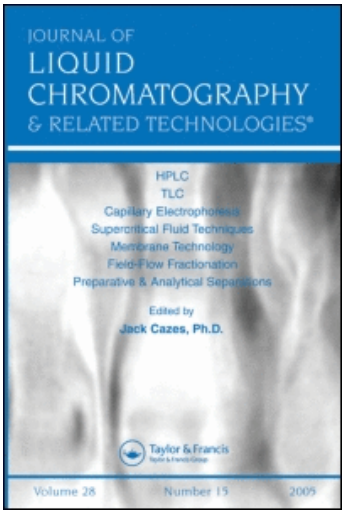

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Use of Hydride-Based Separation Materials for Organic Normal Phase Chromatography

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Abstract: The chromatographic properties of two hydride-based stationary phases (a material with a hydride surface and one with a hydride surface modified by the addition of C_{18}) are tested with several types of compounds that are typically retained in the organic normal phase (ONP) mode. Both phases display ONP retention, but to differing degrees. Various mobile phases are investigated, as well as some gradient elution profiles. Efficiency, peak shape, and reproducibility for the several solutes in both isocratic and gradient elution, as well as the effects of water in the mobile phase, are assessed.

Keywords: Hydride, Normal phase, Chromatography, Separation materials

INTRODUCTION

The organic normal phase (ONP) mode dates to the origins of liquid chromatography and the pioneering experiments of Tswett. Classic normal phase chromatography developed using silica as the stationary phase, but with the advent of chemical modification materials, having organic moieties with polar functional groups such as amino, cyano and diol were also found to be practical for ONP. The mechanism for solute retention by this method is adsorption of the solutes on the polar sites of the stationary phase.[1–5] One of the prime uses of normal phase chromatography is for preparative applications, due to the ease of removing the solvent, in comparison to

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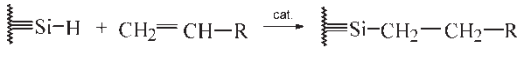
reversed-phase methods. However, reliable and rugged methods for analytical determinations in ONP have often been difficult to develop. Variability in retention, as well as broad and/or asymmetric peaks on silica-based phases for ONP, are often ascribed to the fact that water strongly adheres to the polar surface. This layer controls both the thermodynamics and kinetics of exchange between the bulk mobile phase and the site of interaction on the stationary phase.[6] Thus, water is often added to the mobile phase at low concentrations in order to, at least partially, mitigate these effects.[7]

A new type of stationary phase has been developed over the last decade that has been fabricated on silica, but resulting in very few silanols on the surface. This material is referred to as silica hydride where Si-H moieties have replaced approximately 95% of the Si-OH groups on the surface[8,9] when compared to the original unmodified silica. One approach for creating a hydride surface and then modifying it with different organic groups is silanization/hydrosilation.[10–12] The hydride surface is put in place through the silanization process and, then, the second step (hydrosilation) attaches the desired organic functionality for the stationary phase. When alkenes are used in the hydrosilation reaction, a monodentate bonded phase is formed.



cat = catalyst, typically hexachloroplatinic acid or free radical initiator

Double attachment of the bonded group occurs when an alkyne is used as the organic moiety in the hydrosilation reaction.[13,14] The underlying structure of the surface is different because the unreacted sites on the surface are Si-H rather than the silanols that are present on stationary phases fabricated with ordinary silica. The presence of silicon hydrides, as opposed to the silanols, leads to different and desirable properties for HPLC separation materials.[8,9,15] A recent study[16] exploring the separation capabilities of hydride-based stationary phases demonstrated that these materials can function in the ONP mode. This investigation provides further documentation that both stationary phases having only a hydride surface, as well as those further modified by the hydrosilation, possess useful properties for normal phase applications.

EXPERIMENTAL

Materials

The hydride-based columns (4.6 × 75 mm) used for the study (Bidentate C18 (BD C_{18}) and Silica-C) were obtained from MicroSolv Technology Corporation (Eatontown, NJ). Two other columns, a Zorbax silica (4.6 × 150 mm)

and an Eclipse XDB-C_{18} (4.6 × 150 mm), were used for comparison tests with the hydride-based materials. The phenols used for normal phase evaluation were provided by Symyx Technologies (Santa Clara, CA). The other test solutes were obtained from Sigma-Aldrich (Milwaukee, WI). Solvents used for the chromatographic runs (for preparing sample stock solutions, mobile phases, wash solvents) were hexane, ethyl acetate, methylene chloride, diethyl ether, tetrahydrofuran, and methanol and were purchased in the highest purity available.

