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THE SYNTHESIS AND CHARACTERIZATION OF ENDCAPPED SILICA

HYDRIDE STATIONARY PHASE

A Thesis

Presented to

The Faculty of the Department of Chemistry

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Prema Gangadhara

August 2011

The Designated Thesis Committee Approves the Thesis Titled

THE SYNTHESIS AND CHARACTERIZATION OF ENDCAPPED SILICA

HYDRIDE STATIONARY PHASE

by

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APPROVED FOR THE DEPARTMENT OF CHEMISTRY

SAN JOSE STATE UNIVERSITY

August 2011

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ABSTRACT

THE SYNTHESIS AND CHARACTERIZATION OF ENDCAPPED SILICA

HYDRIDE STATIONARY PHASE

by Prema Gangadhara

The silanization/hydrosilation process was developed to overcome some of the drawbacks associated with the use of organosilane reagents for the synthesis of high pressure liquid chromatography (HPLC) stationary phases. The first step is called silanization, in which silica or other oxides react with triethoxysilane (TES), resulting in the formation of a silica hydride surface. Endcapping the stationary phase using trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) eliminates the unreacted and accessible silanols on the silica hydride intermediate.

This research employed the modification of bare silica to silica hydride via silanization. Endcapping after silanization eliminated accessible and unreacted silanols. Endcapping was confirmed by using diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy. The stationary phase was characterized for its hydrophobicity and silanophilicity properties. The effectiveness of the stationary phase was determined for its aqueous normal phase and reversed-phase chromatographic behavior. The performance was compared with the performance of C8 silica hydride column under similar conditions. This research confirmed that endcapped columns could be used in the aqueous normal phase and reversed-phase modes. Endcapping increased the hydrophobicity of the surface, which in turn increased the efficiency of separation for nonpolar solutes.

ACKNOWLEDGEMENTS

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1. INTRODUCTION

A. Chromatography

Chromatography is a separation technique that has applications in all branches of science. The separation is based on a physical property in which the stationary and mobile phases are brought into contact and the sample mixture is separated based on its affinity towards a mobile phase and/or a stationary phase. The technique can be classified by the types of mobile and stationary phases used and the levels of equilibrium involved during the transfer of solutes between the two phases. Separation by chromatography can be classified into many different types on the basis of physical state of the mobile phase, the separation mechanism, and the shape of the chromatographic bed. A few of the separation techniques are adsorption chromatography, partition chromatography, size exclusion chromatography, affinity chromatography, and ion-exchange chromatography. The applications of some of the separation techniques are as follows: gas chromatography is used mainly for volatile compounds; ion exchange chromatography is used mainly for separating ions; liquid chromatography is used for separating non-volatile compounds; size exclusion chromatography is used for separating compounds based on molecular weight [1-4].

B. Modes of Separation

1. Normal Phase High Performance Liquid Chromatography

In normal phase chromatography, the stationary phase is strongly polar and the mobile phase is nonpolar. The stationary phase usually consists of silica, cyano, diol, or amino groups; therefore polar compounds are retained longer than the nonpolar compounds. The retention of the compounds is governed by an adsorption mechanism on the column. The mobile phase usually consists of an organic solvent combination of a very nonpolar liquid with a slightly more polar solvent such as hexane/ethyl acetate or hexane/isopropanol. The main limitation of this separation is that it cannot be used with aqueous solvents [1-4].

2. Reversed-Phase High Performance Liquid Chromatography

In reversed-phase (RP) HPLC, the stationary phase is nonpolar in nature, whereas the mobile phase is polar. The stationary phase is usually made of nonpolar ligands bonded to a polar matrix; therefore nonpolar compounds that interact with the stationary phase are retained longer in the column and are better separated. In reversed phase, the separation is mainly governed by a partitioning process rather than by adsorption [1-4].

3. Hydrophilic Interaction Liquid Chromatography

Hydrophilic interaction liquid chromatography (HILIC) is normal phase chromatography that is used to separate polar compounds. HILIC provides the advantage

of having aqueous and organic components in the mobile phase. This unique feature

distinguishes it from normal phase chromatography. The chromatographic surface used

for the stationary phase can be neutral, polar, or ionic. The main draw back with HILIC

separation is that it can only separate polar compounds and cannot be used for nonpolar

compound separation [1-4].

4.   Aqueous Normal Phase Chromatography

A silica hydride (Si-H) stationary phase is used in aqueous normal phase (ANP)

as an alternative to other types of silica materials. The technique is used for separating

both polar and nonpolar compounds. This unique stationary phase has many important

advantages over ordinary silica columns in which most of the adsorptive properties are

controlled by silanol groups. Si-H can separate both acidic and basic compounds because

of its unique capability to use a wide range of mobile phase compositions, from 100%

aqueous to pure nonpolar organic solvents.

Reversed-phase and aqueous normal phase separations are complementary to each

other as water is common in both modes of separation. ANP displays rapid equilibration

of the separation materials and, in some cases it is possible to operate both separation

modes simultaneously. This unique character helps in separating both hydrophobic and

hydrophilic compounds in a single isocratic or gradient run. A number of silica

hydride-based stationary phases have been studied because, it is possible to obtain

reversed-phase and aqueous normal phase capabilities by varying the surface composition

for a range of applications. The transition between ANP and RP is possible only with

silica hydride stationary phases by the use of water in the mobile phase.

The HILIC separation mode cannot undergo this transition. Therefore it can only

separate polar compounds. This unique property of ANP makes it different from HILIC

in which only polar compounds are separated [1-4].

C. Resolution and Column Efficiency

Resolution is one of the main factors that define the efficiency of HPLC columns.

It describes how well separated two peaks are from each other.

$$R = 1.18 \, \Delta t_R / W_1 + W_2$$

Where Δt_R is the difference in retention times between two peaks and W_1 and W_2 are the

widths of the two peaks.

The other factors on which HPLC separation and efficiency depend are column

selectivity (α), the sample capacity factor (k'), and column efficiency (N).

Column selectivity is defined as

$$\alpha = (t_{R2} - t_0) / (t_{R1} - t_0)$$

Where t_{R2} is the retention time of the second peak, t_{R1} is the retention time of the first

peak and t_0 is the retention time of the void volume. Separation is achievable for $\alpha > 1$.

The sample capacity factor is defined as

$$k' = (t_R - t_0) / t_0$$

Where t_R is the retention time of a retained peak and t_0 is the retention time of the void

volume.

The column efficiency (N) is based on the number and height of the theoretical plates, which can be determined by the equation

$$N = 5.54 \, (t_R/W)^2$$

Where t_R is the retention time of a solute and W is the width of the peak.

The height equivalent to a theoretical plate (HETP) can be calculated by using the Van Deemter equation

$$HETP = L/N$$

Where L is the column length and N is the number of theoretical plates. A low HETP value represents high column efficiency [1, 5].

D. Column Material

The solid supports that can be used for surface modification are polymers, titania, alumina, zirconia, thoria, and silica. Silica is the most widely used solid support in liquid chromatography because it is inexpensive and is commercially available in almost any particle size. It has a very high mechanical stability, is very rigid, and can resist swelling. It also provides high efficiency and comes in different pore sizes which make it easy to modify in various ways.

Although silica has been one of the dominant choices as a solid support for stationary phases, numerous attempts have been made to find an alternative material to overcome some of the deficiencies associated with it. The major issues associated with silica while using it as a stationary phase are pH instability and its strong affinity for basic compounds. Silica has problems when measuring samples with a pH less than 2 or

greater than 8, as at a lower pH the stationary phase undergoes decomposition, while at higher pH it begins to dissolve. It is possible to operate columns at a higher pH, provided the silica support is densely bonded, extensively endcapped, or has a longer chain column packing that allows the use of organic buffers as part of the mobile phase.

The silica surface can have different kinds of active groups. These groups are shown in Figure 1 [6]. The different active groups are siloxane, free silanols, geminal silanols, vicinal silanols, and bonded water. Each of these active groups has advantages and disadvantages depending on the type of analysis conducted. These active groups can help in the separation of some compounds and interfere with others. The functionality that is most widely used in the modification of silica surfaces is free silanol [6].

There are different types of silica that are available and are distinguished based on pore size, specific surface area, particle size, etc. The important factor to consider when choosing silica is the pore size, since it determines the interacting surface of the stationary phase. In general, larger pore size silica is useful for the analysis of large-sized or macromolecules, whereas smaller pore size silica is useful for the analysis of small molecules. It is possible to operate silica at different pH values, under high pressures, and in both aqueous and organic mobile phases used during the analysis [7].
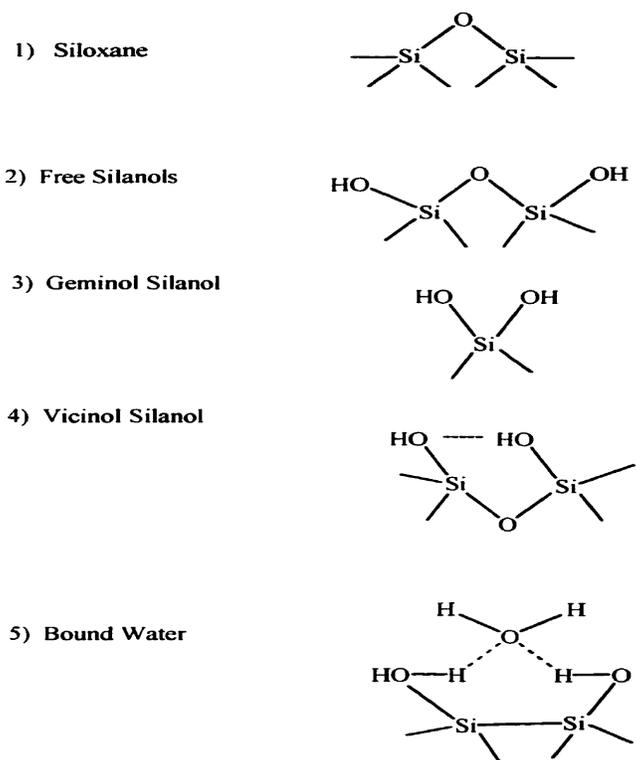
Figure 1. Active groups on a silica surface.

The type of silica used for this research was Astrosil type B silica, which has a narrow pore size distribution (100 Å). It is not possible to convert all the silanol groups during bonded phase reactions due to steric effects. These unreacted silanol groups can cause problems like peak tailing and loss of chromatographic resolution. These groups can also interact with analytes via hydrogen bonding [7]. The main goal of this research was to first convert silica to silica hydride via a silanization reaction, and then cap the unreacted silanol groups with a methyl group and characterize it through chromatograpic techniques.

E. Silica Surface Modification

Silica is the most extensively used stationary phase material, but it cannot be used in its native form, because of its limited pH stability and peak tailing when analyzing basic compounds. The prolonged exposure to strongly acidic and basic aqueous mobile phases can deteriorate the silica surface. Making modifications to the surface of silica can be advantageous in terms of pH stability, as well as peak tailing, and allows the silica support to be used with aqueous solutions for a longer period of time. Silanol groups on the silica surface react with bonding groups to form the bonded phase [8]. Silica surfaces can be modified using several methods. Some examples are:

1. Esterification

The reaction is achieved between a silanol group and an alcohol group. The reaction can be written as

$$Si\text{-}OH + R\text{-}OH \text{ ---> } Si\text{-}OR + H_2O$$

The link between the organic moiety and the silica surface is silicon-oxygen-carbon (Si-O-C). This method is very simple and the bonded phase is thermally stable. The drawback of this modification is that the bonded phase is very unstable in the presence of water. Therefore it is not possible to use stationary phases based on this modification for many HPLC applications with aqueous mobile phases [8].

2. Organosilanization

The majority of commercially available columns use the organosilanization method for silica surface modification. The reaction involves an organosilane reagent with a halide, methoxy, or ethoxy group and the silica surface. The organosilane reagent is covalently bonded to the silica surface and the reaction can be used to produce monomeric or polymeric stationary phases, depending on the type of silane used. An organosilane reagent with three reactive groups forms a multilayered polymeric stationary phase due to crosslinking between the adjacent organosilane groups. The general disadvantage of organosilanization is that an acid by-product is formed during the reaction which can hydrolyze the Si-O-Si-C bonds. Monomeric organosilane stationary phases have poor hydrolytic stability under acidic and basic conditions [9-10].

The organosilanization reaction can be written as

$$Si\text{-}OH + X\text{-}Si\ R'_2\ R \longrightarrow Si\text{-}O\text{-}Si\ R'_2\ R + HX$$
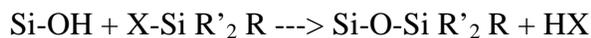
X-halide and R-alkyl

$$Si\text{-}OH + X_3\text{-}Si\text{-}R \longrightarrow Si\text{-}O\text{-}Si\text{-}R + 3HX$$
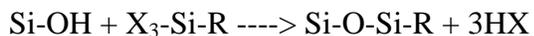
3. Chlorination Reaction Followed by a Reaction with Grignard Reagents or an Organolithium Compounds

In this two step reaction, the first step is carried out by reacting silica with thionyl chloride to form an unstable chlorinated intermediate. In the second step the chlorinated intermediate reacts with an organometallic compound leading to the formation of a Si-C linkage. The disadvantages of this reaction are that the byproduct metal salt which is

9

formed in the second step is very difficult to separate from the original product, and the

chlorinated intermediate is very unstable, hence the reaction has to be carried out in dry

environment [8-9].

The reaction can be written as:

$$Si\text{-}OH + SOCl_2 \text{-----}> Si\text{-}Cl + SO_2 + HCl$$

$$Si\text{-}Cl + BrMgR \text{----}> Si\text{-}R + MgClBr$$

or

$$Si\text{-}Cl + Li\text{-}R \text{-----}> Si\text{-}R + Li\text{-}Cl$$
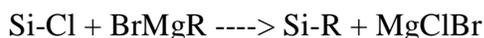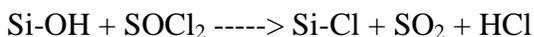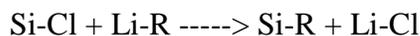
4. Silanization/Hydrosilation

The recent approach for the modification of silica surfaces involves the use of

triethoxysilane (TES) in a reaction called silanization, which is followed by a

hydrosilation reaction to attach an alkyl group to the silica surface. The silanization

reaction involves the formation of a stable hydride monolayer by the reaction of silica

with TES in the presence of an acid catalyst. The hydride intermediate formed can be

stored for longer periods of time, as it is stable in air or water without degradation. The

reaction has been reported to yield a 95% surface coverage with a nonpolar Si-H

monolayer [11].

a. Silanization
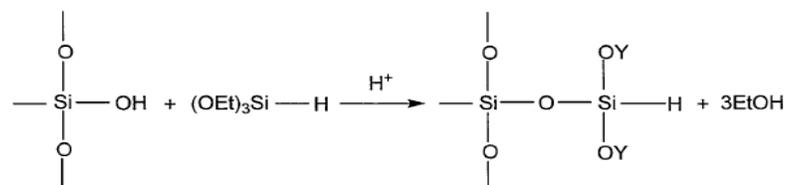
b. Hydrosilation
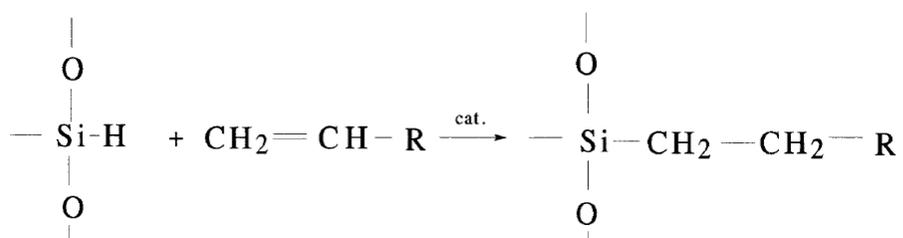
cat. = catalyst, typically hexachloroplatinic acid

The attachment of an organic group at the silica hydride surface to form a Si-C bond occurs in the hydrosilation step. The reaction is facilitated in the presence of a transition metal complex catalyst, usually Speier's catalyst, a 2-propanol solution of hexachloroplatinic acid. The drawback of this reaction is that the transition step while attaching an olefin to the silica hydride forms a complex with the catalyst. The use of a transition metal complex catalyst forms the intermediate that can be of very large in size. This might result in a lower yield of organic group on the surface; even though having a smaller size olefin in comparison to the organosilane species in the conventional

organosilanization bonding methods. The other problem is that the catalyst can undergo

reduction by itself. The silica hydride and metal complex catalyst react together and

form Pt (0) deposition on the surface. The presence of the metal on the surface affects

the chromatographic performance. The use of free radical initiation instead of a

transition metal complex might solve the problem. But hydrosilation via a metal complex

has been proven to be successful on silica hydride, and limited studies have been done on

free radical initiation [12]. The formation of the Si-C bond at the surface seems to be

more thermodynamically and hydrolytically stable than the commercially available

stationary phases that are formed after an organosilation reaction.

An important advantage of hydrosilation method is that the silica hydride

intermediate can be used by itself as a stationary phase for many chromatographic

applications. It is possible to use silica hydride columns in reversed-phase conditions,

organic normal phase conditions, and aqueous normal phase conditions. The research

focused on the aqueous normal phase and reversed-phase conditions using the endcapped

Si-H stationary phase and comparing it with a C8 silica hydride column. As shown in

Figure 2 [2], in reversed-phase chromatography the composition of the mobile phase

water/acetonitrile varies from 0% to 100% of organic constituents, which decreases the

retention of hydrophobic analytes as the composition of less polar organic solvent is

increased [2].
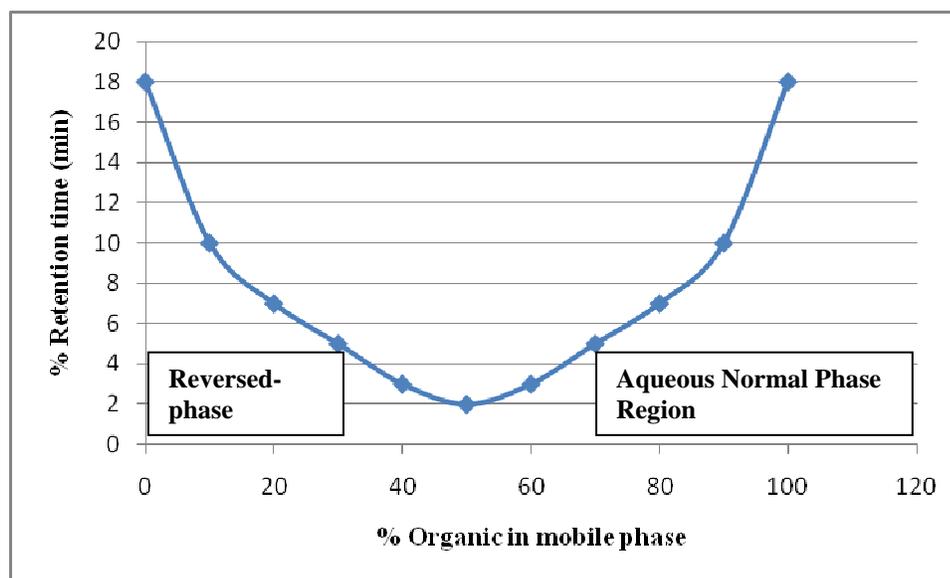
Figure 2. Retention map for RP and ANP region on a Si-H based stationary phase.

The goal for this research was to use a silica hydride intermediate and endcap the surface with trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). The endcapping was performed for the remaining unreacted silanol groups which were not converted to Si-H groups. This enhanced the column efficiency by minimizing the amount of silanol groups on the silica surface. Endcapping is defined as the secondary derivatization reaction that can be used to shield and reduce silanol groups on a modified silica surface [9, 13-14].

F. Endcapped Silica Hydride Stationary Phase

Endcapping can be done using polar reagents with an amide group or an alcohol group, or using nonpolar reagents with a methyl group depending on the type of separation that needs to be achieved. The use of an organosilane reagent replaces many of the unreacted silanol groups on silica and also increases the hydrophobicity of the stationary phase surface. The organosilane reagent reacts with only 50% of the available silanol groups and the remaining groups can cause problems by interacting with the analyte molecules. Endcapping overcomes problems with the interactions between the analyte and silanol groups by converting Si-OH groups to Si-O-Si-C groups. Endcapping of the silica hydride stationary phase uses a 1:1 molar ratio of trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS). TMCS-HMDS eliminates the few remaining unreacted and accessible silanols on the silica hydride intermediate which increases hydrophobicity of the surface. It is these strongly adsorbing sites on the surface of silica that are the targets for any endcapping [15].

For this research, Astrosil silica was first modified to silica hydride using triethoxysilane (TES), a silanization reaction. Then the silica hydride was endcapped with a 1:1 molar ratio of TMCS and HMDS to minimize the amount of silanol groups on the silica surface. The endcapping of silica hydride increased the hydrophobicity of the stationary phase which in turn was useful for the separations of nonpolar compounds. The results of endcapped Si-H were compared with a Si-H C8 column for chromatographic separations under aqueous normal phase and reversed-phase conditions.

The obtained results help to understand the polarity difference between the two columns and its applications. These studies determine whether endcapping is sufficient after silanization or further modification is required for the separation of nonpolar compounds.

G. Advantages of Using Hydride-based Stationary Phases

Silica hydride-based columns are desirable because of their high mechanical strength, high column efficiency, and ease of fabrication. It is possible to develop several bonded phases via silica derivatization with different compounds having different functional groups. Silanol groups are the main reaction sites for silica and modification to a silica matrix can be measured using magic-angle-spinning (MAS) ^{29}Si NMR and diffuse reflectance infrared Fourier transform spectroscopy [4].

Acidic silanol groups interact with basic analytes which leads to poor resolution, and peak tailing. Metals such as Fe, Al, and Zn which are found in silica can also contribute to peak tailing and retention properties during separation. Silica that is highly acidic and contains lower amounts of metals is a better starting material for stationary phase synthesis [4].

A comparison of the chemical composition between silica and silica hydride is shown in Figure 3 [2]. An ordinary silica surface has silanol groups (Si-OH) while the hydride silica surface has Si-H groups. The fundamental difference between the two materials leads to differences in their surface properties, which are responsible for the

significant differences in chromatographic separation between the two surfaces. Silanol

groups are highly polar while the Si-H is weakly hydrophobic, and it has also been

proved that silica hydride columns can be used for a wide range of applications [4].
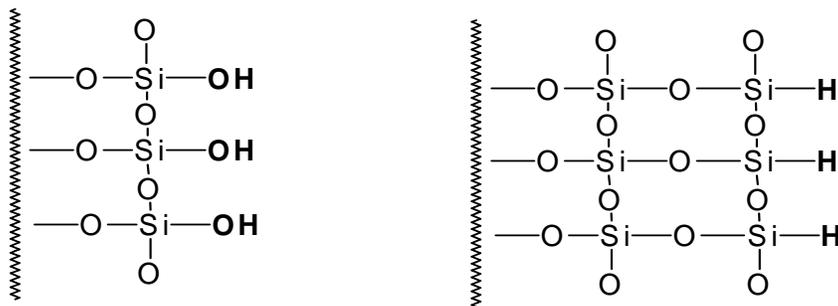
**Figure 3. Idealized structures of a normal silica surface (left) and silica hydride
surface (right).**

It is possible to synthesize silica hydride in two ways, one with the use of thionyl

chloride to form an Si-Cl intermediate. The main drawback to this process is that Si-Cl is

very sensitive to moisture and the reaction is easily reversible once exposed. Another

way of synthesizing silica hydride is via a silanization reaction. The process is done by

the condensation reaction between silanols and TES. This is a single step reaction and is

not sensitive to water as it uses an aqueous acid as a catalyst. Previous work has proven

that it is possible to get up to 95% silica hydride surface under carefully controlled

synthetic conditions [2, 4].

H. Characterization Techniques

Diffuse Reflectance Fourier Transform Infrared Spectroscopy, hydrophobicity, silanophilicity tests, and high performance liquid chromatography were used to characterize the endcapped stationary phase and the results were compared with a C8 silica hydride column.

1. Diffuse Reflectance Fourier Transform Infrared Spectroscopy

The spectroscopic characterization of the bonded material is done by using diffuse reflectance Fourier transform infrared spectroscopy (DRIFT), which determines the presence of organic moieties or functional groups on any silica surface including silica hydride. The measurement gives qualitative information about the structure of the molecule, and the fingerprint region of the molecule is often used to distinguish between one molecular structure and another. The Fourier transform process averages the signal, which increases the sensitivity of the measurement, and thus allows for the characterization of samples with weak absorption bands. DRIFT, like other infrared methods, measures molecular vibrations. The infrared region is divided into near, mid, and far regions with a frequency ranging from 12,800 cm^{-1} to 10 cm^{-1}. The mid-infrared region ranges from 4000 cm^{-1} to 200 cm^{-1} is used to obtain structural information of the molecules. The molecules undergo vibrational or rotational motions upon absorption of infrared radiation which changes the dipole moment of the molecule. The molecules absorb radiation when the natural vibrational frequency of the molecule matches the frequency of the incident radiation. The radiation on the surface undergoes partial

reflectance and partial transmittance and the light absorbed from the material undergoes

reflectance and transmittance. DRIFT is used in this research to obtain specific structural

information of the starting material as well as the synthesized product, which can be used

to confirm the success of a specific reaction [1-2].

2. Neue Test

Neue test is used to determine the hydrophobicity and silanophilicity of a

stationary phase material. The physico-chemical properties (hydrophobicity and

silanophilicity) of the columns are measured at pH 7. The Neue test is described below.

a. Hydrophobicity Parameter (u)

The hydrophobicity test is used to determine the retention of nonpolar

compounds; the greater the value of hydrophobicity of the column the greater the

retention of nonpolar solutes.

 The hydrophobicity parameter(u) is calculated as follows:

$$\ln(k) = u * V - 3.068$$

where V = the molar volume of the analyte (acenaphthene or naphthalene).

$$k = (t_r - t_0)/t_0$$

k = retention factor and t_0 = retention time of the void volume.

b. Silanophilic Interaction Parameter (S)

The silanophilic test is used to measure strong polar/adsorptive interactions with the surface. The lower the value from the silanophilic test the lower the surface adsorption, which results in better peak shape for polar compounds. The compounds used for this test are amitryptiline, acenaphthene, and uracil.

The silanophilic interaction parameter(S) is calculated as follows:

$$S = lnk_{amitryptiline} - 0.7124 * lnk_{acenaphthene} + 1.9748$$

$$k = (t_r-t_0)/t_0$$

k = retention factor and t_0 = retention time of the void volume [16].

3. Elemental Analysis

The quantitative micro-combustion method determines the amount of compound bonded to the silica surface. The measurement is based on a high temperature oxidation of the organic compounds by converting the elements of interest to gaseous molecules. The percentage carbon obtained indicates the amount of organic moiety that has been successfully attached on the silica surface [17].

4. High Performance Liquid Chromatography

As shown in Figure 4 [18] the concept of any chromatographic separation technique is that the sample mixture is injected onto the packed analytical column and the mobile phase is then pumped through the column. This carries the sample mixture along

the stationary phase. Different compound mixtures have different interactions with the stationary phase and the mobile phase based on their properties. The separation is mainly based on their interaction towards the stationary phase or mobile phase. The compounds which are separated eventually elute from the column and are detected using an appropriate measurement apparatus. Different detectors are chosen based on the sample properties and the technique can be used for a large variety of mixtures.

