Summer 2016

Evaluation of a Silica Hydride Based Diol Stationary Phase For High Pressue Liquid Chromatogrphy

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DOI: <https://doi.org/10.31979/etd.uqb8-6x7z>
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EVALUATION OF A SILICA HYDRIDE BASED DIOL STATIONARY PHASE FOR
HIGH PRESSURE LIQUID CHROMATOGRAPHY

A Thesis

Presented to

The Faculty of the Department of Chemistry

San José State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Kathleen Talbott

August 2016

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The Designated Thesis Committee Approves the Thesis Entitled

EVALUATION OF A SILICA HYDRIDE BASED DIOL STATIONARY PHASE FOR
HIGH PRESSURE LIQUID CHROMATOGRAPHY

by

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Abstract

EVALUATION OF A SILICA HYDRIDE BASED DIOL STATIONARY PHASE FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY

by Kathleen Talbott

The impact of chromatography across many scientific fields and applications is limitless. It is a vital everyday separation, characterization, and purification tool for many scientists worldwide. Innovations in high performance liquid chromatography (HPLC) stationary phases have led to more diverse separations essential to many fields including the pharmaceutical industry and research. Silica hydride based stationary phases have been shown to display both reverse phase (RP) and aqueous normal phase (ANP) chromatographic behavior. This is a result of both the silica hydride surface and the bonded phase. The goal of this work was to characterize silica hydride based diol stationary phases for HPLC. A wide range of compounds with varying polarities were analyzed. Retention was observed under ANP and RP conditions. Two representative silica hydride based diol stationary phases were compared to demonstrate the effect that the length of the bonded phase has on the separation capabilities of the column. The diol bonded phase with a longer carbon chain retained analytes with more hydrophobic (or non-polar) characteristics longer than analytes with more hydrophilic properties. As part of a larger study the effect of buffer concentration on the ANP retention of model compounds was investigated. Retention dramatically decreased when the concentration of some buffers was increased. This trend is opposite of what has been observed in hydrophilic interaction liquid chromatography (HILIC), indicating a clear distinction in the retention mechanism for HILIC and ANP.

Acknowledgments

During my graduate research I had the opportunity to develop skills that are crucial to being a research scientist. I am very thankful for this opportunity and would like to take this time to recognize those that gave me support during my journey to complete my thesis work. Dr. Joseph Pesek and Dr. Maria Matyska for the patience and guidance they showed me. Without them the research described in this work would not have been possible. Their knowledge and expertise have always impressed me. My committee members, Dr. Stone and Dr. Terrill, gave me their valuable time and support. The discussions we shared about this work helped focus and encourage me.

I also want to thank MicroSolv for supplying their silica hydride based diol column.

For all the scientific discussions and company during my Masters work I'd like to give a special thanks to my friends and colleagues at San Jose State University: Harshada Natekar, Joshua Topete, and Micheal Lim.

I want to express my deepest gratitude to my family, husband, and friends who patiently listened and supported me. Especially to my parents that have always been there to support and encouraged me throughout my 20 years of education. To my husband that has given me comfort and has been my anchor for seven years. He has always encouraged me and walked along side me on my journey.

Table of Contents

INTRODUCTION	1
Historical Background	1
High Performance Liquid Chromatography	2
Detectors	5
Stationary Phase.....	6
Type B Silica.....	6
Type C Silica.....	7
Modes of Separation	8
Reversed-Phase Liquid Chromatography	8
Hydrophilic Interaction Liquid Chromatography	8
Aqueous Normal Phase.....	9
Concentration of Mobile Phase Modifiers	10
In Hydrophilic Liquid Interaction Chromatography	11
In Reversed-Phase Liquid Chromatography	11
Research Goals	12
EXPERIMENTAL	13
Materials	13
HPLC: Mobile Phase Preparation	13
Bonded Stationary Phases	14

Analytes Used for Column Evaluation	17
Structures	20
Bonded Stationary Phases	20
Polar/ Hydrophilic Analytes Used for Column Evaluation.....	20
Nonpolar/ Hydrophobic Analytes Used for Column Evaluation	23
Select Applications: Bisphenols.....	23
Select Applications: Blood Pressure Medications	24
Select Applications: Illicit Drugs	24
Select Applications: Isolation of Hydroxyproline for ¹⁴ C Dating.....	24_Toc453407100
Organic Acids Found in Wine	25
Methods and Instrumentation	26
Sample Preparation	26
Perkin Elmer Flexar FX-10 UHPLC.....	26
Hewlett-Packard 1090 Series II	27
HP 1050 Series.....	28
RESULTS	29
Retention Maps of Select Analytes	29
ANP Retention of Analytes	30
Reversed-Phase Retention of Analytes	39
Analytes With No Retention in ANP or RP.....	41

Mobile Phase Modifier Concentration Studies	42
Formic Acid	44
Ammonium Acetate	50
Comparison of Two Silica Hydride Based Diol Bonded Phases	54
Select Applications of Silica Hydride Based Diol	56
Bisphenols.....	56
Drugs and Medications	57
Amino Acids in Carbonless Mobile Phase	59
CONCLUSIONS.....	61

List of Tables

Table 1. Mobile phase solvents and mobile phase additives.	14
Table 2. MicroSolv Diol EXP 1.0.....	15
Table 3. MicroSolv Diol 2.0.	16
Table 4. Vydac Diol.	16
Table 5. Manufacturer and CAS numbers for analytes used in studies	18, 19
Table 6. Retention times of nucleobases and nucleosides.	31
Table 7. Retention times of amino acids.....	33
Table 8. Retention times of two purine alkaloids.	35
Table 9. Retention times of miscellaneous positively charged analytes.....	37
Table 10. Retention times of nonpolar analytes.....	40
Table 11. Summary of pH ranges for 20:80 mixtures of DI water and acetonitrile with an added buffer over a range of concentrations.	43
Table 12. Analyte pKa values and corresponding net average charges at pH 3 and 6.	44
Table 13. Retention of polar/hydrophilic compounds as a function of formic acid concentration.....	48
Table 14. Retention of thiamine as a function of formic acid concentration.....	48
Table 15. Retention times of nonpolar analytes as a function of formic acid concentration.	49
Table 16. Retention times of polar analytes as a function of ammonium acetate concentration in the mobile phase.....	51
Table 18. Retention times of nonpolar analytes as a function of ammonium acetate concentration in the mobile phase.....	52
Table 17. Retention times of thiamine as a function of ammonium acetate	

concentration in the mobile phase.....	53
Table 19. Capacity factor (k) of theobromine using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.	55
Table 20. Capacity factor (k) of tryptophan using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.	55
Table 21. Capacity factor (k) of fluorene using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.	55

