a good knowledge of the bonded phase chemistry?
- It's also very important to realize that, even if the bonded phase chemistry is known, a significant contribution to retention and selectivity will be made by the underlying silica and any surface treatments which are carried out after the stationary phase is bonded



Section 1

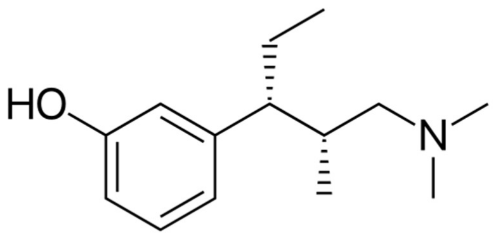
Mechanisms of Interaction and Retention in RP HPLC

Interactions in RP HPLC

- Although reversed phase retention and separation are, mainly, contributed by **hydrophobic interactions**, there are three primary mechanisms of interaction that determine overall chromatographic behavior
- This includes:
  - **Hydrophobic interactions**
  - **Polar** (H-bonds; dipole - dipole) **interactions**
  - **Ionic** (ion exchange) **interactions**
- In addition to these three, **steric selectivity**, or shape selectivity, can sometimes play a role

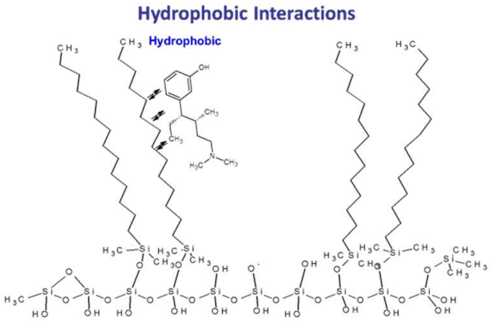
Interactions in RP HPLC

- Let us explore the 3 major mechanisms of interaction using the example of **tapentadol** as a typical small-molecule pharmaceutical compound
- This molecule has polar, hydrophobic, and ionic components in its structure



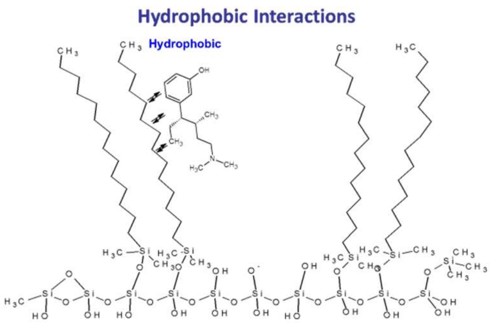
Hydrophobic Interactions

- In RP HPLC, the primary mechanism of retention is a weak and transient **hydrophobic interaction** which includes **hydrophobic** effect and **van Der Waals** forces between the non-polar stationary phase ligand (C18) and the hydrophobic part of the molecule (carbon backbone)



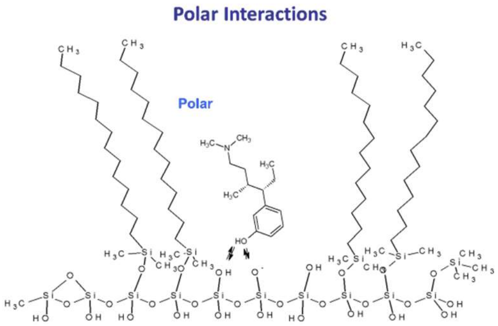
Hydrophobic Interactions

- A fair estimate of retention can be predicted based on **Log P** value, which is octanol/water partition coefficient
- The more hydrophobic a molecule is, the higher **Log P** value it has, which translates to a longer retention in RP HPLC



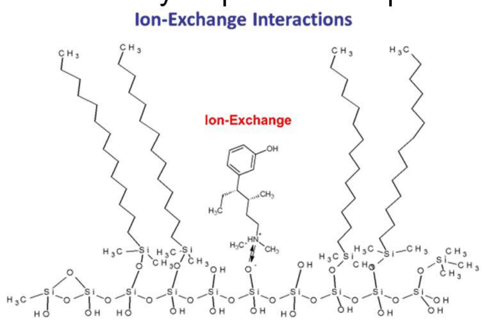
Polar Interactions

- These interactions (relatively weak and transient) occur between polar functional groups of analytes and residual silanols or other surface polar groups in the stationary phase through hydrogen bonding and dipole-dipole interactions



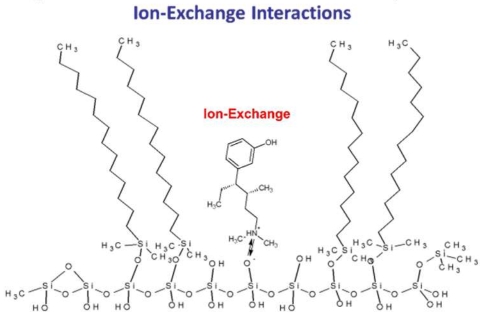
Ion Exchange Interactions

- These silanols can become deprotonated and acquire a negative charge, then can interact ionically with positively charged basic analyte molecules
- These ion-exchange interactions are very strong and slow in contrast to hydrophobic and polar interactions



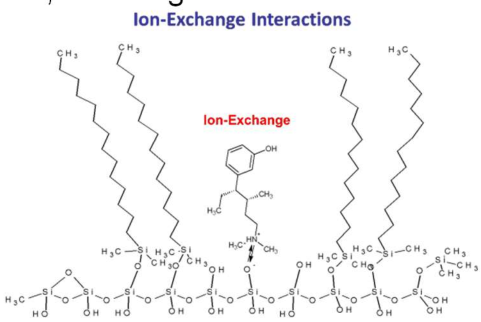
Ion Exchange Interactions

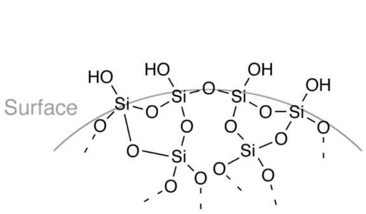
- Therefore, analytes experience different rates of interaction occurring which can cause peak distortion
- This is a classic example of basic analytes interacting with residual silanols, which can be controlled by suppressing silanol ionization at low pH

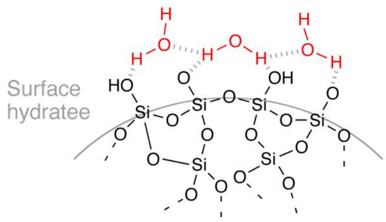


Ion Exchange Interactions

- Most RP media is based upon silica bonded with a non-polar stationary phase, such as C18
- Chromatographic manufacturers try to achieve complete end-capping of all silanol groups, however, it cannot reach 100%, resulting in residual surface silanol groups





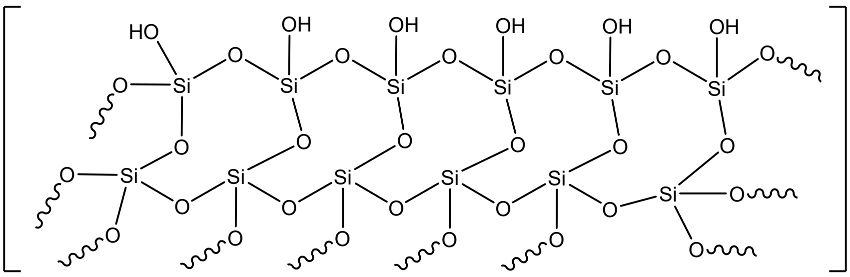


Section 2

Chemistry of Silica Gel

Chemistry of Silica Gel

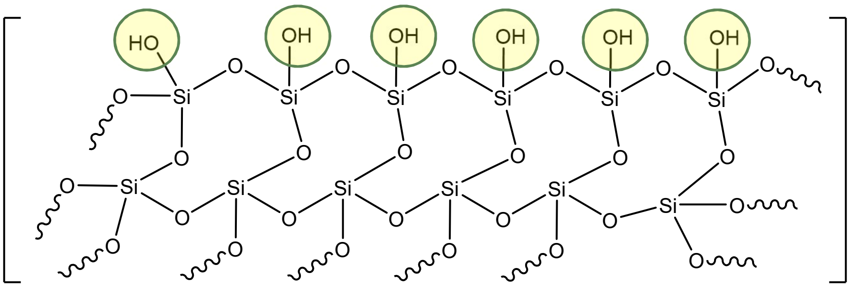
- Unsubstituted high purity silica
  (also used as a stationary phase for NP and HILIC HPLC)



- Various types of silica are used to construct stationary phase particles
- Traditional polysiloxane is a fully inorganic polymer having reasonable pH and mechanical stability

Chemistry of Silica Gel

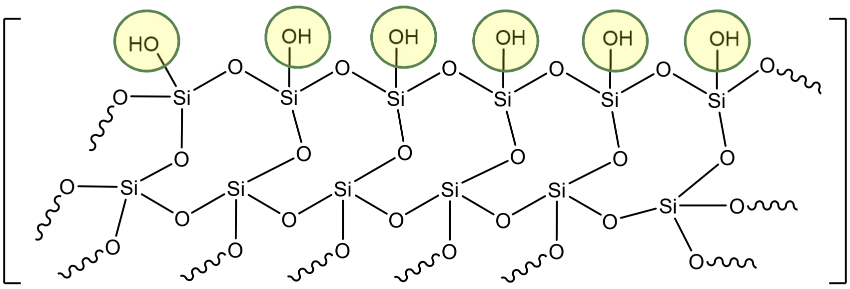
- Surface silanol (Si-OH) groups of a polysiloxane matrix are used to chemically attach the bonded phase ligand



- Chemical nature and environment of these siloxanes can have influence on the retention and especially the selectivity of a stationary phase