There are two possible elution modes: the isocratic separation mode and the gradient separation mode. In isocratic elution the mobile phase composition is held constant throughout the separation, whereas in gradient elution the composition is changed throughout the process. It is possible that the gradient can be step or linear based on the mixture to be analyzed. Gradient elution is often used in separating biological samples of high molecular weight or to separate complex mixtures. The one disadvantage of using the gradient mode is that it shows prominent baseline drift for some detectors as the gradient proceeds.
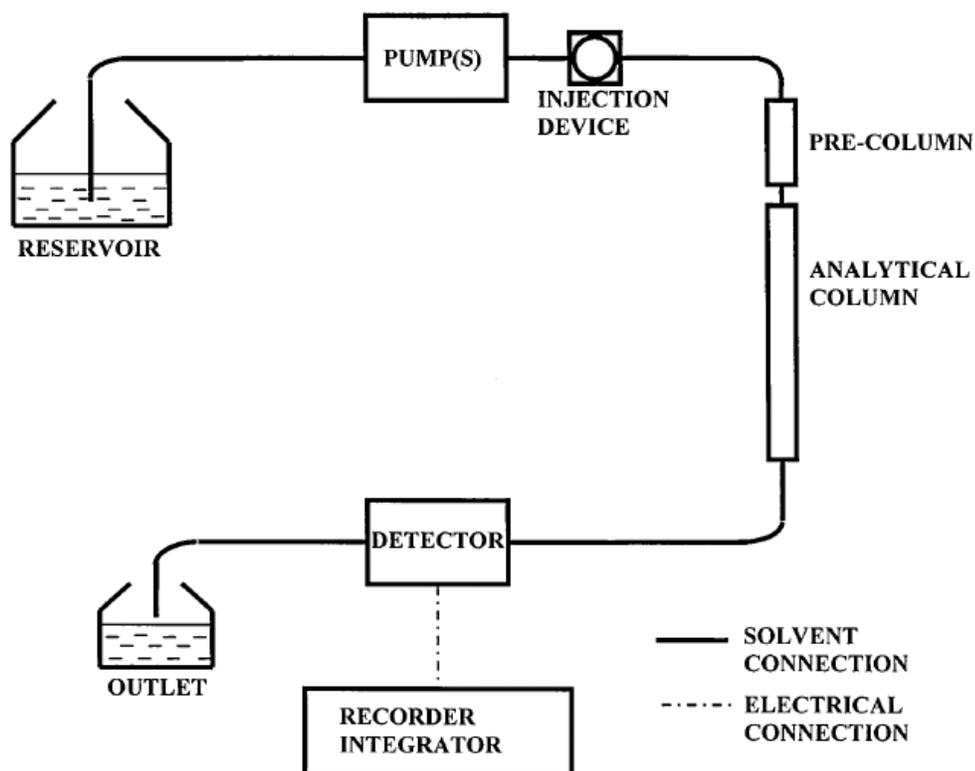
Figure 4. Schematic diagram of HPLC system.

The detectors also play a very important role in the HPLC system. They are

chosen based on the sample characteristics and the kind of analysis. Some of the

detectors available are UV, fluorescence, refractive index, charged aerosol detector,

evaporative light scattering, and mass spectrometry. The mass spectrometer is the most

sensitive detector and is extensively used in almost all industries. It is considered the

universal detector as it provides molecular information of the sample. UV detectors are

commonly used, as they are less expensive and used for the compounds that have a

chromophore to absorb the UV radiation. The fluorescence detector is used for the

compounds which have a flurophore, and refractive index detectors are used for

compounds which cannot be detected by UV. The drawback of the refractive index

detector is that it cannot be used for gradient elution conditions. Charged aerosol

detectors are used for compounds which are easily evaporated and are much more

sensitive than UV detectors [4, 18].

a. Evaporative Light Scattering Detector

The evaporative light scattering detector (ELSD) is one of the detectors used for

this research. This detector is used for compounds which cannot be analyzed using UV,

compounds that do not have a chromophore and also it is gradient compatible. Therefore

it can be used as an alternative to the refractive index detector. The eluent stream passes

through the nebulizer into the evaporation chamber. The nebulizer transforms the

column eluent into small droplets by the use of the inert gas nitrogen. Then the droplet is

allowed to evaporate to form fine particles suspended in the atomizing gas. The

suspended particles pass through a laser beam and, based on the light scattering of the

incident beam, the particles are detected by means of an optical fiber.

The three simple steps on which the ELSD operates are nebulization, evaporation,

and detection. During nebulization, the column eluent passes through a nitrogen gas

spray needle and forms small droplets of aerosols. The aerosols then pass through a

heated drift tube, where it is evaporated to form a fine uniform mist of sample particles in

a solvent vapor. The evaporated particles in the solvent vapor pass through a cell and

scatter the laser light beam. The scattered light is then detected with the help of a

photodiode generating a signal. ELSD is useful for the detection of amino acids because a traditional UV detector is insensitive to these compounds since most do not have a chromophore.

ELSD requires that a part of the aerosol be diverted to waste in order to measure effectively under high HPLC flow rates. The earliest design used a nebulization chamber with a restricted flow caused by a sharp turn, in which only smaller particles would successfully pass through and move towards the detection cell. The larger particles fail to pass and are directed toward the waste line. This design is appropriate for high flow rates but has difficulty in evaporating the mobile phase. Therefore, most of the sample is sent to waste which in turn compromises the detector sensitivity. To overcome the drawbacks, a plate impactor is designed. Larger particles hit the plate, condense, and go to waste whereas the smaller particles make it through the annular spaces of the impactor. The plate impactor can be either on or off and there are no settings to moderate the flow rates or to moderate the evaporation of mobile phases.

The SofTA Model 400 ELSD detector is used for this research. The use of thermo-split technology provides a smooth variation of an aerosol cloud over a wide range of conditions. The split ratio of the aerosol can be controlled by an aerosol splitter that combines a gentle bend with temperature controlled walls. It is easy to evaporate mobile phases with high organic content by heating the walls of the chamber. The increased temperature results in the rapid evaporation of solvent which allows all the particles to negotiate the bend. It is difficult to evaporate mobile phases with high aqueous solvents or at high flow rates; therefore the walls of the chamber are cooled, and

some fraction of the eluent is sent to waste. These experimental conditions result in part

of the aerosol condensing to form larger particles which fail to negotiate the bend, and are

sent to waste. The one advantage of using this system is the possibility of controlling the

split ratio in a smooth analog fashion by varying the temperature of the spray chamber.

The temperature can be varied from 0°C to 80°C in 1°C increments and is very easy to

operate with a user-friendly touch screen that allows online monitoring as well. The

schematic representation of the thermo-split chamber is shown in Figure 5. The top

diagram represents the heated surface and bottom diagram represents the cooled surface

where the solvent can be condensed and passed to the drain.

The advantages of using an evaporative light scattering detector are that it is a

universal detector which can be used for all samples that are less volatile than the mobile

phase. Qualitative analysis of a sample is more accurate than the other commonly used

detectors. It does not require any sample preparation which eliminates the sample

derivatization step. It has a very high sensitivity which can detect samples in the

nanogram range. The baseline is relatively stable even with a rapidly changing gradient.

The cost of operating the instrument is cheaper when compared to a mass spectrometer.

The disadvantage of using the ELSD detector is that it cannot be used for quantitative

measurements because the concentration of the sample is non-linear with respect to the

peak area. The light scattering measurement is a function of particle size rather than the

concentration of the sample. ELSD can be used on samples which are less volatile than

the mobile phase; therefore, only volatile mobile phase modifiers can be used. The use of

non-volatile mobile phase modifiers leads to detector noise [4, 19-20].
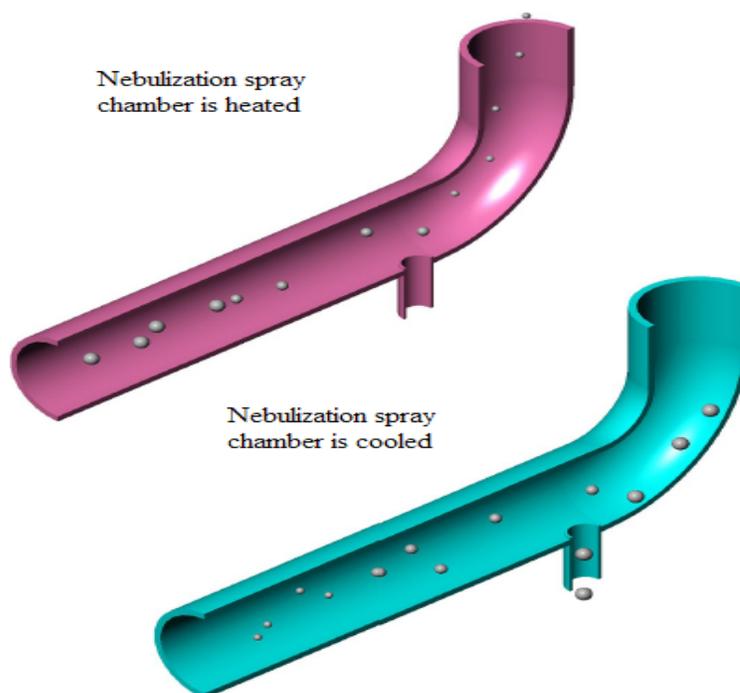
Figure 5. Schematic diagram of nebulization spray chamber.

I. **Goals**

The initial step was to synthesize a silica hydride stationary phase using Astrosil silica and TES via a silanization reaction. Then characterization of the silica hydride material was done using DRIFT which confirms the success of the reaction. The silica hydride material was endcapped using TMCS and HMDS for the unreacted silanol groups to increase the efficiency and peak symmetry of the column. The endcapped silica hydride material was again characterized using DRIFT to confirm the success of the reaction.

The initial objective of this research was to synthesize an endcapped silica hydride stationary phase followed by spectroscopic characterization to confirm the endcapped silica hydride stationary phase formation. The final goal of this research was

to determine the ability of the endcapped silica hydride column to operate in the aqueous normal phase mode for selected small molecules such as amino acids, weak organic acids, and vitamins. The study was done using the retention of analytes based on hydrophilicity in ANP chromatography and hydrophobicity in RP high performance liquid chromatography. The same experiments were repeated with a C8 Si-H column under the same conditions and using the same instrument. The results from C8 were compared with the results of the endcapped Si-H column to determine the separation capabilities of the endcapped column in comparison with the C8 Si-H column.

II.   EXPERIMENTAL

A. Materials

1. Chemicals

The chemicals and compounds used for this research in the preparation and characterization of stationary phases are listed in this section.

Table 1. Type of silica used for the silica hydride synthesis

Type of silica	Particle size	Pore size	Lot number
Astrosil spherical silica	4.2 µm	108 Å	7-122 F2

Table 2. Chemicals used for synthesis and characterization

Chemical Name	Supplier Name
Acetonitrile	Fisher chemical, Pittsburg, PA
Trifluoroacetic acid	Thermoscientific, Barrington, IL
Formic acid	Thermoscientific, Barrington, IL
Deionized water	Millipore
Dioxane	Fisher chemical, Pittsburg, PA
Triethoxysilane	Aldrich Chemical Co., Milwaukee, WI
Toluene	Fisher chemical, Pittsburg, PA
Diethyl ether	Fisher chemical, Pittsburg, PA
Trimethylchlorosilane	Sigma Aldrich, St Louis, MO
Hexamethyldisilazane	Sigma Aldrich, St Louis, MO

2. Compounds Used for Chromatographic Characterization

Table 3. Chemicals used for hydrophobicity and silanophilicity test

Chemical Name	Supplier Name
Sodium monobasic phosphate	Spectrum, Newark, CA
Sodium dibasic phosphate	Spectrum, Newark, CA
Methanol	J. T. Baker Chemical Co., Phillipsburg, NJ
Uracil	Matheson Coleman & Bell, Cincinatti, Ohio Lot number=UX55-5985
Naphthalene	J. T. Baker Chemical Co., Phillipsburg, NJ
Acenaphthene	J. T. Baker Chemical Co., Phillipsburg, NJ, Lot number=A006
Amitryptiline hydrochloride	J. T. Baker Chemical Co., Phillipsburg, NJ

Table 4. Compounds used for analysis

Chemical Name	Supplier Name
Phenylalanine	J.T.Baker, Phillipsburg, NJ
Niacinic acid	Merck & Co., Inc, Rahway, NJ
Citric acid	Mallinckrodt, St Louis, MO 63147
Lisinopril	Sigma-Aldrich, St Louis, MO
Raloxifene	Sigma-Aldrich, St Louis, MO
Dimethylthiourea	Sigma-Aldrich, St Louis, MO
Lipoic acid	Sigma-Aldrich, St Louis, MO
Triphenylphosphine	Sigma-Aldrich, St Louis, MO
Melamine	Sigma-Aldrich, St Louis, MO

3. Structures of the Compounds Used for Neue Test

The structures of the compounds used for Neue test are shown in

Figure 6 [21-24]
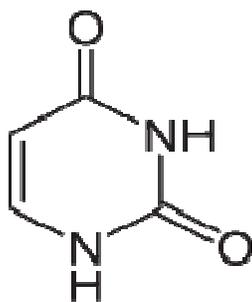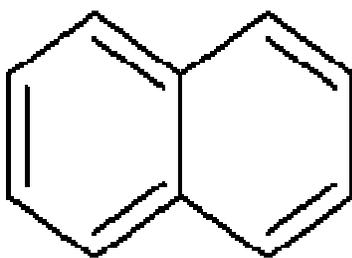
Uracil   **Naphthalene**   **Acenaphthene**
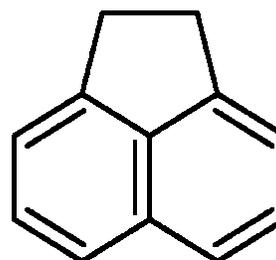
Amitriptyline hydrochloride
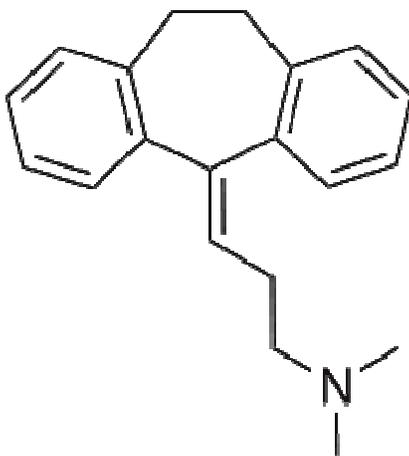
Figure 6. Structures of Neue test compounds.

4. Structures of Compounds Used for Chromatographic Characterization

The structures of the compounds used for chromatographic characterization are shown in Figure 7 [25-33].
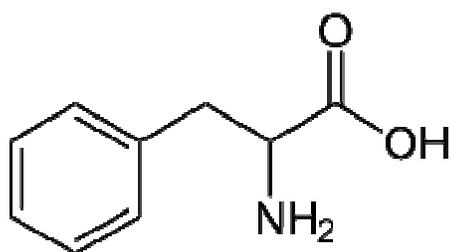
Phenylalanine   **Melamine**
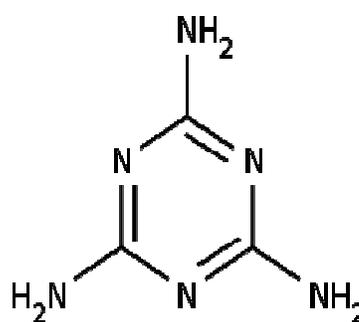
Lisinopril
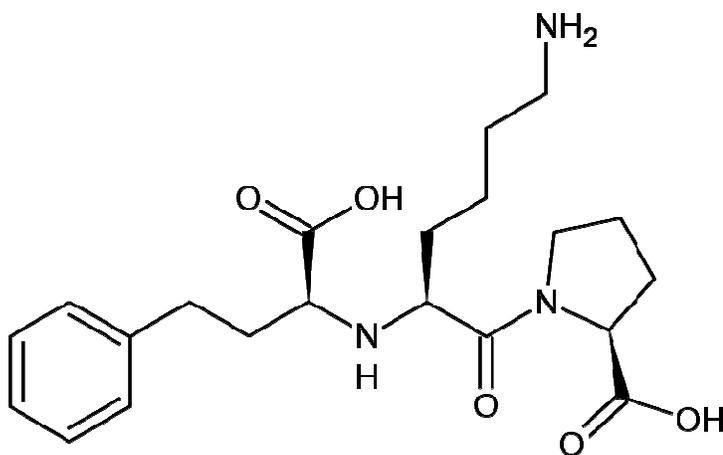
Niacinic acid
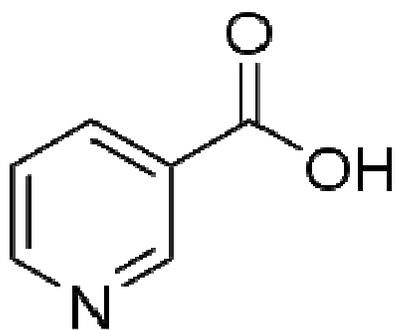
Dimethylthiourea
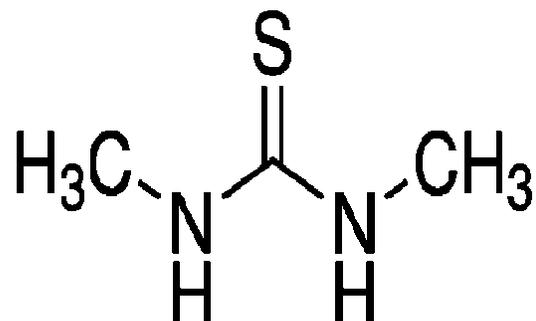
Raloxifene
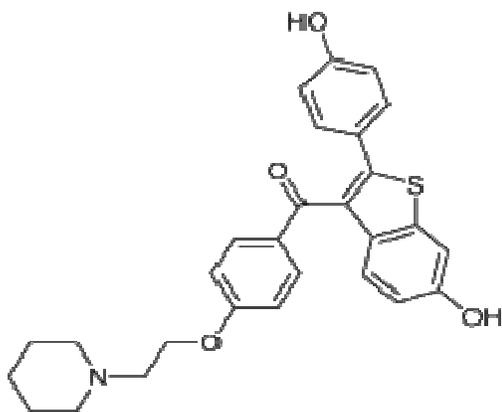
Citric acid
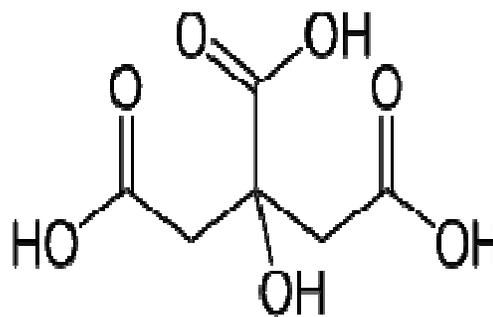
Lipoic acid
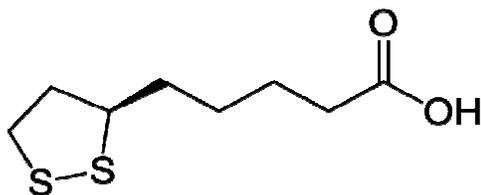
Triphenylphosphine
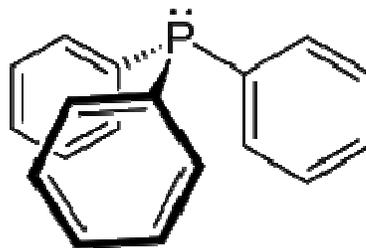
Figure 7. Structures of compounds used for chromatographic Characterization.

B. Synthetic Procedures

1. Synthesis of Silica Hydride via Silanization

All the glassware required for the silica hydride synthesis was dried overnight in an oven at 110°C. The Astrosil silica was also dried overnight in a vacuum oven at 120°C prior to synthesis. The TES mixture was prepared by adding 90.15 mL of dioxane to a round bottom flask, attached to an addition funnel and a stopper and then added 20.85 mL of TES in an argon atmosphere. On a heating mantel and a magnetic stir plate, a 3-necked 1000 mL round bottom flask fitted with a water condenser and a drying tube, a magnetic stir bar, a thermometer to monitor the temperature during the synthesis, a glass stopper, and an addition funnel to add solvent were set up. To the round bottom

flask, 300 mL of dioxane was added through an addition funnel followed by the silica, which was removed from the vacuum oven. The addition funnel was rinsed off with 300 mL dioxane to minimize the loss of silica and then 7.35 mL of 2.3 M HCl was added , and then the reaction mixture was heated to 70°C. When the reaction mixture reached 70°C, the TES mixture was added drop-wise through the addition funnel for 20 to 30 min with constant stirring. After adding all of the TES mixture, the temperature was raised to 90°C and the mixture was allowed to reflux for 90 min. After 90 min, the reaction mixture was cooled down to room temperature and was transferred to a medium sized filter crucible, attached to a filtering flask. The mixture was filtered using vacuum suction and then the solid silica was collected in the filter crucible. It was then washed twice with dioxane, three times with toluene, and three times with diethyl ether, using 50 mL of each solvent each time. The final silica hydride product was dried overnight at room temperature by placing the beaker in the hood to evaporate the ether used during washing cycles. The final product was then dried overnight in the vacuum oven at 110°C.

2. Endcapping of Silica Hydride

The experimental setup was identical to the one used for silica hydride synthesis. The set-up involved 3 necked round bottomed flasks equipped with a water condenser and drying tube, a thermometer, a glass stopper, a magnetic stir bar, a magnetic stirrer, and a heating mantle. Like silanization, prior to endcapping, all the required glassware was dried overnight in the oven at 110°C. The Astrosil silica hydride was kept in the

vacuum oven overnight at 100°C prior to synthesis. Endcapping was carried out by adding silica hydride, trimethylchlorosilane, and hexamethyldisilazane to toluene, and the reaction mixture was heated to 60°C. The reaction mixture was heated and refluxed for 120 min at 100°C. Upon completion of the reaction, the reaction mixture was cooled rapidly and the silica hydride was separated by filtration.

Endcapping procedure: A 3-necked flask with a condenser was placed on the heating mantle which was set-up on a stir plate (used for stirring the reaction mixture) and 30 mL of toluene was added to the 3-necked flask and heated to 60°C. While it was being heated, three other components were added to the toluene in the order listed:

1. 3 g of silica hydride

2. 3.38 mL of TMCS-TMCS trimethylchlorosilane 97.0%

3. 5.67 mL of HMDS 1,1,1,3,3,3-hexamethyldisilazane 99.0%

The reaction flask was flushed with argon and the reaction mixture was heated to 100°C and refluxed for 120 min for the reaction to complete. After the reaction period, the heating mantle was turned off and the mixture was cooled as rapidly as possible. However, removal of the reaction solution began within 5 min after completion of the procedure. The silica hydride was separated from the reaction solution by filtration using a filtering funnel (90M). It was then washed two times with dioxane, three times with toluene, and three times with diethyl ether, using 50 mL of each solvent each time. The final endcapped silica hydride product was dried overnight at room temperature by placing the beaker in the hood to evaporate the ether used during wash cycles. The finished product was dried overnight in a vacuum oven at 110°C.

C. Instrumentation Procedures

1. Diffuse Reflectance Infrared Fourier Transform Spectroscopy

Spectroscopic characterization was used to confirm the formation of Si-C bonds after the endcapping process. An ATI Mattson Infinity Series FT-IR spectrophotometer with a Hewlett Packard FX-2 computer was used to collect the spectrum of Si-H and endcapped Si-H surfaces. Prior to the sample scan, a measurement of pure KBr was taken as a reference. The sample was measured by using a 95:5 ratio of solid sample to IR-grade KBr. The sample was ground finely using a pestle and placed in a sample cup 2 mm in diameter and 2 mm in depth, and the powder was leveled with a spatula. The sample cup was placed in the DRIFT sample holder and scanned. The sample was scanned 200 times at a resolution of 2 cm^{-1} over a range of 4000-450 cm^{-1} and normalized to 100% transmittance.

2. Column Packing

The stationary phase material after synthesis was packed in-house by a slurry method using a CCl_4-methanol (9:1 v/v) mixture. The dimensions of the stainless steel tubes used for packing the bonded phase material were 75 x 4.6 mm i.d, which were provided by Alltech located at Deerfield of Illinois. A pneumatic pump, operated at 6000 psi pressure with HPLC grade methanol as the driving solvent, was used to pack the

column. The column was held in the above condition for about 30 min before being removed.

3. High Performance Liquid Chromatography

The HPLC system used for the research was a SoftA Corporation Model 400 Evaporative Light Scattering Detector (ELSD) instrument equipped with an LDC Milton Roy CM400 binary pump system, a Rheodyne Model 7126 manual injector with a 20 µL injection loop, and a Waters in-line degasser. The ELSD detector operated with the supply of nitrogen at 65 psi for nebulization of the column eluent. The ELSD used had a 670 nm laser diode and hermetically sealed photodiode for detection. The data collection system consisted of Chemstation software, an HP35900C multichannel interface, and an HP Deskjet 693C printer. The mobile phase was vacuum filtered using a 0.2 µm Nylon 66 membrane (Alltech Associates, Inc. Deerfield, IL) as a means of removing any particles from the solvent system before the analysis. The HPLC separations were done using a flow rate of 0.5 mL/min. A Milli-Q^{TM} purification system (Millipore Corp., Bedford, MA) was used for the preparation of deionized water.

The other HPLC system used was an 1100 series Agilent instrument equipped with a binary pump, autosampler, degasser, and a UV detector. Chemstation software was used for the data analysis. The pump flow rate used was 0.5 mL/min and the column temperature was maintained at ambient.