Instrumentation

Chromatographic runs were performed with a Waters LC system interfaced with a Micromass ZQ detector. The LC component consisted of two Waters pumps, an LC 515 used to deliver methanol to the interface and an LC 600 controller used to deliver mobile phases to the column. A Waters 2996 photo diode array, the primary detector, was interfaced with a Micromass ZQ, a quadrupole mass analyzer with a dynolite photomultiplier tube serving as the second detector. The mass spectra were obtained using the atmospheric pressure chemical ionization (APCI) mode.

Sample Preparation

Stock solutions of all the samples were made by dissolving approximately 20 mg of each compound in 20 mL of 5% ethyl acetate in hexane. These solutions were filtered using 0.2 micron syringe filters prior to use.

Chromatographic Procedures

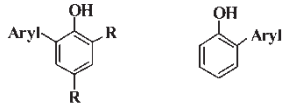
Chromatographic separations were performed using the following mobile phase compositions: 3%, 4%, 5%, and 10% ethyl acetate (EtOAc) in hexane; 100% hexane; 10% and 20% methylene chloride (CH_2Cl_2) in hexane; 5% and 10% diethyl ether ($(C_2H_5)_2O$) in hexane; and 5% and 10% tetrahydrofuran (THF) in hexane, in order to evaluate phenol retention. The mobile phase flow-rate used was 1 mL/min. The sample injection volume was set at 10 μL and the concentration used was 1 mg/mL for each run. For gradient analyses, the optimum conditions are described in the appropriate figures.

RESULTS AND DISCUSSION

Evaluation of Phenol Compounds

Several mixtures of substituted phenols were evaluated on two hydride-based columns (silica hydride and bidentate C_{18}), as well as in some cases, on

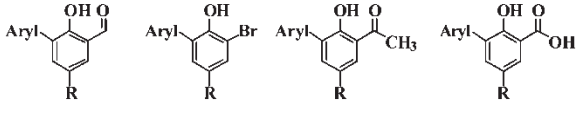
ordinary commercial silica and C_{18} columns. The first test mixture consisted of two components as shown below:



Compound A MW 324 Compound B MW 228

Figure 1 shows the chromatograms obtained with various columns under some representative mobile phase conditions. Figure 1A is the data for a commercial silica column that is typically used in normal phase applications. As can be seen, only the mobile phase of 5% ethyl acetate in hexane gives acceptable separation with no peak asymmetry or distortion. Figure 1B involves testing the same two compounds with a typical commercial C_{18} material generally used in reversed-phase applications. As might be expect under normal phase conditions, this column provides little (only in 100% hexane is there partial separation) or no resolution of the two compounds. These results can be compared to columns based on silica hydride instead of ordinary silica, such as the two tested above. For the case of the unmodified silica hydride, the results under the same conditions are shown in Figure 1C. In all four tests, the two compounds are baseline resolved with generally good peak shape (there is some asymmetry for the most retained peak in 100% hexane). The longest retention is obtained with 100% hexane, as expected and, when comparing two modifier concentrations, shorter retention is found for the case with the highest amount of modifier in the mobile phase (10% CH_2Cl_2). Perhaps the most surprising result is presented in Figure 1D, showing the chromatogram of these two compounds on a hydride-based C_{18} phase under these normal phase conditions. For each mobile phase composition tested, the hydride–based C_{18} displays retention and resolution of the two compounds, in contrast to the ordinary silica-based octadecyl phase, where very little, if any, retention for these solutes is obtained.

A second test mixture contained the following four phenol compounds, with each having an additional functional group or halide as shown below:



Compound C with an aldehyde MW 255 Compound D MW 407 Compound E with a ketone MW 277 Compound F with an acid MW 321

The presence of these additional groups should lead to stronger retention in the organic normal phase. The chromatograms for these four compounds at various mobile phase compositions, using a commercial silica column, are shown in Figure 2A. As can be seen, these compounds are retained longer than the first

test group under comparable mobile phase conditions. Compound F, the phenol that has a carboxylic acid group, is the most strongly retained solute, eluting after 10 minutes under each of the mobile phase compositions tested. In most cases, the peaks display reasonable symmetry, but resolution of all components is not seen for every mobile phase. For the C_{18} stationary phase based on ordinary silica, the four components are not resolved under any mobile phase