List of Figures

Figure 1. Basic schematic of an HPLC.	2
Figure 2. Example of three overlaid liquid chromatograms showing void volume (t_0) and retention time of the third peak (t_r).	4
Figure 3. Surface of type B and type C silica. Strong water association with type B silica.	7
Figure 4. Ideal analyte retention on silica hydride stationary phase.	10
Figure 5. Retention profile of one nucleoside and four nucleobases.	32
Figure 6. Retention map of amino acids.	34
Figure 7. Caffeine and theobromine retention profiles.	36
Figure 8. Retention profile of Histamine.	38
Figure 9. Retention profile for thiamine hydrochloride.	38
Figure 10. Retention profile of nonpolar compounds.	41
Figure 11. Retention profile of amino acids and thiamine as a function of formic acid concentration.	45
Figure 12. Retention profile of nonpolar analytes as a function of formic acid concentration.	47
Figure 13. Retention profiles of analytes as a function of ammonium acetate concentration.	51
Figure 14. Retention profiles of thiamine as a function of ammonium acetate concentration.	53
Figure 15. Comparison of the capacity factor of analytes L-tryptophan and fluorene on two diol columns; 1,2-dihydroxybutyl (short diol) and 1,2-dihydroxyoctyl (long diol).	56
Figure 16. Chromatograms of bisphenol S (top), bisphenol F (middle), and bisphenol A (bottom).	57
Figure 17. Overlaid chromatograms of the blood pressure medications: Amiloride (left) and triamterene (right).	58
Figure 18. Overlaid chromatograms of three illicit drugs: hydroxybupropion (left),	

LSD (middle), and codeine (right).....	59
Figure 19. Retention of hydroxyproline using 100% water as mobile phase with water blank overlaid.	59
Figure 20. Chromatogram of L-histidine using water (0.1 mM phosphoric acid) as mobile phase.	60

INTRODUCTION

Historical Background

The impact of chromatography across many scientific fields and applications is limitless. It is a vital everyday separation, characterization, and purification tool for many scientists worldwide. There are many types of chromatography, but in general the technique is used to separate the matrix from isolated analytes in a sample. The analytes are the target species and the matrix is the remaining components of the sample. This separation occurs due to the unique physical and chemical properties of the many components of the mixture. Chromatography was first reported in 1903 by Tswett, a Russian botanist. Tswett separated plant pigments using solvents and filter paper.¹ This was later referred to as paper chromatography (PC). He theorized that chlorophyll had preferential adsorption onto the filter paper that was weaker than the dissolving power of the solvent. Depending on how polar the solvent was, the plant pigments would travel farther down the filter paper. Later, Tswett would use calcium carbonate as his adsorbent material, which he packed into a thin glass column. Using this column and solvent he separated different plant pigments from each other. He was the first to refer to this technique as “chromatography,” which literally means “color writing”.¹

When the mobile phase is liquid, the type of chromatography is classified as liquid chromatography (LC). In LC, the sample is introduced into the solvent, called the mobile phase, which is passed over a column packed with porous material, called the stationary phase. The analyte(s) and the matrix will interact with the two phases to varying degrees. If the analyte “prefers” to interact with the stationary phase over the

mobile phase, it will retain on the column and will have a slower velocity than an analyte that interacts more strong with the flowing mobile phase. This causes analytes to come out of the column, or elute, at different times based on their unique chemi-physical properties.²

High Performance Liquid Chromatography

The stationary phases are composed of small porous particles, typically 3-5 μm , that are tightly packed into a column. If the mobile phase is “pushed” through the porous stationary phase using a pump at high pressures, the term high performance liquid chromatography (HPLC) is used to describe the technique. This method of chromatography was originally named high pressure liquid chromatography in the 1960s and was changed in the 1970s to high performance liquid chromatography with advances in the instrumentation and stationary phases. The basic schematic can be seen in figure 1.

Figure 1. Basic schematic of an HPLC.

The instrumentation of HPLC has five major components: high pressure pump, injector, HPLC column, detector, and data system. The pump forces the various solvents, which make up the mobile phase, in precise amounts through the instrument at a constant flowrate. HPLC experiments can operate under two different mobile phase compositions. A constant mobile phase composition is called isocratic, while varying the amounts of solvents over time is called a gradient. The injector introduces a small volume of sample into the constant flow of the mobile phase. Separation occurs within the column that contains the stationary phase. After the target analyte elutes, the detectors will optimally be able to quantify the target with high levels of precision and accuracy. This signal is then sent to the computer, or data system, that records the signal strength starting upon time of injection of the sample. Those data make up the chromatogram.

There are various features of an HPLC chromatogram that should be mentioned. Time is recorded on the x-coordinate and detector signal is recorded as the y-coordinate. The time it would take a compound with no preferential interaction with the stationary phase to reach the detector is referred to as the void time or dead time (t_0). This is the shortest possible time that a compound can appear in the chromatogram and is largely dependent on the flow rate, volume of connecting tube in the instrument, and internal volume of the column.² When a compound is retained by the stationary phase, the velocity of this compound down the column is slowed. The stronger a compound is retained on the column, the later its signal will reach the detector after injection. In Figure

2, three chromatograms are overlaid with peak C being retained the strongest of the three

Figure 2. Example of three overlaid liquid chromatograms showing void volume (t_0) and retention time of the third peak (t_r).

peaks. The retention time (t_r) of a peak corresponds to the time the peak experiences the maximum signal.

Using retention times to compare experiments where all experimental conditions are kept constant can be done. However, it is not useful to compare retention times from multiple experiments that have different void times. This happens in a number of cases including changing columns, instruments, temperature, or flow rate. In these instances the capacity factor or retention factor (k) can be used.² The capacity factor is a dimensionless ratio of the time retained on the column and the void volume. It is expressed as follows:

$$k = \frac{t_r - t_0}{t_0} \quad \text{or} \quad k = K \frac{V_m}{V_s}$$

Where, K is the partitioning ratio for a particular analyte, V_s is the volume of the stationary phase, and V_m is the volume of the mobile phase.

Detectors

After passing through the column, the mobile phase and analytes enter a detector. This detector should be capable of producing some sort of response signal from the analyte that is well above the signal from the mobile phase. Many types of detectors have been used in HPLC instrumentation. Ultimately, the detector used in an experiment depends on the type of analysis and the target compound(s) signal strength. Two main types of detection used in academia and industry are ultraviolet (UV) detection and mass spectrometry (MS). Mass spectrometry has been very useful to scientists all over the world. This method of detection allows for simultaneous detection of multiple compounds with different mass to charge ratios. The limit of detection for many compounds is very low when using this method; however, analytes need to be ionizable to be detected.²

Ultraviolet detection is considerably cheaper than MS, but requires that the analyte has an active chromophore and that the solvents that make up the mobile phase do not. The concentration of analytes is proportional to the area under the curve, as described by Beer-Lambert's Law. Quantification of analytes can be done using a calibration curve or standard addition methods. UV light sources emit a wide range of wavelengths that can be selected using a monochromator and filters. With a diode array detector multiple wavelengths can be detected. This is especially useful when the strongest absorbed wavelength is unknown or the sample contains two or more

compounds that do not absorb similar wavelengths. The majority of experiments and results described in this paper were performed using ultraviolet detection with a diode array detector, or DAD.

Stationary Phase

The efficiency of the column is dependent on several characteristics of the stationary phase that change the theoretical plate number (N). These parameters include particle dimensions, uniformity of particles, as well as the quality of the packing process.³ This value can be calculated for a column using the following equation:

$$N = 5.5452 t_r^2 / W_{0.5}^2$$

Where, t_r is the retention time and $W_{0.5}$ is the peak width at half height.

