

Review

The development of silica hydride stationary phases for high performance liquid chromatography from conception to commercialization

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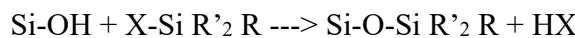
Abstract: The development of a stationary phase material for high performance liquid chromatography based on a surface of silica hydride as opposed to silanols on ordinary silica is discussed including synthetic approaches, characterization and applications. There are several synthetic approaches available to create a silica hydride surface. Modification of the Si-H moiety on the silica surface can be accomplished through use of a hydrosilation reaction. Both the intermediate silica hydride and the material modified with an organic moiety can be characterized by a number of spectroscopic as well as a variety of other methods. Further insights into the retention mechanism are provided through chromatographic measurements. The ultimate utility of any chromatographic stationary phase material is determined by its success for solving challenging analytical problems. A broad range of applications are reviewed to illustrate the versatility and usefulness of silica hydride-based stationary phases.

Keywords: Hydride surface, silicon-carbon bond, aqueous normal phase, retention mechanism

1. Introduction

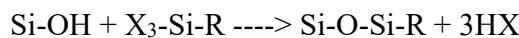
The fundamental properties of silica as a support material for high performance liquid chromatography were described by Unger [1] quite some time ago. The information provided with respect to silica in this monograph is essentially unchanged after several decades. Among these properties two of the most significant are the presence of silanols on the surface and the ability to modify these entities with a number of known chemical reactions. The former defines the surface as a strongly polar medium while the latter provides a means of modifying this polarity with suitable organic moieties in order to produce a stationary phase of the desired chemical properties. The most common type of chemical modification, that is used for virtually all commercially available products for HPLC based on silica, is organosilanization. There are two possible approaches for this reaction scheme as shown below:

MONOMERIC BONDING



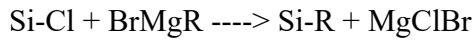
X-halide and R-alkyl

POLYMERIC BONDING

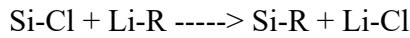


In the case of monomeric bonding, an organosilane compound with one reactive group reacts with the silanol on the silica surface to produce a single point of attachment via silicon-oxygen-silicon-carbon bonds. For polymeric bonding, an organosilane compound with three reactive groups forms a bond to the surface but also crosslinks with adjacent organosilane reagents to form a polymeric network on the surface. While the latter usually leads to higher carbon loads, i.e. more organic moieties bonded to the surface, the reaction is difficult to control and often results in considerable variation in the stationary phase from batch to batch. More reproducible materials are made from the monomeric bonding process and thus it is the approach used for a significant number of commercial HPLC stationary phases based on silica.

A drawback of the organosilanolization approach is the Si-O-Si-C linkage at the surface between the silica support material and the bonded organic moiety. While stable in a variety of organic-aqueous mobile phases it has limitations based on pH stability. At low pH, the O-Si-C point of attachment is subject to hydrolysis while at higher pH the support silica is prone to a dissolution process. Thus a goal for improvement of stationary phase stability is to fundamentally alter this bonding reaction and to produce a direct silicon-carbon bond at the surface. An early approach to achieve this goal was the use of a two-step process involving a chlorination reaction followed by either Grignard or organolithium attachment to the surface [2] as shown below:



Or

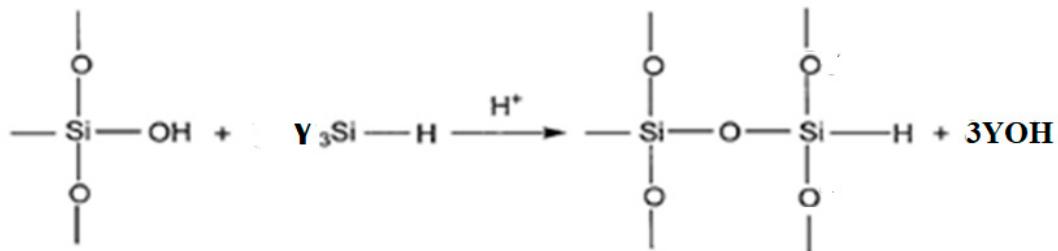


While this reaction scheme achieves the desired result of having a direct silicon-carbon bond between the silica surface and the attached organic moiety, it has some significant drawbacks. The reaction with thionyl chloride produces a material that is hydrolytically unstable. Thus the synthesis must be done under scrupulously dry conditions or otherwise the chlorinated silica reverts back to the original material. In the second reaction with a Grignard reagent or an organolithium compound, the by-products are salts that can easily be trapped on the stationary phase surface and thus result in erratic chromatographic behavior. Thus, while feasible, this is not a practical approach to make a commercially viable stationary phase based on a material with a direct bond between the surface and the organic moiety.

2. Evolution of Silica Hydride Stationary Phases

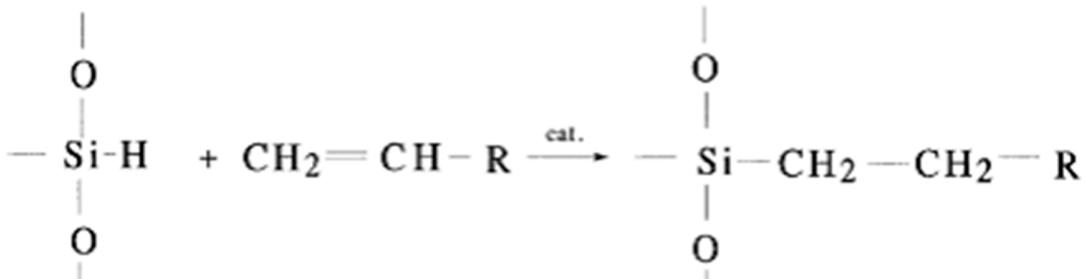
In the late 1980s and early 1990s a new reaction protocol was developed in an attempt to use silica as a support material and having the organic moiety attached through a direct silicon-carbon bond [3-5]. This synthetic approach also involved a two-step process but is considerably less stringent with respect to reaction conditions than the previously described chlorination/Grignard or

organolithium method. The first step is to change the surface morphology from one that is dominated by silanols to another reactive site for the attachment of organic groups. This goal is achieved by reacting silica with a trifunctional silane so that through controlled polymerization a monolayer is formed on the surface where the new functionality is an Si-H group as shown below.