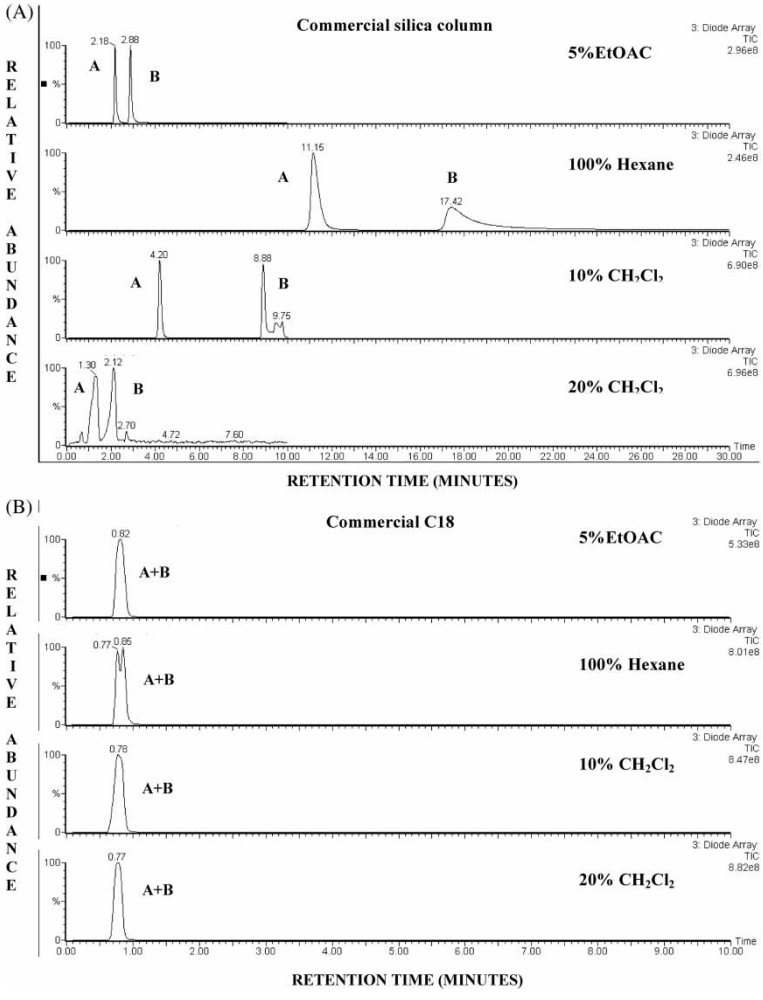


Figure 1. Chromatograms of compounds A and B on: A) a commercial (Zorbax) silica column; B) a commercial Eclipse XDB-C_{18} column; C) silica-C (hydride) column; and D) a Bidentate C_{18} (hydride) column. Mobile phases, from top to bottom: 5% EtOAc in hexane, 100% hexane, 10% and 20% CH_2Cl_2 in hexane. Flow rate 1 mL/min. Detection by mass spectroscopy in the APCI+ mode.

(*continued*)