III.   RESULTS AND DISCUSSIONS

A. Spectroscopic Characterization of Bonded Stationary Phases

1. Diffuse Reflectance Infrared Fourier Transform Spectra

a. Silanization

The DRIFT spectra were used in this research to confirm the Si-H bonded stationary phase. The DRIFT spectra of Si-H using Astrosil silica is shown in Figure 8. The very intense absorption band near 2250 cm^{-1} was assigned to the Si-H stretching frequency which confirmed Si-H formation after the silanization reaction with TES. The broad band between 3000 and 3700 cm^{-1} was assigned for the absorbed water and OH groups from the silica.
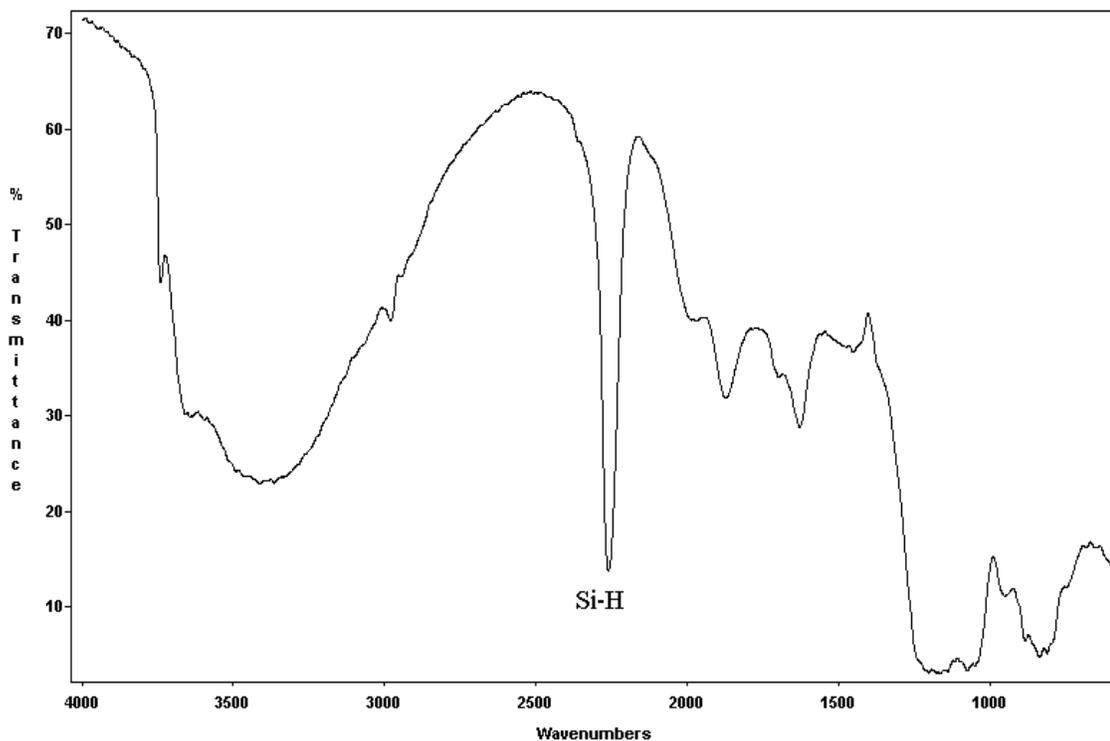
Figure 8. DRIFT spectrum of silica hydride stationary phase.

b. Endcapped Silica Hydride

Figure 9 shows the DRIFT spectrum of the endcapped silica hydride. The endcapped Si-H material gave a lower but significant intensity absorption band near 2250 cm^{-1}, which confirmed the Si-H stretching band. The broad band between 3000 and 3700 cm^{-1} was assigned for the absorbed water and OH groups from the silica, this was seen in the silanization reaction step as well. The strong C-H stretching vibration band between 2800 to 3000 cm^{-1} indicated again the formation of an organic bonded phase or the attachement of an oganic group to Si-OH surface through the endcapping process.
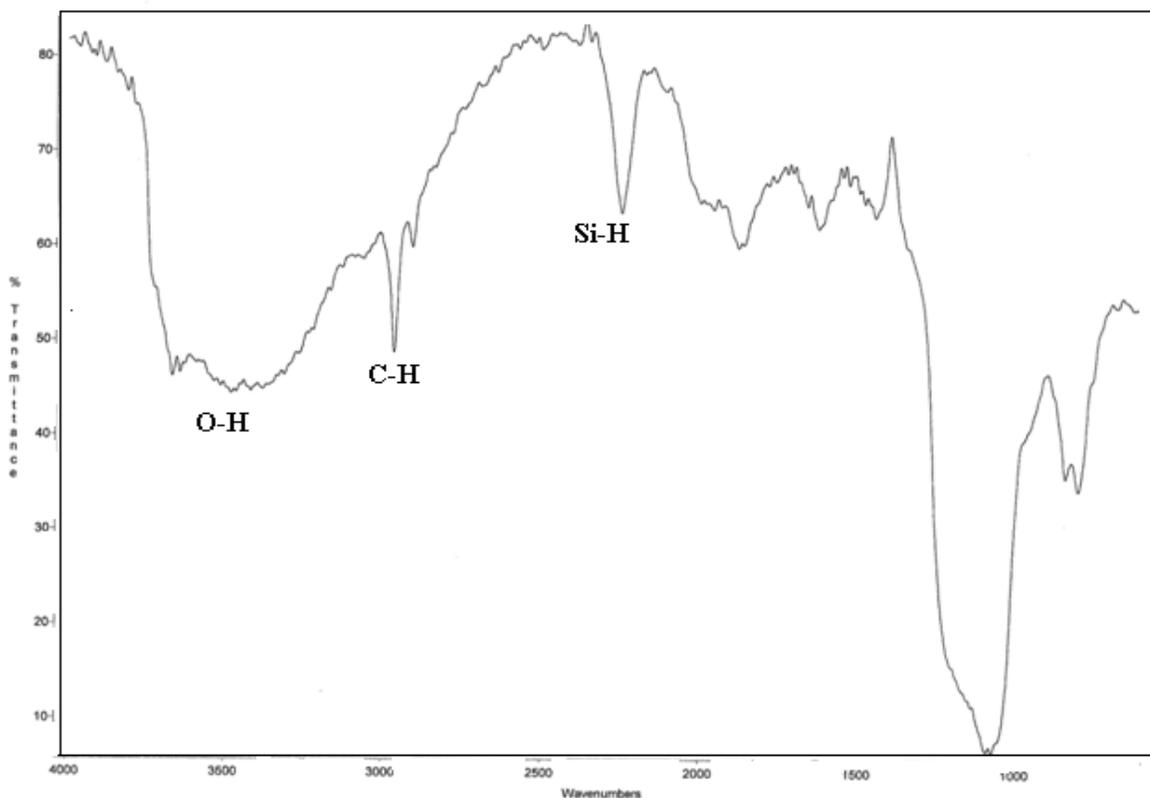
Figure 9. DRIFT spectrum of endcapped Si-H stationary phase.

B. Chromatographic Characterization of Bonded Stationary Phase

The spectroscopic characterization confirmed the success of bonding after

silanization and endcapping process. The Neue test confirmed the hydrophobicity and

silanophilicity of the stationary phase. The information obtained was helpful in

determining the type of compounds which could be used for chromatographic

characterization. Chromatographic characterization helped in determining the potential

applications of the new stationary phase material, and the technique used for

chromatographic characterization was HPLC with either an ELSD detector or UV detector.

1. Characterization of HPLC Packing by Neue Test

The hydrophobicity and silanophilicity were determined using four compounds. The information obtained helps to determine the type of compounds that could be retained on the column.

Experimental Conditions: The mobile phase used was 35:65 20 mM phosphate buffer/methanol, pH 7. The flow rate used was 0.5 mL/min and the injection volume was 1 µL. The compounds were detected at 214 nm. Two sample mixtures were made. The first mixture contained uracil (16 mg/mL), naphthalene (60 mg/mL), and acenaphthene (200 mg/mL) in methanol. The second mixture contained amitryptiline hydrochloride (100 mg/mL) and acenaphthene (200 mg/mL) in methanol. Uracil in the first mixture was used as a marker for the column void volume. The solutes used and their retention times at both conditions are shown in Table 5.

Table 5. Results of Neue test

Compounds	Endcapped Si-H Column (min)	C8 Si-H column (min)
Uracil	1.0	1.8
Naphthalene	2.2	4.3
Acenaphthene	2.3	6.0
Amitryptiline hydrochloride	2.1	1.7

a. Hydrophobicity Result of Endcapped Si-H Stationary Phase

The mixture of uracil, naphthalene, and acenaphthene in methanol was run on the endcapped column to measure the hydrophobicity of the stationary phase.
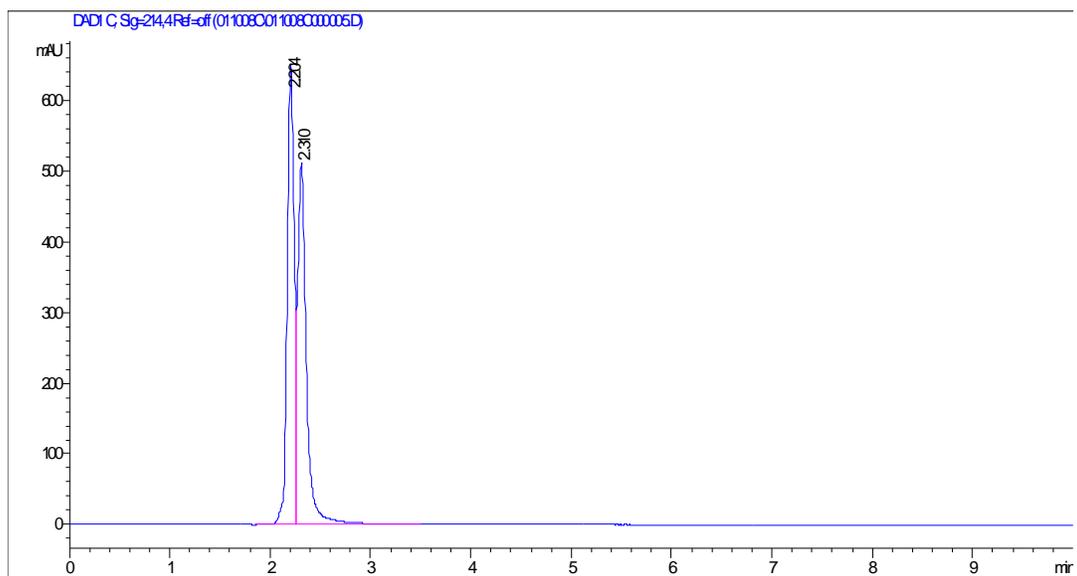
Figure 10. Hydrophobicity test on endcapped silica hydride phase. 2.204 min is naphthalene and 2.310 min is acenaphthene.

Naphthalene eluted at a retention time 2.204 min, acenaphthene at 2.31 min, and uracil eluted at the solvent front which did not show up for the endcapped silica hydride stationary phase as shown in Figure 10. The hydrophobicity parameter was calculated for the endcapped column as shown below:

Hydrophobicity Parameter(u)

$$\ln(k) = u * V - 3.068$$

V = Molar volume of the analyte (acenaphthene or naphthalene), which is 200 mM

$$k = (t_r - t_0)/t_0$$

t_r = 2.31 min, the retention time of acenaphthene and t_0 = 1.0 min, the retention time of the void volume that was obtained separately by running uracil.

$$k = (2.31 - 1.0)/1.0 = 1.31$$

Hydrophobicity of endcapped Si-H: ln (1.31, Acenaphthene) + 3.068/200 = 0.016

The hydrophobicity of endcapped Si-H column was 0.02 in comparison to C18 silica hydride column which is 0.04 [19] as shown in Table 6.

b. Silanophilicity Result of Endcapped Si-H Stationary Phase

The mixture of amitryptiline and acenaphthene dissolved in methanol was run on the endcapped column to measure the silanophilicity of the stationary phase.
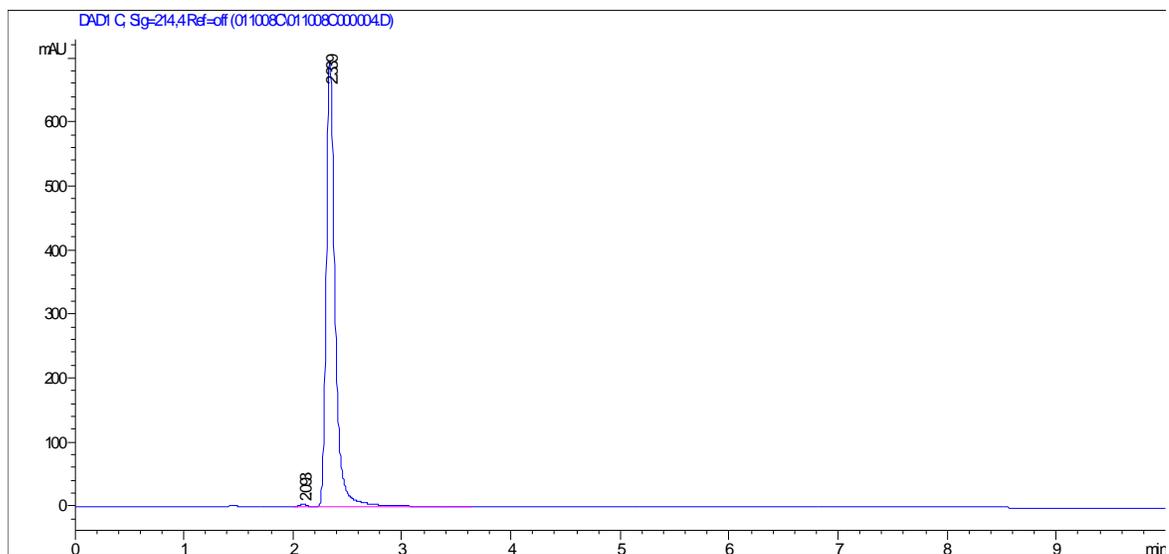
Figure 11. Silanophobicity test on endcapped silica hydride. 2.093 min is amitryptiline and 2.310 min is acenaphthene.

Amitryptiline eluted at a retention time 2.093 min and acenaphthene at 2.310 min as shown in Figure 11. The silanophilicity was calculated for the endcapped column as shown below:

$$S = \ln (k_{amitryptiline}) - 0.7124 * \ln (k_{acenaphthene}) + 1.9748$$

$$k = (t_r - t_0)/t_0$$

$t_r = 2.093$ min, the retention time of amitryptiline and $t_0 = 1.0$ min, the retention time of the void volume that was obtained separately by running uracil.

$$k = (2.093 - 1.0)/1.0 = 1.09$$

$k = 1.09$ for amitryptiline

$t_r = 2.310$ min, the retention time of acenaphthene

$k = 1.31$ for acenaphthene

$$\ln (1.09, \text{Amitryptiline}) - 0.7124 * \ln (1.31, \text{Acenaphthene}) + 1.9748 = 1.80$$

The silanophilicity of endcapped Si-H column was 1.80 in comparison to C18 silica hydride column which is 1.43 [19] as shown in Table 6.

c. Hydrophobicity Result of C8 Stationary Phase

The mixture of uracil, naphthalene, and acenaphthene in methanol was run on the C8 column to measure the hydrophobicity of the stationary phase.
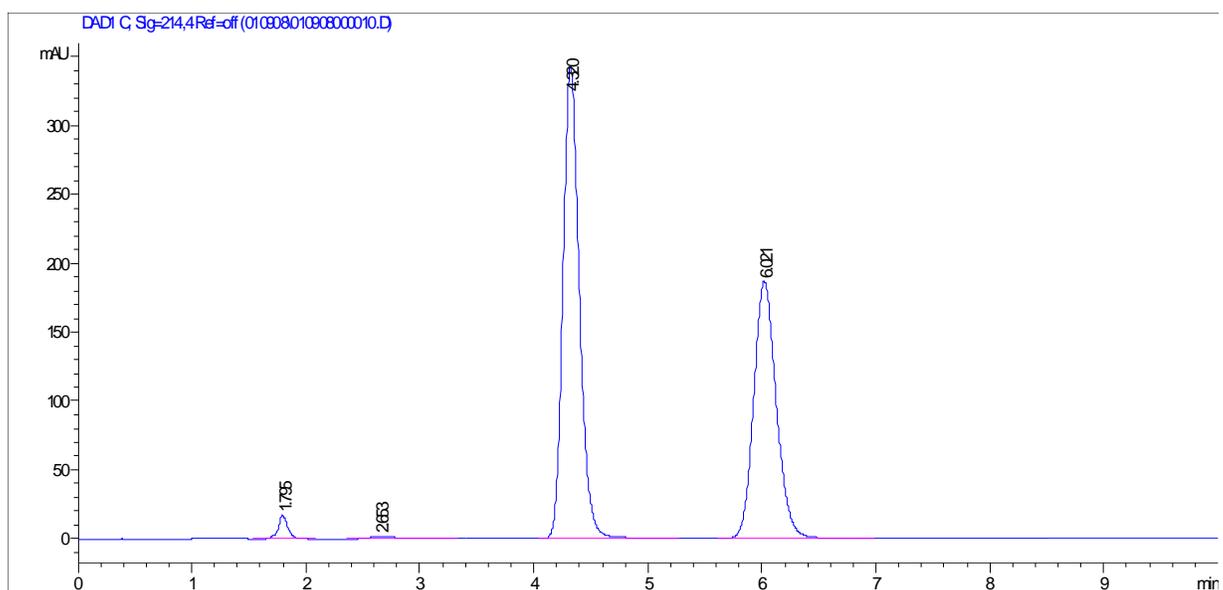
Figure 12. Hydrophobicity test on C8 phase. 1.795 min uracil, 4.320 min naphthalene, and 6.021 min acenaphthene.

Naphthalene eluted at a retention time 4.320 min, acenaphthene at 6.021 min, and uracil eluted at 1.795 min at the solvent front as shown in Figure 12. The calculation of the hydrophobicity parameter for the C8 column:

Hydrophobicity Parameter(u)

$$\ln(k) = u * V - 3.068$$

V = Molar volume of the analyte (acenaphthene or naphthalene), which is 200 mM

$$k = (t_r - t_0)/t_0$$

$t_r = 6.02$ min, the retention time of acenaphthene and $t_0 = 1.795$ min, the retention time of the void volume.

$$k = (6.02 - 1.795)/1.795 = 2.35$$

Hydrophobicity of C8 column:

47

$$\ln (2.35, \text{Acenaphthene}) + 3.068/200 = 0.02$$

The hydrophobicity of C8 Si-H column was 0.02 in comparison to C18 silica hydride

column which is 0.04 [19] as shown in Table 6.

d. Silanophilicity Result of C8 Stationary Phase

The mixture of amitryptiline and acenaphthene in methanol was run on the C8

column to measure the silanophilicity of the stationary phase.
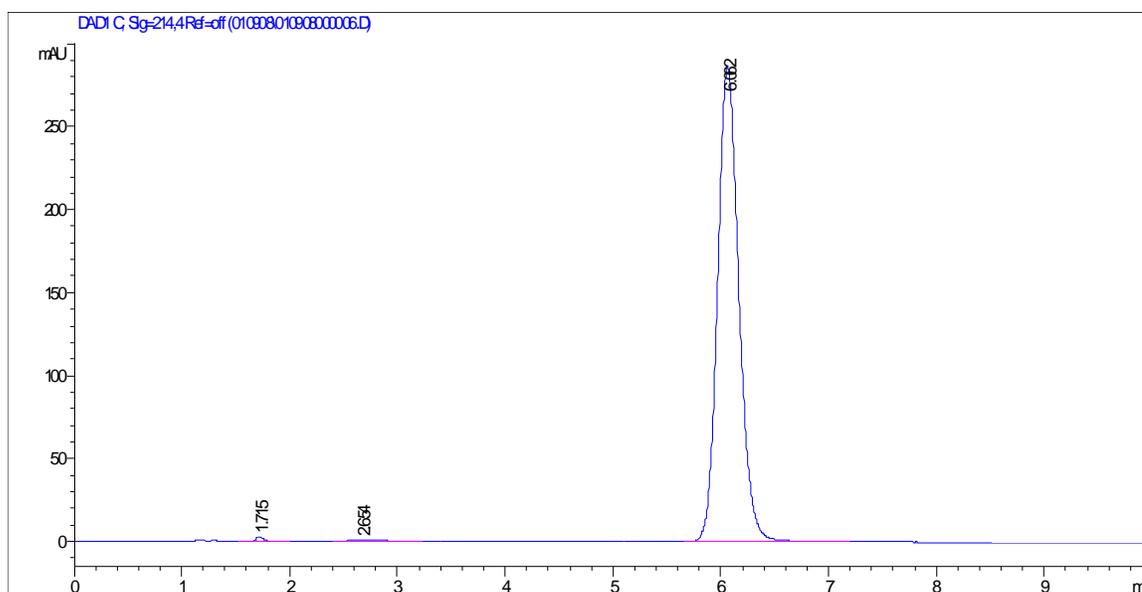
Figure 13. Silanophobicity test on C8 column. 2.654 min amitryptiline and 6.062 min acenaphthene.

Amitryptiline eluted at a retention time 2.654 min and acenaphthene at 6.062 min

as shown in Figure 13. The calculation of the silanophilicity factor for the C8 column:

$$S = \ln (k_{\text{amitryptiline}}) - 0.7124 * \ln (k_{\text{acenaphthene}}) + 1.9748$$

$$k = (t_r - t_0)/ t_0$$

t_r = 2.654 min, the retention time of amitryptiline and t_0 = 1.795 min, the retention time for the void volume.

$$k = (2.654 - 1.795)/1.795 = 0.478$$

k = 0.478 for amitryptiline

t_r = 6.062 min, the retention time of acenaphthene

k = 2.377 for acenaphthene

ln (1.715 min, Amitryptiline) – 0.7124 * ln (6.06 min, Acenaphthene) + 1.9748 = 0.399

The silanophilicity of C8 Si-H column was 0.4 in comparison to C18 silica hydride column which is 1.43 [19] as shown in Table 6.

Table 6. Summary of hydrophobicity and silanophilicity results of C8 and endcapped silica hydride stationary phases

			From article [19]	
Columns	**Hydrophobicity**	**Silanophilicity**	**Hydrophobicity**	**Silanophilicity**
C18	NA	NA	0.0397	1.429
C8	0.02	0.399	-	-
Endcapped Si-H	0.016	1.8	-	-

- = The information is not available.

The comparison of hydrophobicity and silanophilicity data between C18, C8, and endcapped Si-H is shown in Table 6. The relative degree of hydrophobicity between the

columns was as expected. The C18 column was higher than C8 column, which was greater than the endcapped Si-H column. This correlates well with the length of the side chain attached to the surface; a longer aliphatic chain results in higher hydrophobicity. The comparison of silanophilicity data between the three columns does not show any trend; however a lower value results in less surface adsorption. Therefore the C8 column has less adsorption when compared to the C18 and the endcapped silica hydride columns.

2. ANP and RP Behavior of Endcapped Si-H Using an ELSD Detector

a. Aqueous Normal Phase Behavior of Citric Acid

Amino acids, vitamins, weak organic acids, chelators, and lipoic acid were used for the chromatographic characterization of the endcapped silica hydride column and the results were compared with the C8 (75 x 4.6 mm i.d) silica hydride column. The endcapped column was packed in a 75 x 4.6 mm i.d. stainless steel tube, which was purchased from Alltech, Deerfield, IL.

The chromatographic characterization of the columns was done using HPLC with UV and an ELSD detector. The parameters used for HPLC with the ELSD detector were a flow rate of 0.5 mL/min, an injection volume of 5 µL, and the column temperature at ambient. The concentration of the samples was 0.5 mg/mL in a 50:50 water/acetonitrile solution. The ELSD parameters were set and stabilized with the starting conditions of the isocratic system. The temperature of the drift tube and spray chamber were adjusted until a smooth baseline was obtained. The spray chamber was kept at room temperature

(25°C) for all the analyses and the drift tube temperature was set at 65°C to evaporate the mobile phase. The composition of the mobile phase used was 0.1% formic acid (FA) because it was compatible with the ELSD detector and was very easy to evaporate during light scattering detection. A water blank was run to eliminate any carryover from the previous measurements. The main goal of this part of the research was to evaluate the endcapped stationary phase for aqueous normal phase and reversed-phase behavior.

The concentration of citric acid used for aqueous normal phase behavior was 0.5 mg/mL in a 50:50 water/acetonitrile solution. The ELSD detector parameters were set and stabilized with the starting conditions of the isocratic system.
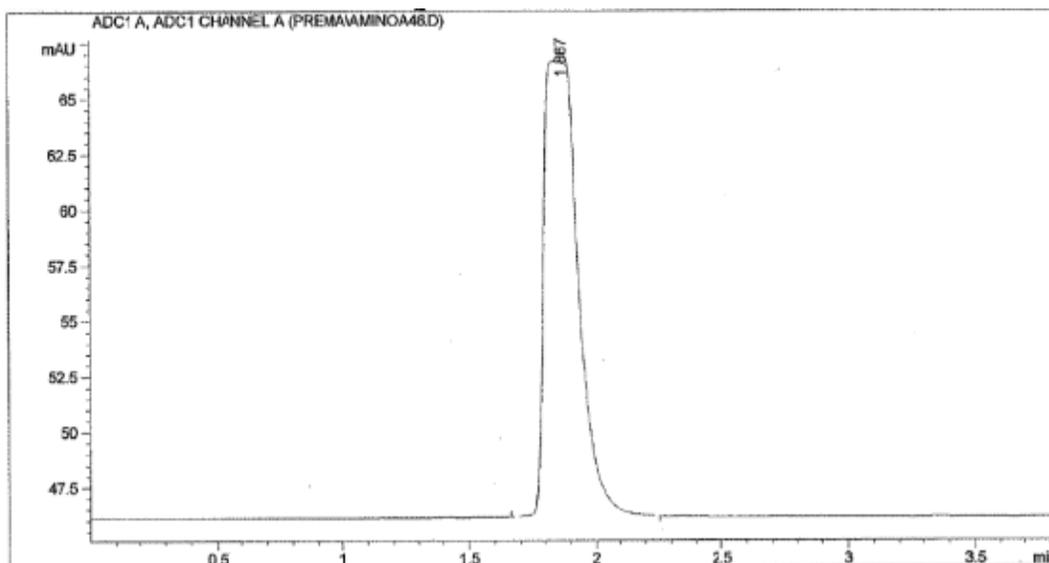
Figure 14. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: acetonitrile + 0.1% formic acid.
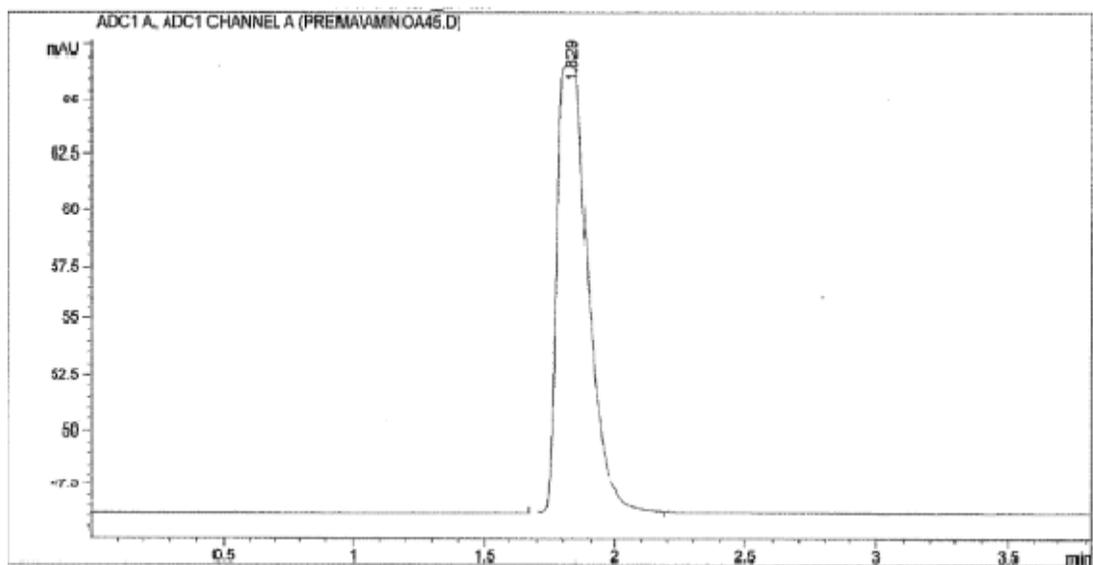
Figure 15. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 10:90 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
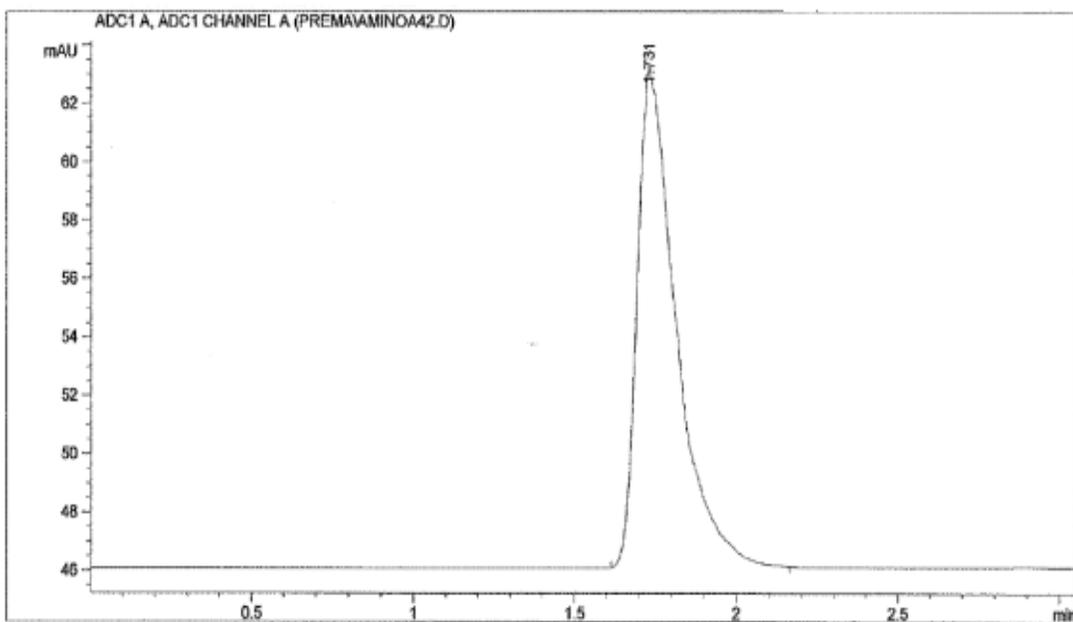
Figure 16. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 20:80 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
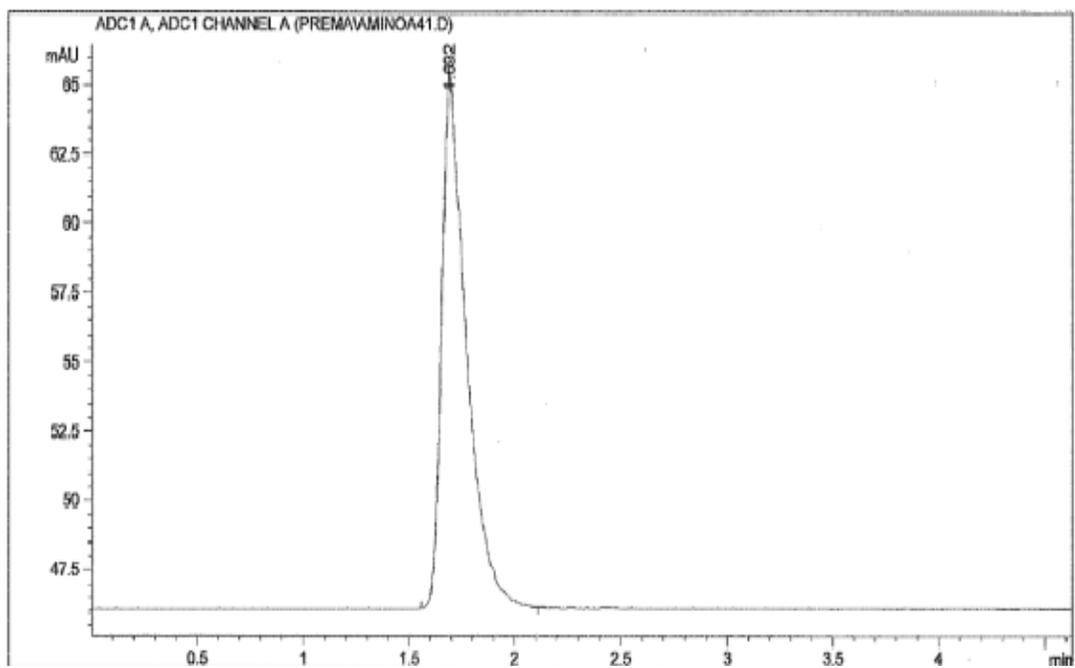
Figure 17. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 30:70 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
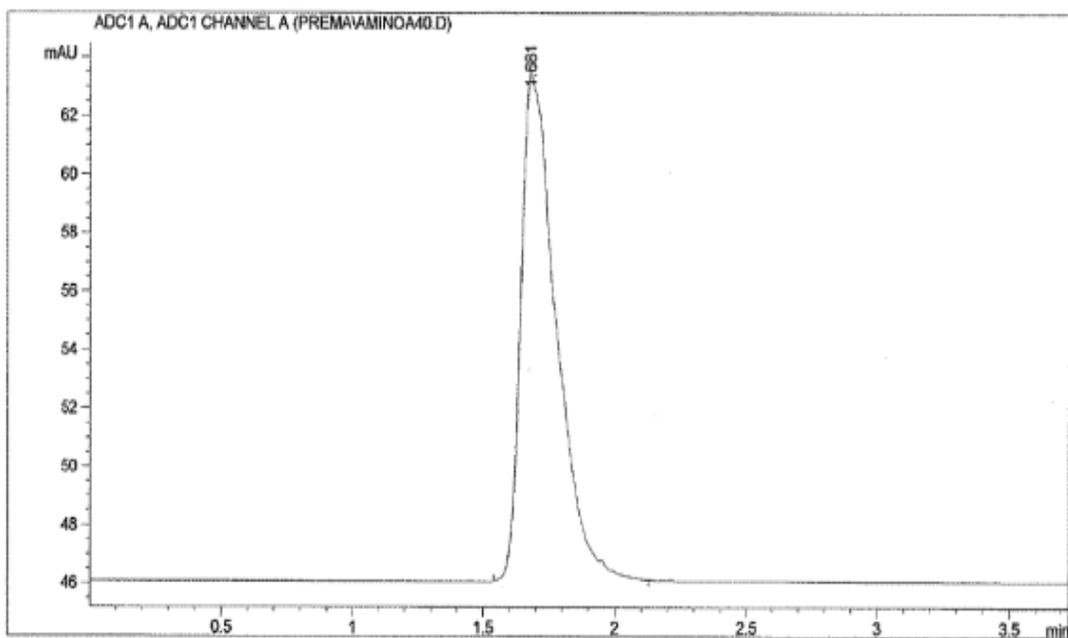
Figure 18. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 40:60 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