Type B Silica

The column, which houses the stationary phase, can be considered the heart of a HPLC instrument. Today, the most common stationary phase support used is type B silica. This is because silica can be porous with a high surface area and has high mechanical strength.⁴ As the surface area of the stationary phase increases the occurrence of an interaction between the stationary phase and the analyte increases. Ordinary silica has a silanol rich surface. Silanol is very polar and therefore not very efficient at retaining nonpolar analytes.^{2,4-6} This can be overcome by covalently modifying the silica surface with an organic functionalization. Residual silanols, unreacted on the surface, can cause secondary interactions with the analyte and the hydrophobically modified surface of the silica. This can lead to peak tailing, especially for basic compounds above pH 5.^{2,4,7} Further end capping of residual silanols is often performed after functionalization to

prevent or reduces these effects. This endcapping can be reversed when operating under standard reversed-phase conditions, but is stable between pH 3 to 9.⁴

Type C Silica

Another solution to these unreacted silanol groups left on the surface of type B silica is to convert the silanol and other oxides to silica hydride, giving the surface of the silica a slightly nonpolar character. This is achieved through a silanization reaction.⁸ The silica hydride surface is stable in aqueous conditions under a larger pH range than endcapped silica, pH 2-9, and does not sacrifice mechanical strength.⁴ Silica hydride can be modified through a hydrosilation reaction to form a silicon-carbon bond with the bonded phase.⁹ This creates a stable bond that has been shown to be more stable silicon-oxygen-carbon bond that is produced when type B silica is modified.¹⁰ As mentioned previously, the surface of the silica hydride is slightly nonpolar, which is important to reduce tailing in the reversed-phase mode. This hydrophobicity also causes a weak association with water when compared to type B silica, see Figure 3. It has been shown

Figure 3. Surface of type B and type C silica. Strong water association with type B silica.

using frontal analysis for multiple silica hydride based stationary phases that the water layer is less than one water molecule thick.^{11, 12}

Modes of Separation

Reversed-Phase Liquid Chromatography

Reversed-phase chromatography is the most popular mode of separation and is used to retain compounds that have hydrophobic character. The stationary phase is largely nonpolar while the mobile phase is polar. Separations are characterized by an increase in capacity factor of an analyte with a decrease in organic solvent in the mobile phase. Today, Type B silica is the most common support for the stationary phase², but type C silica can be used as well.

Hydrophilic Interaction Liquid Chromatography

Hydrophilic interaction liquid chromatography (HILIC) is a type of normal phase chromatography that uses mainly organic solvents in the mobile phase with a small amount of an aqueous component. The stationary phase has a polar surface. Polar moieties can be added to a type B silica surface to create different bonded phases. The strong association of water from both the silanol rich surface and the polar bonded phase create a water rich layer at the surface of the stationary phase. This causes a partitioning effect of analytes between the water rich layer adsorbed onto the surface of the stationary phase and the mainly organic mobile phase.^{13–15} This separation mode has been shown to be effective for separating polar compounds. These stationary phases are rarely useful in reversed-phase separations, because the bonded phase is polar.

Derivatives of diol bonded phases, particularly relevant to this study, have been extensively used on type B silica in HILIC separations. Historically, it was one of the first bonded phases to be successfully derivatized onto type B silica. Many variations of

diol have been used in literature,¹⁶ but have not been based on silica hydride support outside of Dr. Pesek's lab at San Jose State University.

Aqueous Normal Phase

Silica hydride based stationary phases are used in aqueous normal phase (ANP). This includes simple silica hydride or a derivatized version of silica hydride. The mobile phase consists of mainly of organic solvents with a small aqueous component. Polar analytes retention will increase in ANP mode as the organic solvent content increases in the mobile phase. This sounds reminiscent of HILIC, but the two modes are based on different separation mechanisms. The reason for this is the water layer adsorbed onto the surface of type C silica is very small due to the replacement of the acidic silanols with slightly hydrophobic silica hydride groups. This prevents the HILIC type mechanism, that relies on this adsorbed water layer, from function. Analytes may interact with the bonded phase or the silica hydride groups. Since the mobile phase in ANP is complimentary to RP, these columns may be used in RP and ANP modes. The retention maps of analytes on silica hydride based columns show a characteristic U-shaped curve, as seen in Figure 4. The ability of ANP capable columns to retain both polar and

Figure 4. Ideal analyte retention on silica hydride stationary phase.

nonpolar compounds will likely lead to more diverse separations that are useful for many biologically relevant and pharmaceutical samples.

Concentration of Mobile Phase Modifiers

The effect of buffer concentration on chromatographic results is a key parameter when considering method development. Ultimately, the magnitudes of trends observed will be determined by the properties of the buffer, stationary phase, and analyte. The concentration and type of buffers can affect peak shape, retention, and resolution of analytes. As such, buffer concentration studies have already been investigated using type B silica. Since, type C silica is a relatively new stationary phase option this area has been unexplored until just recently.

In Hydrophilic Liquid Interaction Chromatography

Buffer concentration studies performed on HILIC columns determined that an increase in retention with an increase in salt, or buffer, concentration may occur. The increase in salt concentration in the adsorbed water layer is believed to increase the water layer at the surface of stationary phase. This will then increase the retention of hydrophilic analytes.^{16–18}

The pH of the mobile phase may affect the ionization of the analyte. This can have a large impact on the retention of ionizable analytes. For acids the higher the pH the more likely the analyte will be ionized and for bases decreasing the pH will increase the likelihood that an analyte will be ionized. If an analyte is ionized, several new interactions with the stationary phase and analyte may be possible. If these interactions are possible, ion-dipole and hydrogen bonding, between the analyte and polar stationary phase should increase retention. However, acids have been shown to have a little retention when ammonium formate was used as a mobile phase additive.¹⁹ This may be explained by the buildup of negative charges at the stationary phase surface due to ionized silanols at higher buffer pH. This causes electrostatic repulsion between an ionized acidic analyte and the stationary phase. Higher pH can also suppress the ionization of bases, which may lead to a decrease in retention of these analytes.

In Reversed-Phase Liquid Chromatography

An increase in buffer concentration in RP has been found to decrease the retention of analytes. It has been proposed that both the analyte and buffer are competing for adsorption sites on the stationary phase. Adsorption is a main source of retention in RP

and therefore an increase in buffer concentration will lead to a decrease in retention of analytes.

If an ionizable analyte is at a pH that is within the buffering range, the retention can be greatly affected. An ionizable acid should prefer the polar mobile phase at lower pHs. Likewise, an ionizable base should prefer the polar mobile phase at higher pHs. This preference for the mobile phase will lead to a decrease in retention.¹⁶

Research Goals

The ability of type C silica-based columns to effectively separate both polar and nonpolar molecules has inspired many studies to investigate their unique properties. Derivatizing the type C silica with either a nonpolar or polar moiety should increase its RP or ANP character respectively.

This study had three main goals. To characterize the column, determine if the alkyl chain length of the diol changes chromatographic behavior of analytes, and to analyze changes in retention with varied concentrations of mobile phase buffer. To characterize the column, many compounds with different polarities and functional groups were run under different mobile phase conditions to determine which factors increased retention. Diol stationary phases with varied alkyl chain lengths of the diol bonded phase were compared. It was expected that an increase in carbon chain length, of the diol bonded phase, would increase the RP capabilities. The buffer concentrations in the mobile phases were varied for select compounds to investigate how retention of different compounds changes. It has been reported in literature that buffer concentration studies performed on HILIC columns determined that there is an increase in retention with an

increase in salt, or buffer, concentration. If the silica hydride based diol uses a different mechanism than HILIC columns this trend should be different.