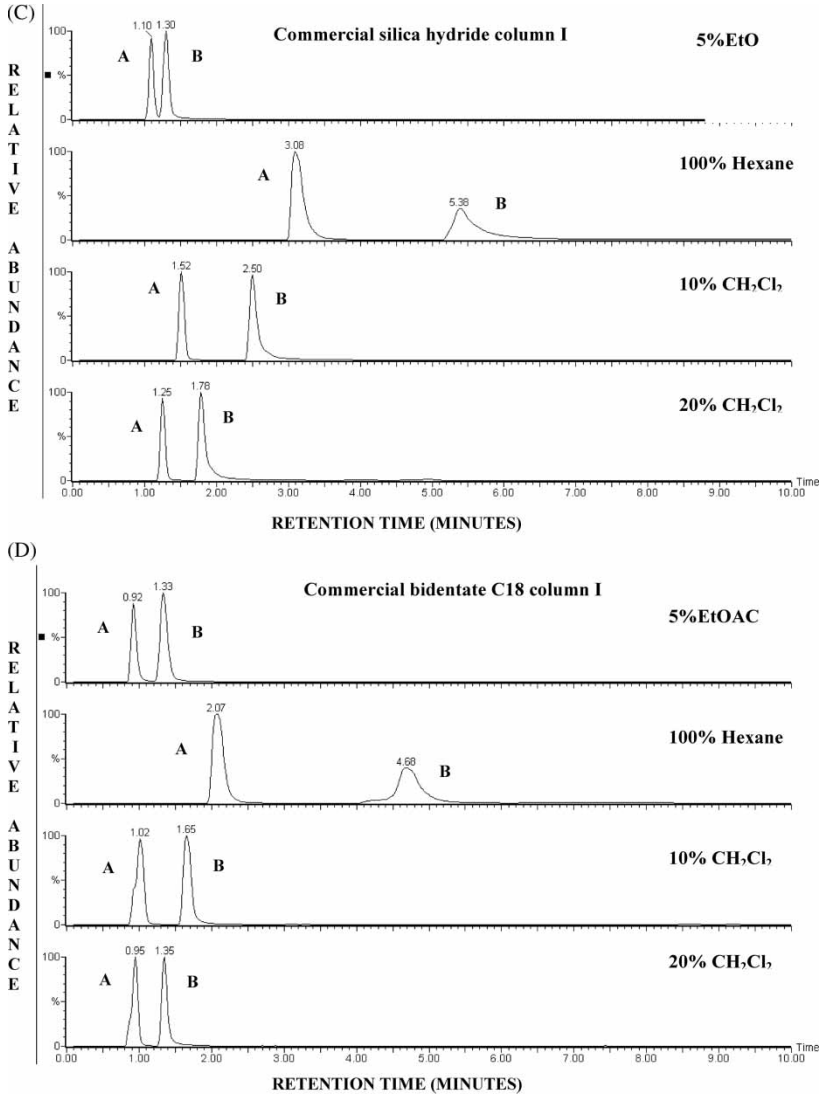


Figure 1. Continued

conditions. Two examples are shown in Figure 2B. This result is consistent with the first test mixture, where no significant resolution of the two components was obtained using either hexane or hexane-modified mobile phases. The chromatograms for the second test mixture on a bare silica hydride stationary phase are shown in Figure 2C. Under three of the four solvent conditions presented, Compounds C, D, and E are resolved and, in all cases, efficiency and peak symmetry are good. In two instances, the retention of Compound F is

reduced significantly and appears in the time frame of the chromatograms. However, with 10% THF as the modifier, the peak is quite broad. For three of the mobile phase compositions, an additional peak (impurity) can also be detected. The chromatograms for the same mixture on the hydride-based BD C_{18} column are shown in Figure 2D. In each case, Compound F is retained