b. Reversed-Phase Behavior of Citric Acid

The concentration of citric acid used for reversed-phase behavior was 0.5 mg/mL in a 50:50 water/acetonitrile solution. The ELSD detector parameters were set and stabilized with the starting conditions of the isocratic system.
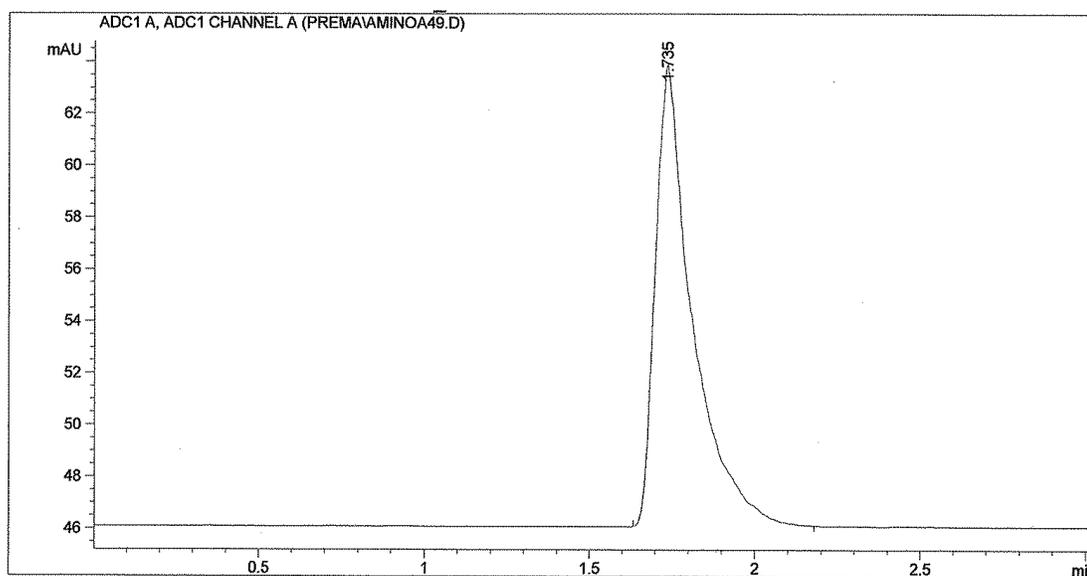
Figure 19. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 60:40 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
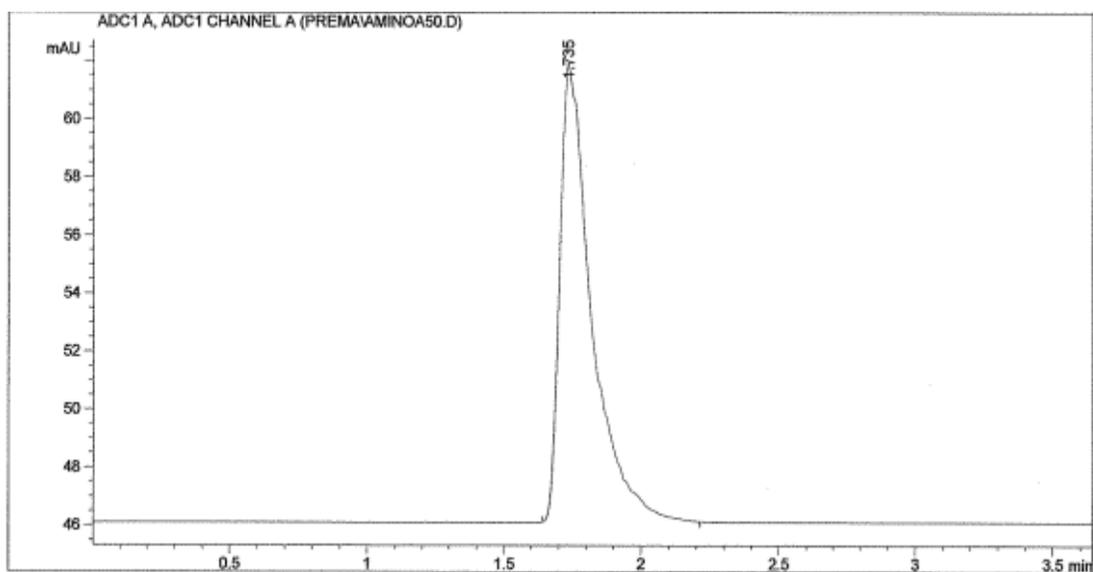
Figure 20. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 70:30 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
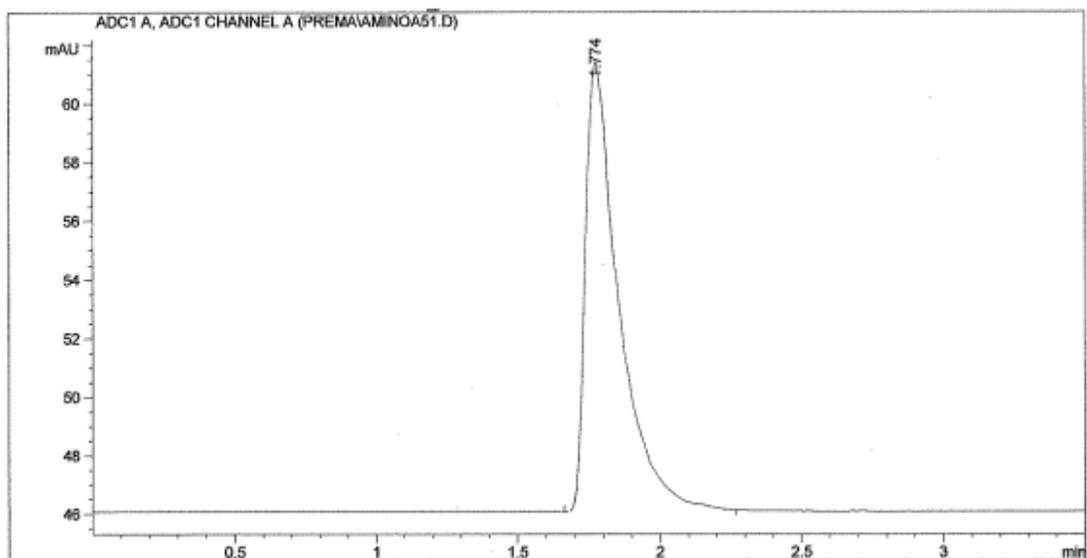
Figure 21. **Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 80:20 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.**
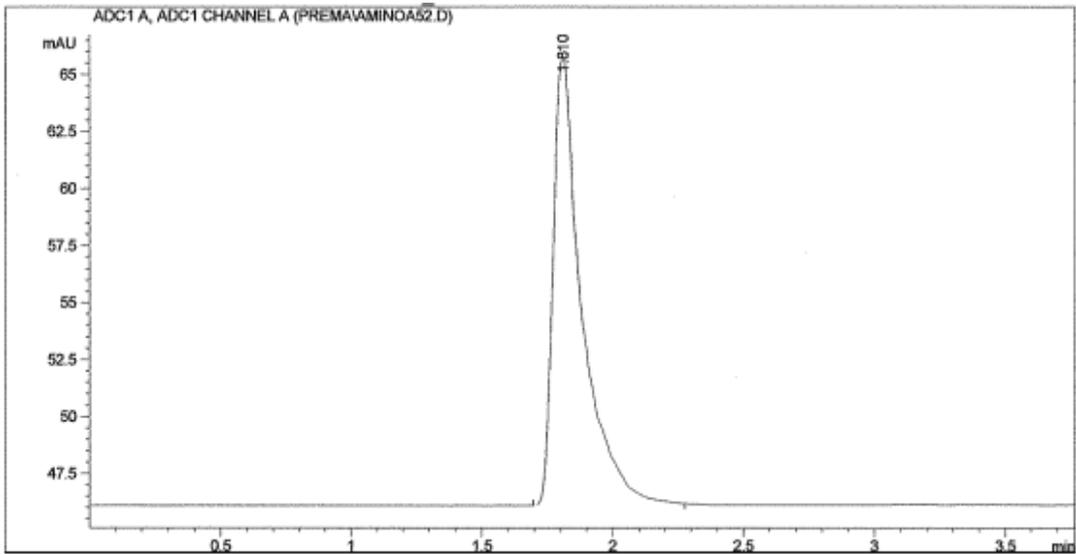
Figure 22. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: 90:10 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
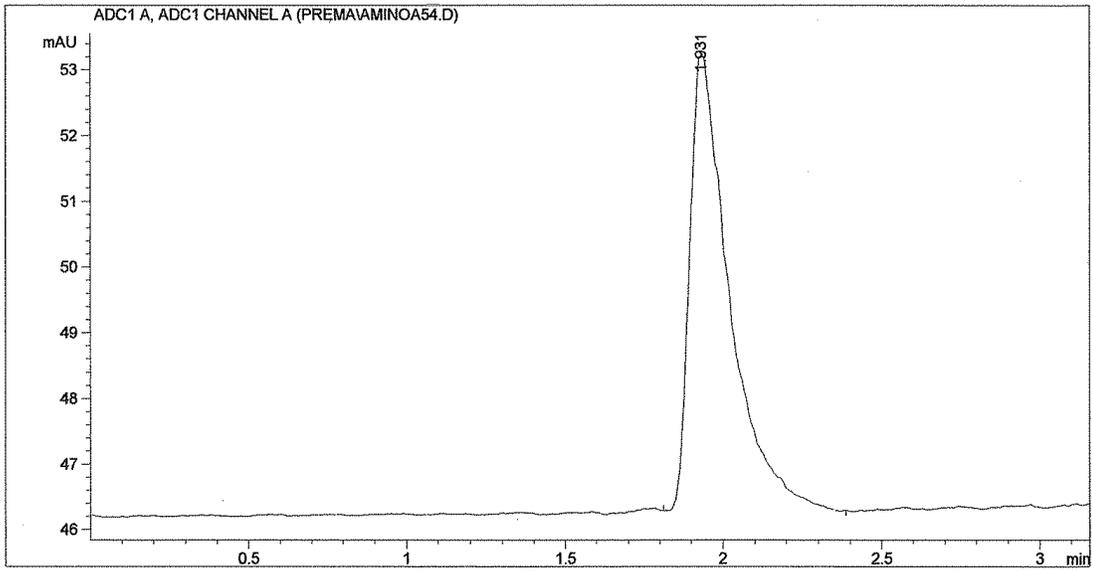
Figure 23. Chromatogram of citric acid on endcapped silica hydride stationary phase. Mobile phase: acetonitrile + 0.1% formic acid.
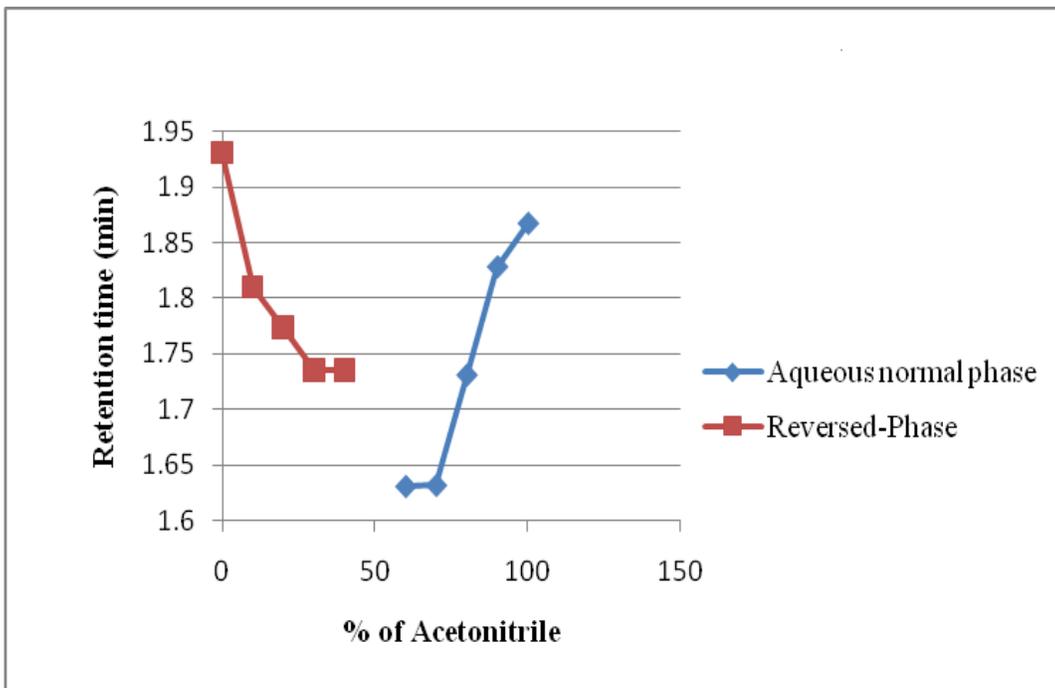
Figure 24. Retention map of citric acid for ANP and RP conditions.

Citric acid was analyzed using aqueous normal phase and reversed-phase

conditions as shown in the chromatograms from Figure 14 to Figure 22. The mobile

phase used was 0.1% formic acid in acetonitrile and 0.1% formic acid in water and, the

detector used for the analysis was the evaporative light scattering detector. As shown in

Figure 24, citric acid can be retained using the aqueous normal phase elution mode and

the reversed-phase elution mode. In the aqueous normal phase mode, the retention of the

compound increased as the percentage of acetonitrile increased. It can be retained using

the reversed-phase elution mode also as it displayed good retention under selected

reversed-phase isocratic conditions. Citric acid was also tested with 0.1% ammonium

acetate in water and 0.1% ammonium acetate in acetonitrile using a UV detector. It was

also measured using 0.1% formic acid in water and 0.1% formic acid in acetonitrile with

a UV detector. The chromatograms for all the isocratic conditions tested had many split

peaks and some conditions had negative peaks. Therefore more investigations need to be

done using different mobile phase compositions.

c. Reversed-Phase Behavior of Melamine

The concentration of melamine used for reversed-phase behavior was 0.5 mg/mL

in a 50:50 water/acetonitrile solution. The ELSD detector parameters were set and

stabilized with the starting conditions of the isocratic system.
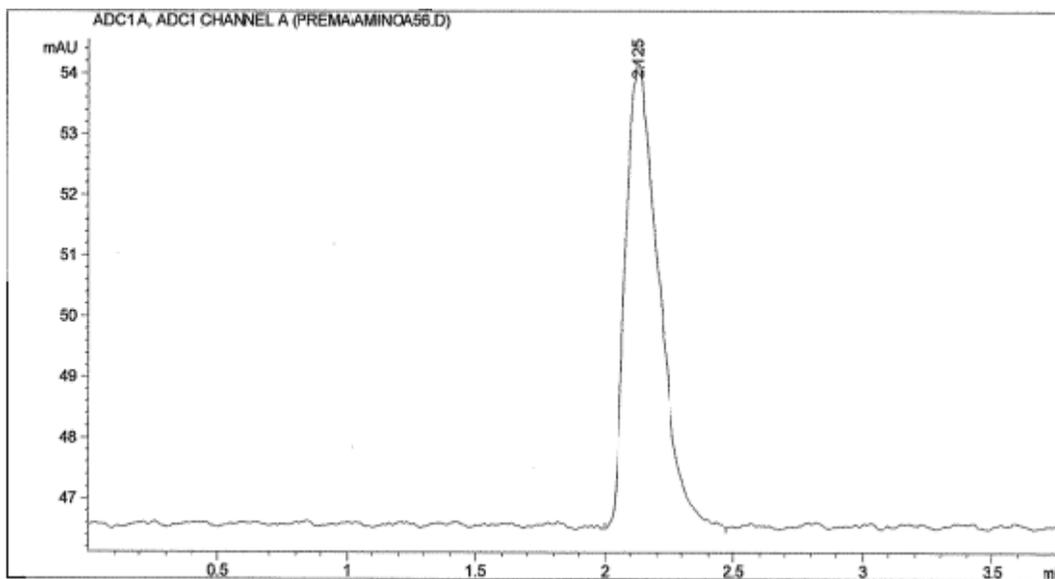
Figure 25. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 90:10 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
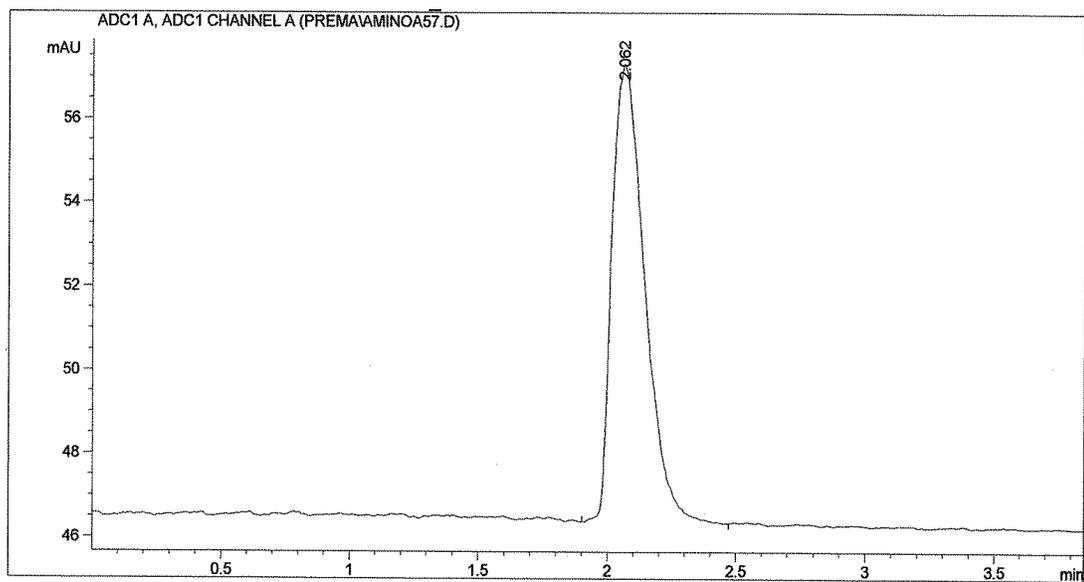
Figure 26. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 80:20 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
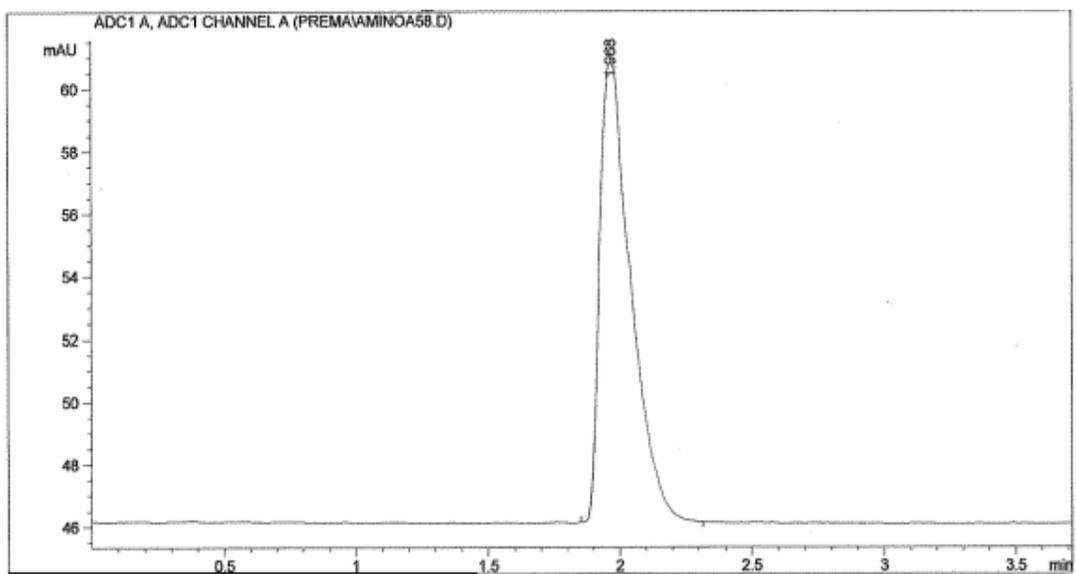
Figure 27. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 70:30 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
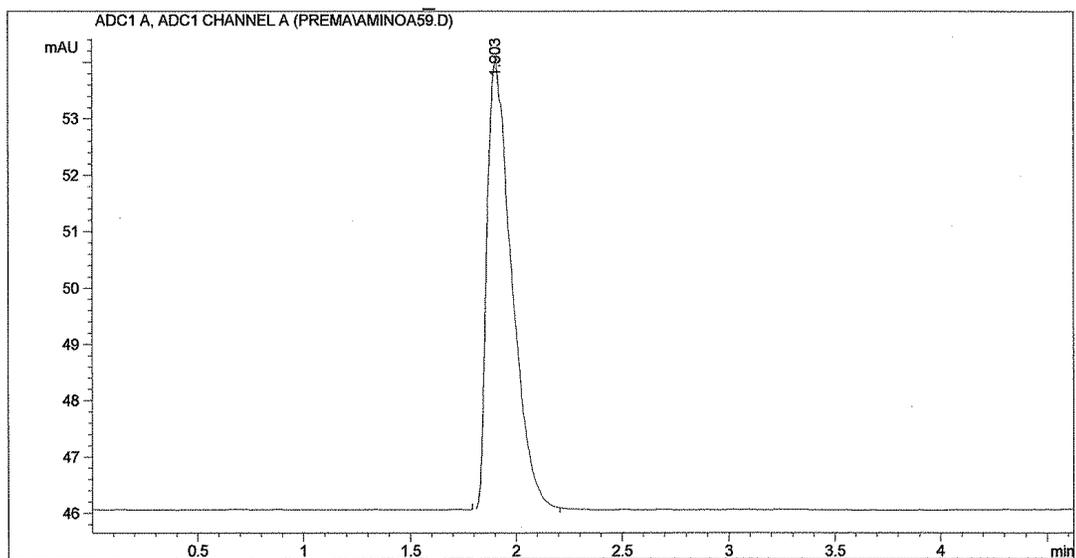
Figure 28. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 60:40 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