It is very probable that there is a very small water layer on the surface of the diol stationary phase. This assumption is supported by observations from reference 11, which was discussed in the Introduction. If there is not a significant water layer at the stationary phase surface, then the partitioning mechanism that type B stationary phases display are likely to play a minimum role in retention. Retention of polar analytes on a type C diol column may occur through several different interactions between the stationary phase and analyte. Possible intermolecular interactions between the stationary phase and analyte are likely to increase retention of analytes. The polar diol may interact with an analyte through hydrogen bonding (if available), dipole-dipole, ion-dipole, and London-dispersion. The silica hydride surface and alkyl chain connecting the diol functional group to the stationary phase surface may interact with an analyte through London dispersion.

EXPERIMENTAL

Materials

HPLC: Mobile Phase Preparation

The mobile phase for most HPLC analysis was a mixture of acetonitrile and MilliQ DI water with small amounts of formic acid or ammonium acetate added. The mobile phases used for the green tea extracts included a mixture of dichloromethane and

ethyl acetate. The specifications for the components that comprised the mobile phases used in this thesis research can be found in Table 1.

Table 1. Mobile phase solvents and mobile phase additives.

Compound	CAS number	Manufacturer
Acetonitrile	75-05-8	Honeywell
MilliQ DI water	N/A	Millipore
Formic acid	64-18-6	Spectrum mfg. corp.
Ammonium acetate	631-61-8	Matheson Coleman & Bell
Ethyl acetate	141-78-6	Not recorded
Dichloromethane	75-09-2	Fisher Chemicals

Bonded Stationary Phases

Three columns were used to analyze the silica hydride based diol stationary phase behaviors and capabilities. The specifications for these three columns can be found in Tables 2 through 4.

Table 2. MicroSolv Diol EXP 1.0.

Batch Number	130605-01
Pore Size	100Å
Surface Area	340+/- 20 m ² /g
Pore Volume	1.00 +/- 0.10 mL/g
Particle Size	4 µm
Carbon Load	9–10%
pH Range	2.0-8.0
Stable Temperature	80°C
Max Pressure	4,500

Table 3. MicroSolv Diol 2.0.

Serial Number	C1516
Pore Size	120Å
Surface Area	340+/- 20 m ² /g
Pore Volume	1.00 +/- 0.10 mL/g
Particle Size	2.2 µm
Carbon Load	9–10%
pH Range	2.0-8.0
Stable Temperature	80°C
Max Pressure	8,000

Table 4. Vydac Diol.

Silica gel	Vydac 101 TP
Lot number for silica gel	900201
Surface Area	106 m ² /g
Pore Size	300 Å
Particle Size	6.6 µm

The Vydac Diol was synthesized in house in 1997. Procedures are described in reference.²⁰

Analytes Used for Column Evaluation

A majority of the samples were prepared in a 40:60 ratio of acetonitrile and water with 0.1% formic acid. Analytes that were not completely soluble in this mixture were prepared in water or acetonitrile, depending on the hydrophobicity of the compound. Samples were then filtered using a 0.45 μm nylon filter. The manufacture and CAS numbers for analytes that were used in experiments can be found in Table 5.

Table 5. Manufacturer and CAS numbers for analytes used in studies.

Compound	CAS number	Manufacturer
L-Histidine	71-00-1	Sigma Chemical Co.
L-Tryptophan	73-22-3	Sigma Chemical Co.
L-Phenylalanine	63-91-2	J.T. & Baker Chemical Co.
L-(+)- α -Phenylglycine 99%	2935-35-5	Not recorded
Uracil	66-22-8	Sigma Chemical Co.
Guanine	73-40-5	NBC National Biochemical Corp.
Cytosine	71-30-7	Nutritional Biochemical Corp.
Adenine	73-24-5	Sigma Chemical Co.
Adenosine	58-61-7	Calbiochem Co.
Histamine	51-45-6	Not recorded
Caffeine	58-08-2	Not recorded
Theobromine	83-67-0	Sigma-Aldrich
Thiourea	83-67-0	Not recorded
Thiamine hydrochloride	337-27	Matheson Inc.
Naphthalene	91-20-3	Sigma-Aldrich
Pyrene	129-00-0	Aldrich
Fluorene	86-73-7	Aldrich
Amiloride	17440-83-4	AK Scientific

Table 5 (continued). Manufacturer and CAS numbers for analytes used in studies.

Compound	CAS number	Manufacturer
Triamterene	396-01-0	AK Scientific
Lysergic acid diethylamide	50-37-3	Cerilliant
Codeine	76-57-3	Cerilliant
(+/-) Hydroxybupropion	357399-43-0	Cerilliant
Vanillic acid	121-34-6	MC Biomedicals
Syringic acid	530-57-4	MC Biomedicals
Protocatechuic acid	99-50-3	MC Biomedicals
p-Coumaric acid	501-98-4	MC Biomedicals
Pyruvic acid	127-17-3	Acros Organics
Ferulic acid	1135-24-6	MC Biomedicals
Caffeic acid	331-39-5	MC Biomedicals
Bisphenol A	80-05-7	Sigma-Aldrich
Bisphenol F	620-92-8	Sigma-Aldrich
Bisphenol S	80-09-1	Sigma-Aldrich

Structures

Bonded Stationary Phases

MicroSolv Diol 1.0™ and Diol 2.0™
(Printed with permission from MicroSolv
Technology Corporation)

Vydac Diol
(Adapted from MicroSolv Technology
Corporation webpage)

Polar/ Hydrophilic Analytes Used for Column Evaluation

Histidine

Tryptophan

Phenylalanine

Phenylglycine

Adenosine

Adenine

Cytosine

Uracil

Guanine

Histamine

Caffeine

Theobromine

Thiourea

Thiamine hydrochloride
(salt form of B₁ vitamin)

Nonpolar/ Hydrophobic Analytes Used for Column Evaluation

Naphthalene

Fluorene

Pyrene

Select Applications: Bisphenols

Bisphenol A

Bisphenol F

Bisphenol S

Select Applications: Blood Pressure Medications

Amiloride

Triamterene

Select Applications: Illicit Drugs

Codeine

Lysergic acid diethylamide

Hydroxybupropion

Select Applications: Isolation of Hydroxyproline for ¹⁴C Dating

Hydroxyproline

Organic Acids Found in Wine

Vanillic acid

Syringic acid

Pyruvic acid

Protocatechuic acid

p-Coumaric acid

Ferulic acid

Caffeic acid

Methods and Instrumentation

Sample Preparation

Sample solutions were prepared by weighing out 1 mg solid standards and dissolving them in a premixed 40:60 mixture of DI water and acetonitrile with 0.1% formic acid. If the resulting solution showed signs of overloading the column, the sample was diluted to an appropriate concentration with a 40:60 premix solution.

Perkin Elmer Flexar FX-10 UHPLC

Separations performed to demonstrate select applications were performed using a Perkin Elmer Flexar FX-10 UHPLC. Baseline separation or partial separations were achieved for the following standards: three bisphenols, two blood pressure medications, and three illicit drugs. Retention of amino acids in a carbonless solvent was achieved using the Flexar FX-10 UHPLC. Organic acids found in wine were also tested under RP and ANP conditions.