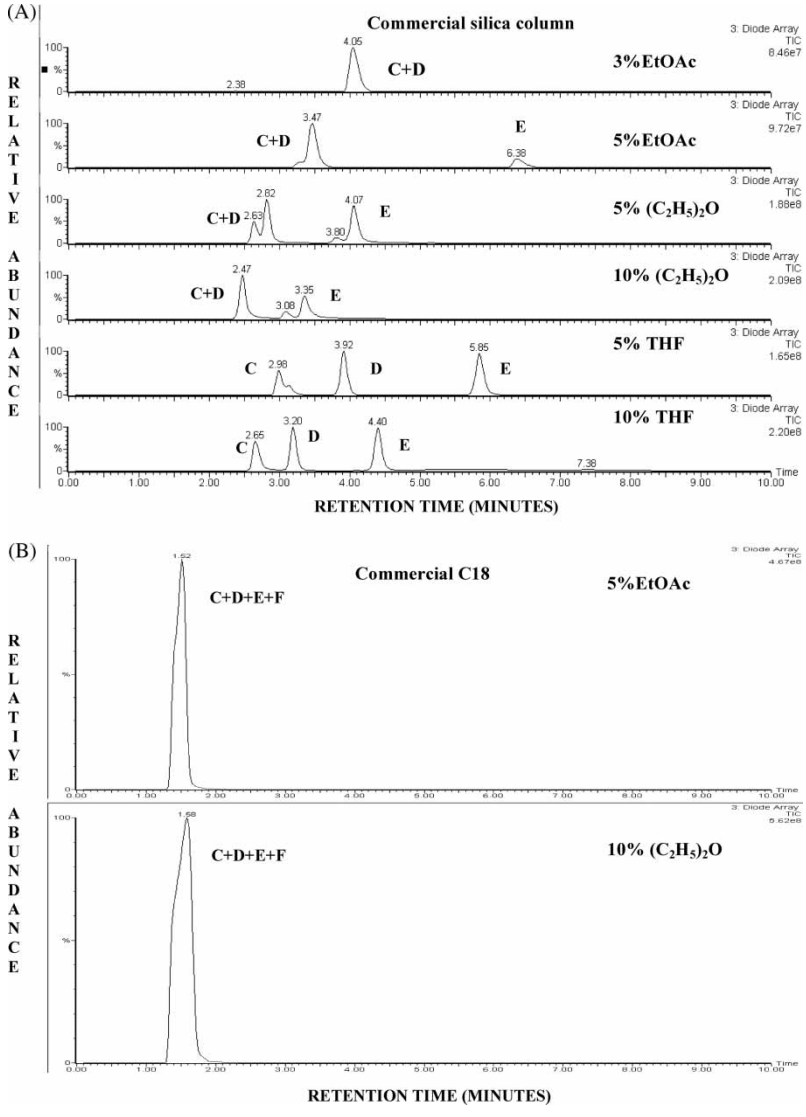


Figure 2. Chromatograms of compounds C, D, E, and F on: A) a commercial (Zorbax) silica column; B) a commercial Eclipse XDB-C_{18} column; C) Silica-C (hydride) column; and D) a bidentate C_{18} (hydride) column. Other conditions, same as Fig. 1.

(*continued*)