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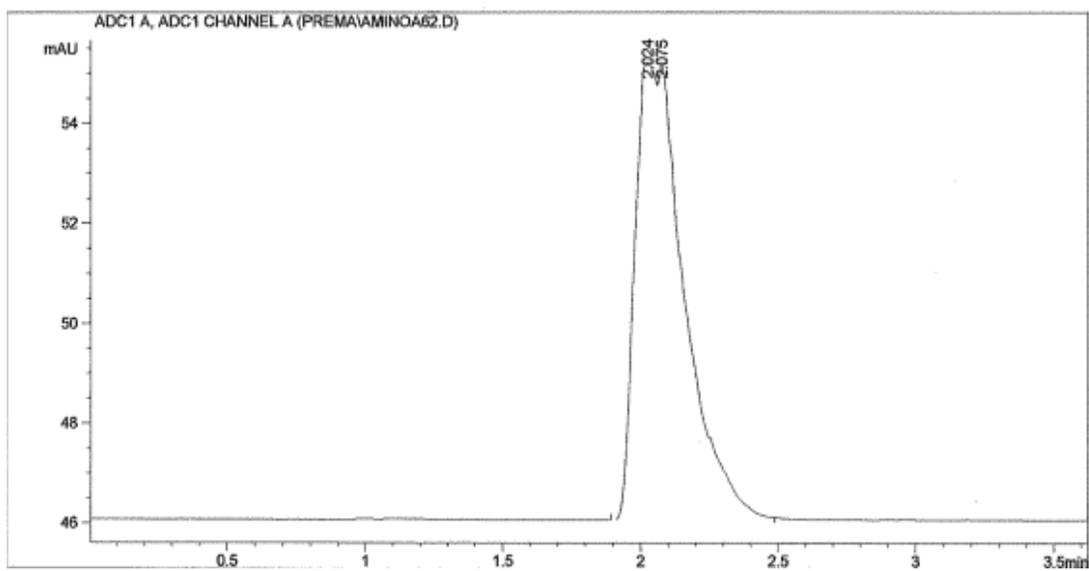
Figure 29. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 50:50 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

d. Aqueous Normal Phase Behavior of Melamine

The concentration of melamine used for aqueous normal phase behavior was 0.5 mg/mL in a 50:50 water/acetonitrile solution. The ELSD detector parameters were set and stabilized with the starting conditions of the isocratic system.
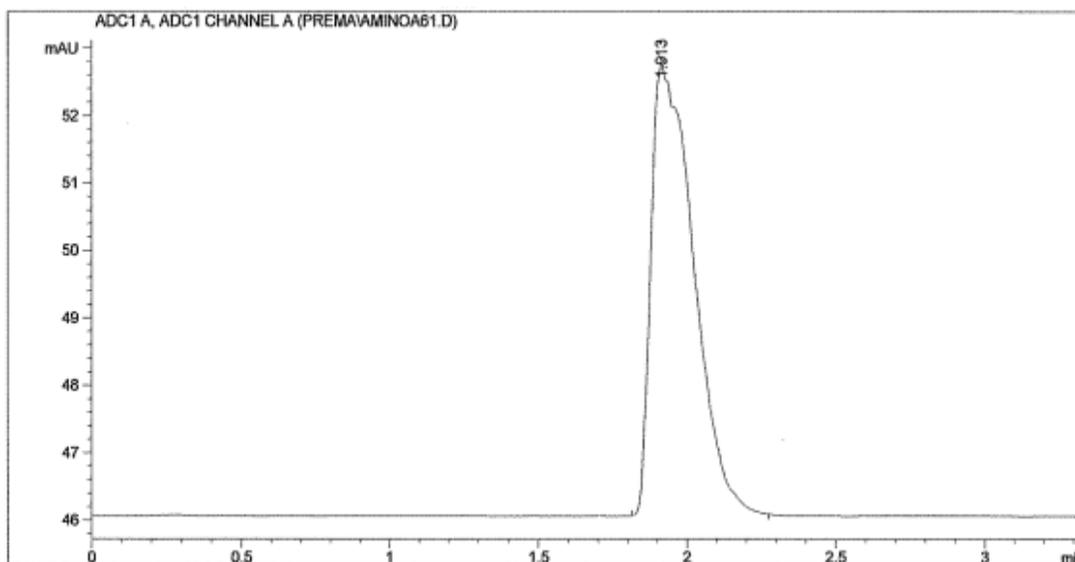
Figure 30. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 40:60 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
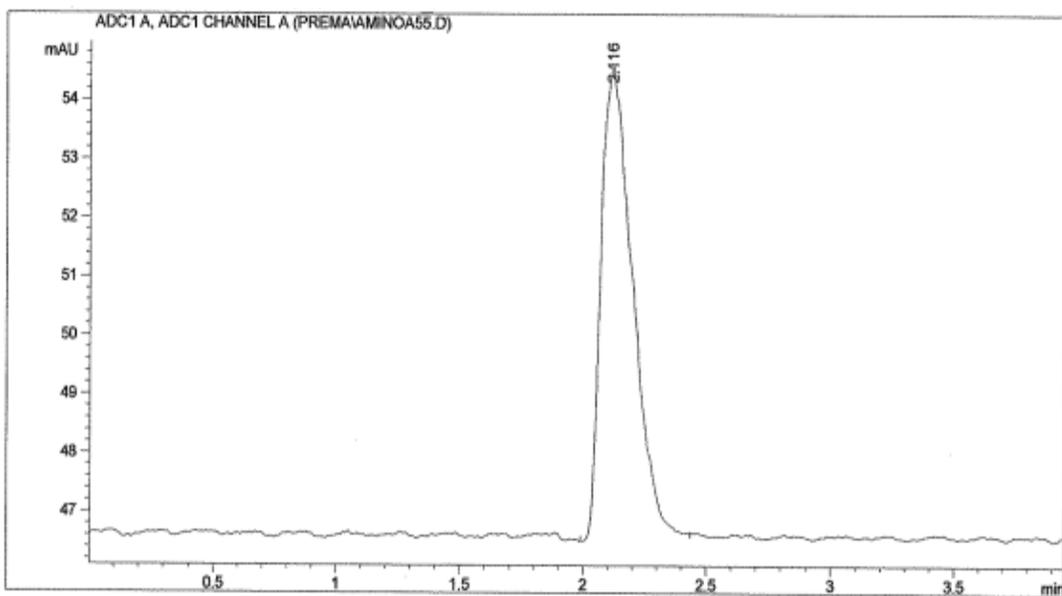
Figure 31. Chromatogram of melamine on endcapped silica hydride stationary phase. Mobile phase: 20:80 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
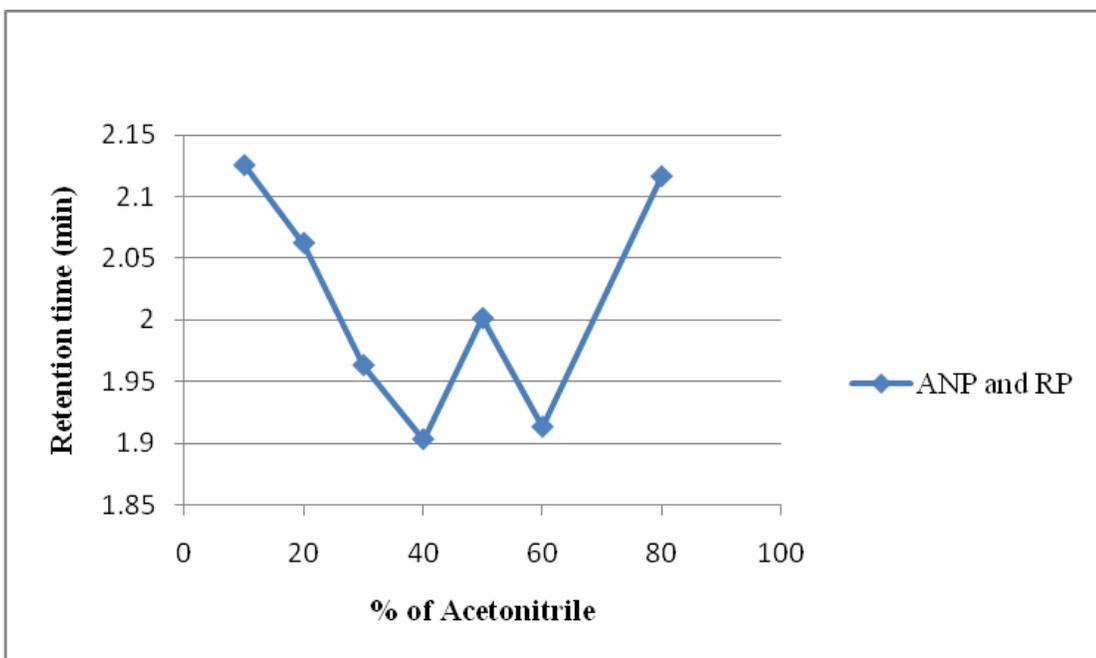
Figure 32. Retention map of melamine for ANP and RP conditions.

Melamine was analyzed using aqueous normal phase as well as using reversed-phase conditions as shown in the chromatograms from Figure 25 to Figure 31. The mobile phase used was 0.1% formic acid in acetonitrile and 0.1% formic acid in water. The detector used for the analysis was the evaporative light scattering detector. As shown in Figure 32, melamine can be retained using the aqueous normal phase elution mode. The basic principle of the aqueous normal phase mode is that retention increases as the percentage of acetonitrile increases. Melamine can also be retained in the reversed-phase elution mode as shown in Figure 32 for the isocratic conditions with high water content.

e. Aqueous Normal Phase Behavior of Phenylalanine

The concentration of phenylalanine used for aqueous normal phase behavior was 0.5 mg/mL in a 50:50 water/acetonitrile solution. The ELSD detector parameters were set and stabilized with the starting conditions of the isocratic system.
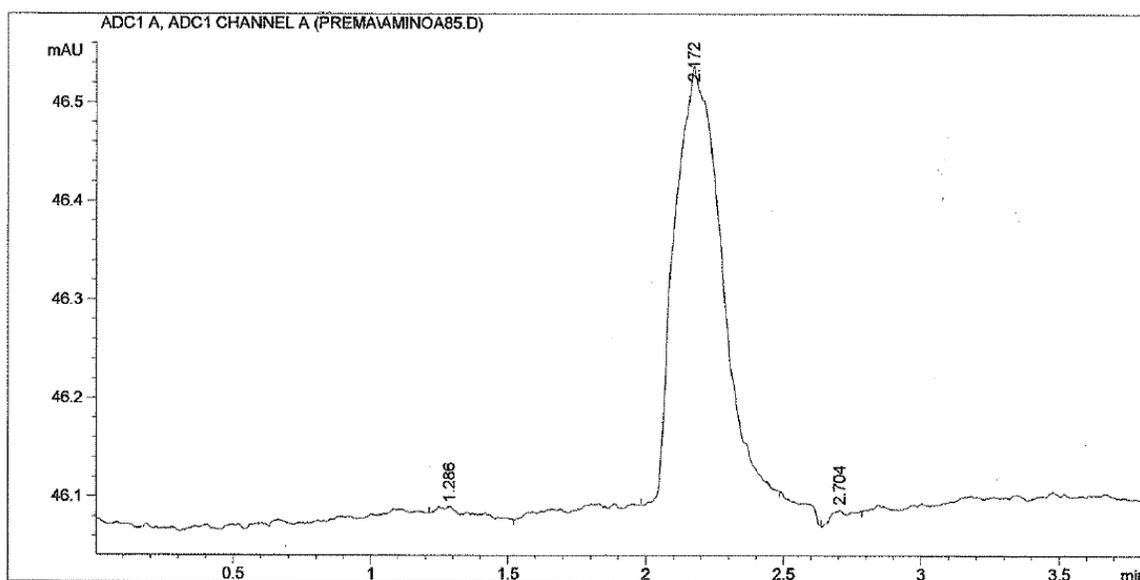
Figure 33. Chromatogram of phenylalanine on endcapped silica hydride stationary phase. Mobile phase: 40:60 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
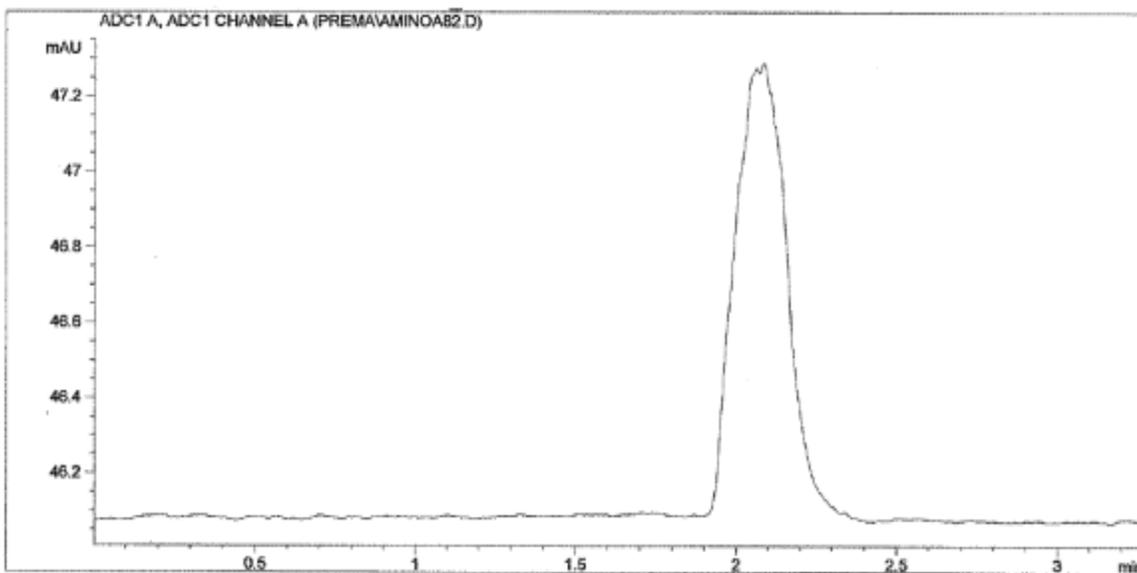
Figure 34. Chromatogram of phenylalanine on endcapped silica hydride phase. Mobile phase: 50:50 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

Phenylalanine was analyzed using 0.1% formic acid in water and 0.1% formic acid in acetonitrile under ANP isocratic conditions with an ELSD detector as shown in Figures 33 and 34. The compound was dissolved in 50:50 water:acetonitrile at a concentration of 0.5 mg/mL. The compound was also analyzed using UV detection which is discussed in a subsequent section. Considerably more work has been done using UV detection in comparison to ELSD.

3. ANP and RP Behavior of Endcapped Si-H Using UV Detection

The chromatographic characterization of the endcapped silica hydride column was done by measuring the retention behavior of the compounds discussed in the experimental section. The results were compared with the results of C8 (75 x 4.6 mm i.d) silica hydride column. The characterization of the column was done under aqueous normal phase mode as well as under reversed-phase mode. The parameters used for HPLC with UV detection were a flow rate 0.5 mL/min, an injection volume 5 µL, and the column temperature at ambient. The concentration of the samples was 0.5 mg/mL in 50:50 water and acetonitrile solution. The wavelengths used for the analysis were 254 nm and 220 nm, and the column was maintained at the starting conditions of the gradient or isocratic system until a stable baseline was obtained. A water blank was run to eliminate any interference from the previous measurements. Raloxifene, dimethylthiourea, lipoic acid, triphenylphosphine, phenylalanine, niacinic acid, and lisinopril were analyzed using HPLC with a UV detector under isocratic and gradient conditions. The gradient program used for this research is shown in Table 7 and Table 8. The main goal of this research was to characterize the endcapped Si-H hydride column using aqueous normal phase and reversed-phase mode under isocratic and gradient conditions.

Table 7. Mobile phase gradients used for reversed-phase analysis

Gradient A			Gradient B		
Time (min)	Water with 0.1% TFA	Acetonitrile with 0.1% TFA	Time (min)	Water with 0.1% TFA	Acetonitrile with 0.1% TFA
0	100	0	0	95	5
25	60	40	5	95	5
35	30	70	35	5	95
40	30	70	40	5	95
45	100	0	45	95	5
50	100	0	50	95	5

Table 8. Mobile phase gradients used for reversed-phase analysis

Gradient C			Gradient D		
Time (min)	Water with 0.1% TFA	Acetonitrile with 0.1% TFA	Time (min)	Water with 0.1% TFA	Acetonitrile with 0.1% TFA
0	75	25	0	75	25
5	75	25	5	75	25
30	55	45	30	50	50
35	50	50	35	0	100
40	5	95	40	0	100
50	5	95	45	75	25
55	75	25	50	75	25
60	75	25			

a. Aqueous Normal Phase Behavior of Phenylalanine

Phenylalanine was analyzed using aqueous normal phase isocratic conditions and concentration used was 0.5 mg/mL in a 50:50 water/acetonitrile solution. 220 nm UV wavelength was selected and stabilized with the starting conditions of the isocratic system.
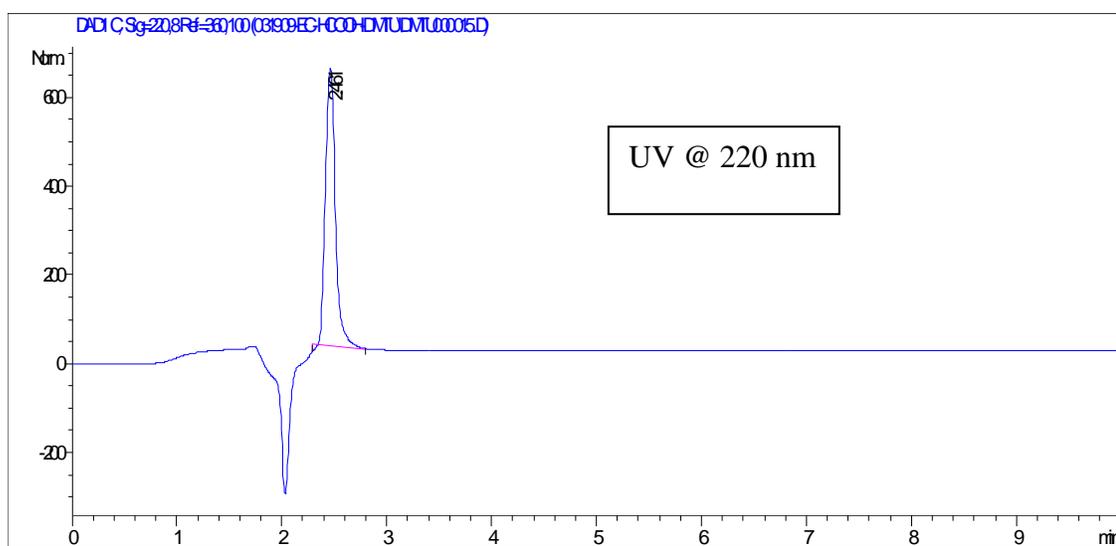
Figure 35. Chromatogram of phenylalanine on endcapped silica hydride stationary phase. Mobile phase: 40:60 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
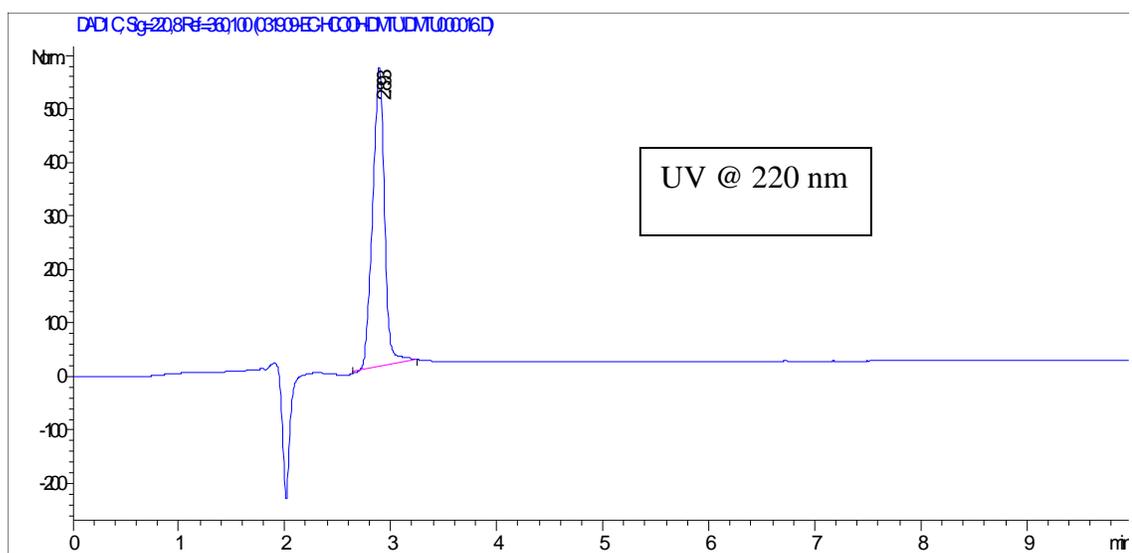
Figure 36. Chromatogram of phenylalanine on endcapped silica hydride stationary phase. Mobile phase: 30:70 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
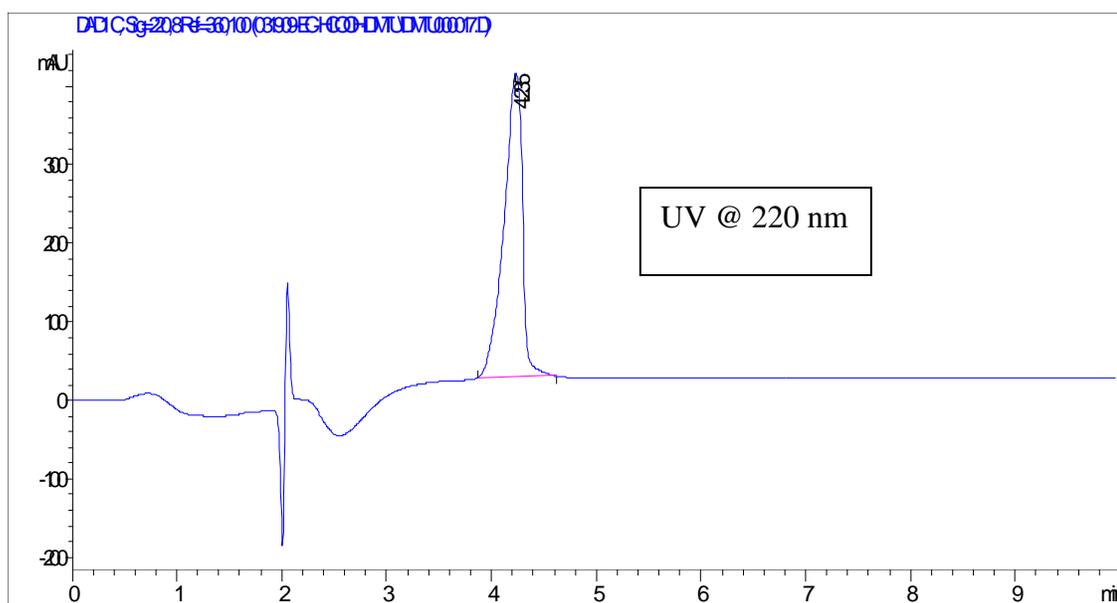
Figure 37. Chromatogram of phenylalanine on endcapped silica hydride stationary phase. Mobile phase: 20:80 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

Table 9. Summary of isocratic elution data for phenylalanine

Phenylalanine Isocratic		
% Water + 0.1% FA	% Acetonitrile + 0.1% FA	Retention time (min)
40	60	2.5
30	70	2.9
20	80	4.2

Phenylalanine was analyzed using aqueous normal phase isocratic conditions. The mobile phase used was 0.1% formic acid in water and 0.1% formic acid in acetonitrile as shown in Figures 35 to 37. The compound was dissolved in 50:50 water/acetonitrile at a concentration of 0.5 mg/mL and the detector used was UV. In ANP mode the retention of the compound increases as the percentage of acetonitrile increases as shown in Table 9.