The software for data analysis and instrument interface used for HPLC separations was Perkin Elmer Chromera data system. The internal volume of this instrument is very small, reducing the diffusion of the sample plug after injection. The instrument was equipped with an auto-sampler, 20 μ L injection loop, peltier column oven, and a FX UV/Vis detector with a 2.4 μ L volume. Injections volumes were 1-5 μ L

depending on the concentration of the sample and corresponding absorbance. The injection program was pick up mode. This fills either side of the injection loop with mobile phase and a 1 μ L air cushion on either side of the sample volume. The air cushion serves to prevent diffusion of the sample into the mobile phase. The mobile phase was delivered with a micro-binary 10,000 psi pump system. Specific information about sample preparation and instrument parameters can be found in the results section for each application.

Hewlett-Packard 1090 Series II

Formic acid studies, ammonium acetate studies, and retention profiles were done using a HP 1090 Series II. The instrument was equipped with an auto-sampler, column oven, ternary pump system, and a UV/Vis diode array detector (DAD). Solvents were continually degassed with helium. This degassing system reduces the volume in the tubing compared with using a separate on-line degasser. This makes solvent changes more efficient. Injection is controlled by a metered syringe. The syringe lowers into a sample vial withdrawing the specified volume of sample. The vial is then moved out of the way and the needle connects to the flow of the mobile phase. Mobile phase is pumped using a DR5, which can deliver solvents in an isocratic or gradient fashion. The software system used to operate the instrument and for data analysis was ChemStation.

Data was acquired using wavelengths of 254 and 210 nm, but data is reported using 254 nm wavelength. Injection volumes were 3 μ L and the flow rate was 0.5 mL/min for all chromatograms collected using this instrument. The oven temperature was

kept at 40° C to reduce the viscosity of the solvents and lower the pressure at high water content in the mobile phase.

Solvent line A consisted of DI water with an added buffer and Solvent B consisted of acetonitrile with an added buffer. The solutions used in the formic acid studies were prepared volumetrically. Formic acid was added to 500 mL of DI water or acetonitrile to make 0.300%, 0.200%, 0.100%, and 0.050% solutions.

For ammonium acetate studies the buffer was not soluble in acetonitrile, so solvent B was prepared by using 90 acetonitrile and 10% water. Desiccated ammonium acetate solid was weighed and added to a 1 L volumetric flask to make 10 mM ammonium acetate. A portion of this solution was set aside for 10.0 mM chromatographic experiments. The remaining solution was used for serial dilutions with a premixed 90/10 acetonitrile/water to make 5.0 mM, 2.5 mM, and 0.5 mM solutions. In a similar fashion 1 L 10.0 mM ammonium acetate was prepared in a 1 L volumetric flask. A portion of this solution was kept and another portion was used for serial dilutions to prepare 5.0 mM, 2.5 mM, and 0.5 mM solutions. These solutions were used within 48 hours after preparation.

HP 1050 Series

The HP 1050 was used to develop a method to identify bisphenol leaching in plastics. This instrument is equipped with a quaternary solvent system, an auto-sampler, online solvent degasser, dual piston pump, and DAD detector. The data processing was handled by a computer with ChemStation (Pascal version) installed. Compressed nitrogen controls operation of the auto-sampler. Sample vials are moved under the injection needle

and a metered volume is withdrawn into a syringe. Vials are then moved and the sample is injected into a flow through sample loop. Two pistons in the pump are used to reduce the fluctuation in pressure. The first piston cycles at twice the speed of the second. This allows the second piston to fill the gap in pumping solvent. The flow through cell in the detector is recommended to operate under 400 bar. Up to five wavelengths and bandwidths can be collected with the DAD. The wavelength and bandwidth are programmable by entering these parameters into the method.

Bisphenol A, S, and F were separated isocratically with 93:7 DI water: acetonitrile on the Cogent DIOL 2.0 column. Both solvents contained 0.1 volume percent formic acid. Signal was collected at 214 nm with 4 nm bandwidth. Flow rate was 0.4 mL/min and injection volume was set to 1 μ L.

RESULTS

Retention Maps of Select Analytes

To evaluate the type C diol stationary phase retention profiles were recorded for analytes with a wide range of polarities and hydrophilicities. For each analyte, a set of isocratic chromatograms were obtained by altering the ratio of the aqueous and organic solvents in the mobile phase. Mobile phase solvent A was DI water and solvent B was acetonitrile. Analytes were run with 0.2 volume percent formic acid in each solvent in the mobile phase (except in two cases, which are noted). The set of chromatograms for each analyte are organized into hydrophilic and hydrophobic analytes. Hydrophilic compounds were used to analyze the ANP characteristics of the column and hydrophobic compounds were used to analyze the RP characteristics.

ANP Retention of Analytes

The silica hydride diol stationary phase bonded phase is polar; therefore it was expected that this stationary phase would have good retention of polar compounds operating under ANP conditions. To test this hypothesis, various polar and biologically relevant compounds were used. The retention times of each of the standards are reported as a function of the percent solvent B in the mobile phase. Many of the compounds tested retained very well and all compounds displayed some ANP behavior at high % organic in the mobile phase.

Nucleobases and nucleosides are the basic components that make up DNA and chromatographic separations of these compounds are useful to many scientists. One nucleoside and four nucleobases were retained on the MicroSolv Diol 1.0™ column. These compounds all showed significant retention under ANP conditions. However, uracil showed less than half the retention of all other nucleobases tested. Those data are reported in Table 6 and can be seen graphically in Figure 5. These results prove ANP retention behavior on the silica hydride based diol stationary phase.

Table 6. Retention times of nucleobases and nucleosides.

Ratio of solvent A:B		Retention times (min)				
A	B	Adenosine	Adenine	Uracil	Cytosine	Guanine
10	90	6.394	8.189	2.602	8.626	7.653
20	80	3.569	4.270	2.266	3.472	3.363
30	70	2.599	2.849	2.029	2.608	2.541
50	50	2.043	2.166	1.895	2.005	1.998
70	30	1.845	1.951	1.847	1.816	1.839
80	20	1.869	1.929	*	*	*

* Not observed.

Figure 5. Retention profile of one nucleoside and four nucleobases.

Amino acids are the building blocks of proteins and are actively studied in research. Four L-amino acids were tested and their retention times were recorded. Three amino acids tested have a nonpolar side chain and the fourth has a polar and positively charged side chain. All the amino acids tested show very good retention in the ANP mode. The amino acids with nonpolar sidechains showed less retention than the polar positively charged histidine. Those data are reported in Table 7 and can be seen graphically in Figure 6.

Table 7. Retention times of amino acids.

Ratio of solvent A:B		Retention times (min)			
A	B	Tryptophan	Phenylalanine	Phenylglycine	Histidine
10	90	9.098	10.420	10.670	*
20	80	3.372	3.431	3.651	14.282
30	70	2.326	2.408	2.512	5.070
50	50	1.945	1.925	1.964	2.332
70	30	*	*	*	1.865

* Not observed

Figure 6. Retention map of amino acids.

Caffeine and theobromine are both very similar in their molecular structures, so it is understandable that they behave similarly in a biological sense. They both are able to cross the blood brain barrier and placental barrier. Much research has been done to determine any negative effects they may have on fetal development.²¹ They are both present in many foods regularly consumed around the world including coffee and chocolate. Many companies are interested in quality control of these compounds, especially caffeine.

The retention time of caffeine and theobromine increased with increasing percent organic in the mobile phase after 50% B in the mobile phase. This is likely due to

preferential interactions with the diol over the increasingly nonpolar mobile phase. ANP behavior is therefore confirmed. As the percent organic decreases in the mobile phase from 50 to 10, the retention time increases. Those data are reported in Table 8 and can be seen graphically in Figure 7. This confirms RP behavior and is likely due to interactions with the nonpolar silica hydride surface or the alkyl chain of the diol.