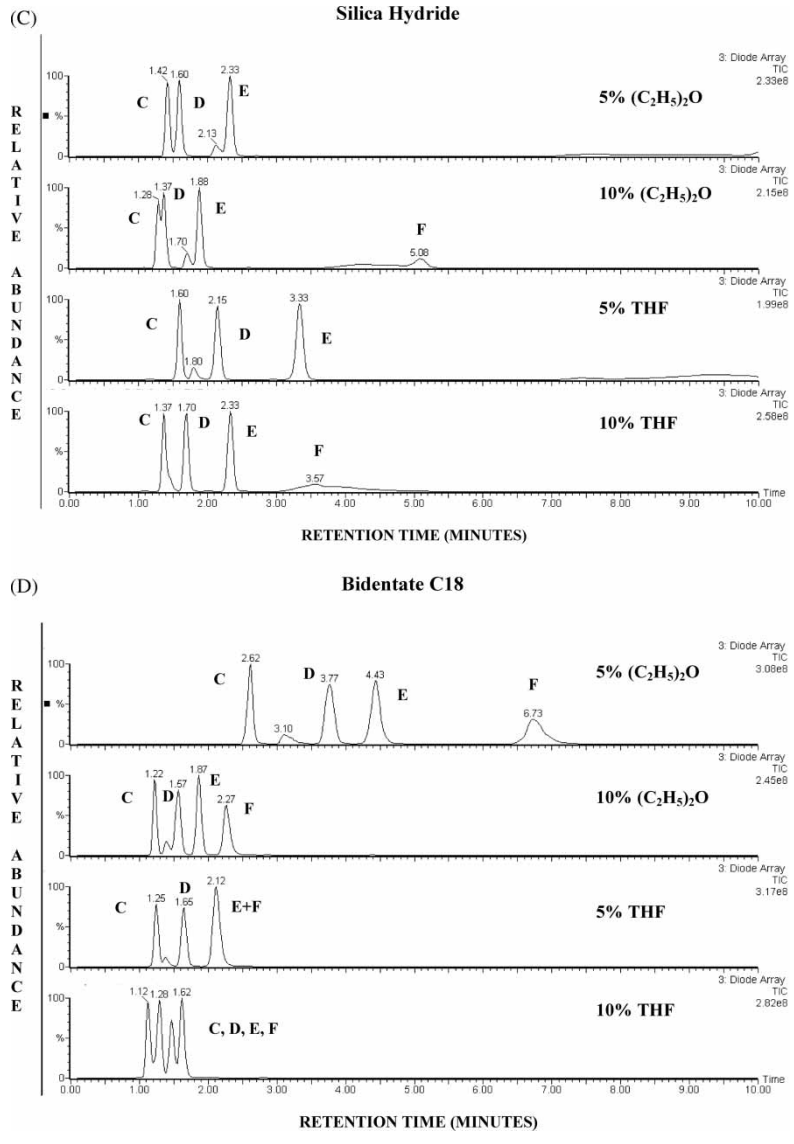


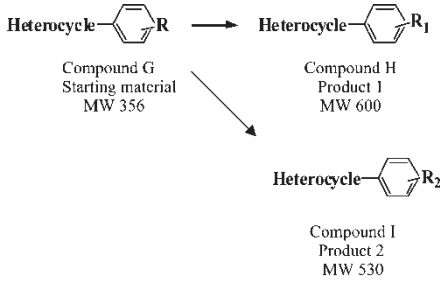
Figure 2. Continued

significantly less than on either the bare silica or bare silica hydride, indicating that the presence of the C_{18} moiety on the surface diminishes the strong polar interactions it has with the surface interaction site. In contrast to the C_{18} on ordinary silica, where no retention of these compounds is observed, the hydride-based C_{18} retains some normal phase retention capabilities. It should be pointed out that the surface coverage of this material is greater than

3.0 μmol/m^2 and is very similar to the standard C_{18} phase tested in Figure 2B. The impurity identified on the hydride-only column (Fig. 2C), as well as with some of the conditions for the standard silica column (Fig. 2A), is also observed with three of the mobile phases shown in Fig. 2D. Of the mobile phases tested, the 10% diethyl ether in hexane on the bidentate C_{18} appears to give the optimum conditions with the shortest analysis time, resolution of all components, and good efficiency and peak symmetry.

Evaluation of Heterocyclic-Aryl Compounds

Two reaction products that resulted from the same starting material were also tested on the hydride-based columns to further define their properties in the normal phase. These test mixtures can be defined as follows:



Due to the basically hydrophobic nature of these compounds, relatively little retention or, in some cases, minimal retention with distorted peak shapes were obtained on the BD C_{18} column for a variety of organic normal phase solvent conditions. However, this was not the case for the stationary phase with an unmodified hydride surface. For some mobile phase conditions, both separation and good peak characteristics were obtained. An example of the separation achieved for Compounds G and H on the silica hydride column is shown in Figure 3A, consisting of the chromatograms of the individual solutes and the resulting chromatogram of the mixture with the 95:5 hexane/ethyl acetate mobile phase. Baseline resolution of the two compounds can also be achieved with 90:10 hexane/CH_2Cl_2 and 90:10 hexane/THF. An example of the separation of Compounds G and I on an unmodified silica hydride column using a 90:10 hexane/THF mobile phase is shown in Figure 3B. In addition to the two primary peaks, at least one additional compound (impurity or reaction by-product) can also be seen in the chromatograms. Baseline resolution of the two compounds and the presence of the third peak can be achieved with a 95:5 hexane/ethyl acetate mobile phase. Baseline resolution of the two primary compounds, but without the presence of the third peak, is found with a mobile phase consisting of 90:10 hexane/CH_2Cl_2.