Chemistry of Silica Gel

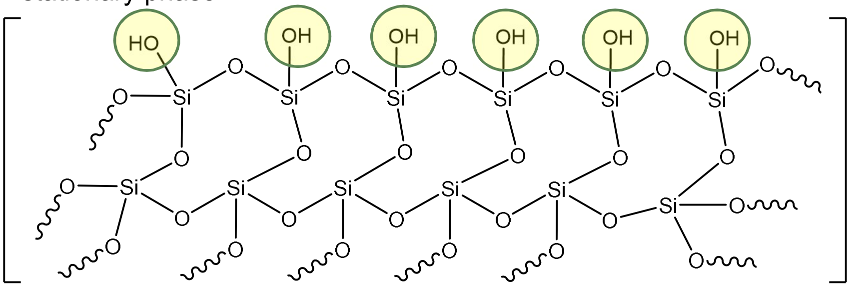
- Surface silanol (Si-OH) groups of a polysiloxane matrix are used to chemically attach the bonded phase ligand



- Silanol groups are inherently acidic (pKa typically 3.5 – 4.5) and are therefore either polar (eluent pH < 2.5) or anionic (eluent pH > 5.5) and can interact with our analytes to alter the interaction of analyte with the stationary phase

Chemistry of Silica Gel

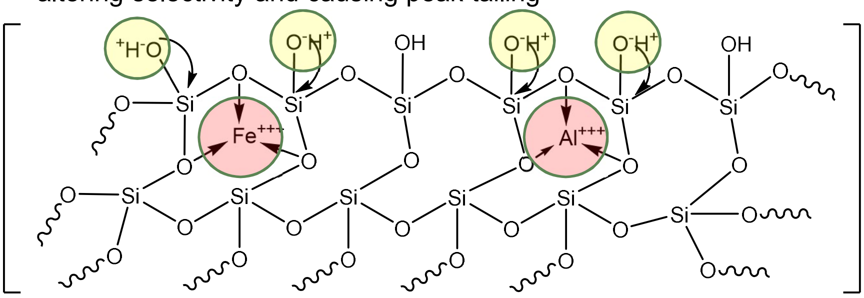
- If analyte contains polar functional groups, the **desired** analyte – stationary phase interactions are supplemented by **secondary** polar-polar interactions, which can significantly affect the selectivity of the stationary phase



- At pH above pKa, silanols will be ionized (Si-O$^-$), **electrostatic interactions** will be strong and can cause significant secondary interactions with basic analytes, which is often seen as **peak tailing** in the chromatogram

Chemistry of Silica Gel

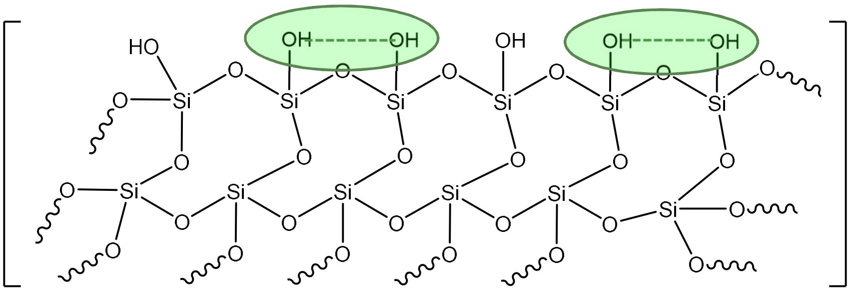
- Inclusion of metal ions close to the surface of the silica matrix can make the silica surface more acidic (due to a co-ordination effect) and thus induce secondary interactions with polar or ionogenic analytes, altering selectivity and causing peak tailing



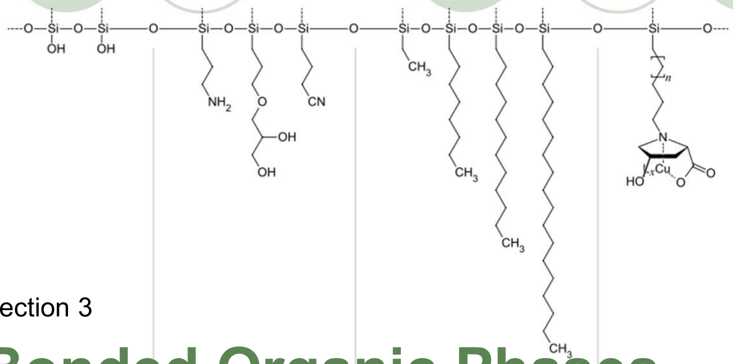
- Most modern "Type B" silicas are washed to remove the majority of metal ions which are known to give rise to the most pronounced secondary effects (**Fe** and **Al** are known to be most significant)

Chemistry of Silica Gel

- A special case in which adjacent silanol groups are hydrogen-bonded with each other thus reducing their acidity, which results in lower tailing but retains ability to provide polar interactions with analytes



- Manufacturers can ensure a high degree of inter-silanol hydrogen bonding and this is a typical feature of modern “Type B” silica.

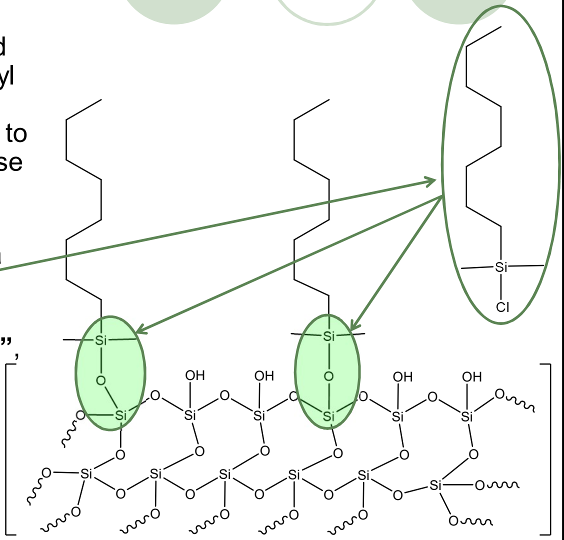


Section 3

Bonded Organic Phases

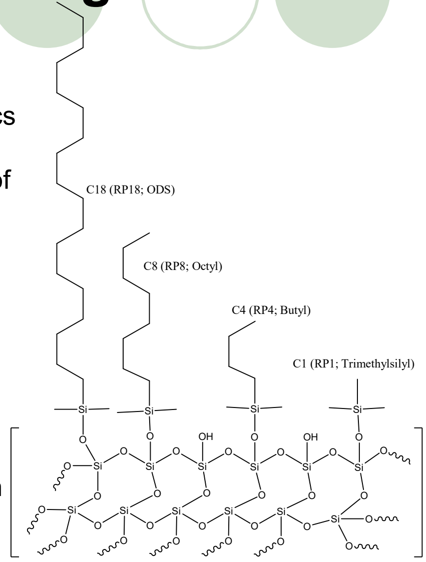
Bonding Organic Phases to Silica

- Alkyl bonded-phase ligand (in this example: “C8”): silyl ether linkage (Si-O-Si), or **siloxane bond**, is formed to anchor the stationary phase to the silica surface
- The reagent to bond the organic phase to the silica surface is on the right
- Such alkyl ligand is called **“monomerically bonded”**, or **“monofunctional”**



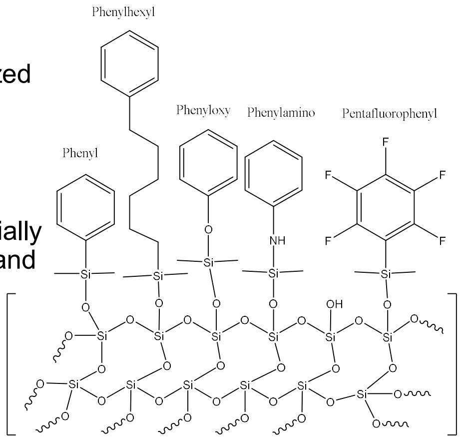
Various Bonded Organic Phases

- There are numerous bonded organic phases having different chemical structures and physical characteristics
- This allows to find the most suitable stationary phase to a wide diversity of analytes thus designing substrate-specific HPLC procedures
- Bonded organic phase may be fully **aliphatic** having a different length of hydrocarbon chain (from one to 30 carbon units)
- Retention mechanism for such columns is based, mainly, on **hydrophobic interactions**
- Different length of hydrocarbon chain provides different lipophilicity / hydrophobicity of a stationary phase



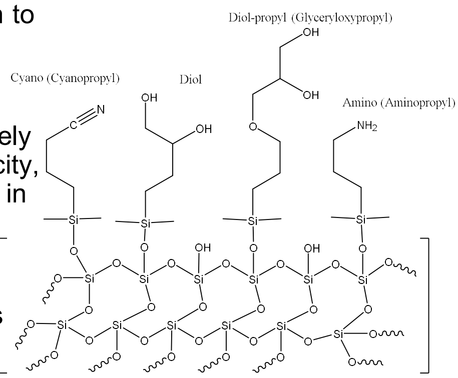
Various Bonded Organic Phases

- Bonded organic phase may contain **aromatic rings**, sometimes with combination of aliphatic chain or additional functionalities
- These substituents are characterized with rich “**π-clouds**”
- For such columns, mechanism of retention is enhanced by **π-π interactions**
- Such stationary phases are especially useful for separation of **aromatic** and **heterocyclic** compounds, as well as analytes containing polyconjugated olefin chains, which also have pronounced systems of **π-electrons**



Various Bonded Organic Phases

- In addition, bonded organic phase may contain **very polar** functional groups, such as cyano (nitrile), amino and hydroxyl (most often, diol)
- Such stationary phases, in addition to “Bare silica” (without any bonded organic phase) are mostly used in Normal Phase (**NP**) separations
- Stationary phases with comparatively low polarity and moderate lipophilicity, such as **Cyano**, may be used both in **NP** and **RP** HPLC, depending on which solvents are comprising the mobile phase
- Bare silica, Diol and Amino phases are also employed in **HILIC**