Table 8. Retention times of two purine alkaloids.

Ratio of solvent A:B		Retention times (min)	
A	B	Caffeine *	Theobromine *
5	95	2.730	3.528
10	90	2.386	2.781
20	80	2.136	2.310
30	70	1.962	2.103
50	50	1.874	1.938
70	30	1.922	1.897
80	20	1.968	1.936
90	10	2.100	1.973

* Caffeine and theobromine retention times were collected using 0.1% formic acid in solvents A and B. All other parameters were consistent with related studies.

Figure 7. Caffeine and theobromine retention profiles.

Two positively charged analytes were tested: thiamine hydrochloride and histamine. Thiamine is the salt form of vitamin B₁, a vitamin important to metabolism. There are numerous thiamine deficiencies, especially in countries dependent on rice as their main carbohydrate source.²² Histamine is a biogenic amine that is vital to many biological processes. Intolerance to elevated concentrations of histamine in humans has recently become an important area of research. Histamine is found naturally in many foods and its production is increased upon ingestion of allergens and other foods, including alcohol. Persons intolerant to histamine may have the following symptoms: vasodilatation, increased vascular permeability, tachycardia, alterations of blood pressure,

arrhythmias, gastric acid secretion, and nociceptive nerve fibers.²³ Determining the concentration of histamine in complex mixtures such as blood and food products is an important piece of information for those interested in the harmful effects of histamine. Their retention times as a function of the percent organic in the mobile phase were recorded and can be found in Table 9. These data are plotted in Figures 8 and 9.

Table 9. Retention times of miscellaneous positively charged analytes.

Ratio of solvent A:B		Retention times (min)	
A	B	Thiamine	Histamine
10	90	29.435	*
20	80	8.098	14.282
30	70	3.861	5.07
50	50	1.952	2.332
70	30	1.847	1.865

* Not observed.

Figure 8. Retention profile of Histamine.

Figure 9. Retention profile for thiamine hydrochloride.

The silica hydride based diol stationary phase clearly displayed ANP behavior for all compounds described above. Slight RP behavior was observed for some of these polar and charged compounds. Retention of these molecules is likely due to the diol functionality and slightly due to the silica hydride and alkyl portion of the bonded phase. Although the retention in RP mode due to the silica hydride was slight, the main benefit of using silica hydride is still present. Meaning, the water adsorbed onto the surface of the silica hydride surface is non-existent compared to the water adsorbed onto the surface containing silanol groups. This water layer adsorbed onto the surface of type-B silica creates a poor environment to retain nonpolar compounds. Type-C silica has repeatedly shown retention in both ANP and RP modes. Functionalizing type-C silica with a highly polar functional group, such as a diol, should still allow for retention in the RP mode.

Reversed-Phase Retention of Analytes

In this section the RP behavior of the silica hydride based diol stationary phase was characterized using three nonpolar compounds. The analytes chosen were two, three, and four membered polycyclic aromatic hydrocarbons (or PAHs). PAHs are a known carcinogen. A major concern regarding this class of compounds is overexposure in workplaces such as factories and places with high levels of exhaust.²⁴

Retention for these compounds was confirmed on the silica hydride based diol stationary phase. Retention profile data can be found in Table 10 and Figure 10. The more aromatic rings on the PAHs led to an increase in retention. This could point to a

possible size exclusion affect, although it is likely due to slower diffusion for larger molecules through a medium.

Table 10. Retention times of nonpolar analytes.

Ratio of solvent A:B		Retention times (min)		
A	B	Naphthalene	Fluorene	Pyrene
50	50	1.444	1.433	1.436
70	30	2.031	2.219	2.801
80	20	2.529	3.349	6.685
90	10	4.496	9.793	*

* Not observed

Figure 10. Retention profile of nonpolar compounds.

Analytes With No Retention in ANP or RP

Seven organic acids did not retain in ANP-like or RP-like conditions. The organic acids tested were: vanillic acid, pyruvic acid, syringic acid, protocatechuic acid, p-coumaric acid, ferulic acid, and caffeic acid. These compounds are found in different varieties of wine. These organic acids eluted within a few seconds of the dead time under high concentrations of acetonitrile and under high concentrations of water.

A Flexar FX-10 UHPLC was used to determine retention times on a MicroSolv Diol 2.0™ column. A flow rate of 0.5 mL/min and injection volume of 2 µL were used. Samples were prepared in DI water at a concentration of 0.1 mg/mL.

Mobile Phase Modifier Concentration Studies

Using mobile phase additives is a very common practice across all scientific disciplines that use chromatography as a tool. Therefore, determining the effect different concentrations of mobile phase additives have across a range of pHs values is valuable information for method development. The silica hydride based diol stationary phase is relatively unexplored. The experiments described below were performed to help elucidate different trends that emerge when varying these parameters. Another key reason for these studies is to compare these observations to the trends described in literature for HILIC separations.

Two additives were used: formic acid and ammonium acetate. The mobile phase consisted of two solvents A and B. Solvent A consisted of DI water. Solvent B consisted of acetonitrile in the formic acid studies and 90:10 ratio of acetonitrile:DI water in the ammonium acetate studies. To these solvents an identical concentration of a buffer was added.

The pH range of these buffers in 20:80 ratio of DI water:acetonitrile was determined using a Fisher Scientific Accumet pH meter. It is important to mention that the calibration standards (one at pH 4.0 and one at pH 7.0) for the pH meter were prepared in DI water and not the 20:80 ratio of DI water:acetonitrile in question. The solvent ratios for the concentration studies vary from this 20:80 mixture. Also, a majority

of the pKa values reported in Table 12 are experimentally determined at temperatures close to 25C and the column oven temperature was kept at 40C. This will perturb the value of the pKa further. For these reasons, the values reported in Table 11 and 12 are approximations. Guidelines for the pH of the ammonium acetate and formic acid buffers are reported in Table 11.

Table 11. Summary of pH ranges for 20:80 mixtures of DI water and acetonitrile with an added buffer over a range of concentrations.

20:80 ratio of DI water to acetonitrile		
Buffer	Concentration range	pH range
Ammonium acetate	2.0 - 10.0 mM	6.13-5.61
Formic acid	0.05 - 3.0 % (v/v)	3.02-2.86

Table 12. Analyte pKa values and corresponding net average charges at pH 3 and 6.

Analyte	pKa1	pKa2	pKa3	Net charge	
				at pH 3	at pH 6
Tryptophan	2.38	9.24	—	0	0
Phenylalanine	2.18	9.09	—	0	0
Cytosine	4.6	12.16	—	2	1
Adenine	4.1	9.8	—	2	1
Adenosine	3.63	12.4	—	2	1
Uracil	9.5	>13	—	1	1
Histamine	6.04	9.75	—	2	1 to 2
Histidine	1.8	6.04	9.33	1	0 to 1
Theobromine	7.89	—	—	1	1

Values taken from reference.²⁵

Formic Acid

The concentration of formic acid was varied for a group of hydrophilic compounds, one permanently charged compound, and a group of hydrophobic compounds. For each of these groups the ratio of solvents A:B was kept constant. The chromatograms of hydrophilic compounds were obtained using 15:85 ratio of A:B. The solvent ratio for the nonpolar compounds was 80:20 of A:B. The solvent ratio of the permanently charged compound, thiamine was 30:70 of A:B.