In this case, the surface of ordinary silica is transformed from a very polar medium to the slightly hydrophobic character of silica hydride. This has profound effects with respect to the chromatographic capabilities of silica hydride that will be described later in this review. The attractive feature of ordinary silica is that the surface could be modified by chemical reactions such as those described above. This made it possible to tailor the surface, and hence the chromatographic properties, from the strong polar and adsorptive character of silica to quite hydrophobic when long chain aliphatic moieties are bonded to the surface.

However, silica hydride can be modified just as easily as ordinary silica through the use of a well-characterized reaction referred to hydrosilation. The general process is shown below.



The reaction can utilize a wide range of organic compounds having a terminal double (alkenes) or triple (alkynes) bond and is easily done in a heterogenous media (solid silica hydride in contact with the organic compound dissolved in an appropriate solvent). Therefore, a new surface can be tailored to the desired chromatographic properties by selection of the appropriate alkene or alkyne. Optimum loading of the organic group is controlled by temperature, solvent and catalyst (many types of metal complexes such as hexachloroplatinic acid or free radical initiators can be used).

An important difference can now be noted between stationary phases based on organosilanization of silica and hydrosilation of silica hydride. In both cases due to steric considerations at best no more than about 50% of the surface reactive groups (silanols on silica and Si-H on silica hydride) can be modified. Thus the remaining groups are polar silanol groups on ordinary silica but mildly hydrophobic Si-H moieties on silica hydride materials. A readily measurable difference between the two surfaces is the amount of water adsorbed. For stationary

phases based on ordinary silica, there are between 4 and 10 layers of water on the surface [6]. However, silica hydride has less than 0.5 of a monolayer of water on the surface due to its hydrophobic nature. This finding has substantial impact on chromatographic differences that are found between stationary phases based on the two support materials that will be discussed later in this review.

In the development of any reaction protocol, reproducibility is a key issue. This is particularly true for the fabrication of stationary phases for HPLC because many types of analyses require retention times from run to run and from column to column to be extremely precise, usually with a standard deviation of a few percent or less. The protocol for the production of silica hydride-based stationary phase has been optimized since its inception and is now extremely reproducible as shown by the chromatograms in Figure 1. Similar chromatograms can be obtained for all of the different

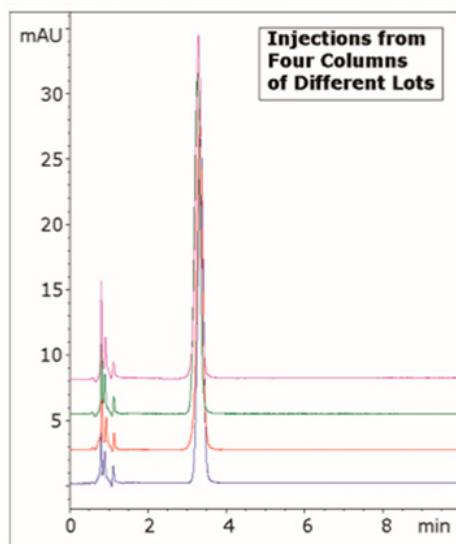
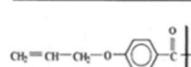
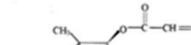
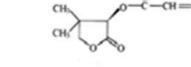
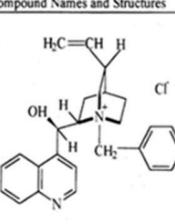
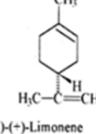


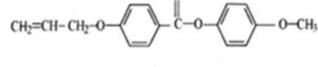
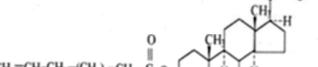
Figure 1. Overlay of chromatograms for the same analyte taken on the same bonded phase of silica hydride columns made from four different synthetic batches.

commercially available columns based on a silica hydride support: Bidentate C18, Bidentate C8, Diamond Hydride, Phenyl, Cholesterol, Undecanoic Acid, Diol, and Amide. The term “bidentate” refers to the double attachment to the surface of the organic moiety due to the use of an alkyne in the hydrosilation reaction [7]. This synthetic approach then provides attachment to the surface by two stable silicon-carbon bonds. This feature is responsible for the long lifetime of these reversed-phase column materials. The versatility of the hydrosilation reaction has been demonstrated over the years by bonding a wide variety of alkenes and alkynes to the silica hydride surface [8-25]. Some examples of organic moieties attached to silica hydride and tested as potential stationary phases are shown in Figure 2. To date only a few of the successful reactions developed have resulted in commercially available columns. In many cases these materials have not been thoroughly tested so it may be possible that useful applications will be found for them in the future.