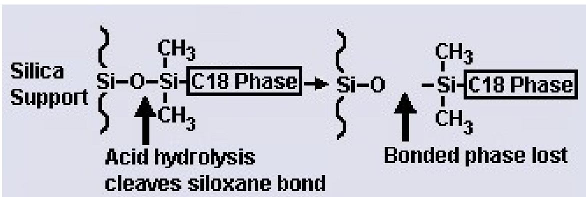
Various Bonded Organic Phases

- The most commonly used types of stationary phases are shown in order of their polarity / hydrophobicity and, as a result, order of retention of non-polar analytes

Stationary Phase							
C18	C8	C4	Phenyl	Cyano	Diol	Amino	Silica

Increasing Polarity / Decreasing Hydrophobicity →

← **Increasing Retention of Non-Polar Analytes**

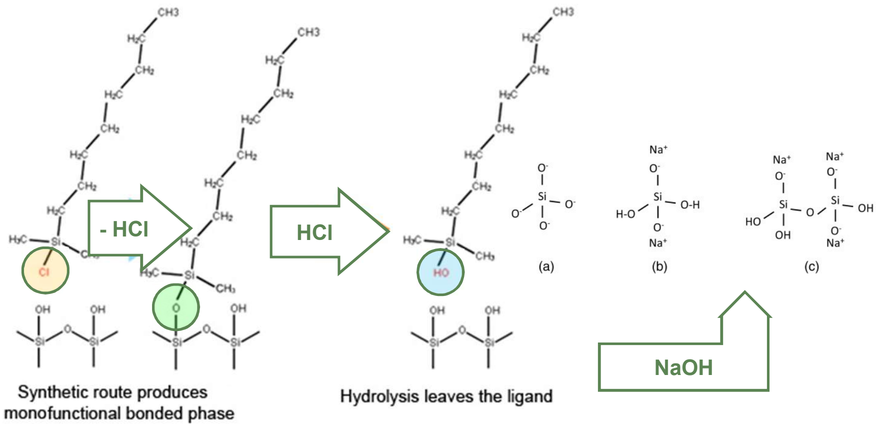


Section 4

Problems of Silica-Based Stationary Phases

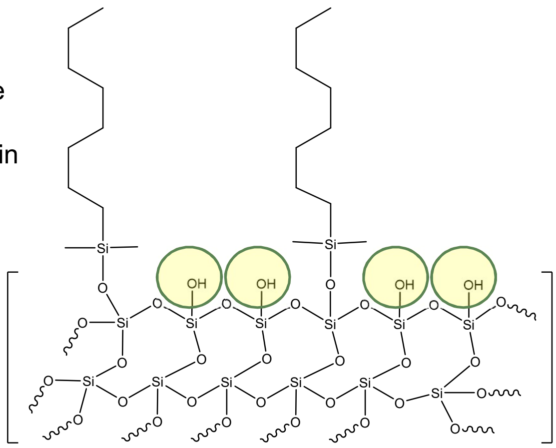
Hydrolytic Stability at Extreme pH

- At **low pH** this ether linkage is susceptible to hydrolysis…
- At **high pH** silica begins to dissolve to form inorganic silicates…



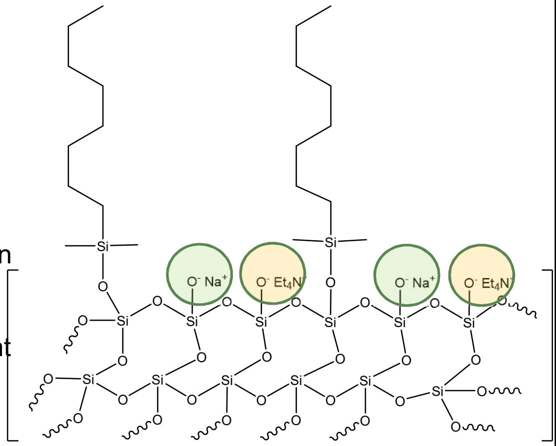
Activity of Residual Silanols

- This “bonding” reaction cannot substitute all the silanol groups of silica surface completely due to “bulkiness” of the molecule of bonded phase
- On the silica surface remain unsubstituted (“residual”) silanol groups which are responsible for secondary polar-polar interactions
- This can **modify** the **retention mechanism** (i.e., add ion exchange to the desired hydrophobic interactions) and, as such, **change selectivity** of the stationary phase



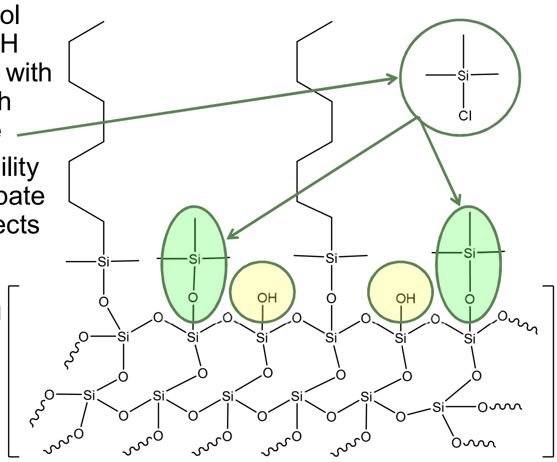
Deactivation of Residual Silanols

- To overcome undesired silanol activity, lone silanol group may be deactivated by adding inorganic or organic bases which will "neutralize" the acidity of silanol groups generating stable salts – silanolates (by non-covalent bonding)
- Such treatment produces stationary phases based on **base deactivated silica** (labeled as "**BDS**")
- Unfortunately, non-covalent bonding is less stable than covalent, and the effect of **base deactivated silica** lasts only for a limited time



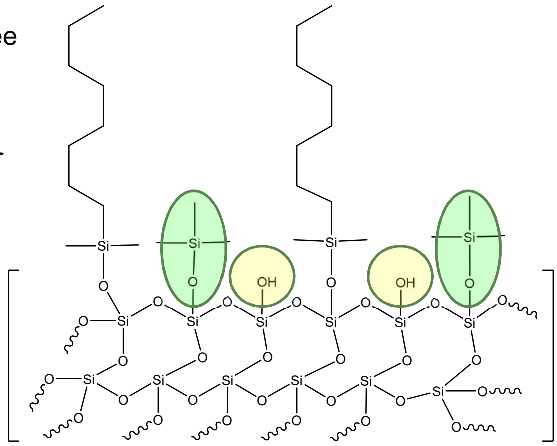
Endcapping of Residual Silanols

- Much more effective way to deactivate undesired silanol activity is substitution of OH (creating covalent bonds) with endcapping reagents, such as **trimethylsilyl chloride**
- This treatment reduces ability of silanol groups to participate in secondary retention effects with polar and ionogenic analytes and therefore will help to reduce peak tailing
- Such treatment is called "**endcapping**"



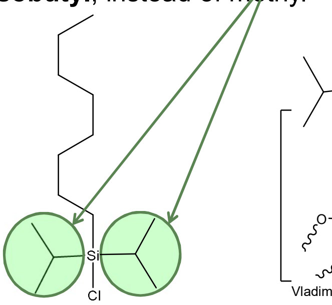
Endcapping of Residual Silanols

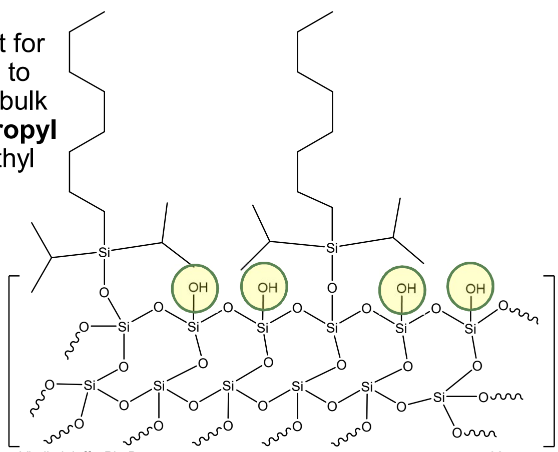
- There are innumerable approaches and reagents used to improve the degree of endcapping
- However, even the most reactive reagents will still leave a significant number of unreacted silanols post treatment
- It is also possible to use more polar non-ionogenic, reagents to alter the selectivity of the phase



Alternatives to Endcapping

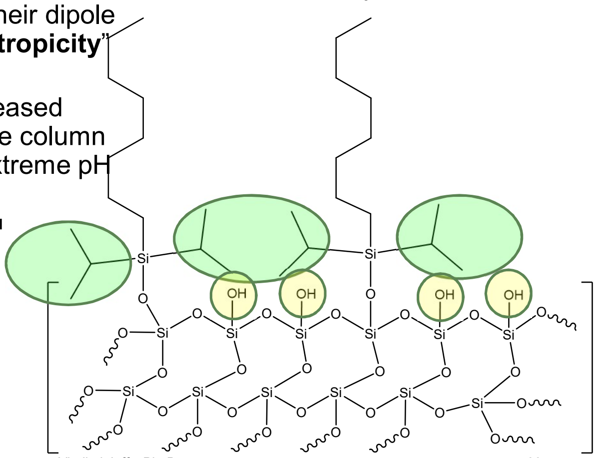
- As an alternative to the "**chemical endcapping**", comes a protective "**steric hindrance**" effect which makes residual silanols inaccessible for direct chemical interaction
- For this purpose, a reagent for bonding the organic phase to the silica surface contains bulk side groups, such as **isopropyl** or **isobutyl**, instead of methyl