All hydrophilic compounds including thiamine had a drastic increase in retention as the concentration of formic acid was decreased. This sharp increase is clearly demonstrated in Figure 11. The opposite trend has been reported in literature for HILIC

Figure 11. Retention profile of amino acids and thiamine as a function of formic acid concentration. type separations. HILIC retention of polar analytes decreases as the buffer concentration decreases. This corroborates the hypothesis that polar analyte retention in HILIC and ANP operate under two separate mechanisms of retention.

Several possible reasons for the increase in retention after a decrease in formic acid buffer concentration are considered here:

- i.** A preferential interaction between the formate anion and the stationary phase. This decreases the number of available sites for the analyte to interact with the stationary phase.
- ii.** A preferential interaction between the formate anion and the polar analytes. This decreases the number of available sites for the analyte to interact with the stationary phase.
- iii.** The ionic strength and pH of the mobile phase ionizes the very few silanols remaining on the silica hydride based diol stationary phase. Under more basic conditions (low buffer concentration) the ionized silanols are likely to retain positively charged species.
- iv.** The ionic strength and pH of the mobile phase ionizes (or unionizes) each analyte into a form that is less favorable for retention. The sharp upward trend occurs for all polar analytes at the same concentration of formic acid. Since the analytes tested have a range of pKa values it is unlikely that this trend is a result of a change in ionization of the analytes. Also, the nonpolar analytes experienced an upward trends as formic acid concentration decreased as well, observed in Figure 12. This is most definitely not due to a change in the ionization state of these compounds.

The data for the retention profiles of polar and nonpolar compounds can be found in Table 13, Table 14, and Figure 12.

Figure 12. Retention profile of nonpolar analytes as a function of formic acid concentration.

Table 13. Retention of polar/hydrophilic compounds as a function of formic acid concentration.

	Retention Times (min.)			
% formic acid	Tryptophan	Phenylalanine	Adenine	Cytosine
0.300%	3.660	4.269	4.777	4.839
0.200%	3.981	4.810	5.006	5.219
0.100%	4.503	5.340	5.242	6.122
0.050%	4.900	5.766	5.378	6.627
0.025%	6.108	6.719	5.604	9.194
0.00%	9.250	8.985	6.687	20.579

Table 14. Retention of thiamine as a function of formic acid concentration.

	Retention Times (min.)
% formic acid	Thiamine
0.200%	3.861
0.100%	4.449
0.02%	7.972
0.00%	27.380

As the concentration of formic acid in the mobile phase increases there is a noticeable decline in the retention for all three nonpolar analytes tested, see Figure 12. These results are not as severe as what was observed with the polar analytes. Also, the

shape of the curves in the two categories are noticeably different. This suggests that the mechanism of retention is different between the nonpolar and polar analytes on the silica hydride based diol stationary phase. If the mobile phase additive is competing with the analytes for adsorption sites this would make sense. The formate anion will likely be much more attracted to the polar bonded phase than the nonpolar silica hydride. Nonpolar analytes are likely retained by interacting with the silica hydride groups while the polar analytes are retained much more strongly on the diol. Similarly, if the formate anion is interacting with the analytes causing a reduction in retention, the strength of this interaction would be expected to be greater with polar analytes. Based on these two assumptions, adsorption of polar analytes onto the stationary phase is likely to be more hindered.

Table 15. Retention times of nonpolar analytes as a function of formic acid concentration.

% formic acid	Retention Times (min.)		
	Naphthalene	Fluorene	Pyrene
0.200%	2.529	3.363	6.685
0.100%	2.636	3.483	6.822
0.02%	2.692	3.567	6.950
0.00%	2.710	3.600	7.049

The retention times of polar and nonpolar compounds showed a dependence on formic acid buffer concentration. Polar compounds experienced a much larger change in

retention times over the concentration range studied than the nonpolar compounds studied.

Ammonium Acetate

The relationship between the concentration of a buffer with a higher pH range and retention times of polar and nonpolar analytes was studied. To investigate this ammonium acetate, another common buffer in HPLC, was used. The same analytes were used to keep consistent with the formic acid buffer concentration studies. The retention times of nonpolar and polar analytes as a function of ammonium acetate concentration are reported in Table 16, Table 17, and Figure 13. No significant change in retention was observed across the ammonium acetate concentration studied for polar and nonpolar analytes. These results are not consistent with what was observed with the formic acid buffer system.

Figure 13. Retention profiles of analytes as a function of ammonium acetate concentration.

Table 16. Retention times of polar analytes as a function of ammonium acetate concentration in the mobile phase.

Ammonium acetate concentration (mM)	Retention time (min.)			
	Tryptophan	Phenylalanine	Adenine	Cytosine
10.0	3.184	3.576	2.794	3.087
2.5	2.995	3.572	2.796	3.009
0.5	3.154	3.414	2.815	3.084

Table 17. Retention times of nonpolar analytes as a function of ammonium acetate concentration in the mobile phase.

	Retention time (min.)		
Ammonium acetate concentration (mM)	Naphthalene	Fluorene	Pyrene
10.0	2.687	3.627	7.690
5.0	2.689	3.664	7.655
0.0	2.675	3.643	7.828

The most obvious difference between the ammonium acetate buffer and formic acid buffer are their operating pH ranges; see Table 11. The analytes have varying pK_as and are unlikely to have such a large increase in retention upon decreasing the amount of formic acid in the mobile phase. Therefore, any pH effects are likely to affect the stationary phase and not the individual analytes.

If this is a result of ionic interactions between the buffer ions with either the analyte or stationary phase (see points **i** and **ii** in section III-B-1), it could be that ammonia and acetate ions do not interact as strongly as the formate ion. Another possibility is the ammonium acetate ions in solution prefer to interact with each other and therefore do not interact with the analytes or stationary phase as strongly as the formate anion.

As observed in Table 18 and Figure 14, the retention of thiamine as a function of ammonium acetate concentration coincided with the formic acid results. This could

possibly due to the permanent charge on the thiamine. If this is an effect due to charge, this would not explain why cytosine and adenine do not follow a trend similar to thiamine. Future studies need to be done to investigate how buffer concentrations affect silica hydride based stationary phases with polar bonded phases. Buffers with common ions as the formic acid and ammonium formate should be done. This should uncover new information to help determine if preferential interactions between the buffer ions and either the stationary phase or analytes affects the retention of analytes. To help determine

Figure 14. Retention profiles of thiamine as a function of ammonium acetate concentration.

Table 18. Retention times of thiamine as a function of ammonium acetate concentration in the mobile phase.

Ammonium acetate concentration (mM)	Thiamine
10.0	11.977
5.0	17.722
2.5	24.205

if pH effects these trends, buffers with pH ranges that are in between the pH ranges for formic acid and ammonium acetate should be studied.