Compound Names and Structures		Catalyst
1-octene	<chem>CH2=CH-(CH2)5-CH3</chem>	Pt, FR
1-octadecene	<chem>CH2=CH-(CH2)15-CH3</chem>	Pt
4-phenyl-1-butene	<chem>CH2=CH-CH2-CH2-phenyl</chem>	Pt
1-docosene	<chem>CH2=CH-(CH2)19-CH3</chem>	Pt
1-triacontene	<chem>CH2=CH-(CH2)27-CH3</chem>	Pt, FR
1H,1H,2H-Perfluoro-1-octene	<chem>CH2=CH-(CF2)5-CF3</chem>	Pt
Squalene		Pt
1-octyne	<chem>CH≡C-(CH2)5-CH3</chem>	Pt
1,9-decadiyne	<chem>CH≡C-(CH2)6-C≡CH</chem>	Pt
3,9-decadiyne	<chem>CH3-CH2-C≡C-(CH2)4-C≡CH</chem>	Pt
Benzonitrile	<chem>N#Cc1ccccc1</chem>	FR

Compound Names and Structures		Catalyst
<chem>CH2=CH-CH2-O-phenyl-C(=O)-NH-CH(=CH2)-phenyl</chem>		Pt
4(Allyloxy)benzyl Chloride + (R)-(+)-1-Naphtho-Dehydroamine		Pt, FR
β -cyclodextrin		Pt, FR
<chem>CH2=CH-C(=O)-CH2-O-C(=O)-CH2-CH=CH2</chem>		Pt, FR
(R)-(+)-Acryloyloxy- β , β , dimethyl- γ -butyrolactone		
Pt - Hexachloroplatinic acid FR - Free radical		

Compound Names and Structures		Catalyst
	Pt	
(R)-(+)-Limonene		Pt

Compound Names and Structures		Catalyst
<chem>CH2=CH-O-phenyl-C(=O)-O-phenyl-O-CH3</chem>		Pt
Cholesteryl 10-Undecanoate		Pt

Pt - Hexachloroplatinic acid
FR - Free radical

Pt - Hexachloroplatinic acid
FR - Free radical

Figure 2. Some examples of alkenes and alkynes that have been successfully bonded to a silica hydride surface.

3. Characterization of Silica Hydride Materials

A crucial part of the fabrication of new materials for chromatographic stationary phases is characterization by physical and spectroscopic methods. There are several approaches that have been used to establish the success of the hydrosilation reaction on silica hydride and to verify the identity of the bonded organic moiety. One of the most frequently used spectroscopic techniques is diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy [8,9,26-28]. This approach results in the infrared spectrum of the surface of the silica hydride particle. An example of such a spectrum is shown in Figure 3 for a butyl phenyl moiety bonded on a silica hydride surface. The

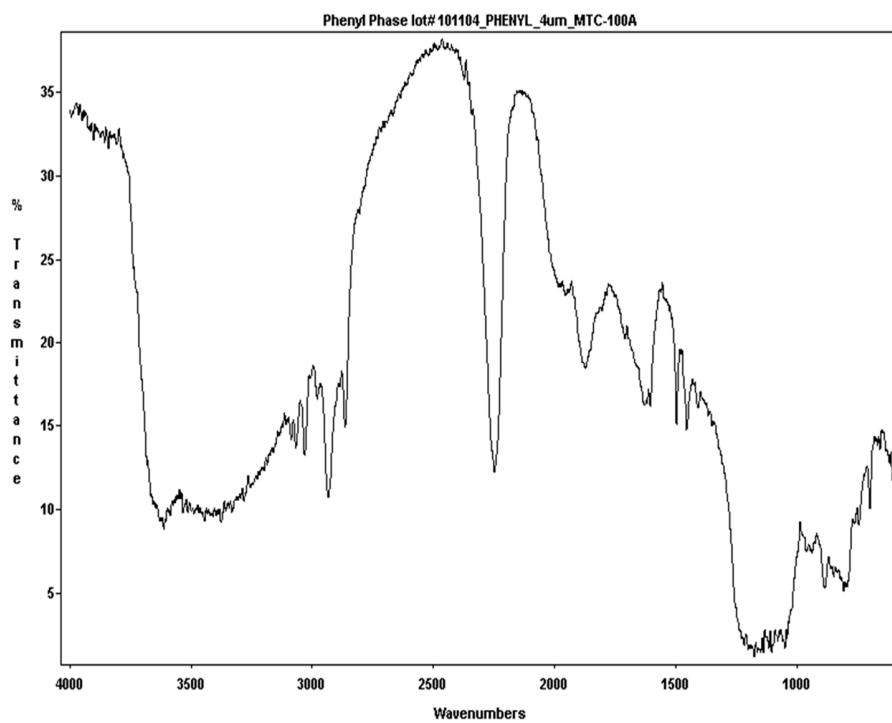
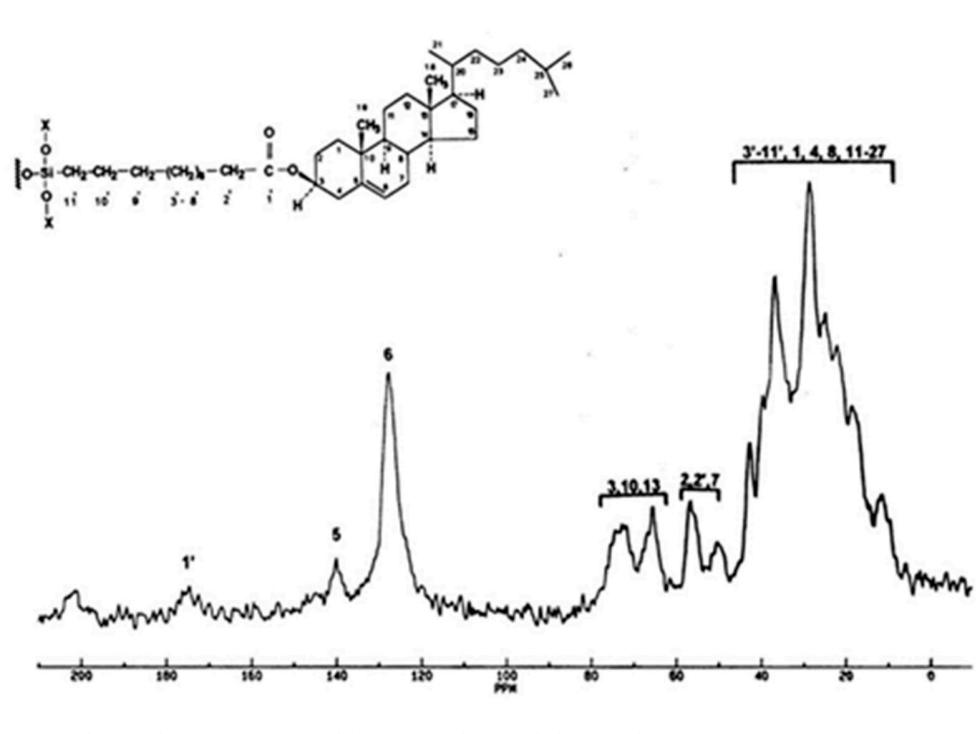