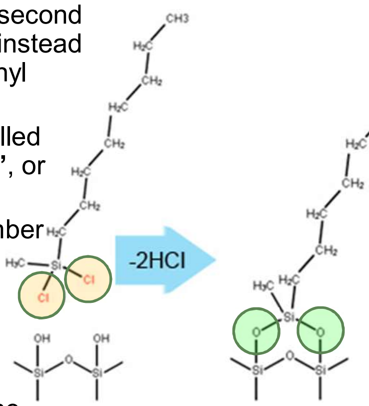
Alternatives to Endcapping

- Such bulk side groups provide effect of "**masking**", or "**shielding**" which prevents residual silanols from direct contact with analytes, but retains them, thus employing their dipole to ensure high "**cosmotropicity**" of the stationary phase
- This also provides increased protection of silica of the column against hydrolysis at extreme pH
- Example: columns **Zorbax Eclipse XDB™** (Extra Dense Bonding)

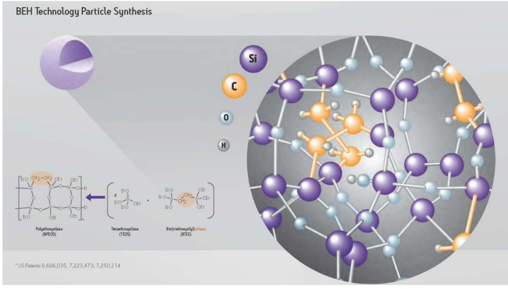


Alternatives to Endcapping

- Another way is a difunctional modification, where a second **Cl** group is employed instead of one of the side methyl substituents
- Such alkyl ligand is called **"dimerically bonded"**, or **"difunctional"**
- It "covers" a twice number of silanol groups, thus being an alternative to a classic endcapping
- Such bonded phases ensure much higher hydrolytic stability of the stationary phase at low pH
- Example: **Zorbax SB™** (Stable Bond) columns



Synthetic route produces difunctional link to the silica surface - protection from hydrolys is at low pH

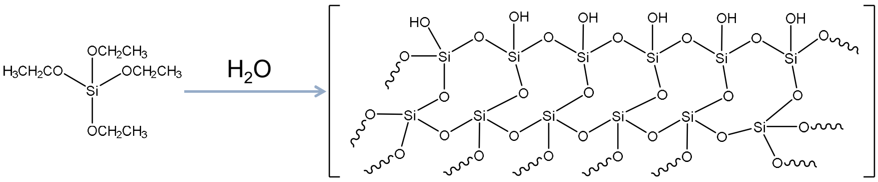


Section 5

High Purity Type B and "Hybrid" (Organic-Inorganic) Silica

High Purity "Type B" Silica

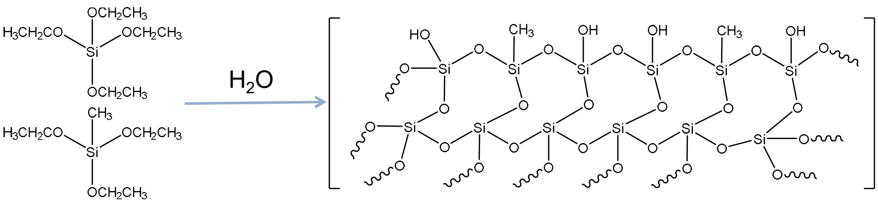
- Most commonly used monomer for production of synthetic silica is **tetraethoxysilane**



- Controlled hydrolysis of this monomer provides high purity "**Type B**" silica with well developed and very uniform polyhydroxylated surface

Chemistry of “Hybrid” Silica

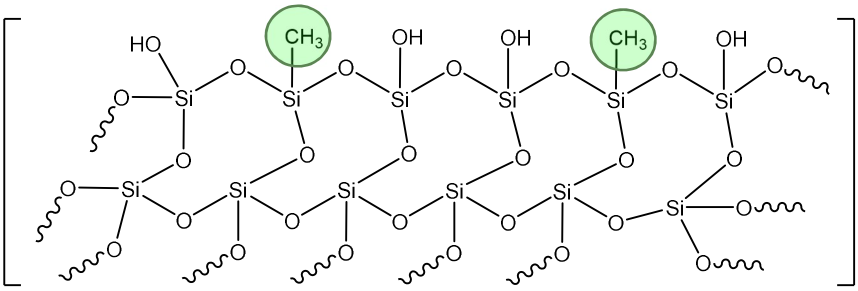
- Some manufacturers use “**hybrid**” organic / inorganic silanes (**methyl**-polysiloxane or **ethyl**-polysiloxane) to impart better pH stability
- For production of such “hybrid” material, to **tetraethoxysilane**, a certain amount of **methyltriethoxysilane** is added



- Controlled hydrolysis of such mixture of monomers provides “**hybrid**” silica with a surface well protected against hydrolysis at **extreme pH**

Chemistry of “Hybrid” Silica

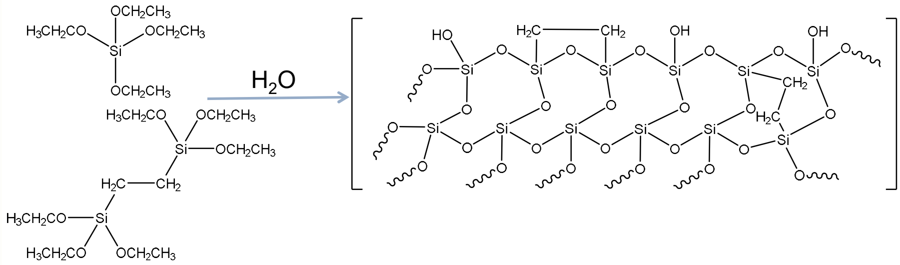
- Stationary phases manufactured (after bonding of organic phase) from such “**hybrid**” silica are, roughly, equivalent to **endcapped** phases (these phases are sometimes called “**forcapped**”)



- “Hybrid” silica for **XTerra™** columns (Waters)
- One of the drawbacks of “**hybrid**” silica of this type is its mechanical stability: it does nor afford very high pressures and may crash…

Chemistry of “Hybrid” Silica

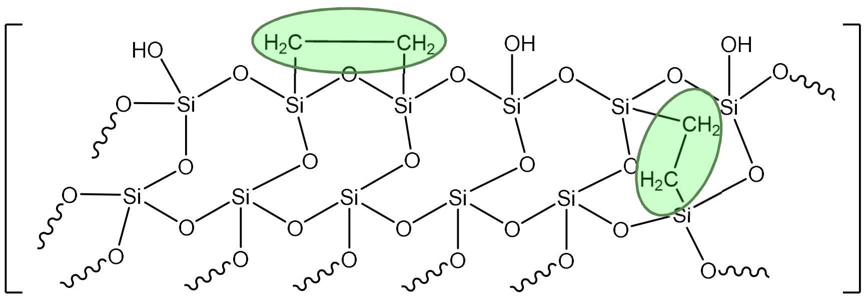
- To overcome the problems of mechanical stability of “**hybrid**” silica, a “dimeric monomer**”, 1,2-bis(thiethoxysilil)ethane**, is taken instead of **methyltriethoxysilane**



- As a result, the organic part of this “hybrid” silica appears in the form of “**ethylene bridges**” which cover and protect the surface and also act as a **cross-link** between the layers of polymeric silica
- Such “**ethylene bridges**” instead of **methyl** groups substantially improve flexibility (and mechanical stability) of the entire structure

Chemistry of “Hybrid” Silica

- Highly improved mechanical stability of “bridged” structures allowed to move to a novel technology in HPLC which requires working at extra high pressures (**above ~600 bar / ~8,000 psi**) - **UHPLC**



- “Hybrid” silica for **BEH (Bridge Ethylene Hybrid)** columns: **XBridge™** and **Acquity™ UPLC** (Waters)

ZirChrom®

SACHTLEBEN

SUPELCO Analytical

Shodex™ HPLC Columns

HAMILTON

Section 6

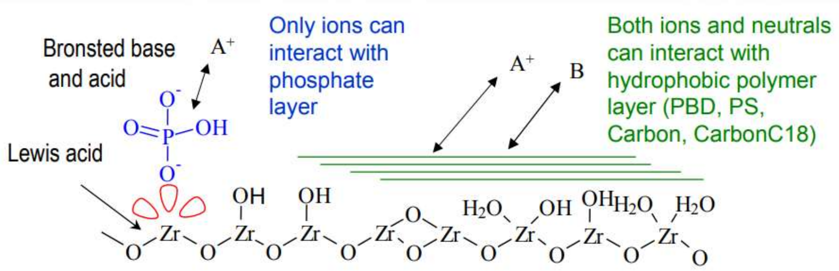
Non-Silica Based Phases

Non-Silica Based Phases?