b. Aqueous Normal Phase Behavior of Niacinic Acid

Niacinic acid was analyzed using aqueous normal phase isocratic conditions and concentration used was 0.5 mg/mL in a 50:50 water/acetonitrile solution. 220 nm UV wavelength was selected and stabilized with the starting conditions of the isocratic system.
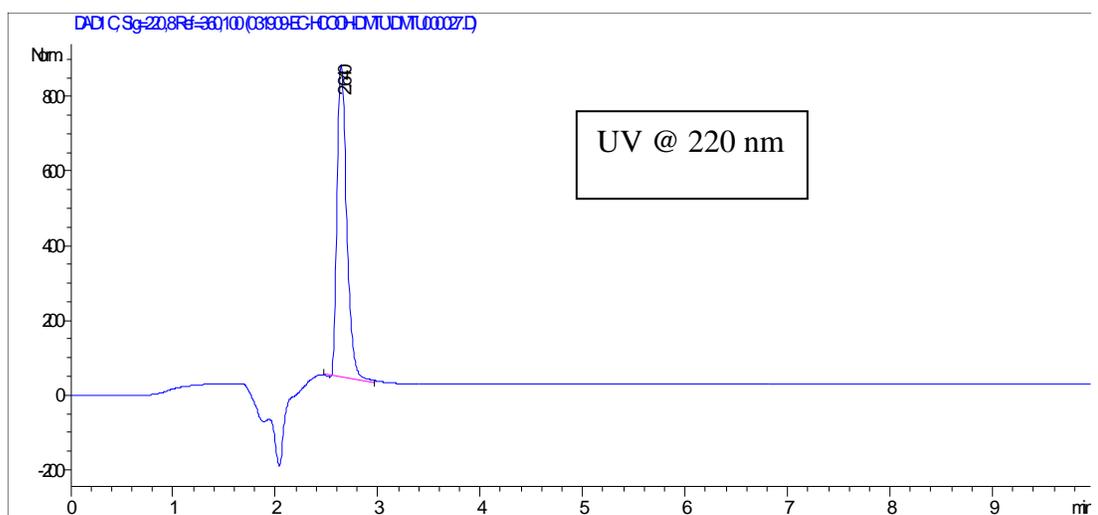
Figure 38. Chromatogram of niacinic acid on endcapped silica hydride stationary phase. Mobile phase: 40:60 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
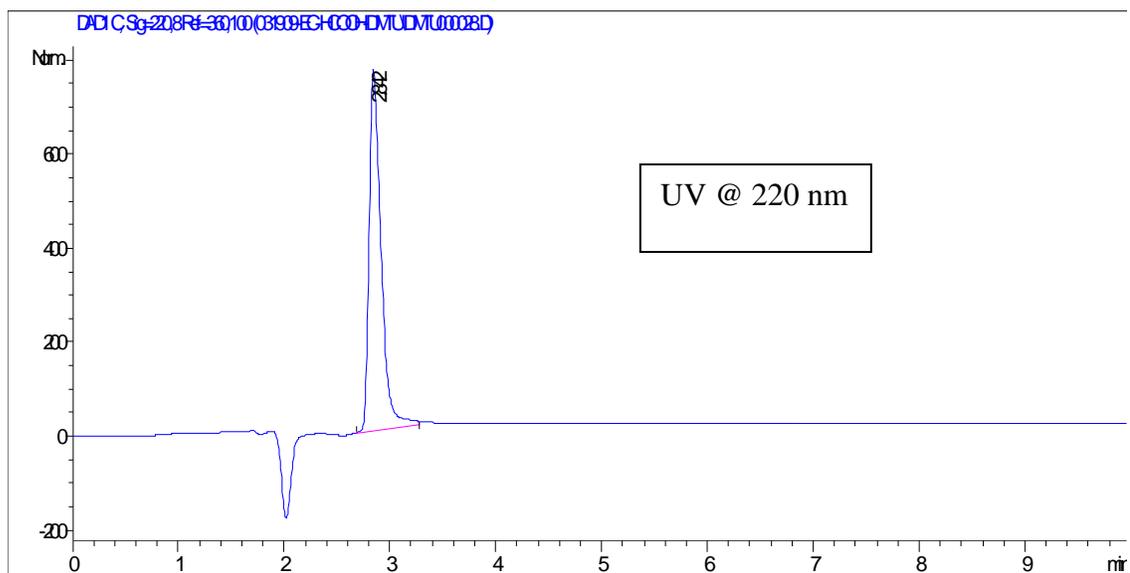
Figure 39. Chromatogram of niacinic acid on endcapped silica hydride stationary phase. Mobile phase: 30:70 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.
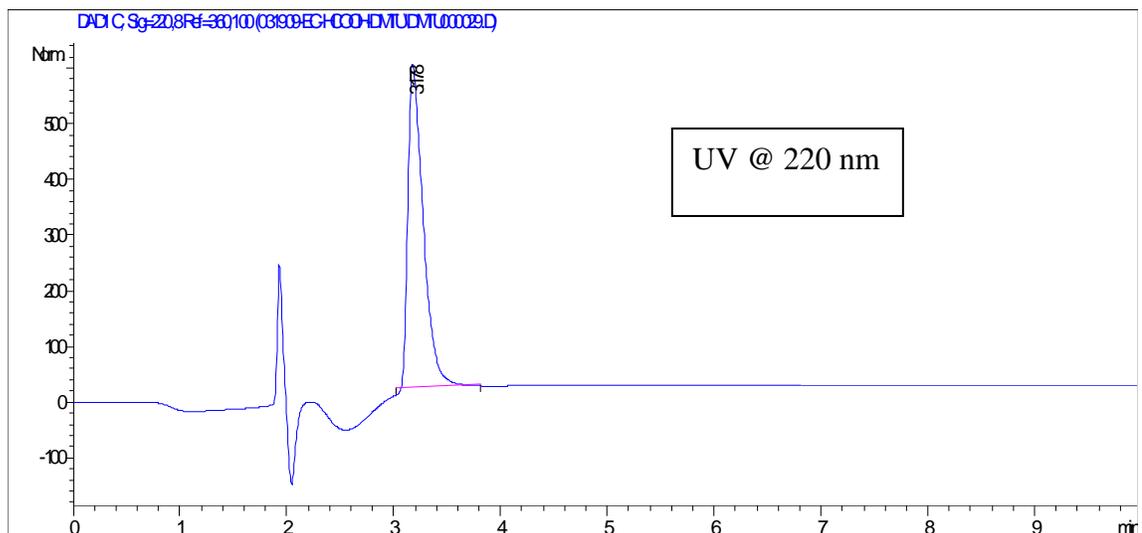
Figure 40. **Chromatogram of niacinic acid on endcapped silica hydride stationary phase. Mobile phase: 20:80 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.**
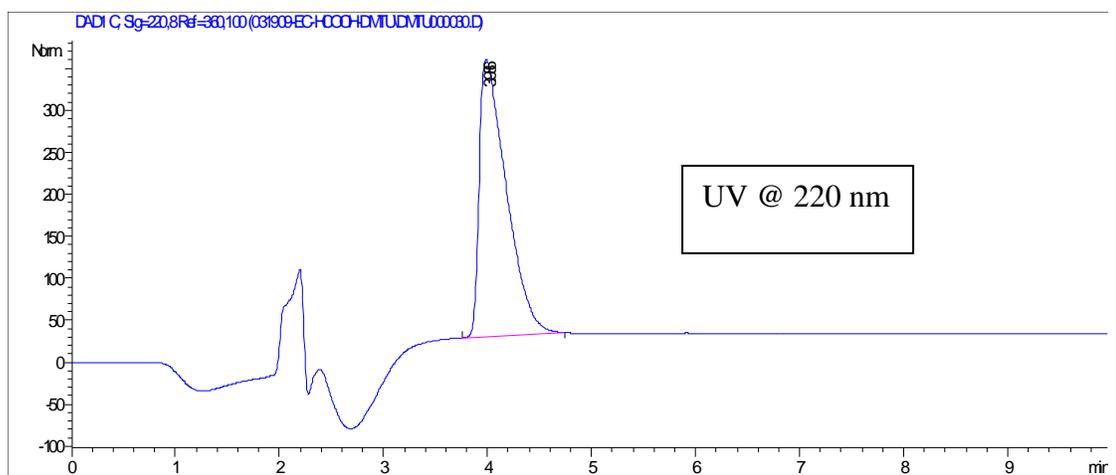
Figure 41. **Chromatogram of niacinic acid on endcapped silica hydride stationary phase. Mobile phase: 10:90 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.**
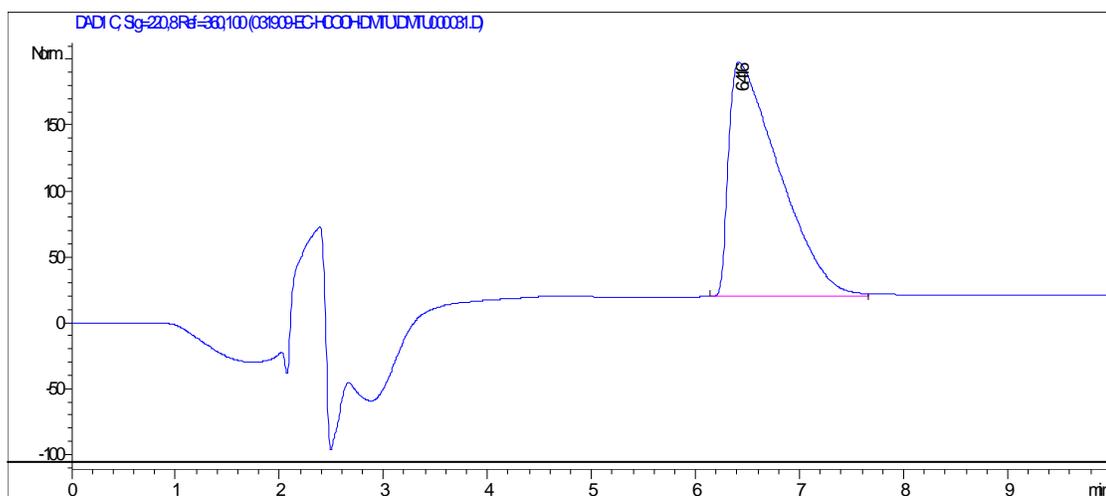
Figure 42. Chromatogram of niacinic acid on endcapped silica hydride stationary phase. Mobile phase: 5:95 water + 0.1% formic acid / acetonitrile + 0.1% formic acid.

Table 10. Summary of isocratic elution data for niacinic acid

Niacinic Acid - Isocratic		
% Water + 0.1% FA	**% Acetonitrile + 0.1% FA**	**Retention time (min)**
40	60	2.2
30	70	2.8
20	80	3.2
10	90	4
5	95	6.4

Niacinic acid was analyzed using UV detection under different isocratic conditions as shown in Figures 38 to 42 under aqueous normal phase conditions. As shown in Table 10, in ANP elution as the composition of acetonitrile increases the retention of the compound increases.

c.   Aqueous Normal Phase Behavior of Lisinopril

Lisinopril was analyzed using aqueous normal phase isocratic conditions and concentration used was 0.5 mg/mL in a 50:50 water/acetonitrile solution.
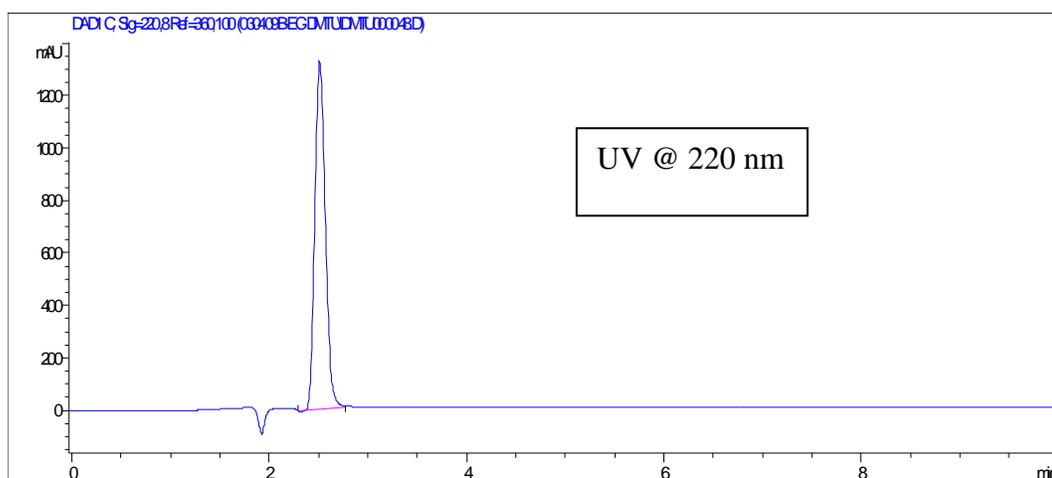
Figure 43. Chromatogram of lisinopril on endcapped silica hydride stationary phase. Mobile phase: 20:80 water + 0.1% trifluoroacetic acid / acetonitrile + 0.1% trifluoroacetic acid.
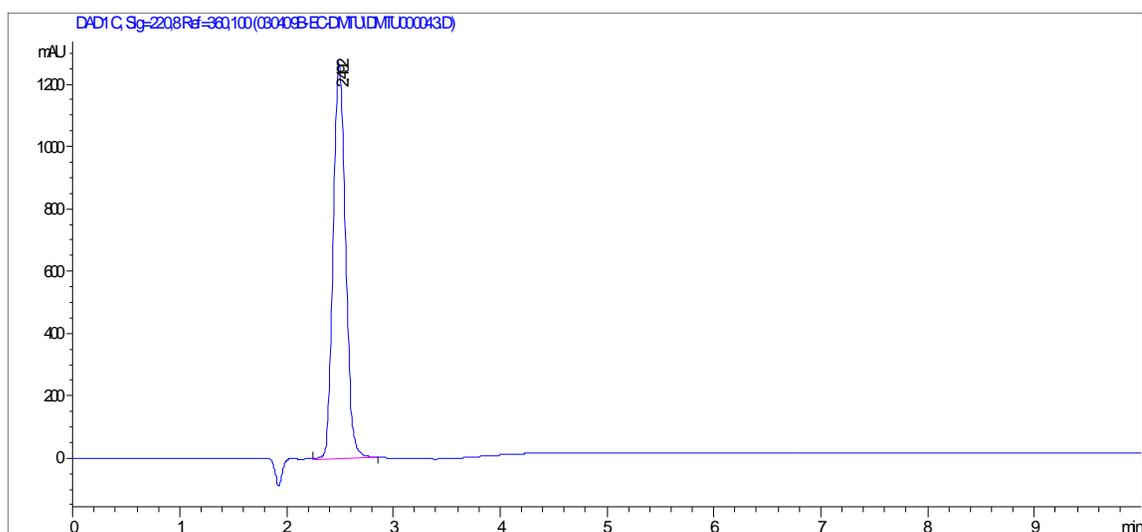
Figure 44. Chromatogram of lisinopril on endcapped silica hydride stationary phase. Mobile phase: 10:90 water + 0.1% trifluoroacetic acid / acetonitrile + 0.1% trifluoroacetic acid.
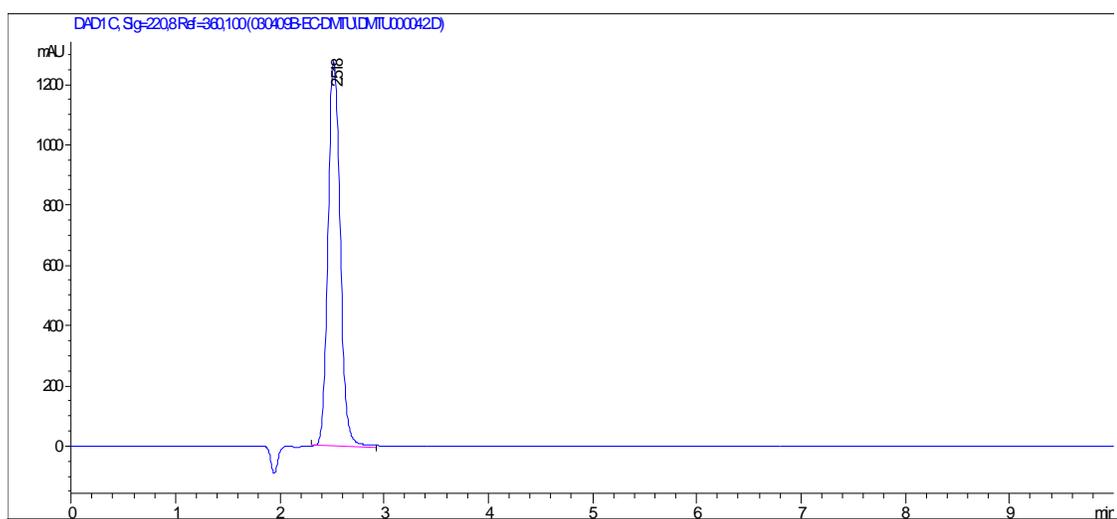
Figure 45. Chromatogram of lisinopril on endcapped silica hydride stationary phase. Mobile phase: 5:95 water + 0.1% trifluoroacetic acid / acetonitrile + 0.1% trifluoroacetic acid.

Table 11. Summary of isocratic elution data for lisinopril

Lisinopril – Isocratic		
% Water + 0.1% FA	% Acetonitrile + 0.1% FA	Retention time (min)
50	50	2.7
30	70	2.0
20	80	2.2
10	90	2.5
5	95	2.5

Lisinopril was analyzed using UV detection under different isocratic conditions. The Figures from 43 to 45 show aqueous normal phase elution and as shown in Table 11 as the composition of acetonitrile increase the retention of the compound increases above a composition of 50% acetonitrile. This data obtained confirmed the aqueous normal phase behavior of lisinopril.

d. ANP Separation of Two Compounds Using an Isocratic Method

Lisinopril and lipoic acid was analyzed using aqueous normal phase isocratic conditions and concentration used was 0.5 mg/mL in a 50:50 water/acetonitrile solution.
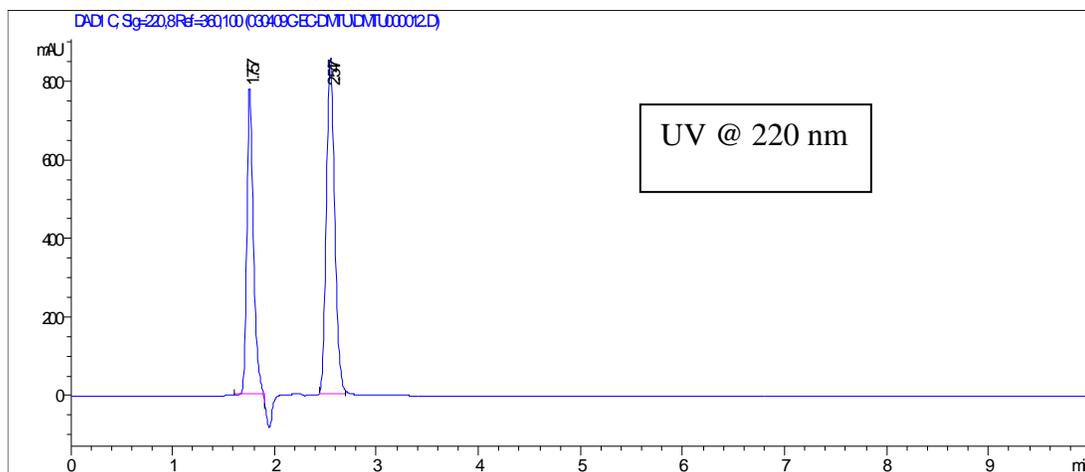
Figure 46. ANP isocratic separation of lipoic acid and lisinopril. Mobile phase: 95:5 acetonitrile/water + 0.1% TFA. The peak at 1.75 min is for lipoic acid and the peak at 2.547 min is for lisinopril.
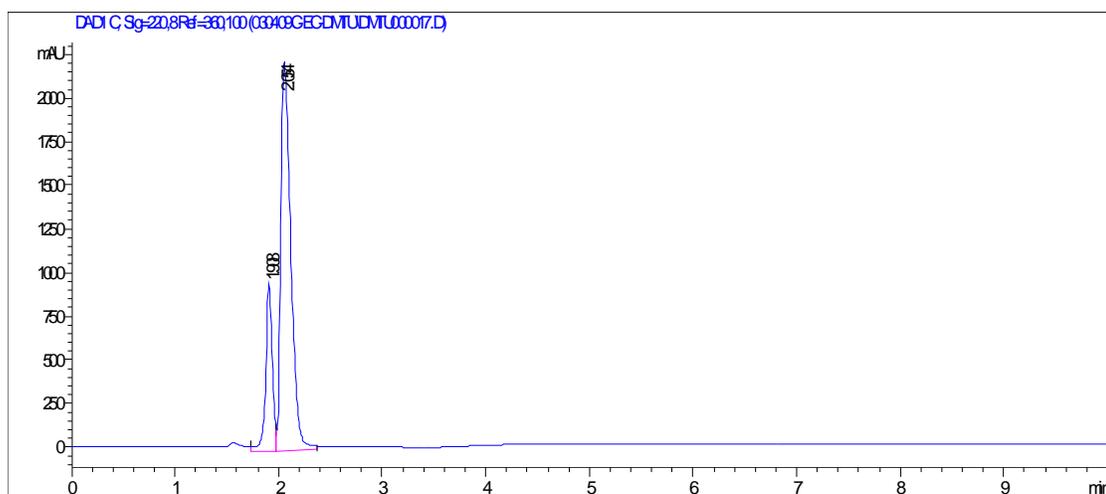
Figure 47. ANP isocratic separation of dimethylthiourea and raloxifene. Mobile phase: 95:5 acetonitrile/water + 0.1% TFA. The peak at 1.978 min is for raloxifene and the peak at 2.547 min is for dimethylthiourea.

Two-compound mixture was tested on the endcapped silica hydride column to determine whether it was be possible to separate them either gradient or isocratic conditions. The first mixture analyzed was lisinopril and lipoic acid as shown in Figure 46. A second mixture analyzed was raloxifene and dimethylthiourea as shown in Figure 47; both mixtures were tested for ANP behavior. The samples were analyzed using UV detection under isocratic conditions. The polar compounds dimethylthiourea and lisinopril were retained longer than the nonpolar compounds raloxifene and lipoic acid, as expected under aqueous normal phase conditions. This was a very good example illustrating typical aqueous normal phase behavior in which nonpolar compounds elute before polar compounds. The polar compounds were retained longer and retention

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decreased as the percentage of polar solvent was increased. Therefore it can be confirmed that endcapped Si-H showed good aqueous normal phase behavior.

e. RP Behavior of Endcapped Si-H Using UV Detection Under Isocratic and Gradient Conditions

In reversed-phase gradient separation the composition of the organic component is increased with respect to time. Nonpolar compounds are preferentially separated in this chromatographic mode.
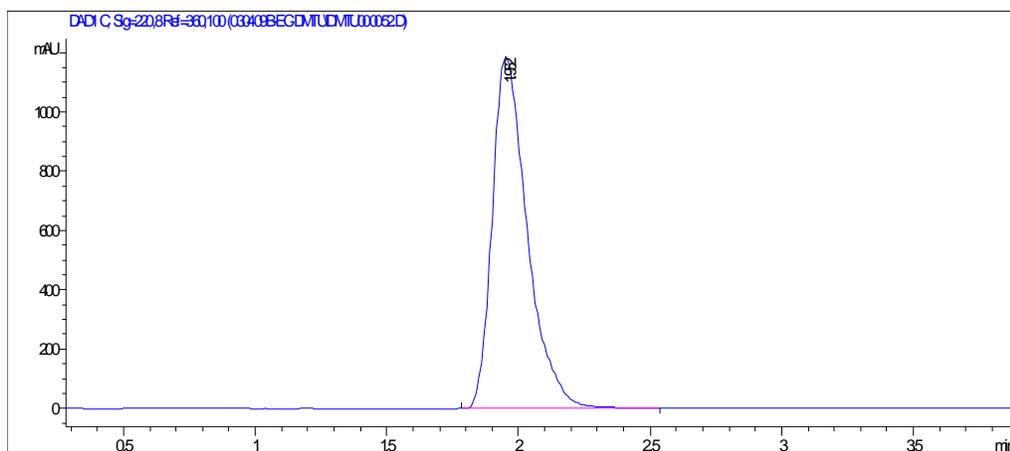
Figure 48. Chromatogram of lisinopril in reversed-phase mode on the endcapped silica hydride phase. Mobile phase: 75:25 water/acetonitrile + 0.1% TFA .
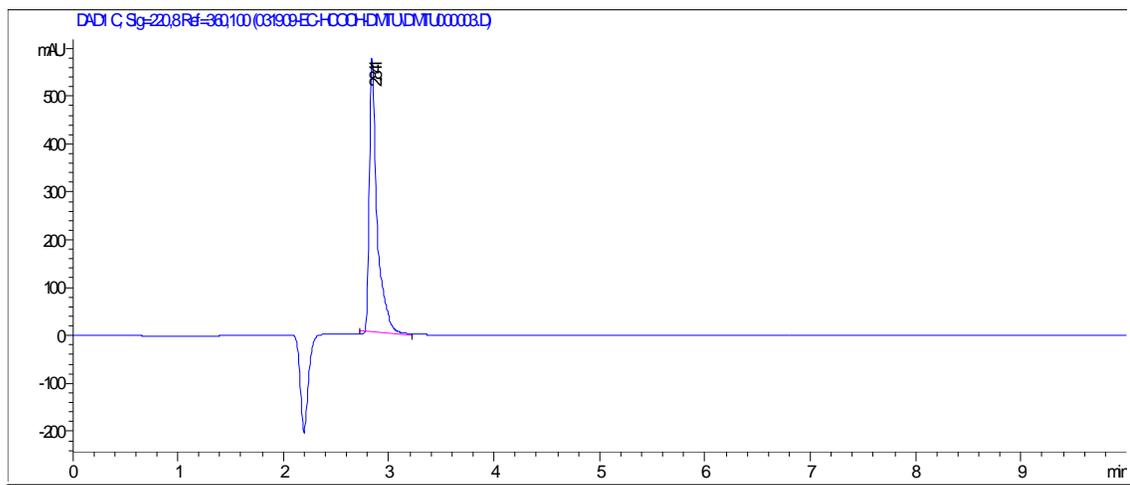
Figure 49. Chromatogram of dimethylthiourea in reversed-phase mode on endcapped silica hydride column using 100% water + 0.1% TFA.

Lisinopril and dimethylthiourea were measured using isocratic reversed-phase conditions as shown in Figures 48 and 49 and detected using UV detection at 220 nm. As mentioned earlier silica hydride columns have the ability to separate both polar and nonpolar compounds. Endcapping the silica hydride enhances the hydrophobicity which will be discussed in a later section.

DADI C, Sig=220,8 Ref=360,100 (030409-EC-DMTU.DMTU000002.D)
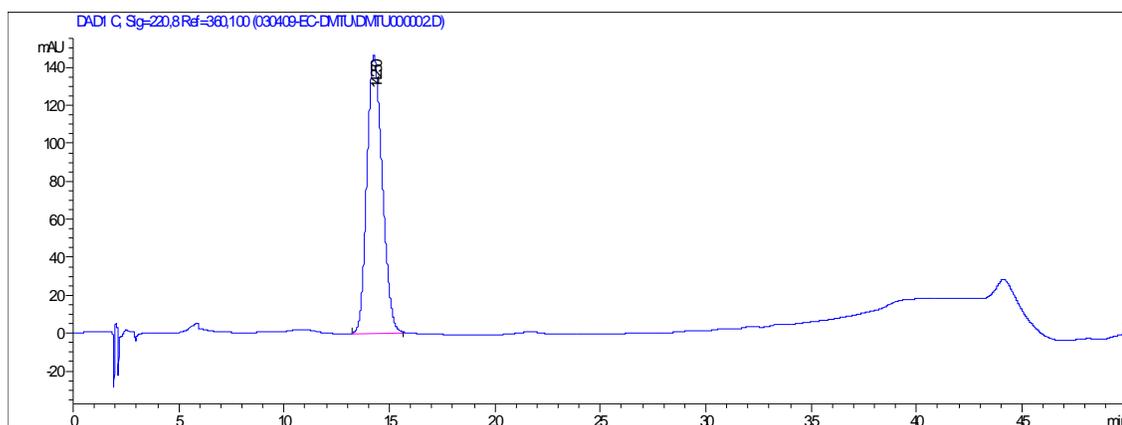
Figure 50. Chromatogram of raloxifene on the endcapped silica hydride column in reversed-phase mode using Gradient A shown in Table 7

DADI C, Sig=220,8 Ref=360,100 (030409-EC-DMTU.DMTU000009.D)
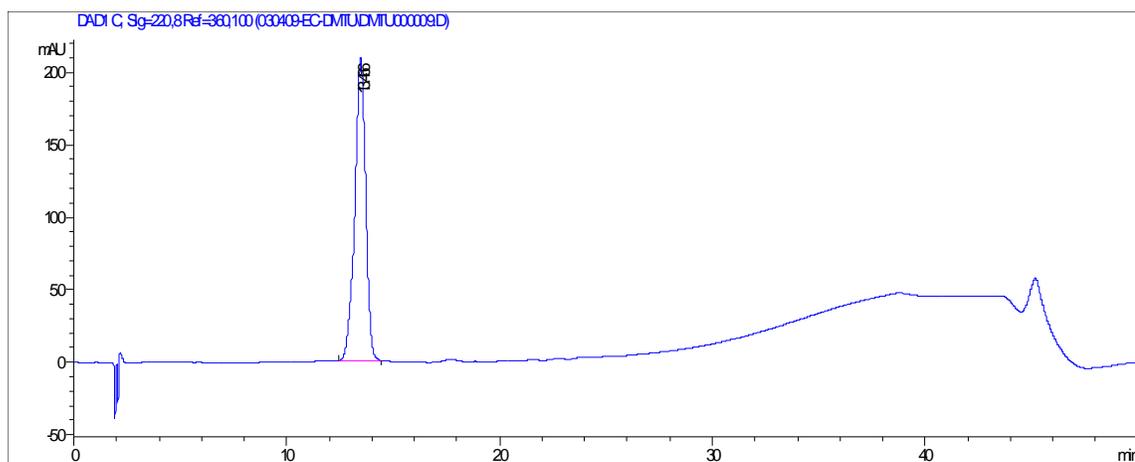
Figure 51. Chromatogram of raloxifene on the endcapped silica hydride column in reversed-phase mode using Gradient B shown in Table 7.

Raloxifene was eluted using Gradients A and B shown in Table 7 and the chromatograms are shown in Figures 50 and 51. The mobile phase used for this analysis

was 0.1% TFA in water and 0.1% TFA in acetonitrile having flow rate of 0.5 mL/min. The concentration of the sample was 0.1 mg/mL dissolved in water. Figures 50 and 51 show the enhancement of the retention capabilities of the endcapped column for nonpolar compounds under reversed-phase conditions. The result was comparable with the C8 data which will be discussed in a subsequent section. The detector used for this data is UV at a wavelength of 220 nm.
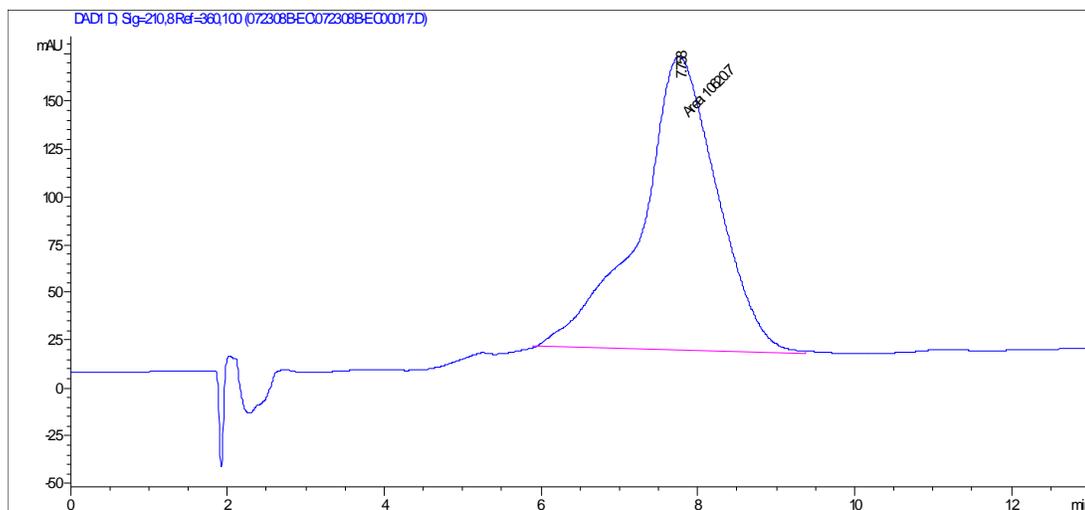
Figure 52. Chromatogram of lipoic acid on the endcapped silica hydride column in reversed-phase mode using Gradient A shown in Table 7.