Figure 3. Diffuse reflectance infrared Fourier transform (DRIFT) spectrum of a butyl phenyl moiety bonded to a silica hydride surface.

essential features are as follows: just above 3000 cm^{-1} peaks are due to aromatic carbon-hydrogen stretch; just below 3000 cm^{-1} are peaks for aliphatic carbon-hydrogen stretch; and the large peak near 2250 cm^{-1} is the silicon-hydrogen stretch from the unreacted Si-H groups on the surface. The remainder of the features in the spectrum are the result silicon-oxygen matrix vibrational frequencies. Another useful spectroscopic technique is cross-polarization magic-angle spinning (CP-MAS) nuclear magnetic resonance (NMR) [29-31]. This method results in the NMR spectrum of the surface of solid particles such as typical chemically modified chromatographic supports. An example of a carbon-13 spectrum obtained for a silica hydride-based material with cholesterol bonded to the surface is shown in Figure 4. While the spectrum is complex, it shows the wide variety of aliphatic carbons, the two olefinic carbons and the carbonyl carbon that are part of the cholesterol moiety. Further corroboration of successful bonding via hydrosilation of silica hydride has also been obtained from the solid state Si-29 CP-MAS NMR spectrum. These two characterization techniques provide sufficient documentation that the organic moiety used in the hydrosilation reaction was successfully bonded to the silica hydride support material. The extent of bonding is determined from elemental carbon analysis [32]. Using the molecular weight on the bonded moiety and the percent carbon, the surface coverage expressed in $\mu\text{mol}/\text{m}^2$ can be calculated. As with organosilanization the extent of bonding (surface coverage) is controlled by the



Cholesterol Stationary Phase

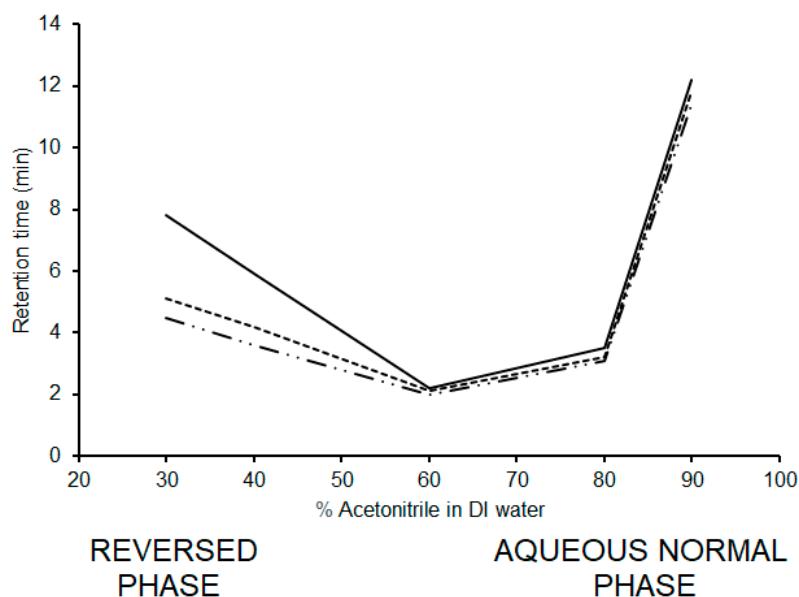
Figure 4. Carbon-13 cross-polarization magic-angle spinning (CP-MAS) NMR spectrum of the silica hydride cholesterol stationary phase.

size of the organic moiety attached. Both ordinary silica and silica hydride have approximately 8 $\mu\text{mol}/\text{m}^2$ of reactive groups (Si-OH for silica and Si-H for silica hydride) on the surface. For very small organic moieties as much as 4–5 $\mu\text{mol}/\text{m}^2$ can be reacted but for very large groups it can be as low as 2 $\mu\text{mol}/\text{m}^2$ or less. The crucial difference between chemical modification of ordinary silica and silica hydride is that the unreacted groups are silanols on silica but Si-H on silica hydride. Thus the underlying surface is very polar on silica but mildly hydrophobic on silica hydride.

An important outcome of the differences in surface chemistry is the amount of water adsorbed by the two chromatographic supports. Table 1 lists a number of silica-based phases (top 4) and silica hydride materials (bottom 5 indicated as Cogent) and their respective layers of adsorbed water [33]. As can be seen, the silica phases have multiple layers (up to 10) of water on the surface while the silica hydride phases have less than one-half of a monolayer on it surface. This property results in a unique chromatographic feature that is shown in Figure 5. All stationary phases based on silica hydride have dual retention mechanism as shown in a plot of retention time vs. mobile phase composition [34]. At high water composition, reversed phase retention for hydrophobic compounds is obtained while at high organic composition normal phase retention is observed for hydrophilic compounds. Therefore, both modes can be tested for a particular sample and the best mode can be selected for developing an analytical format. Virtually all other common stationary phases offer only one mode for doing separations; either reversed-phase or normal phase.

Table 1. Number of water layers on chromatographic Stationary phases

Column	N_w
ZIC HILIC	6.11
ZIC cHILIC	9.48
Luna HILIC	4.72
Triart DIOL	3.06
Cogent Silica C	0.45
Cogent Diamond hydride	0.43
Cogent UDC Cholesterol	0.32
Cogent Bidentate C18	0.28
Cogent Phenyl hydride	0.23

**Figure 5.** Retention map of typical silica hydride-based stationary phases illustrating the dual retention properties of these materials.