- Free silanols on the surface of silica are responsible for undesirable interactions of basic and acidic compounds and the stationary phase (i.e., bad peak shape, low efficiency) as well as low chemical stability
- To overcome this, non-silica based stationary phases have been developed, which can afford increased thermal and chemical stability and sometimes have selectivity different from those based on silica, such as:
  - Zirconium oxide (Zirconia)
  - Titanium oxide (Titania)
  - Aluminium oxide (Alumina)
  - Porous graphitized carbon (PGC)
  - Organic polymers

Zirconia Phases

- Zirconia, as a transition metal oxide, has very rich, reproducible surface chemistry
- Coated zirconia (Carbon and PBD) has mixed-mode surface properties (RPC and IEC) which allow simultaneous nonpolar and polar interaction
- Significant Lewis acid character exists on zirconia phases

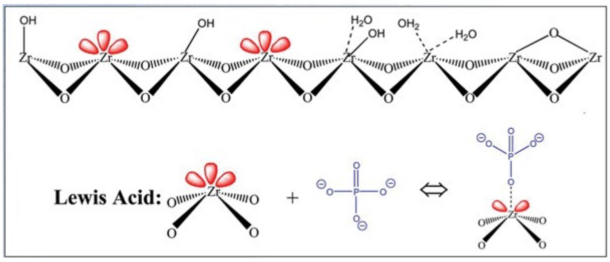


Zirconia Phases

- The primary advantage of zirconia over either silica or polymeric stationary phases is its extreme chemical and thermal stability
- Unlike silica, zirconia is completely stable over the entire pH range and at column temperatures as high as 200°C
- It has very high mechanical strength and high efficiencies

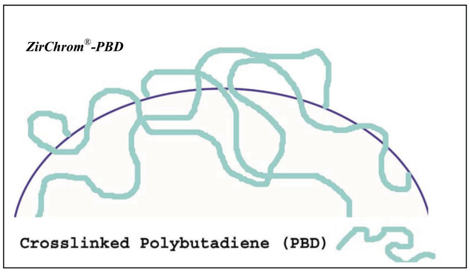
Zirconia chemistry is dominated by Lewis acid-base reactions

Lewis Acid: $Zr^{4+}: H_2O + RPO_3^{2-} \rightleftharpoons Zr^{4+}: RPO_3^{2-} + H_2O$



Zirconia Phases

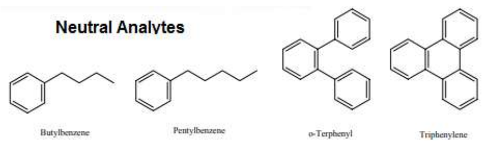
- ZirChrom®-PBD offers an ultra-stable reversed-phase alternative to a C-18 silica phase.

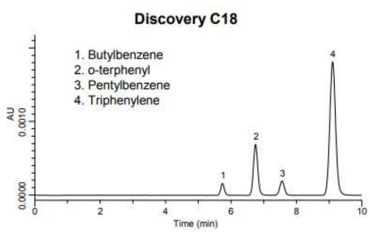


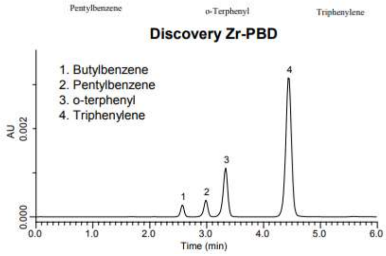
- It is not as hydrophobic as typical C18-silica phases, so less organic solvent is required.
- ZirChrom®-PBD is more stable than any silica phase — both chemically and thermally (pH 1-14, up to 200°C) and is superior to C-18 for the separation of basic compounds.

Zirconia Phases

- Strong efforts have been made by the silica-based column manufacturers to mask the effects of silanols
- There is an increasing need for columns which have different selectivity than silica-based bonded phases
- Zirconia-based stationary phases provide unique selectivity toward acidic, basic and neutral analytes

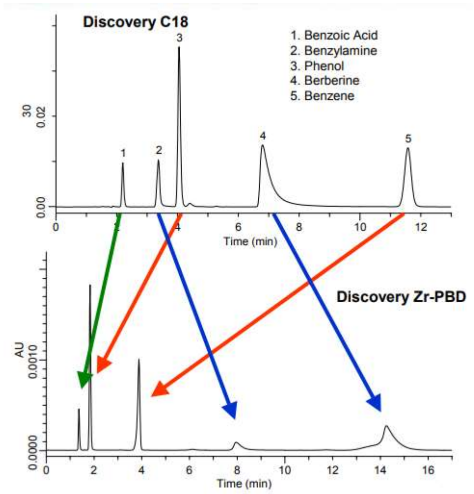






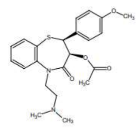
Zirconia Phases

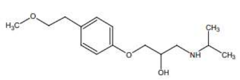
- The unique selectivity arises from alternative analyte-stationary phase interactions
- The combination of ion-exchange and partition mechanisms results in very different chromatography
- Examples of Zirconia-based phases:
  - ZirChrom PBD
  - ZirChrom C18
  - DiamondBond C18
  - Discovery Zr-PBD



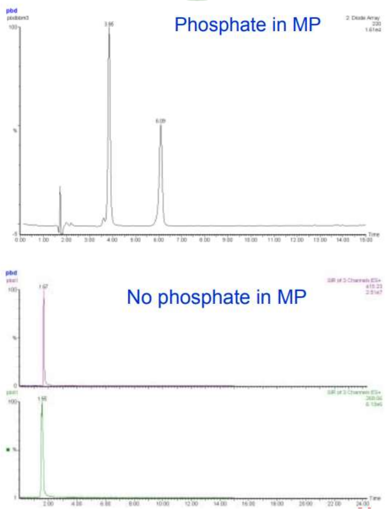
Zirconia Phases

- Buffers play an additional role in the chromatography compared to silica-based systems
- Phosphate is responsible for providing a negative charge on Zr surface resulting in significant ion-exchange capabilities
- Retention of bases is shown to be minimal without the addition of phosphate



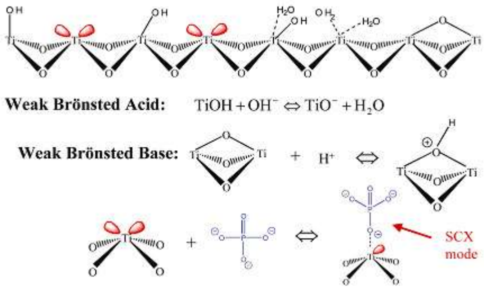


Example: Diltiazem and Metoprolol



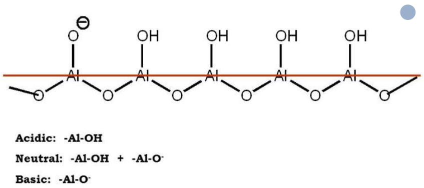
Titania Phases

- Sachtopore is HPLC stationary phase comprised of titanium dioxide (Titania)
- The surface chemistry of titania is quite different from more traditional silica and polymer stationary phases, but is closely chemically related to zirconia. Like zirconia, the dominant surface features of titania includes: Brönsted acid, Brönsted base, and Lewis acid sites



Alumina Phases

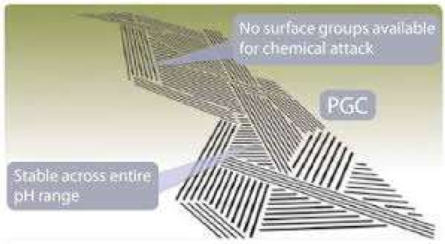
- Alumina has a higher surface area than zirconia and titania
- Examples:
  - GammaBond RP-1 and RP-8 (ES Industries)
  - Aluspher RP-Select B (Merck)
- Alumina-based columns show exceptional stability at high pH levels
- Aluspher RP-Select B possesses high ion-exchange properties, and it is highly retentive for aromatic compounds and for aromatic bases – pyridines – in neutral mobile phases with phosphate buffer

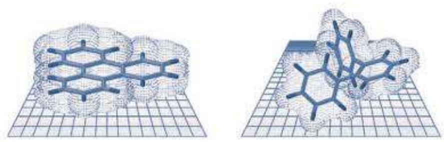


- Basic pharmaceuticals: desipramine, amitriptyline and chlorpromazine – were separated using gradient elution under alkaline conditions, using 15 mM NaOH buffer

Porous Graphitic Carbon Phases

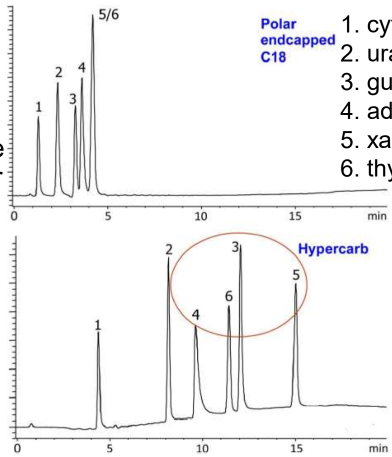
- The PGC stationary phase is commercialized by Thermo-Fisher under the brand name **Hypercarb**
- Advantage of using PGC for analysis of basic compounds is due to its high chemical and thermal stability and high retentivity
- These properties allow to separate basic compounds in their neutral form at high pH and provide increased retention for hydrophilic compounds





Porous Graphitic Carbon Phases

- Typical reversed phase materials are hydrophobic: they were developed for the retention and separation of hydrophobic compounds
- On these phases, polar ionizable compounds are eluted at or near the dead volume of the column
- Hydrophilic drugs, which are poorly retained in the RP mode, could be analyzed in reversed-phase using Hypercarb
- This stationary phase is highly retentive because it consists of 100% carbon



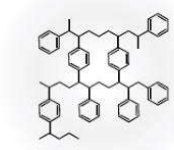
1. cytosine
2. uracil
3. guanine
4. adenine
5. xanthine
6. thymine

Organic Polymer-Based Phases

- Polymer-based materials have been on the market for more than 30 years Crosslinked styrene-divinylbenzene and methylmethacrylate copolymers are the most widely used
- These materials show high pH stability and chemical inertness
- Their rigidity and resistance to the swelling in different mobile phases is dependent on the degree of crosslinkage
- Problems arise when labile samples become irreversibly bound to the silanol groups present on C8 and C18 HPLC columns