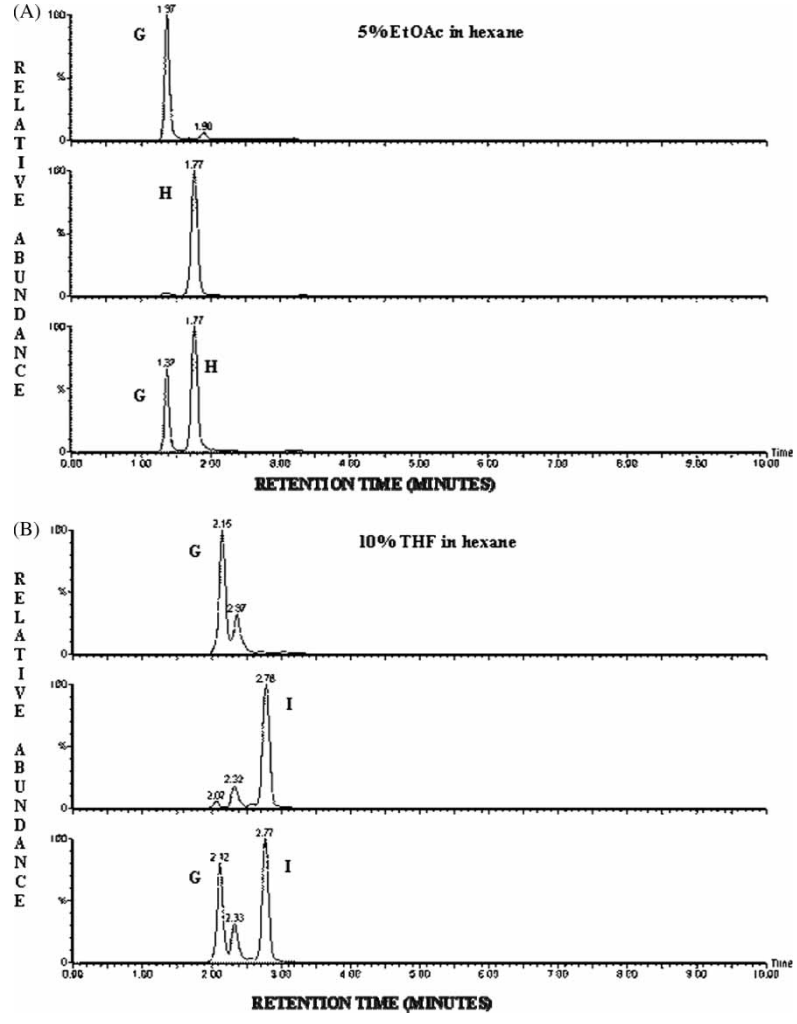
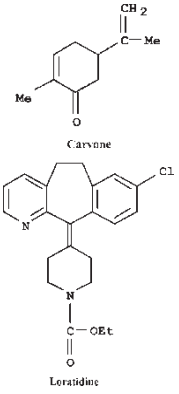


Figure 3. A) Separation of compounds G and H on the Silica-C (hydride) column using a mobile phase of 5% EtOAc in hexane. B) separation of compounds G and I on Silica-C (hydride) column using a mobile phase of 10% THF in hexane. Other conditions, same as Figure 1.

Evaluation of Carvone and Loratidine

A few additional experiments, using a mixture of carvone and loratidine, were conducted in order to gain further insight into the retention capabilities of hydride-based phases. Figure 4A shows the result of injecting carvone onto the Silica C column with a mobile phase of 100% methylene chloride.



The compound is eluted in a reasonable time (7.21 min) with good peak shape. However, under these conditions, loratidine is very strongly retained; its elution time is >30 min. In order to achieve separation of these two compounds in a reasonable time frame, a gradient must be used. An optimized gradient that elutes these two compounds on this stationary phase is shown in Fig. 4B. The initial mobile phase conditions involve adding 5%

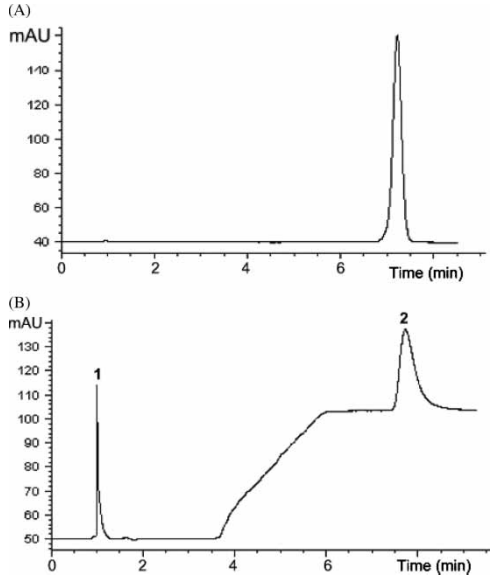


Figure 4. A) Retention of carvone on Silica-C with a 100% methylene chloride mobile phase. Detection at 255 nm B) Separation of carvone (**1**) and loratidine (**2**) on a Silica-C column with a gradient. Gradient: 0–2.0 min 95:5 methylene chloride/ethyl acetate; 2.0–4.0 min to 100% ethyl acetate; 4.0–10.0 min 100% ethyl acetate; 10.0–10.1 min to 95:5 methylene chloride/ethyl acetate. Detection at 272 nm. Flow rate in both chromatograms is 1 mL/min.

ethyl acetate, which considerably shortens the elution of carvone from the previous example (Fig. 4A), resulting in a k′ of approximately one. In order to elute the loratidine in a reasonable time, the gradient goes to 100% ethyl acetate very rapidly (2 min) This produces a desirable result for UV detection, as shown in the figure. The loratidine peak appears at a point where the baseline is flat after completion of the gradient. The peak shape for the loratidine is good and, under these conditions, both compounds could be determined quantitatively, or such a gradient profile could be used in a