4. Separation Mechanisms on Silica Hydride

An interesting challenge with respect to silica hydride-based stationary phases is determining the mechanism that allows these materials to operate in both the reversed-phase and normal-phase modes. The reversed-phase capability is easily explained by ordinary hydrophobic interactions. If the bonded moiety has any hydrophobic properties (and most have at least a minimal amount) plus the mildly hydrophobic nature of the hydride surface, then at least some reversed-phase capabilities are to be expected. However, a more challenging problem is to determine what is responsible for the normal-phase retention properties with respect to polar compounds. The first clue to solving

this problem was obtained by measuring the zeta potential of various silica hydride-based stationary phases [35]. The zeta potential provides a measurement of the surface charge on the stationary phase material. In the case of all silica hydride based stationary phases, it was determined that each had a substantial negative charge on the surface when it is in contact with a high organic content liquid. Such a result was not expected because silica hydride is fabricated by removing more than 95% of silanol groups with the resulting surface being hydrophobic as measured chromatographically under reversed-phase conditions. However, recent investigations [36] have determined that the negative charge on the silica hydride surface is due to hydroxyls from the auto-dissociation of water. This result is similar to those obtained for oil droplets in mixed organo-aqueous solvents [36]. Since the silica hydride particle is hydrophobic in a way that is similar to an oil droplet, the assumption that two behave similarly in comparable environments is a good one. This model accounts for the fact that there is only a small amount of water on the surface but some of it auto dissociates to give OH^- where the negative charge is measured and the H^+ is below the surface and does not effect the chromatographic properties. Thus, retention in the normal phase mode is due to either charge attraction for positive or positively polarized analytes and charge displacement for negative or negatively polarized analytes.

A particularly good example of the unusual properties of the silica hydride-based phases is demonstrated by chromatographic results obtained for the highly polar chemotherapy agent and immunosuppressant methotrexate. A representative chromatogram for this compound is shown in Figure 6. The unique feature of the analysis shown is that this hydrophilic compound is strongly

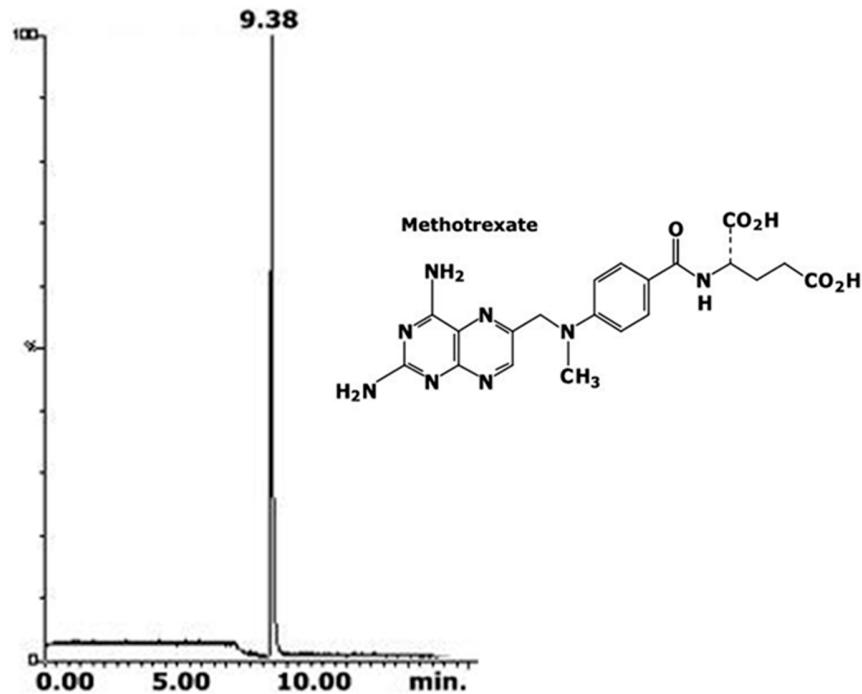


Figure 6. Analysis of methotrexate using a silica hydride C18 stationary phase in the aqueous normal phase mode.

retained on a silica hydride material modified with an octadecyl (C18) moiety. This result would not be possible on an ordinary silica C18 stationary phase that only retains compounds in the reversed-phase mode. Using a mobile phase with a high aqueous content would produce some retention based on weakly hydrophobic character of the molecule but would be much weaker than the strong interaction of methotrexate with the silica hydride phase in the aqueous normal phase mode.

While characterizing and identifying the unique properties of a new separation material is important, the final step and ultimately most relevant is to determine how such a stationary phase can provide analytical capabilities that are useful in practical applications. The U-shaped retention behavior shown in Fig. 5 can be used advantageously for complex samples particularly when the two modes overlap. Thus, there is the ability to retain both hydrophobic and hydrophilic compounds in a single analysis. A simple example is shown in Figure 7 where two compounds, one polar (phenylglycine) and one nonpolar (sertraline), both exhibit significant retention under same analytical conditions. The column used in this analysis was the Diamond Hydride, a silica hydride

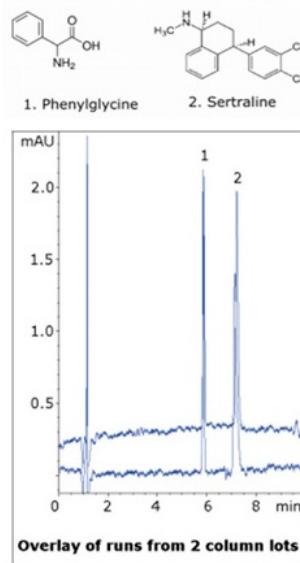


Figure 7. Separation of a polar and nonpolar compound in a single run on a silica hydride-based (Diamond Hydride) HPLC column.

stationary phase with only a minimal amount of an organic moiety (~2% carbon) on the surface. Retention of both of these diverse compounds illustrates the hydrophobic properties of the silica hydride group and the presence of the hydroxyl ions for polar retention. Another more challenging application is illustrated in Figure 8. Here a mixture of peptides ranging from very hydrophobic to