Organic Polymer-Based Phases

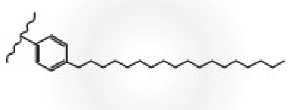
- Hamilton polymers are made entirely of polystyrene-divinylbenzene, there are no silanol groups to cause sample loss
- Recovery and quantitation of labile and reactive samples is enhanced
- Unlike silica-based C8 or C18 columns, PRP-1 has no stationary phase coating
- The integral reversed-phase characteristics of the PRP-1 column eliminate the need for special coating techniques
- Since there is no stationary phase to hydrolyze, the column maintains its performance characteristics longer than many C8 or C18 columns

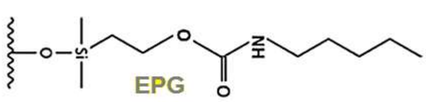


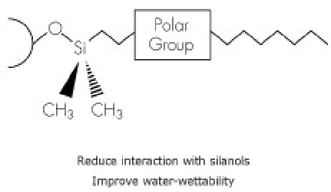
PRP-1

Organic Polymer-Based Phases

- In Shodex columns Asahipak, a polymer particle made of polyvinyl alcohol, with 4 or 5 µm size, is modified with C18 alkyl chains as functional groups
- Such HPLC column has a greater long term pH stability, from 2 to 13
- It contains no silanol groups and provides higher resolution for basic substances, with no peak tailing due to unwanted interactions
- Polymer-based RP material can be used in 100 % water or buffer and a variety of organic solvents
- A C18 column with a stationary phase made of polymer offers more than double the life time compared to silica, because it is chemically more resistant
- The modern column Asahipak ODP (octadecyl-bonded polymer) combines all the advantages of a polymeric RP-C18 column







Section 7

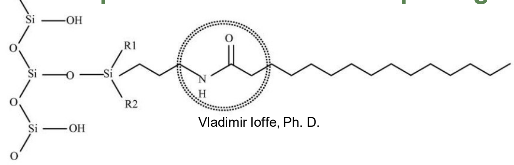
Phases with Embedded Polar Groups

Embedded Polar Groups

- Manufacturers of columns based on deactivated silica try to minimize interactions between polar solutes and silanol groups on the surface of the stationary phase support
- This produces stationary phases with low polarity – and such low polarity phases will all have similar selectivity for polar compounds
- This means that if one brand of deactivated column lacks selectivity to adequately separate a pair of polar solutes, any other brands of similarly deactivated columns will also probably lack adequate selectivity
- To optimize the separation, a more polar stationary phase may be necessary
- Unfortunately, stationary phases that have polar characteristics (due to high silanol activity) will always exhibit poor peak shape and uncertain reproducibility when separating polar compounds

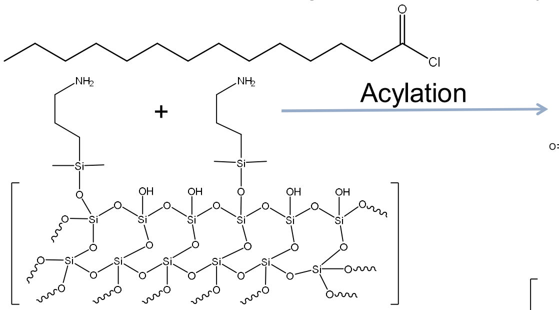
Embedded Polar Groups

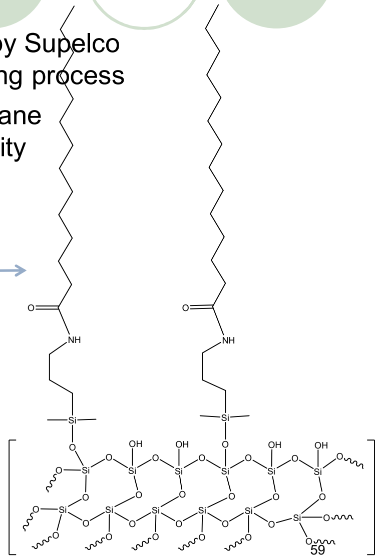
- To design a RP column of higher polarity and to solve the problem of interaction with residual silanols, a new type of stationary phase was developed, with **polar groups**, such as **amides** or **carbamates, "embedded"** in the **bonded phase**
- These **"polar embedded"** phases provide polar selectivity without the poor chromatographic performance associated with stationary phases that have high silanol activity
- The amide or carbamate group shields the silica surface and prevents solutes from directly interacting with silanol groups
- The effect is similar to adding an amine modifier to the mobile phase, thus, there is no need of adding "ion-pair reagents" when developing separations on the **phases with embedded polar groups**



Embedded Polar Groups

- Early generation EPG phases (invented by Supelco in 1988) were based on a two-step bonding process
- The first step – bonding of aminopropylsilane to silica surface creating amine functionality

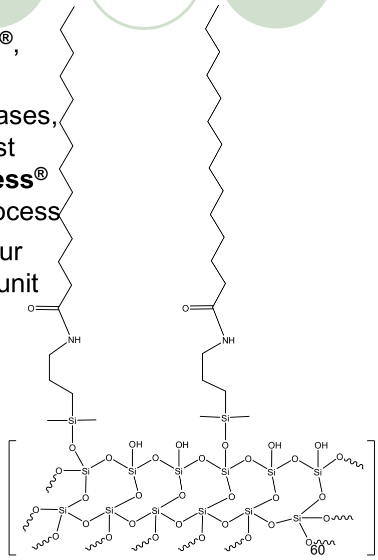




- Then myristoyl chloride acylated amino groups to create a long chain amide

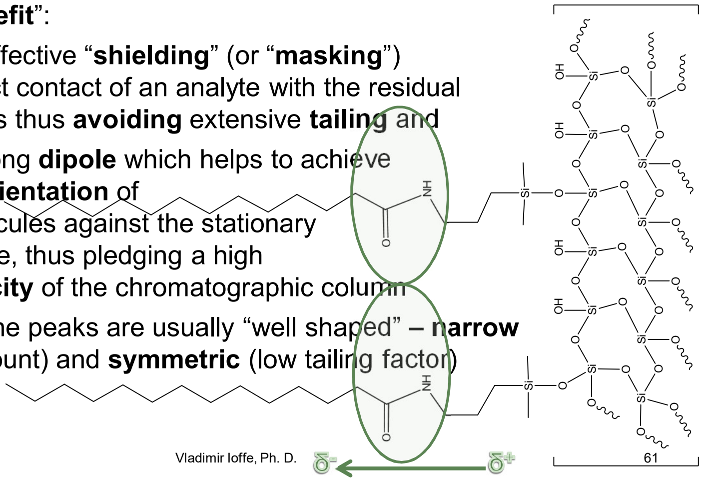
Embedded Polar Groups

- Such early generation EPG phases, **ABZ** ®, suffered from poor reproducibility
- Next generation Supelco "**RP-Amide"** phases, starting from **ABZ+** ® **and** through the most advanced **Ascentis**® and **Ascentis Express**® **RP-Amide**, are produced via one-step process
- In this process, no free amino ligands occur since the amide is introduced as a whole unit
- This one-step bonding process yields excellent batch-to-batch reproducibility



Embedded Polar Groups

- Rich electron cloud of amide group (a lone pair of electrons on **N** atom conjugated with **C=O** double bond) of EPG RP columns suggests a "**double benefit**":
- Provide an effective "**shielding**" (or "**masking**") to avoid direct contact of an analyte with the residual silanol groups thus **avoiding** extensive **tailing** and
- Ensure a strong **dipole** which helps to achieve a **uniform orientation** of analyte molecules against the stationary phase surface, thus pledging a high **cosmortopicity** of the chromatographic column
- As a result, the peaks are usually "well shaped" – **narrow** (high plate count) and **symmetric** (low tailing factor)



Embedded Polar Groups

- A table provides examples of **EPG** phases of various manufacturers which uses the same chemistry based on **amide bond**

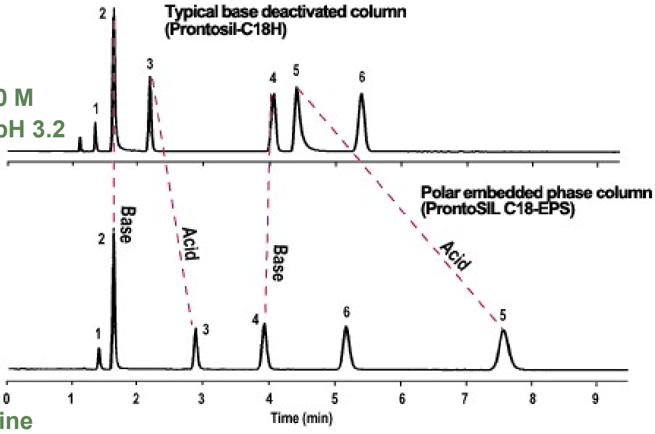
Manufacturer	EPG phases brand names
Supelco	**ABZ+ Plus; Discovery RP-Amide C16; Ascentis RP Amide**
Thermo	**Hypersil HyPURITY Advance; Keystone Prism**
Agilent	**Zorbax Bonus-RP**
Bischoff	**ProntoSIL C18-EPS**
GL Sciences	**Inertsil ODS-EP**
VWR	**Chromegabond ProTech-RP**
Ace	**C18-Amide; Excel C18-Amide**
AMT	**Halo RP-Amide**

Embedded Polar Groups

- Columns packed with polar embedded phases are used in the same way as "conventional" reversed phase columns and the typical separation strategy is also appropriate for these columns
- However, one should expect significant differences in selectivity from polar embedded phases due to their polar selectivity
- The polar selectivity of polar embedded phases comes from the interaction between the embedded polar group and polar solutes
- Generally, polar embedded phases are expected to retain acids **longer** and bases **slightly less** if compared to typical base deactivated reverse phase columns