Comparison of Two Silica Hydride Based Diol Bonded Phases

The alkyl chain length of the bonded phase may be a parameter scientists want to consider during the method development process. Two silica hydride based columns with different alkyl chain lengths were compared by retaining tryptophan, theobromine, and

$$k = \frac{t_r - t_o}{t_o}$$

fluorene under three mobile phase compositions of solvents A and B. The formic acid concentration was kept constant at 0.1% v/v. The capacity factor (k) was calculated using an approximation of the void time and the retention times. In Tables 19-21 the capacity factor for each analyte is reported for the long and short diol columns at each mobile phase composition. In Figure 15 the capacity factor for the long and short diol are graphically compared at three different mobile phase compositions for tryptophan (polar) and fluorene (nonpolar). It is apparent that the short diol column retains polar compounds better and that the longer diol column retains nonpolar compounds better. This shows that

the retention of nonpolar compounds (or compounds containing large nonpolar regions) will increase by increasing the alkyl chain of the diol.

Table 19. Capacity factor (*k*) of theobromine using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.

%B in Mobile Phase	Long Bonded Phase	Short Bonded Phase
95	0.181	0.806
90	0.145	0.472
80	0.074	0.266

Table 20. Capacity factor (*k*) of tryptophan using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.

%B in Mobile Phase	Long Bonded Phase	Short Bonded Phase
90	1.11	7.475
80	0.631	1.24
70	0.161	0.466

Table 21. Capacity factor (*k*) of fluorene using bonded phases 1,2-dihydroxyoctyl and 1,2-dihydroxybutyl.

%B in Mobile Phase	Long Bonded Phase	Short Bonded Phase
30	0.820	0.215
20	2.67	0.927
10	6.89	4.18

Figure 15. Comparison of the capacity factor of analytes L-tryptophan and fluorene on two diol columns; 1,2-dihydroxybutyl (short diol) and 1,2-dihydroxyoctyl (long diol).

Select Applications of Silica Hydride Based Diol

Bisphenols

Bisphenols A, F, and S are hormonally active chemicals found in plastic products. They are classified as endocrine-disrupting chemicals (EDCs). Bisphenols have been reported to have “altered organ weights, reproductive end points, and enzyme expression” in *in vivo* and *in vitro* studies.²⁶ This is especially important in developing fetuses and infants. BPA is regulated in plastic baby bottle products by law in many states, but unfortunately BPS and BPF have not been treated with the same level of scrutiny despite being classified as an EDC.²⁶ The chromatograms below show a method using the silica

hydride based diol stationary phase that could potentially be used to determine leaching of BPA, BPF, and BPS in plastic products. This includes products designed for newborns and infants.

The separation of these bisphenols was achieved isocratically on the Diol 2.0™ column; observed in chromatograms in Figure 16. A HP 1050 HPLC was used with a wavelength of 214 nm and injection volume of 1 µL. The mobile phase consisted of 93% DI water and 7% acetonitrile each with 0.1% formic acid and was delivered at a rate of 0.4 mL/min.

Figure 16. Chromatograms of bisphenol S (top), bisphenol F (middle), and bisphenol A (bottom).

Drugs and Medications

The blood pressure medications amiloride and triamterene were successfully separated isocratically in under 1.5 minutes on the Diol 2.0™ column using a Perkin Elmer Flexar FX-10 UHPLC. This separation is observed in the over lapping

chromatograms seen in Figure 17. The mobile phase was 90% DI water and 10% acetonitrile with each solvent containing 0.1% formic acid. This shows another successful RP application using the silica hydride based diol stationary phase.

Figure 17. Overlaid chromatograms of the blood pressure medications: Amiloride (left) and triamterene (right).

The Diol 2.0TM column was also used to retain three illicit drugs that many forensic scientists may be interested in: hydroxybupropion, lysergic acid diethylamide, and codeine. The overlaid chromatograms is pictured in Figure 18. The instrument used was the Perkin Elmer Flexar FX-10 with a wavelength of 210 nm and a flow rate of 0.5 mL/min. Solvent A was DI water with 0.1% formic acid. Solvent B was acetonitrile with 0.1% formic acid. The mobile phase conditions were 90% solvent B from time 0-0.5 min

and then gradually decreased to 10% solvent B over times 0.5-5.5 min. The initial conditions in the gradient were operating in ANP mode and gradually increased the aqueous component over time to more RP-like conditions.

Figure 18. Overlaid chromatograms of three illicit drugs: hydroxybupropion (left), LSD (middle), and codeine (right).

Amino Acids in Carbonless Mobile Phase

Scientists have successfully used semipreparative HPLC to isolate hydroxyproline in bone samples to be used for accelerator mass spectrometry (AMS) dating with relatively low background noise. To do this it is necessary to use a carbonless solvent (water with the possibility of buffers). Unsurprisingly, this is not an easy task. Hydroxyproline is very hydrophilic and typically elutes very quickly.

The Diol 2.0™ column on the Perkin Elmer Flexar FX-10 UHPLC was used to retain hydroxyproline with a 100% water mobile phase. The overlaid chromatogram of a blank (water) and 0.5 mg/mL hydroxyproline in DI water can be found in Figure 19. Histidine was also retained on the HP 1090 HPLC using the Diol 1.0™ column with

water and 0.1 mM phosphoric acid mobile phase, see Figure 20. Radio carbon dating

Figure 19. Retention of hydroxyproline using 100% water as mobile phase with water blank overlaid.

using histidine is not mentioned in literature, but the chromatogram was run as a possible alternative to using hydroxyproline; pictured in Figure 20. The capacity factor of hydroxyproline was 0.65 and histidine was 2.2.

Figure 20. Chromatogram of L-histidine using water (0.1 mM phosphoric acid) as mobile phase.

CONCLUSIONS

This work proved that silica hydride based diol stationary phases were able to retain analytes in both ANP and RP modes. This dual retention allows nonpolar and polar compounds to be retained using a single stationary phase. It has been reported in literature that the water layer adsorbed onto the surface of silica hydride based stationary phases is less than one water molecule thick.¹¹ Based on this, it is unlikely that retention of polar molecules is due to a HILIC-like mechanism (partitioning). Instead, a combination of adsorption and ionic interactions is likely.

Two silica hydride based columns were used to retain test analytes. The bonded phases of these two diols, referred to as “long diol” and “short diol,” correspond to the bonded phases 1,2-dihydroxybutyl and 1,2-dihydroxyoctyl, respectively. The capacity factors were significantly larger for polar analytes and smaller for nonpolar compounds on the short diol. This is a result of the larger nonpolar region on the long diol bonded phase. Adsorption of nonpolar compounds on the stationary phase is increasingly encouraged as the nonpolar character of the bonded phase increases.

A dependence of retention times of polar and nonpolar analytes on the concentration of formic acid buffer was observed. As concentration increased, retention times of analytes decreased on the silica hydride based diol stationary phase. This trend is reverse of what has been observed with HILIC retention on type B silica-based columns. It is highly likely that ANP and HILIC retention occurs through different mechanisms. Polar test compounds experienced a much larger change in retention time over the concentration range of formic acid than the nonpolar test compounds. It is possible that

ionic interactions are responsible for this trend. In another study, analyte retention did not change significantly with increased concentration of ammonium acetate buffer (with the exception of thiamine, a permanently charged compound). This buffer operates over a higher pH range. This indicates that pH may alter the environment of the stationary phase, changing the retention mechanism, or mechanisms. Further studies should be done to characterize the specifics of pH dependence and ionic interactions. To test pH dependence, buffers with pH ranges in between those of the formic acid and ammonium acetate buffers should be used. To determine if the buffer ions are preferentially interacting with either the analytes or stationary phase, buffers with common ions to formic acid and ammonium acetate should be used. Ammonium formate and acetic acid buffers should be included in this future study.

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