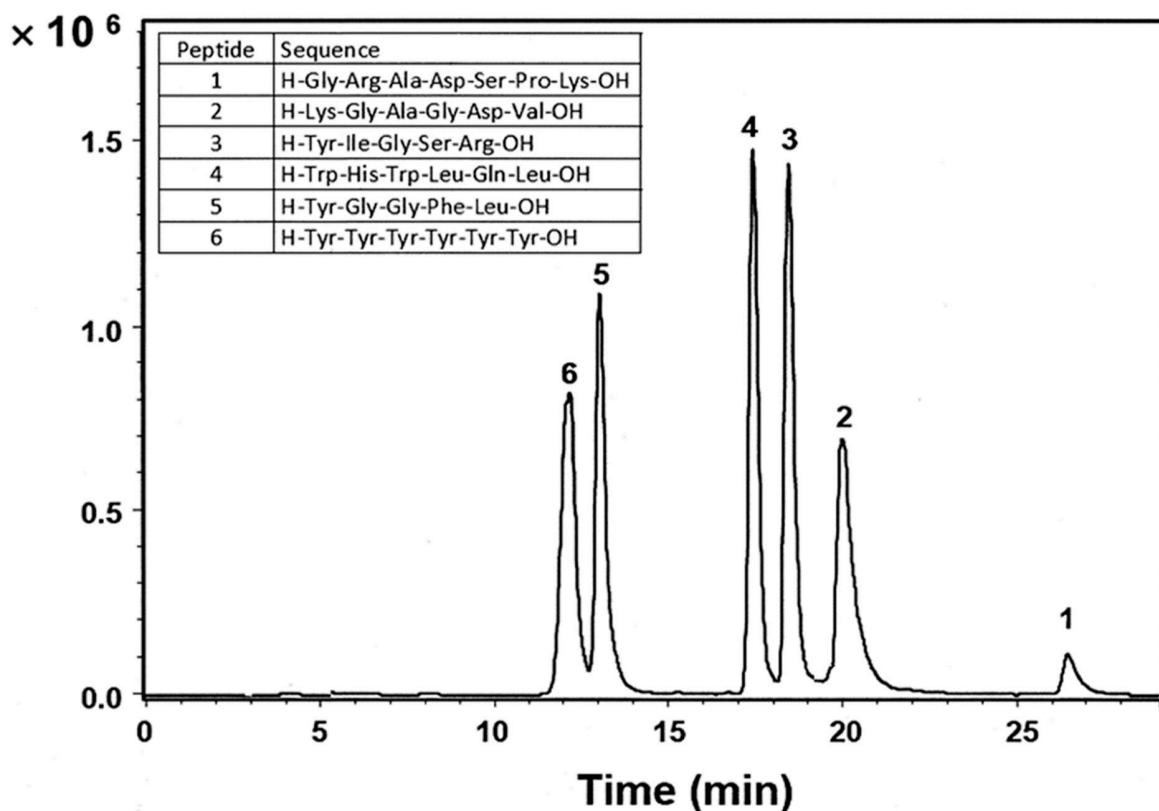


Figure 8. Separation of a mixture containing both hydrophobic and hydrophilic peptides in a single run on the Diamond Hydride column under aqueous normal phase conditions.

very hydrophilic are separated in a single run on the same Diamond Hydride column [37]. Such a result is not possible on other columns and in fact most proteomic analyses are done using a two-dimensional approach; one run done on a hydrophobic phase such as C18 and the second run done on a more polar medium such as ion-exchange. Because of the versatility of the silica hydride columns, many analyses can be attempted by three possible approaches: reversed-phase, aqueous normal-phase, or a method that combines some of both modes so that both hydrophobic and hydrophilic compounds can be retained simultaneously.

5. Applications of Silica Hydride HPLC Columns

Because of the extensive work already done on Type C columns, such as some of those found in the literature [38-61], a summary of all possible applications is not possible. A few examples will be presented to illustrate some of the capabilities of these unique stationary phases.

An area of growing importance is that of food analysis. Methods have been developed for food quality, purity and possible health benefits. The determination of polyphenols is an active area of investigation for many food products. Polyphenols fall into the general category of anti-oxidants, which have been shown to have potential for the preventing and curing cancer as well as other

diseases. Figure 9 shows the analysis of two polyphenols, rutin and quercetin, that have been identified as having potential health and medicinal benefits [62]. Rutin is a citrus flavonoid