Lipoic acid was eluted using Gradient A and the mobile phase used for this analysis was 0.1% TFA in water and 0.1% TFA in acetonitrile as shown in Figure 52. The peak shape was not symmetric under this gradient condition but it eluted at 8 min which was a good retention for the compound.
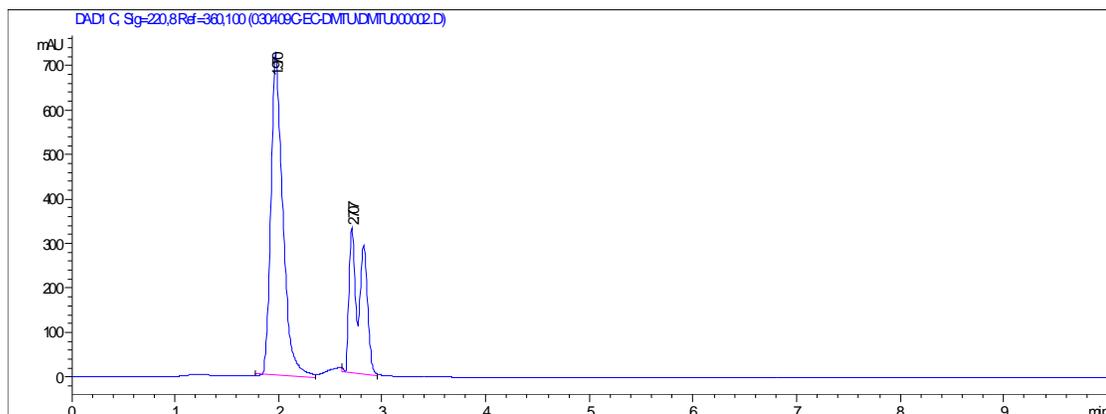
Figure 53. Reversed-phase isocratic separation of lisinopril and lipoic acid. The peak at 1.90 min is lisinopril and the peak at 2.8 min is lipoic acid. The isocratic composition used is 75:25 water/ acetonitrile + 0.1% TFA.

The lisinopril and lipoic acid sample mixture were separated using a reversed-phase isocratic condition as shown in Figure 53. The mobile phase flow rate was 0.5 mL/min and with UV detection at 220 nm. Lisinopril being polar compared to lipoic acid eluted at 1.97 min and lipoic acid eluted at 2.8 min. Lipoic acid is a stereoisomer, so it is possible that the isomers are being separated under the given conditions. In order to determine whether the endcapped silica hydride column can be used for the separation of stereoisomers, more work needed to be done using different isomeric compounds.
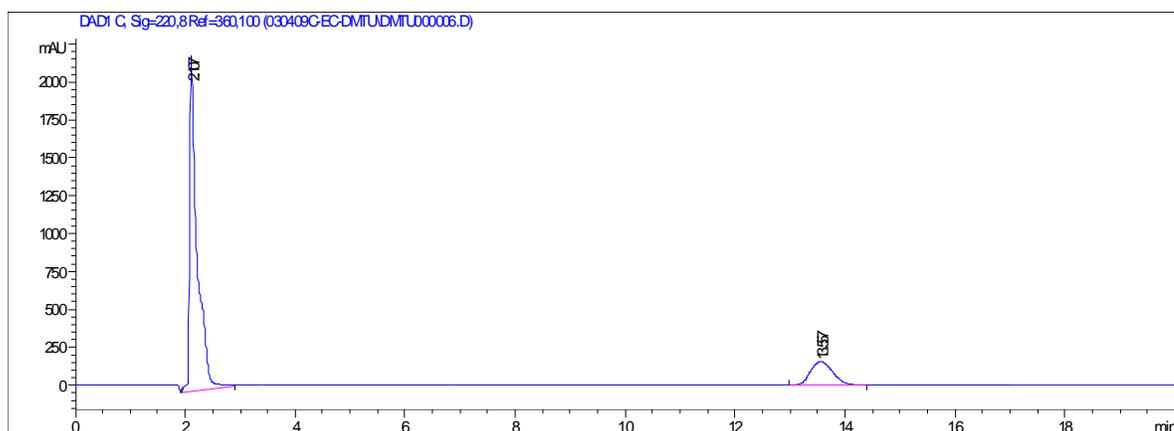
Figure 54. Reversed-phase gradient separation of dimethylthiourea and raloxifene. The peak at 2.07 min is for dimethylthiourea and the peak 13.557 min is for raloxifene. Gradient B used for the separation is shown in Table 7.

Raloxifene and dimethylthiourea were separated under reversed-phase conditions as shown in Figure 54. The gradient used for the separation was Gradient B shown in Table 7. The mobile phase flow rate was 0.5 mL/min with UV detection at 220 nm. Dimethylthiourea being very polar eluted at the beginning of the gradient and raloxifene being very nonpolar eluted at the latter part of the gradient.

From the results of this study, it can be interpreted that endcapping the silica hydride stationary phase with trimethylchlorosilane and hexamethyldisilazane increases the ability of the stationary phase for nonpolar compound separation. The separation between the compounds occurs by the hydrophobic interaction between the analytes and the stationary phase. The data obtained was comparable with the results obtained from

the C8 column which will be discussed in a subsequent section. This confirms the fact that endcapping the silica hydride stationary phase increases the hydrophobic capabilities which can be used for the separation of nonpolar compounds.

As mentioned earlier that after silica modifications with TES there are some residual silanol groups present on the surface which may interact with the analyte molecule. This interaction might result in peak tailing, band broadening, and poor separation of basic compounds. One goal of this research was to reduce the amount of residual silanol groups on silica, and to prevent the remaining silanol group interaction with the samples of interest by endcapping Si-OH.

4. ANP and RP Behavior of Si-H based C8 Column Using UV Detection

a. Aqueous Normal Phase Behavior

Phenylalanine, raloxifene, dimethylthiourea, and lipoic acid were analyzed under aqueous normal phase conditions on Si-H based C8 column as shown in Figures 55 to 60. The concentrations of the samples are 0.5 mg/mL and the detector used was UV.
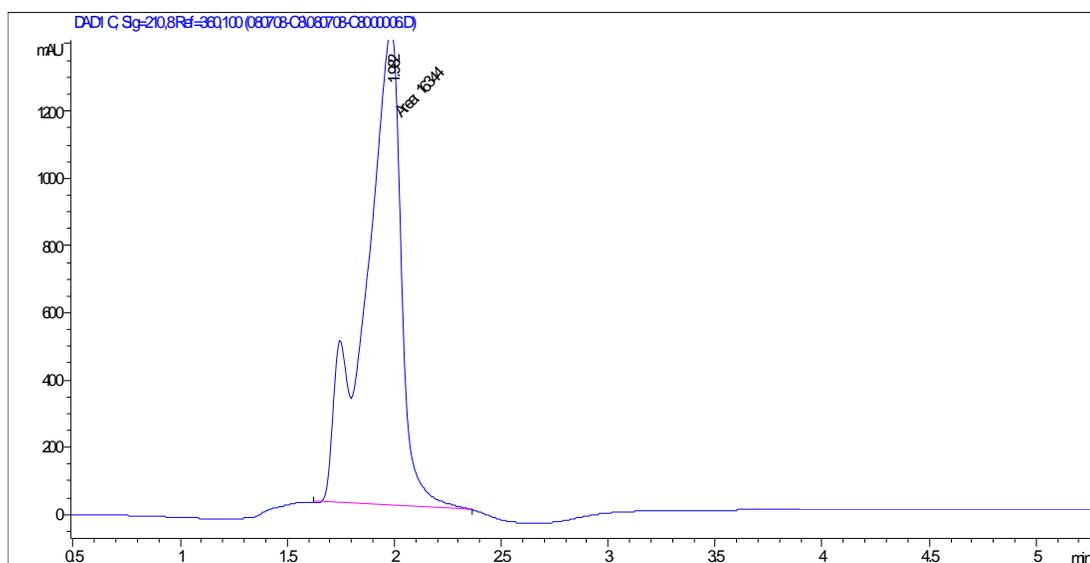
Figure 55. ANP isocratic retention of phenylalanine on silica hydride-based C8 column. The isocratic composition used is 60:40 acetonitrile/water + 0.1% TFA.

Table 12. Summary of isocratic elution data for Phenylalanine

Phenylalanine-Isocratic condition		
Water + 0.1% TFA	Acetonitrile + 0.1% TFA	RT (min)
50	50	1.7
40	60	2
5	95	1.9
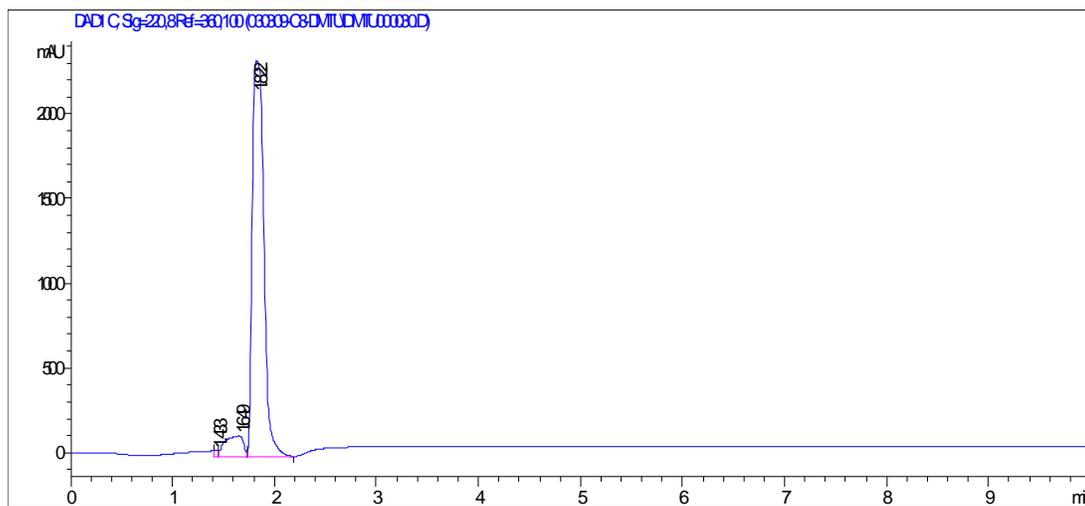
Figure 56. ANP isocratic retention of dimethylthiourea on silica hydride-based C8 column. The isocratic composition used is 90:10 acetonitrile/water + 0.1% TFA.
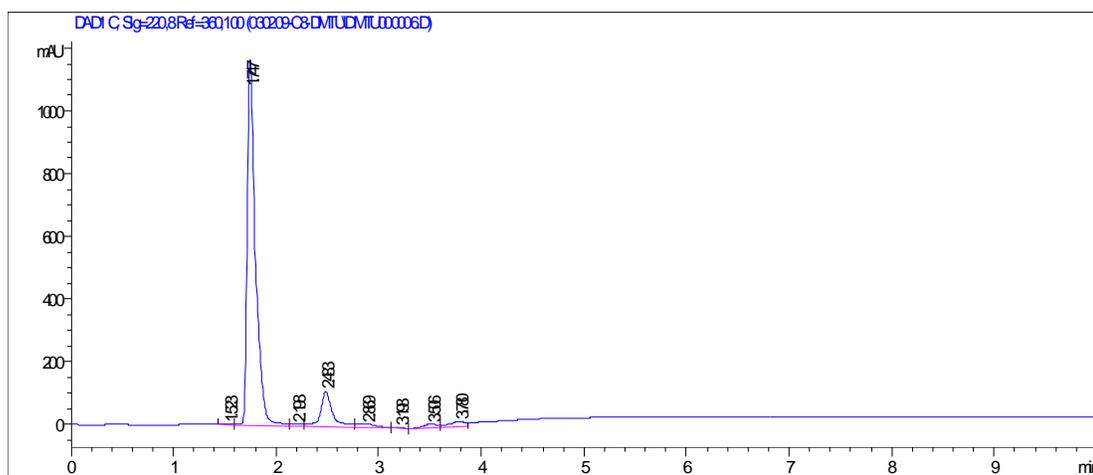
Figure 57. ANP isocratic retention of raloxifene on silica hydride-based C8 column.

The isocratic composition used is 95:5 acetonitrile/water + 0.1% TFA.
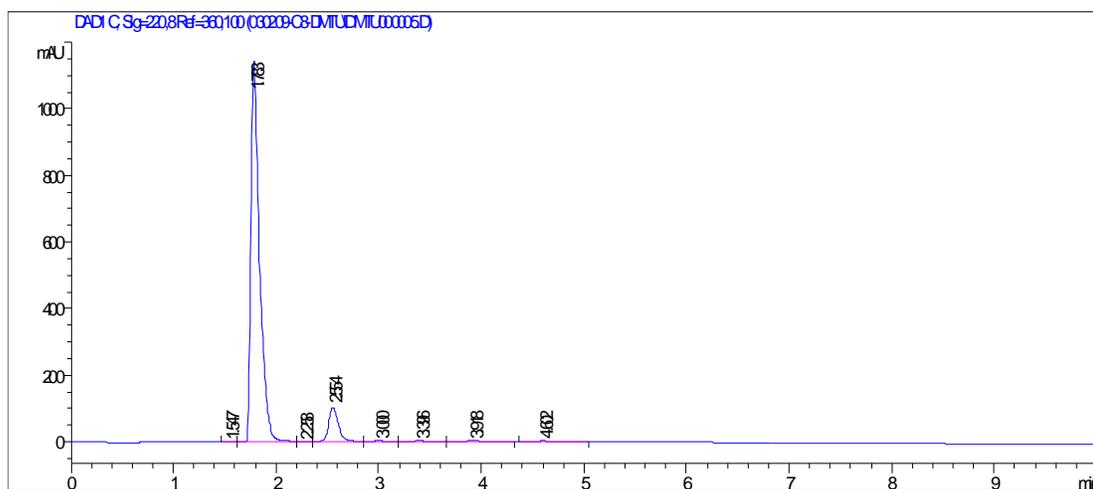
Figure 58. ANP isocratic retention of raloxifene on silica hydride-based C8 column.

The isocratic composition used is 80:20 acetonitrile/water + 0.1% TFA.
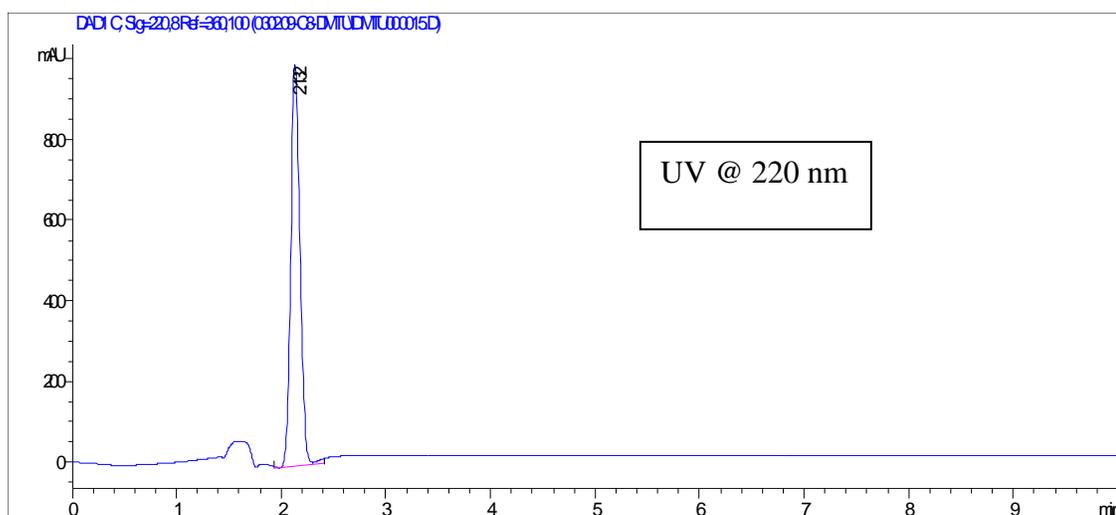
Figure 59. ANP isocratic retention of lipoic acid on silica hydride-based C8 column. The isocratic composition used is 80:20 acetonitrile/water + 0.1% TFA.
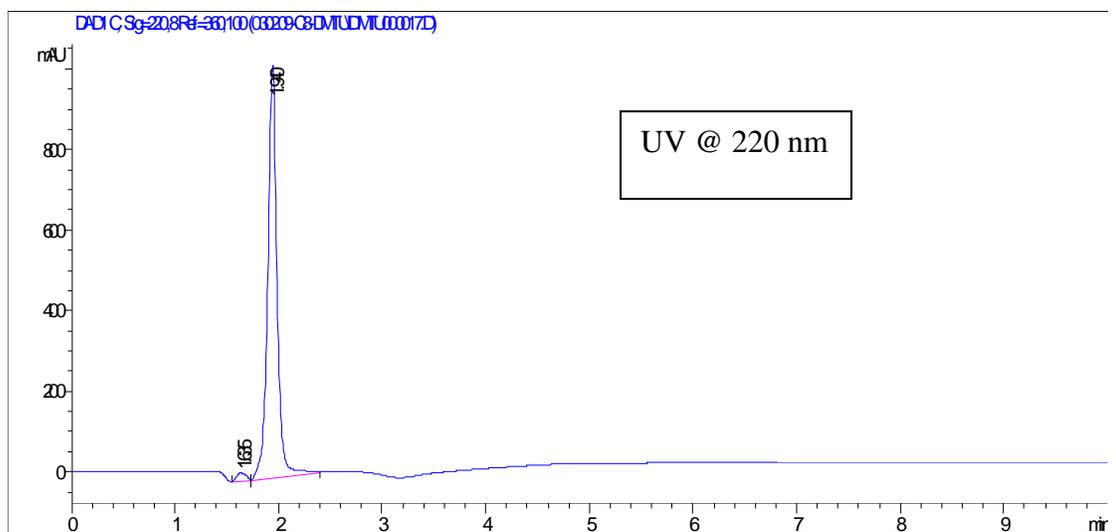
Figure 60. ANP isocratic retention of lipoic acid on silica hydride-based C8 column. The isocratic composition used is 95:5 acetonitrile/water + 0.1% TFA.

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Raloxifene being nonpolar eluted early in the aqueous normal phase mode. The aqueous normal phase behavior data obtained for the C8 column confirms that the stationary phase was not suitable for aqueous normal phase separation of these compounds.

b. Reversed-Phase Behavior

Raloxifene, triphenylphosphine, lisinopril, and niacinic acid were analyzed under reversed-phase gradient conditions using silica hydride based C8 column as shown in Figures 61 to 66. The concentration of the sample was 0.5 mg/mL and the detector used was UV.
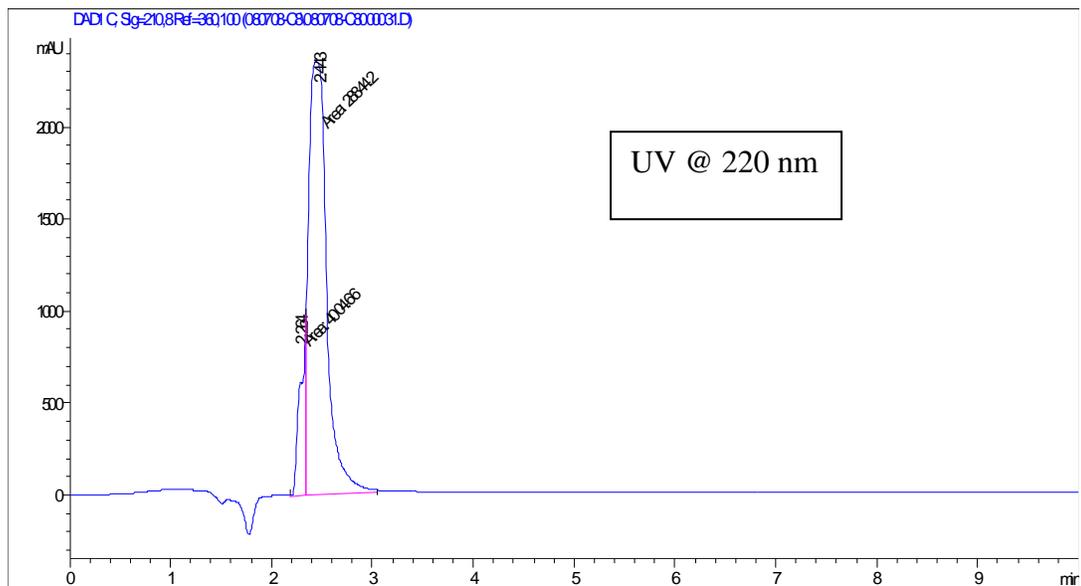
Figure 61. RP isocratic retention of niacinic acid on silica hydride-based C8 column. The isocratic composition used is 30:70 acetonitrile/water + 0.1% TFA.
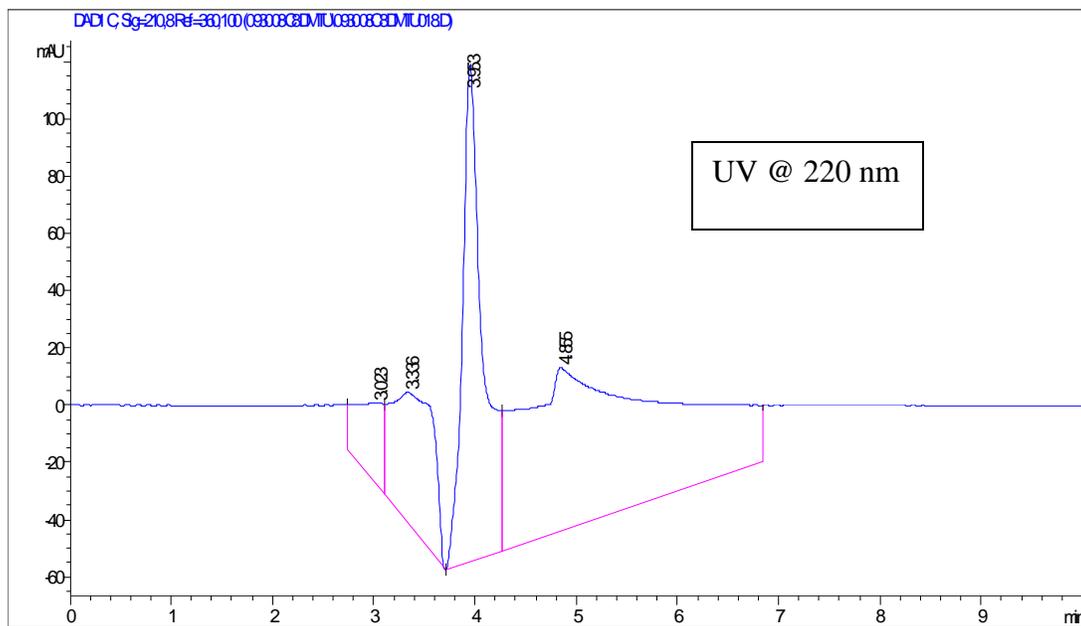
Figure 62. RP isocratic retention of lisinopril on silica hydride-based C8 column.

The isocratic composition used is 45:55 acetonitrile/water + 0.1% TFA.
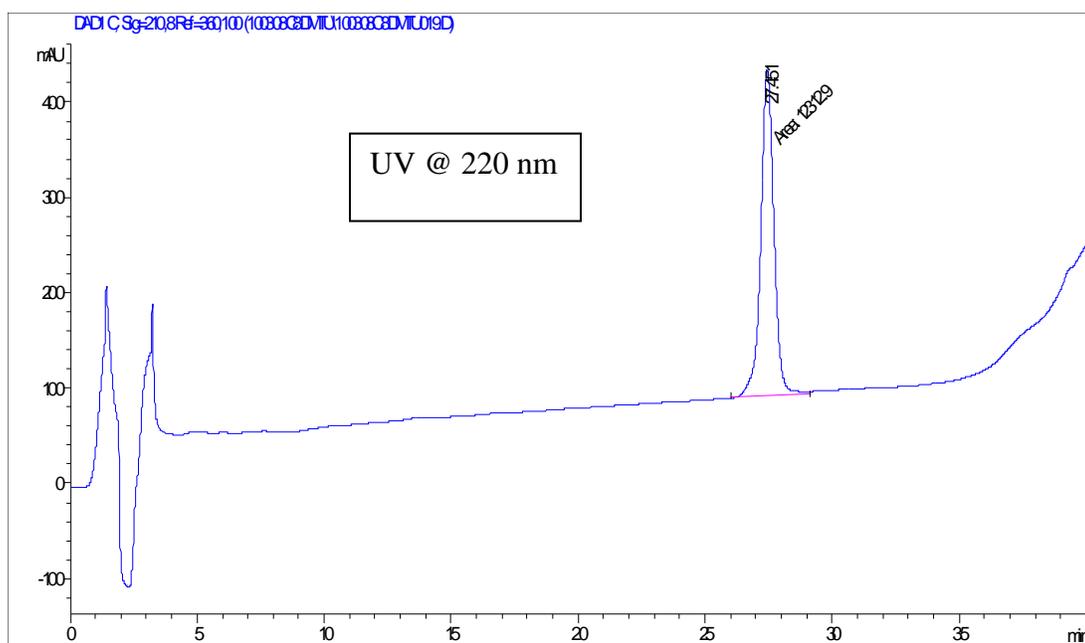
Figure 63. RP gradient retention of triphenylphosphine on the silica hydride-based C8 column. It elutes at 27.5 min with Gradient C as shown in Table 8.
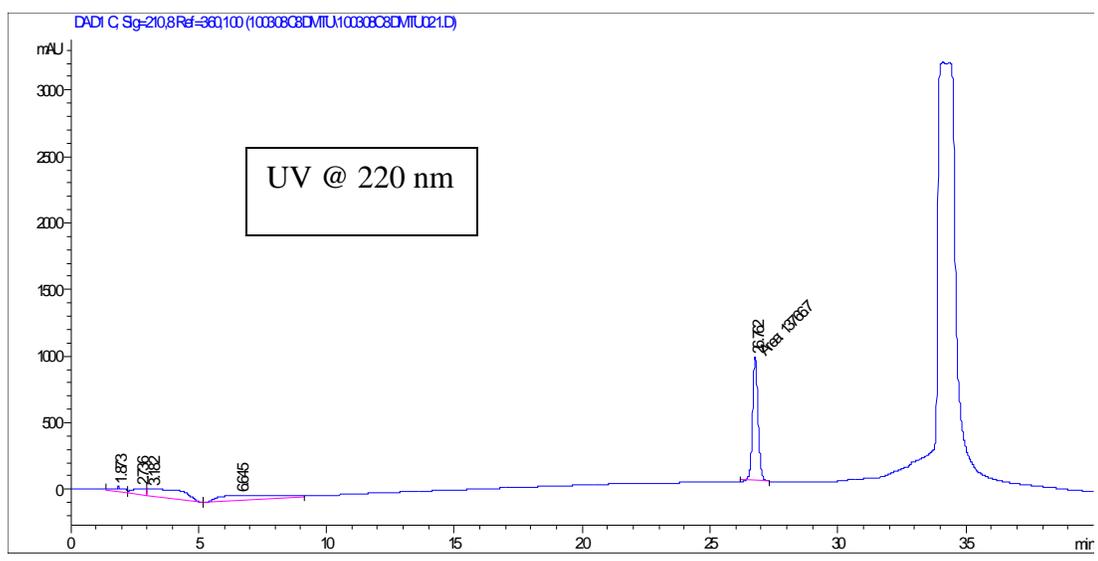
Figure 64. RP gradient retention of triphenylphosphine on silica hydride-based C8 column. It elutes at 26.7 min with Gradient B as shown in Table 7.