Embedded Polar Groups

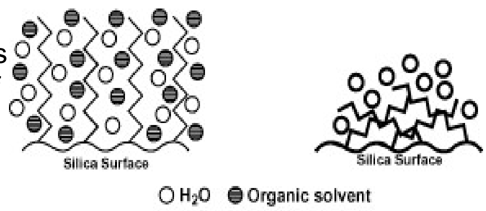
Mobile Phase:
65% Acetonitrile
35% Aqueous 0.050 M
Phosphate buffer, pH 3.2



Sample:
1. Uracil
2. Pyridine
3. Phenol
4. N,N-Dimethylaniline
5. p-Butylbenzoic acid
6. Toluene

Embedded Polar Groups

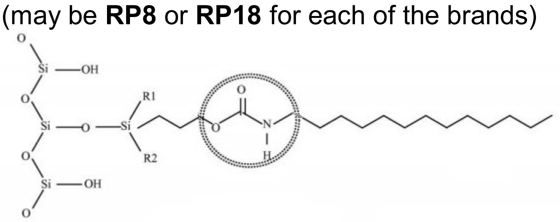
- “Classical” (“conventional”) RP columns (typical phases having C18 or C8 chains bonded), when operating with less than 10% of organic modifier in the mobile phase, usually undergo what many researchers call "**phase collapse**", or "**matting**“
- As phase collapse progresses, the availability of alkyl phase to interact with solutes decreases and retention time decreases
- Additional benefit of columns with embedded polar groups is improved **water-wettability** of the stationary phase: they do not have this problem and are preferred over other reverse phases for high aqueous mobile phase composition



Phase Collapse

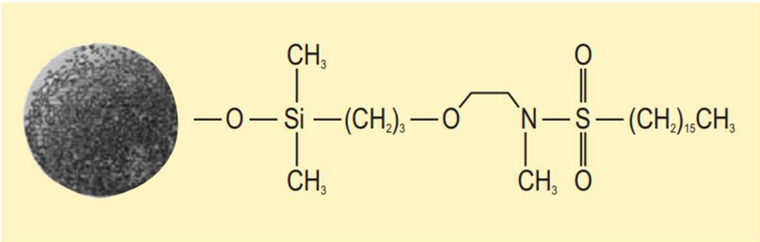
Embedded Polar Groups

- Amide bond has one substantial drawback: it has low hydrolytic stability at elevated pH
- Therefore, EPG columns based on amide function cannot work above pH 8 – 8.5
- At **Waters**, the problem of pH-stability of the columns with EPG has been solved by introducing the more pH-resistant polar embedded group: **carbamine**
- Examples: **Symmetry™ Shield**; **XBridge™ Shield** and **Acquity™ UPLC Shield** (may be **RP8** or **RP18** for each of the brands)



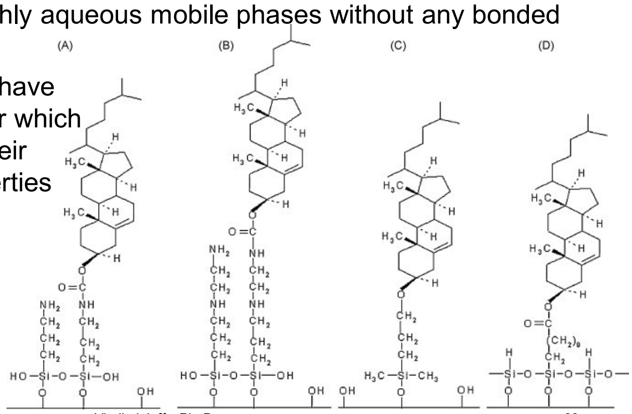
Embedded Polar Groups

- There are also some alternative types of EPG RP columns
- Similar to amide bond, EPG columns based on sulfonamide function, have been introduced by Dionex
- Example: **Acclaim™ Polar Advantage**
- These sulfonamide-embedded, reversed phase columns provide unique selectivity and excellent peak shape for acidic, basic, and neutral analytes.



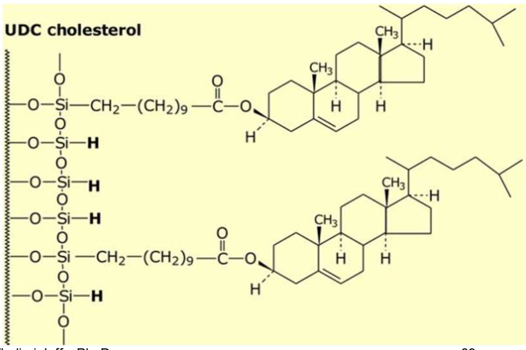
Embedded Polar Groups

- Additional alternative is cholesterol bonded phases with different linkage spacers
- One of the advantages of cholesterol stationary phases is a possibility to use them with highly aqueous mobile phases without any bonded phase collapse
- Cholesterol phases have high resolving power which may be related to their "liquid crystal" properties



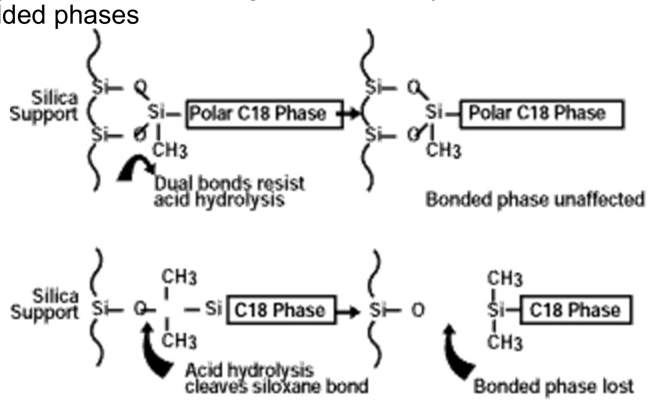
Embedded Polar Groups

- An example of commercially available cholesterol stationary phase is **Cogent UDC-Cholesterol™** of MicroSolve, based on **TYPE-C Silica™** (silica hydride surface) with direct silicon-carbon bonds, which makes such columns hydrolytically stable
- These columns can work in three different chromatographic modes:
  - Reverse phase
  - Normal phase, and
  - Aqueous normal phase (which is not HILIC!)
- A note:
  "UDC" stands for ursodeoxycholic acid



Embedded Polar Groups

- Stationary phases with embedded polar groups, which use dual siloxane bonds to increase bonded phase stability (such as **ProntoSIL C18-EPS**), ensure much higher hydrolytic stability at low pH and actually demonstrate much greater durability than other C18 and polar embedded phases

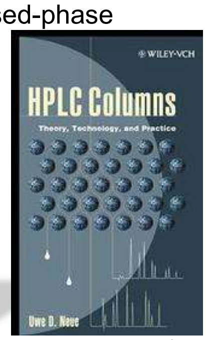


Section 8

“Equivalent” Columns? Alternative Columns?

“Equivalent”, or Alternative Columns

- **Column "equivalency" is actually a can of worms**
- If you have a complex separation, say an impurity profile with closely eluting analytes, your chances are slim to none to find an equivalent column
- If you are doing a USP procedure for content uniformity, with one peak and one internal standard, nearly every reversed-phase column will work for you
- The rest of the world is in between
- **Uwe D. Neue**



"Equivalent", or Alternative Columns

- A useful tool for comparison of the columns having the "same" chemistry was suggested by **Nobuo Tanaka** in 1989

Journal of Chromatographic Science, Vol. 27, December 1989

Chromatographic Characterization of Silica C_{18} Packing Materials. Correlation between a Preparation Method and Retention Behavior of Stationary Phase

Kazuhiro Kimata, Kazufusa Iwaguchi, and **Seiichiro Onishi**
Nacalai-tesque Co., Kaide-Ishibashi, Muko
Kiyokatsu Jinno
Toyohashi University of Technology, School of Materials Science, Toyohashi
Roy Eksteen
Toso Haas Technical Center, Woburn, MA 01801
Ken Hosoya, Mikio Araki, and **Nobuo Tanaka***
Kyoto Institute of Technology, Department of Polymer Science and Engineering, Matsugasaki, Kyoto

"Equivalent", or Alternative Columns

- The "Tanaka Diagram" takes into account 6 critical characteristics of the columns:

Table II. Characterization Scheme for Commercial Packing Materials

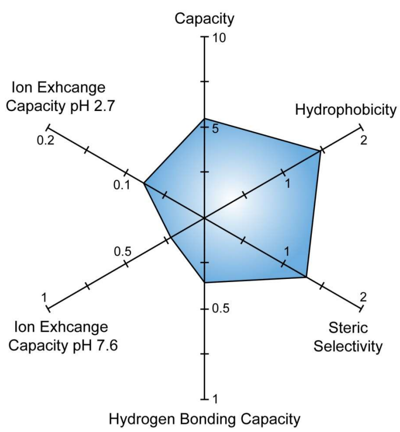
Parameter	Measurement (Mobile phase)	Property of stationary phase	Factors in preparation of the stationary phase
A: k'_{AB}	k' (amylbenzene) 80% CH_3OH	amount of alkyl chains	surface area of silica, surface coverage
B: $\alpha(CH_2)$	k' (amylbenzene) / k' (butylbenzene) 80% CH_3OH	hydrophobicity	surface coverage
C: $\alpha_{T/O}$	k' (triphenylene) / k' (o-terphenyl) 80% CH_3OH	steric selectivity	functionality of silane, surface coverage
D: $\alpha_{C/P}$	k' (caffeine) / k' (phenol) 30% CH_3OH	hydrogen bonding capacity	amount of silanols, endcapping, surface coverage
E: $\alpha_{A/P}$	k' (benzylamine) / k' (phenol) 30% CH_3OH, pH 7.6*	ion exchange capacity at pH > 7	amount of silanols and ion exchange sites
F: $\alpha_{A/P}$	k' (benzylamine) / k' (phenol) 30% CH_3OH, pH 2.7*	ion exchange capacity at pH < 3	amount of ion exchange sites at pH 3, silica pretreatment

* Mobile phase: 30% methanol, 0.02 M phosphate buffer.