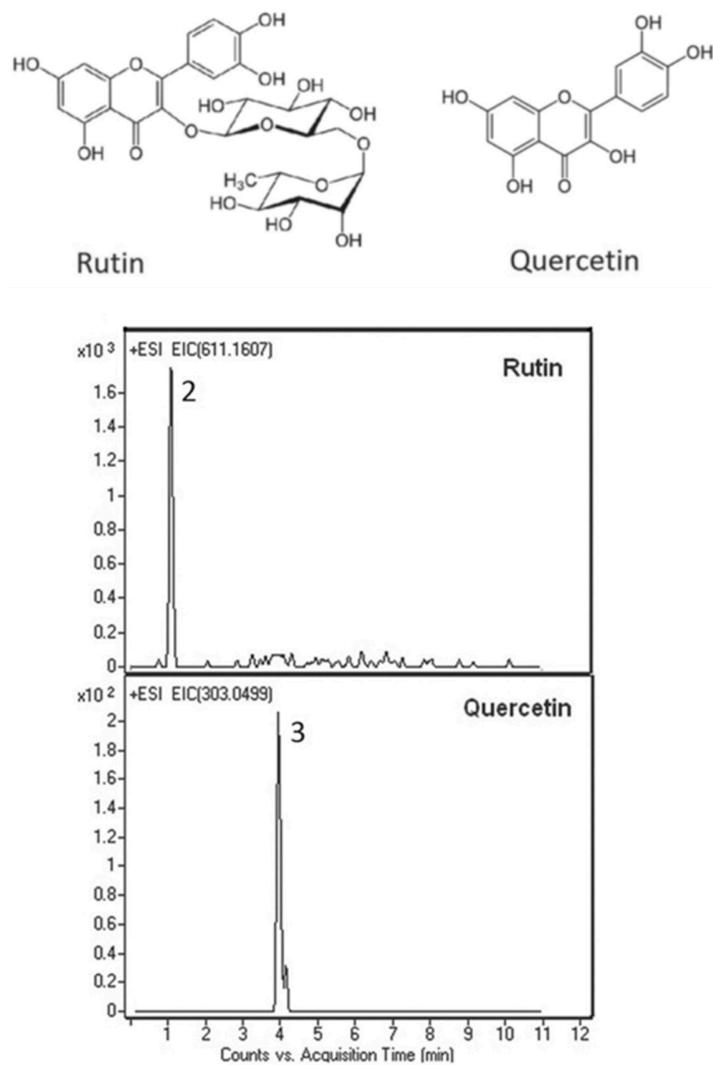


Figure 9. Analysis of the polyphenols rutin and quercetin on the Diamond Hydride column in the aqueous normal-phase mode.

glycoside found in many plants including buckwheat and asparagus. Rutin is also found in citrus fruits such as orange, grapefruit, lemon, and lime, apple, berries such as mulberry, clingstone' peaches and green tea infusions. Quercetin is found a wide variety of food sources including red kidney beans, cilantro, dill, red onion, kale, cranberry, sweet potato, blueberry, broccoli and tea. Both of these compounds are analyzed on the Diamond Hydride column in the aqueous normal-phase mode because of the multiple polar hydroxy groups in their structures.

The analysis of metabolites is an important aspect of pharmacokinetic studies, understanding various physiological and biological systems, clinical tests and food production. These determinations are almost always made in complex matrices with hundreds and in some cases even

thousands of compounds in many samples. Figure 10 shows the analysis of some crucial components in the uric acid cycle along with the internal standard tyrosine [63]. This metabolic is crucial in a number of physiological functions and the concentrations of these compounds can

METABOLITE ANALYSIS

COMPONENTS IN URIC ACID METABOLIC CYCLE

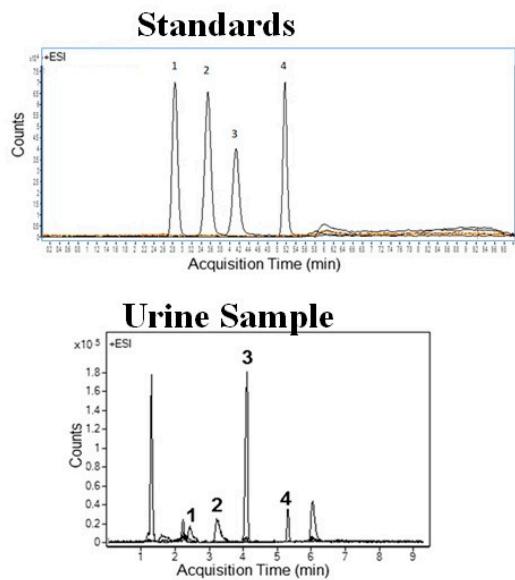


Figure 10. Analysis of components in the uric acid on the Cogent Diol stationary phase in the aqueous normal-phase mode.

potentially be used in disease diagnosis. As shown in the two extracted ion chromatograms, the analysis of these compounds in a real physiological sample parallels the data obtained with standards. Thus, matrix effects for this silica hydride-based (Type C) column (Cogent Diol) are minimal for this analysis in the aqueous normal-phase mode.

Another area of growing importance in chemical analysis are investigations and method development related to the forensic field. Of the various aspects of forensic analysis, methods related to illicit drugs are among the most important. Figure 11 is an LCMS analysis of several of the street drugs classified as “bath salts”. The chemical category of these compounds is cathinones and the basic structure is also shown in the Fig. 11. In this analysis, the column used is the phenyl phase operating in the reversed-phase mode [64]. The phenyl column is used because as can be seen the cathinone moiety has an aromatic ring as a primary constituent. Thus pi-pi interactions between the analyte and the stationary phase can offer a means for discriminating among the various analytes. The chromatogram shown in Fig. 11 illustrates the excellent selectivity and efficiency that silica hydride-based stationary phases offer in the reversed-phase mode. The unique

Peaks:

Allantoin: $181.0332 \text{ m/z } [\text{M}+\text{Na}]^+$
 6-aminouracil: $128.0455 \text{ m/z } [\text{M}+\text{H}]^+$
 Uric acid: $169.0356 \text{ m/z } [\text{M}+\text{H}]^+$
 Tyrosine: $182.0812 \text{ m/z } [\text{M}+\text{H}]^+$

Solvents:

A: DI water (0.1% formic acid)
 B: Acetonitrile (0.1% formic acid)

Gradient:

Time	%B
0.00	95
6.00	30
7.00	30
8.00	95

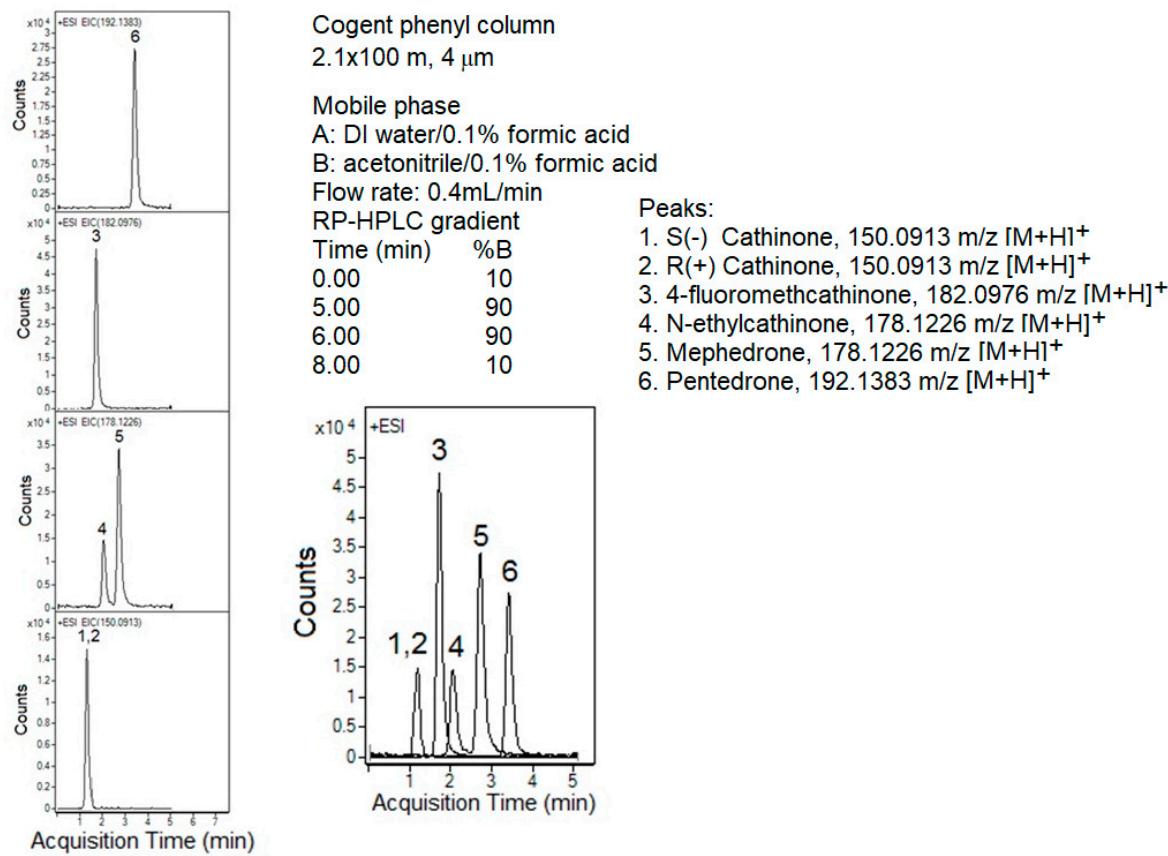


Figure 11. Separation of a mixture of the illicit street drugs “bath salts” on the silica hydride-based phenyl column in the reversed-phase mode.

surface composed of Si-H moieties in contrast to ordinary silica phases where residual silanols are present results in enhanced chromatographic capabilities in both reversed-phase and normal-phase applications.

The analysis of pharmaceuticals, both name-brand and generic, has been an active area of investigation using silica hydride columns in both the reversed-phase and aqueous normal phase modes. Because a significant number of pharmaceuticals are polar compounds, the aqueous normal phase mode using the Diamond Hydride column provides an excellent tool in order to develop reliable analytical protocols for these types of drugs. Figure 12 illustrates the typical results that are obtained with a polar pharmaceutical compound (atenolol) on the Diamond Hydride in the aqueous normal phase mode [65]. Both significant retention and excellent peak shape can be achieved for

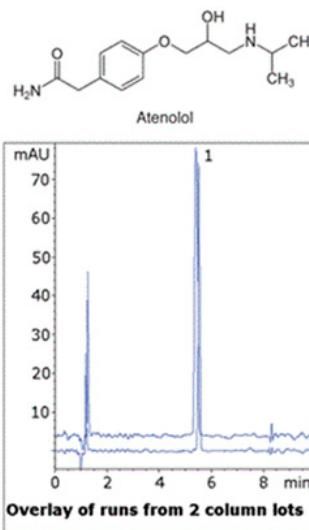


Figure 12. Analysis of the pharmaceutical compound atenolol on the Diamond Hydride column using the aqueous normal phase mode.

this hydrophilic compound. Also demonstrated in this figure is the batch-to-batch reproducibility of fabricating this stationary phase as the analyte peak from the two lots are nearly superimposable in the chromatograms. These unique capabilities have made silica hydride-based stationary phases a superior approach to HILIC when developing analytical protocols for polar compounds. In addition, the hydride surface is less adsorptive than ordinary silica. This property results in less contamination of the stationary phase when complex biological matrices are analyzed and when some deterioration of performance does occur it is much easier to remove the contaminants with column washing than with ordinary silica-based materials.

A more exhaustive compilation of applications can be found in the Knowledge Base section of the Microsolv Technology web site (<http://kb.mtc-usa.com/>).

6. Conclusions

The use of silica hydride-based stationary phases (Type-C silica) have grown steadily over the last 15 years based on the unique properties these materials possess. The dominant feature of silica hydride with respect to ordinary silica is the presence of Si-H groups on the surface in contrast to silanols. This results in a very thin water layer (< 0.5 monolayer) on silica hydride while on ordinary silica there is more substantial amount of water (3 – 10 monolayers). This difference gives silica hydride properties that can be exploited for solving difficult analytical problems. In particular for the analysis of polar compounds using the aqueous normal-phase mode in comparison to HILIC, there is excellent run-to-run, day-to-day and batch-to-batch reproducibility, good peak shape and fast re-equilibration between runs particularly for gradients and easy clean-up when analyzing biological matrices. All of the silica hydride phases can be used in either the reversed-phase or aqueous normal-phase modes. The extent of retention in each of the modes is determined by the organic moiety bonded to the surface. With little or no modification, or with a very small or polar moiety bonded, the aqueous normal-phase mode is dominant. With more hydrophobic bonded

groups, the reversed-phase mode is more prevalent. Differences in selectivity when compared to reversed-phase materials on ordinary silica are also observed. Overall, silica hydride-based separation materials offer a new approach to solving challenging analytical problems in complex samples and matrices.

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