DADI C, Sig=210,8 Ref=360,100 (100308C8DMTU.100308C8DMTU.024.D)

UV @ 210 nm

24.064

Area: 16280.9
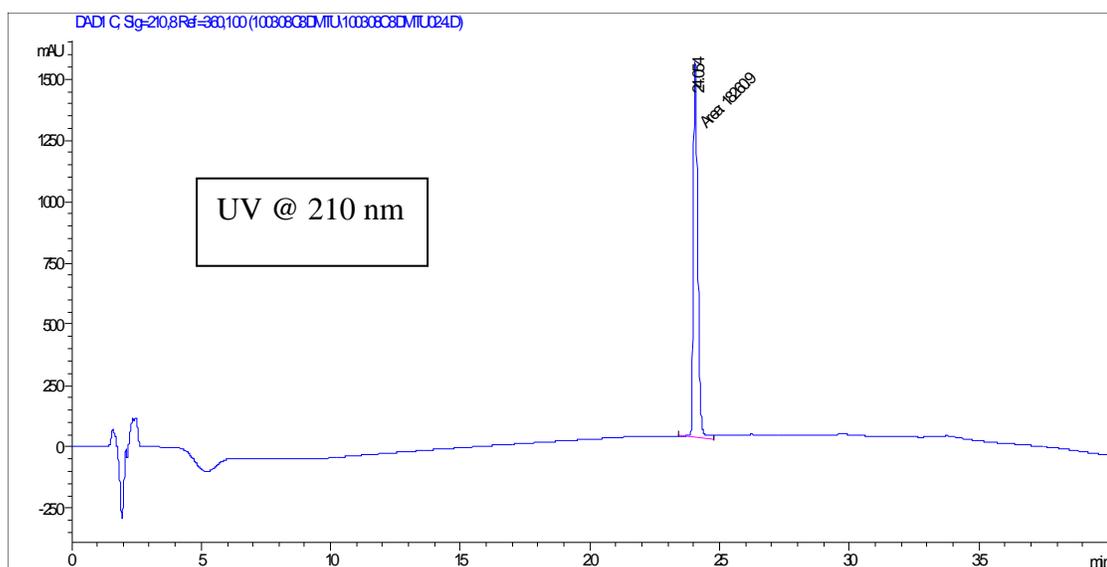
Figure 65. RP gradient retention of raloxifene on the silica hydride-based C8 column. It elutes at 24.06 min with Gradient B as shown in Table 7.
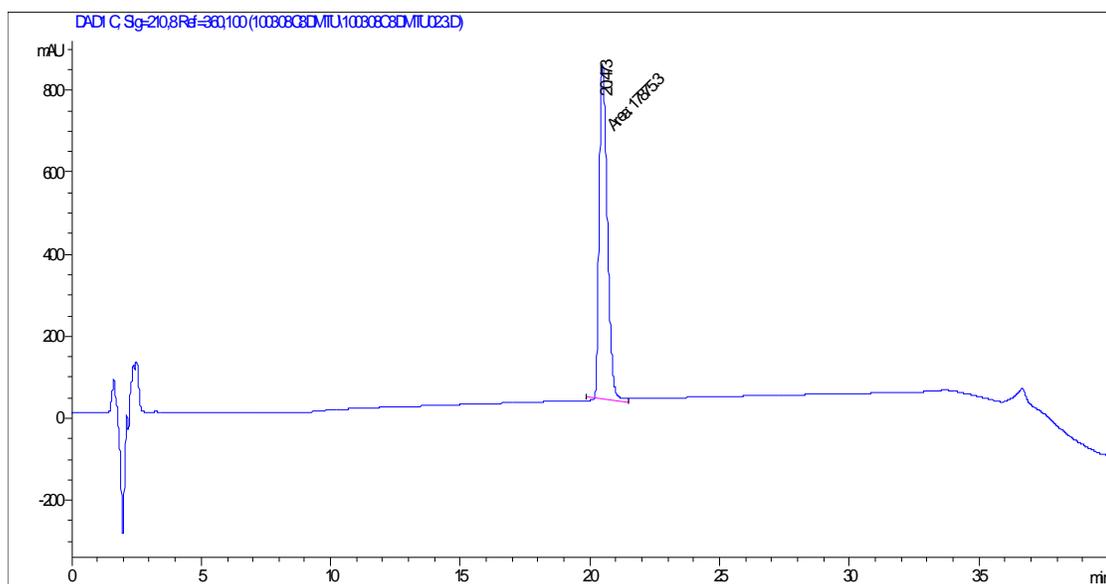
Figure 66. RP gradient retention of raloxifene on silica hydride-based C8 column. It elutes at 20.07 min with Gradient D as shown in Table 7.

Raloxifene and triphenylphosphine being very nonpolar were retained very well on the C8 column. The retention time varied from gradient to gradient depending on the increment of the percent acetonitrile composition in the mobile phase. Triphenylphosphine being very nonpolar does not dissolve in any solvent except cyclohexane. The peak eluting at 35 min in Figure 64 was due to cyclohexane. Gradient B was used as shown in Table 7. Gradient C shown in Table 8 was used for the elution of triphenylphosphine as shown in Figure 63. Lisinopril and niacinic acid were measured using reversed-phase isocratic conditions as shown in Figures 61 and 62. The lisinopril chromatogram has a number of negative peaks that could be from the mobile phase. If the mobile phase has greater absorbance than the compound being analyzed,

then it was possible to observe negative peaks. These negative peaks can be eliminated

by choosing a different mobile phase or by optimizing the gradient conditions. Therefore

more work needs to be done in order to improve the appearance of the chromatogram.
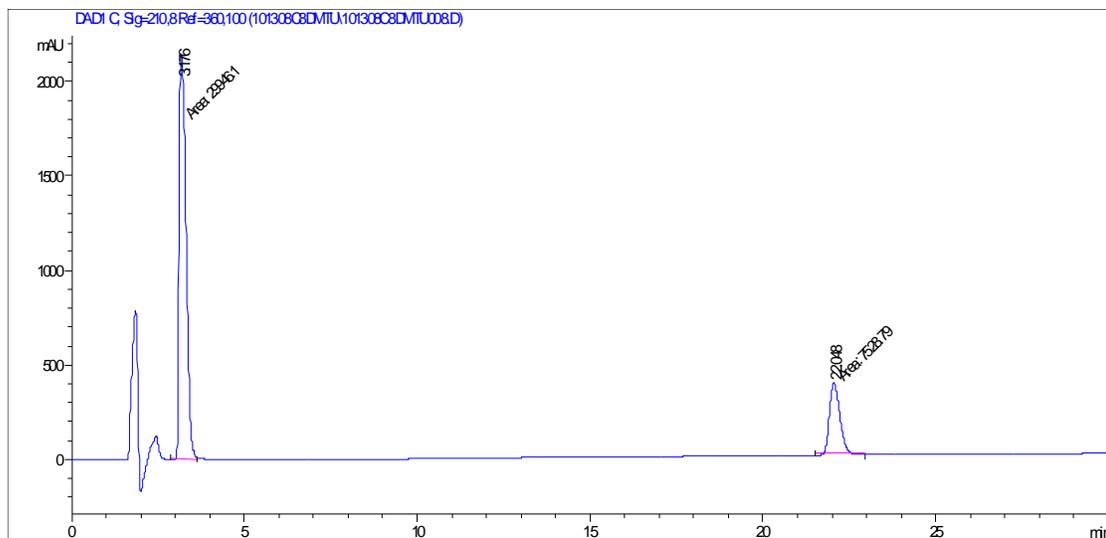
Figure 67. RP gradient separation of lisinopril and raloxifene on silica hydride

based C8 column. Lisinopril elutes at 3.18 min and raloxifene elutes at 22.05 min.

Gradient C used for the separation is shown in Table 8.

A mixture of lisinopril and raloxifene was separated using Gradient C described

in Table 8 under reversed-phase conditions as shown in Figure 67. The mobile phase

flow rate was 0.5 mL/min and UV detection at 220 nm was used. Lisinopril being less

nonpolar compared to raloxifene eluted earlier in the gradient at 3.2 min, and raloxifene

being more nonpolar eluted at 22.05 min. The retention time for each of the compounds

was confirmed by running the single analyte using the same gradient, mobile phase, and
instrument.
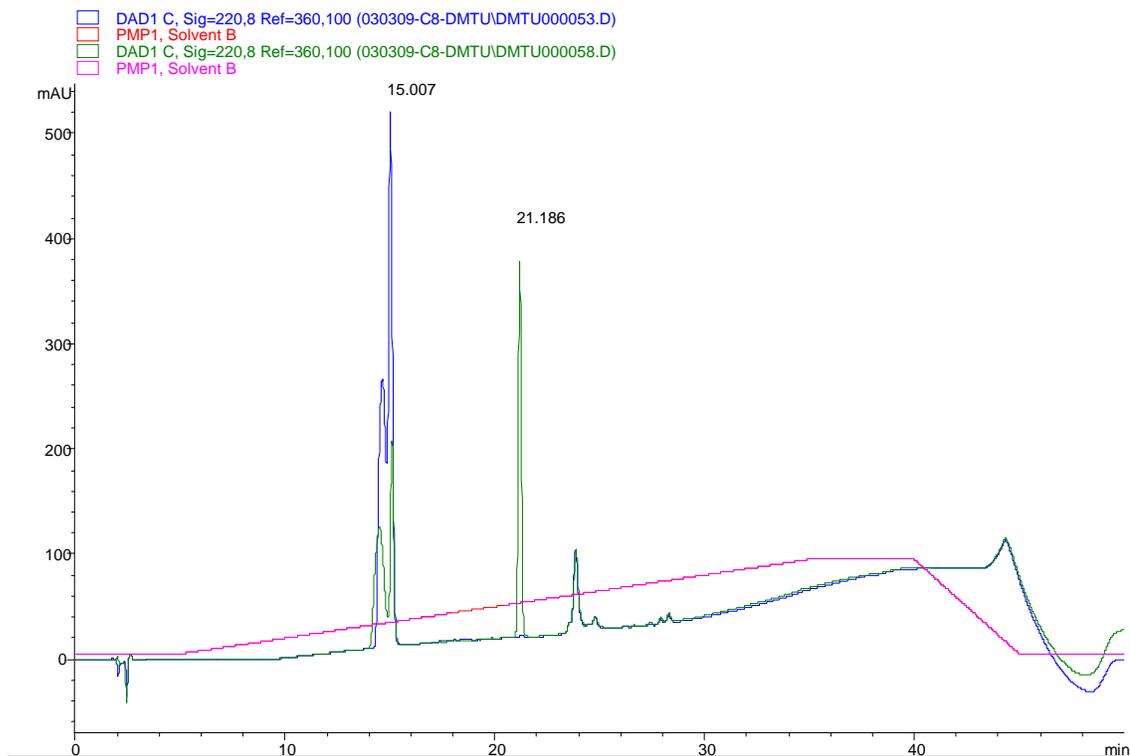
Figure 68. RP gradient separation of lisinopril and lipoic acid on silica hydride
based C8 column. Lisinopril elutes at 15 min and lipoic acid elutes at 21.2 min.
Gradient B used for the separation is shown in Table 8.

The overlay of reversed-phase chromatograms of lisinopril and the mixture of
lisinopril and lipoic acid was as shown in Figure 68. Lisinopril being less nonpolar
compared to lipoic acid eluted earlier at 15 min, while lipoic acid eluted at 21.2 min. The

lisinopril retention time was confirmed by injecting it as a single compound and

overlaying the chromatogram with a mixture of two compounds. Gradient B was used

for the separation and detection was done by UV.

5. Comparison of Endcapped Si-H Column and C8 Column Separation Under RP Conditions

The overlay of the chromatograms of raloxifene on the endcapped silica hydride

column and the C8 column are shown in Figures 69 and 70.
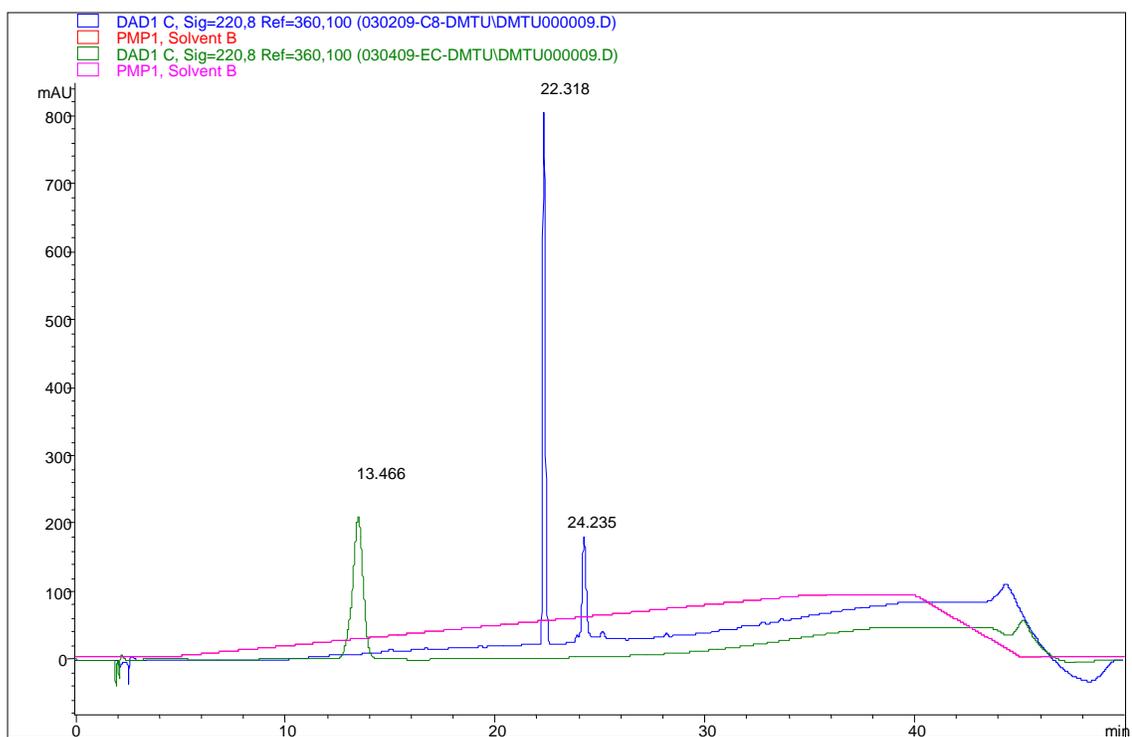
Figure 69. The overlay of RP chromatograms of raloxifene for endcapped Si-H and C8 column. The peak at 13.5 min is on the endcapped Si-H column and the peak at 22.32 min is on the C8 stationary phase. Gradient B used is shown in Table 7.
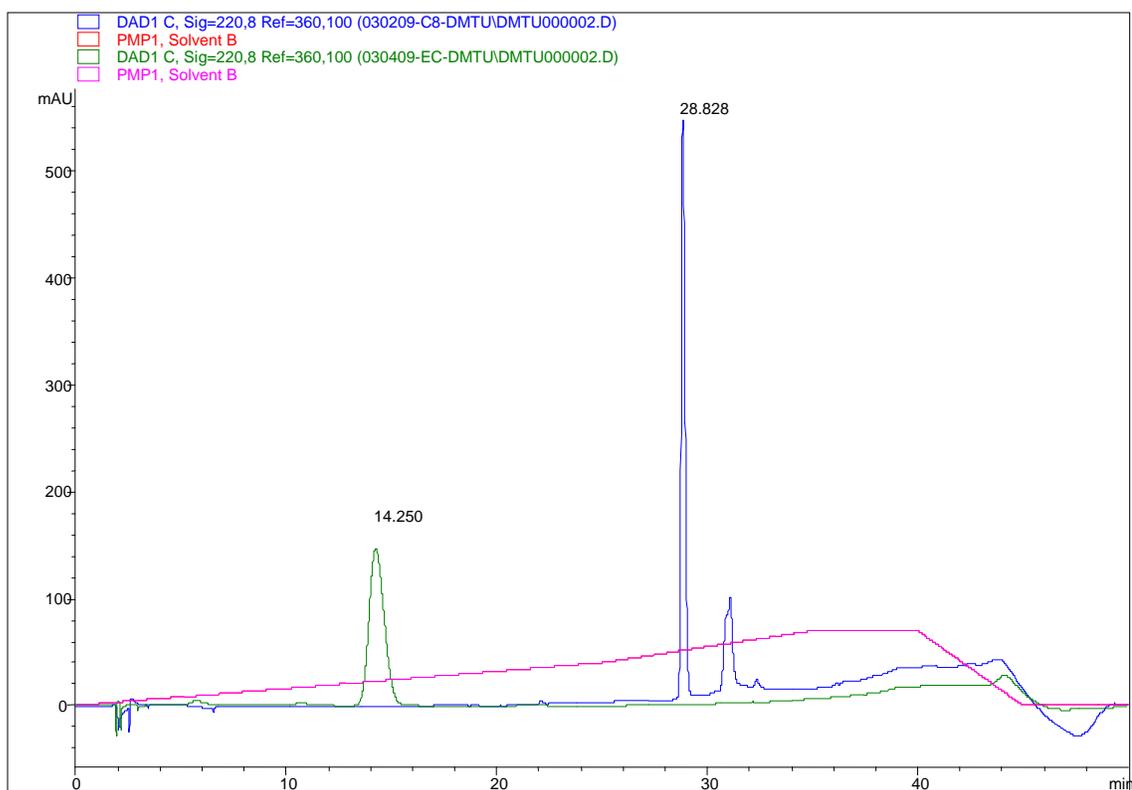
Figure 70. **The overlay of RP chromatograms of raloxifene for endcapped Si-H and C8 column. The peak at 14.3 min is on the endcapped Si-H column and the peak at 29 min is on the C8 stationary phase. Gradient A used is shown in Table 7.**

Raloxifene being nonpolar in nature, so as expected it was retained longer on the C8 column than on the endcapped silica hydride column. Endcapping has definitely increased the hydrophobicity of the silica hydride, because it was able to reasonably retain the nonpolar compound, but to a lesser extent than on the C8 Si-H column. Raloxifene eluted at 13.5 min for the endcapped silica hydride column, and at 22.32 min for the C8 column as shown in Figure 69. The gradient used for the analysis was B as

described in Table 7. Using gradient A as shown in Table 7, raloxifene was retained

longer eluting at 14.3 min for the endcapped Si-H column, and at 29 min for the C8

column as shown in Figure 70.

6. Peak Symmetry Factor

Chromatographic characterization also included an evaluation of the peak

symmetry on the endcapped silica hydride column. The presence of residual silanol

groups after silanization or after endcapping the Si-H stationary phase, sometimes leads

to peak tailing effects. The evaluation of the endcapping process, in regard to an increase

in the efficiency of the silica hydride column separation, was determined by calculating

the peak asymmetry factors of the endcapped silica hydride column. The samples when

injected on the column using the HPLC system undergo a normal dispersion of the

component molecules; this was represented by a bell-shaped Gaussian peak. In an ideal

chromatographic elution the peak shape should be symmetric, but it is not possible to get

symmetric peak shapes, because of tailing caused by unreacted silanol groups. The

unwanted interaction of compounds with the residual silanol groups, causes the

compounds to be retained in the column for longer periods of time, which causes peak

tailing [12]. The peak asymmetry factor is defined as

$$As = b/a$$

Where As is the asymmetry factor with the values a and b determined at 10%

peak height. If the peak is symmetrical then a=b and the asymmetry factor is one or close

to one. As the asymmetry factor approaches one, then the peak symmetry is better. The

determination of peak symmetry is shown in Figure 71 [34].
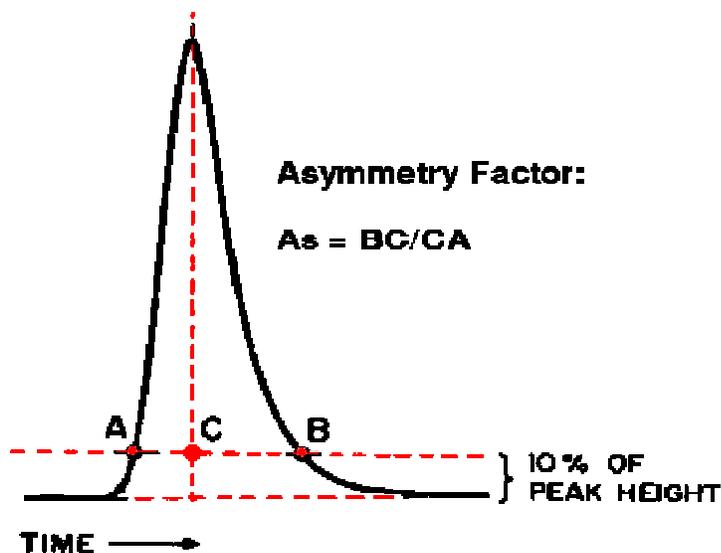
Figure 71. Determination of peak symmetry factor.

Figure 72 shows the plot of peak symmetry factor vs. % acetonitrile for niacinic

acid, citric acid, melamine, and phenylalanine on the endcapped silica hydride column

using the ELSD detector. Figure 73 shows the plot of peak symmetry factor vs. %

acetonitrile for niacinic acid and phenylalanine for the endcapped silica hydride column

using the UV detector. As shown in the figures, the peak symmetry factor is close to one

for most percentages of acetonitrile. This indicates that the column efficiency was indeed

greatly enhanced after the endcapping of the silica hydride stationary phase with

trimethylchlorosilane and hexamethyldisilazane. As shown in Figure 72, melamine has a

symmetry factor close to 5 for a 70% acetronitrile/30% water mobile-phase composition.

Melamine being very basic in nature, could be retained well under reversed-phase

conditions as well as in the ANP mode. For phenylalanine as shown in Figure 73, the

symmetry factor was close to 3 when the composition of the acetonitrile used was 95%,

but other concentrations it was close to one. It appeared to be that to retain phenylalanine

and to get symmetry peak 10% water was required in the mobile phase.
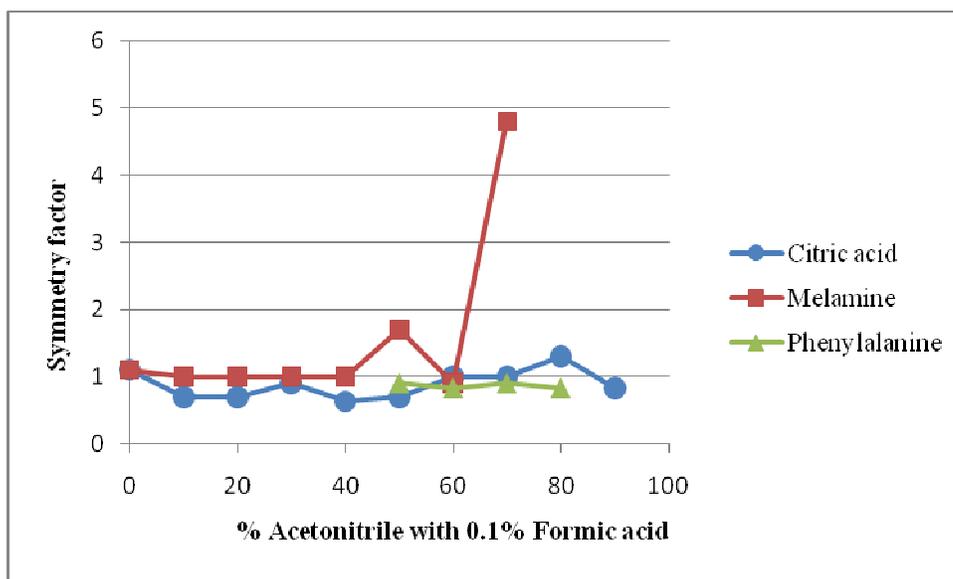
Figure 72. Chromatographic determination of asymmetry factors of compounds melamine, citric acid, and phenylalanine on endcapped silica hydride column using ELSD detection under isocratic conditions.
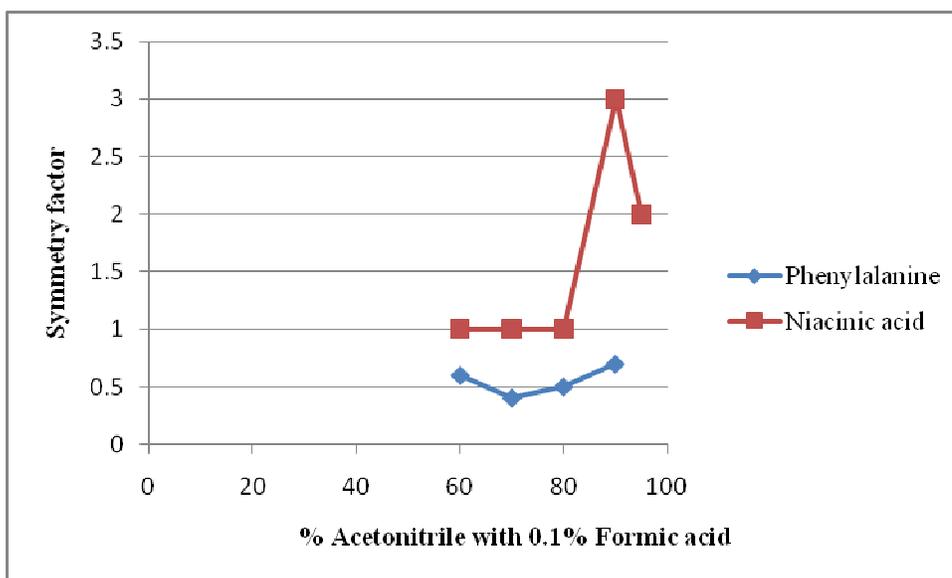
Figure 73. Chromatographic determination of asymmetry factors of the compounds niacinic acid and phenylalanine on endcapped silica hydride column using UV detection under isocratic conditions.

IV.   CONCLUSIONS

For this research a silica hydride stationary phase was synthesized and endcapped with TMCS and HMDS. Spectroscopic characterization by DRIFT confirmed the success of the endcapping process. A Neue test evaluated the level of hydrophobicity to retain nonpolar compounds. The silanophilicity of the endcapped Si-H material confirmed the level of polar/adsorptive interactions with the surface. The hydrophobicity results of endcapped Si-H column were comparable to the C8 Si-H column.

The endcapped Si-H stationary phase showed good aqueous normal phase capabilities as well as some reversed-phase behavior. The results obtained on the endcapped column for the separation of nonpolar compounds were comparable with those from the C8 Si-H column. The endcapped column showed a unique capability to use a wide range of mobile phase compositions starting from 100% aqueous to 100% nonpolar organic solvents. The endcapped column has the capability to rapidly change from reversed-phase to aqueous normal phase. Therefore, it was possible to operate both separation modes simultaneously, which helped in separating both hydrophobic and hydrophilic compounds. The peak symmetry factors calculated for some of the compounds confirmed that the column efficiency was greatly enhanced after the endcapping of the silica hydride stationary phase. Based on the results obtained from this research, it can be concluded that endcapping Si-H material with TMCS and HMDS was successful and showed comparable hydrophobicity with C8.

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