“Equivalent”, or Alternative Columns

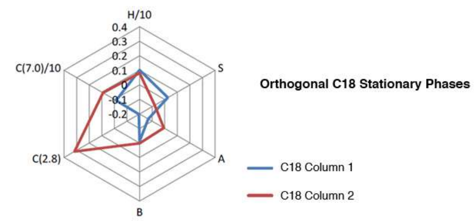
- “Tanaka Diagram” is designed in the shape of six-ray radar, or “Wind Rose”
- Each axis is graduated (gauged) in the units of one of the corresponding stationary phase characteristics:
  - Capacity
  - Hydrophobicity
  - Steric selectivity
  - Hydrogen bonding capacity
  - Anion exchange capacity
  - Cation exchange capacity

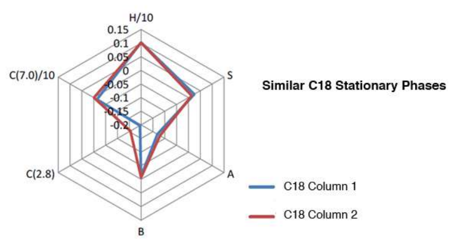
Parameters of the Tanaka Diagram



“Equivalent”, or Alternative Columns

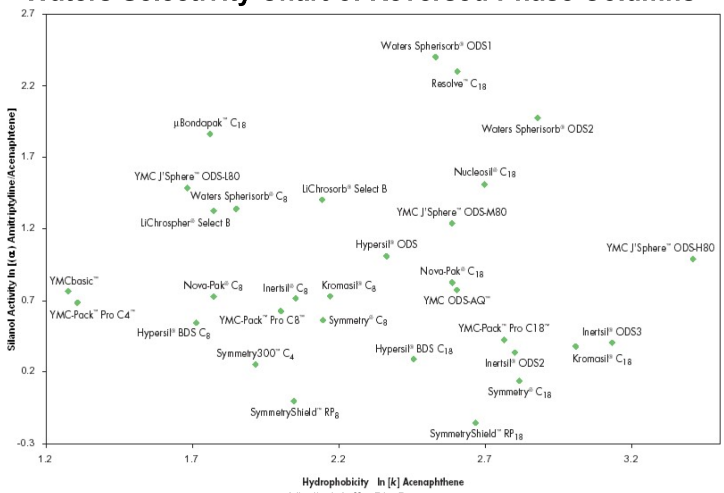
- An example of evaluating similarity / dissimilarity of the pairs of different C18 HPLC columns using the Tanaka Diagrams





"Equivalent", or Alternative Columns

- **Waters Selectivity Chart of Reversed Phase Columns**



"Equivalent", or Alternative Columns

- USP, in association with FDA, NIST and representatives of the "Big Pharma" analyzed various scientific approaches for column comparison and issued a "Stimuli" article in the *Pharmacopeial Forum* in 2005:

Pharmacopeial Forum
Vol. 31(2) [Mar.–Apr. 2005]

STIMULI TO THE REVISION PROCESS
Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

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HPLC Column Classification

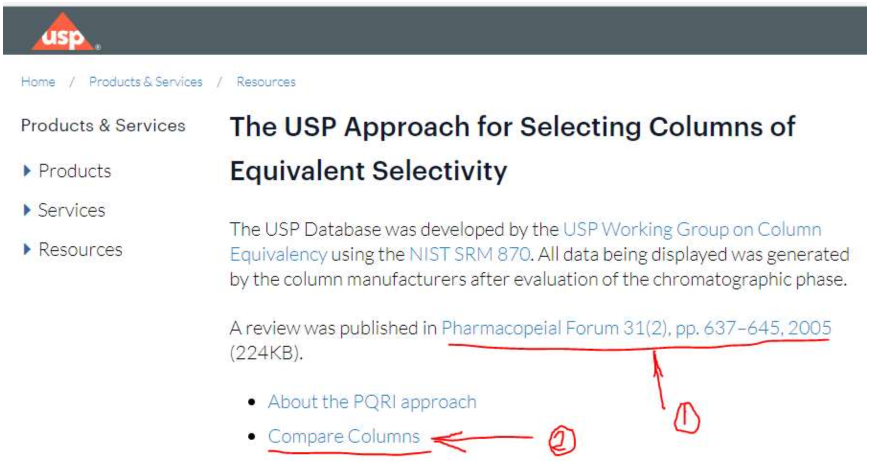
Brian Bidlingmeyer,* *Agilent;* Chung Chow Chan,† *Eli Lilly and Company;* Patrick Fastino,† *FDA;* Richard Henry,* *ThermoHypersil Keystone;* Philip Koerner,* *Phenomenex;* Anne T. Maule,† *3M Pharmaceuticals;* Margareth R.C. Marques,‡ *U.S. Pharmacopeia;* Uwe Neue,* *Waters;* Linda Ng* *USP Pharmaceutical Analysis 2 Expert Committee;* Horacio Pappa,‡ *U.S. Pharmacopeia;* Lane Sander,* *NIST;* Carmen Santasania,* *Supelco;* Lloyd Snyder,† *LC Resources;* Timothy Wozniak,* *USP Pharmaceutical Analysis 2 Expert Committee*

ABSTRACT This *Stimuli* article represents the conclusions and recommendations of the USP Working Group on HPLC Columns. The working group included the five largest manufacturers of HPLC columns in the United States, along with the National Institute of Standards and Technology (NIST) and USP. This work attempts to facilitate the selection of HPLC columns by the analyst when performing a USP test.

“Equivalent”, or Alternative Columns

- Later, based on the analysis performed, USP developed a web tool which can be used for free
- How to use it?
- First, follow the link: http://www.usp.org/resources/usp-approach-column-equiv-tool
- You will open a starting page with the links to
  - The concept paper in “Stimuli” of PF (1)
  - Comparison tool (2)
- Click on the link (2), confirm that you agree with USP requirements – and you will be readdressed to the USP database (with 124 columns) and to PQRI database (with 709 columns) which serve as very useful tools for comparison of similarity / dissimilarity of most commercially available columns
- (The link to PQRI approach is found on the same starting page)

“Equivalent”, or Alternative Columns



“Equivalent”, or Alternative Columns

U.S. Pharmacopeial Convention

USP Database

About USP approach

To find an alternative column for your column of interest, please select this column in the list of columns already evaluated. If your column is not listed, it means that the data from the manufacturer has not been received yet.

218TP 300 C18 (Grace/Vydac)

Then select which parameters are more important for your chromatographic procedure:

CTF: ☑ CFA: ☑ TFA: ☑ BD: ☑

The database will automatically display the first 10 columns that, theoretically, could be equivalent to your column. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically.

Rank	F	Column	Hy	CTF	CFA	TFA	BD	USP Designation	Manufacturer
0	0	**218TP 300 C18**		1.4	7	1.3	5	L1	Grace/Vydac
1	1.75	**Bio Basic 18**	0.9	1.3	2.2	2.1	4.2	L1	Bio-Rad
2	1.77	**Everest C18 300A**	0.8	1.5	2.3	3	4.3	L1	Grace/Davison
3	2.02	**Hypersil PAH**	1.2	2.2	12	2.6	4.6	L1	Thermo Scientific

“Equivalent”, or Alternative Columns

PQRI Database

About the PQRI approach

Select the column that is under evaluation in the list of columns already evaluated. If your column is not listed, it means that the column manufacturer has not sent it for evaluation yet.

Acclaim 120 C18 (Dionex)

You have the option to see the columns that are the most similar to the column of your interest, or the columns that are the most different (for applications in orthogonal methods), by selecting View Different or View Similar.

You are viewing similar columns.

View Different

Select the option Acids present, if there are acids present in the sample, or Bases present, if there are bases present in the sample. Select the pH of the mobile phase. The default is from 2.8 up to 7.0. pH values outside this range are not going to be accepted.

Acids present: ☐ Bases present: ☐ pH of mobile phase: 2.8 Update

The database will automatically display the first 10 columns that, theoretically, could be equivalent or very different to/from your column, depending on the option you selected. The column with rank 0 is your column. The smaller the F value more similar are the columns, at least theoretically. The higher the F value more different are the columns.

Rank	F	Column	H	S	A	B	C(2.8)	C(7.0)	Type	USP Designation	Manufacturer
0	0	**Acclaim 120 C18**	1.032	0.018	-0.143	-0.027	0.086	-0.002	B	L1	Dionex
1	0.24	**TSKgel ODS-100Z**	1.032	0.018	-0.135	-0.031	-0.064	-0.161	B	L1	Tosoh Bioscience
2	0.67	**Inertsil ODS-3**	0.99	0.022	-0.146	-0.023	-0.474	-0.334	B	L1	GL Sciences
3	0.74	**LaChrom C18**	0.993	0.013	-0.151	-0.006	-0.278	-0.12	B	L1	Hitachi High-Tech

Conclusions

- The type of silica used by the manufacturer, the bonding chemistry, post-bonding chemical derivatization (endcapping, etc.), washing and heat treatment will all affect the selectivity of the stationary phase
- This may go some way to explaining why there are almost 700 different (!) C18 stationary phases available to chromatographers, and that defining the column as simply a C18 (or even L1, as per the USP classification) is too little information to reproduce a separation

Thank you for your interest, attention and patience

Questions?
Suggestions?
Which themes, in your opinion, were missing?
(to be added in the next edition of this presentation)