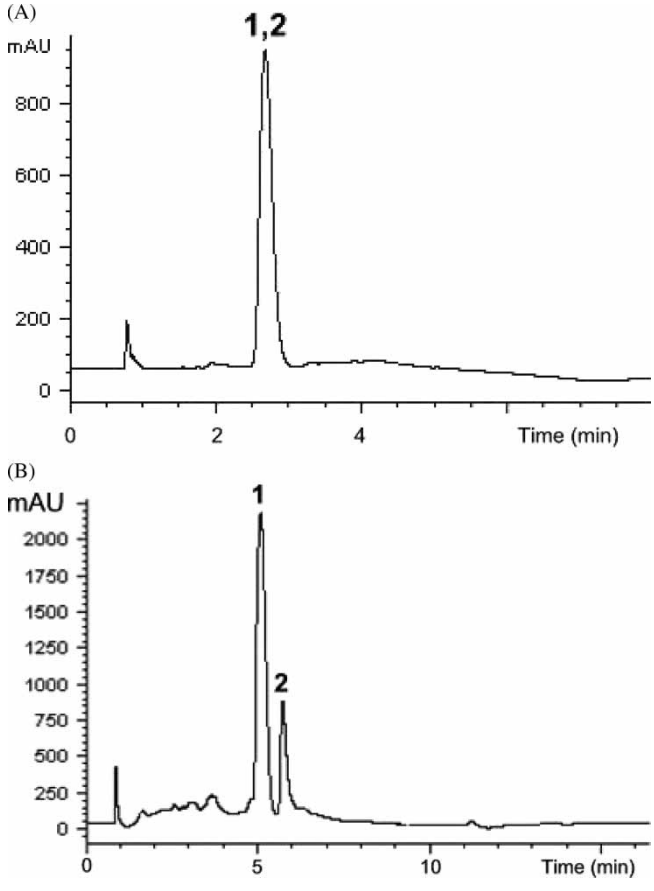


Figure 5. Chromatograms for gradient elution of a mixture of carvone (**1**) and loratidine (**2**) on a BD C_{18} column. A) Gradient: 0–1.0 min 80:20 hexane/dichloromethane; 1.0–7.0 min to 40:60 hexane/dichloromethane; 7.0–10.0 min 40:60 hexane/dichloromethane; 10.0–10.1 min to 80:20 hexane/dichloromethane. B) Gradient: 0–0.1 min 100% hexane; 0.1–7.0 min to 40:60 hexane/dichloromethane; 7.0–10.0 min 40:60 hexane/dichloromethane; 10.0–10.1 min to 100% hexane. Flow rate 1.0 mL/min. Detection at 255 nm.

preparative application. The reproducibility of retention ($n = 5$) for the two compounds is a $\Delta t_R < 1.5\%$.

In order to diminish the strong organic normal phase retention of loratidine on the silica hydride material, and to simplify its separation from carvone, the stationary phase was changed to BD C_{18}. Figure 5A shows the chromatogram obtained when the strong solvent initially comprises 20% of the mobile phase. The two solutes co-elute under these conditions and confirm that the BD C_{18} column has weaker ONP retention than the Silica-C stationary phase. In order to separate carvone and loratidine on BD C_{18}, it is necessary to increase the amount of hexane at the start of the gradient. When the amount of hexane is increased from 80% to 100% and the gradient modified slightly, the two compounds are separated as shown in Figure 5B. Both peak shape and efficiency are good at significant solute concentration levels ($\sim$1 mg/mL). Reproducibility is comparable to that obtained for the gradient used on the Silica-C column.

In all cases, water is not added to a particular level in the mobile phases and, during operation, no special precautions are taken to prevent atmospheric contact with the solvents other than degassing. Thus, in contrast to many ONP separations with other stationary phases, water does not have a significant impact on retention and reproducibility when using hydride-based materials. This property could make the development of normal phase methods simpler and more transferrable than is currently possible.

CONCLUSIONS

Silica hydride-based materials appear to offer a viable alternative to silica, as well as amino, cyano, and diol modified silicas as stationary phases for use in organic normal phase chromatography. Good ONP retention has been observed for several different types of compounds on at least two phases, with good reproducibility and peak shape. Gradient analyses are also possible with good reproducibility. The insensitivity to the presence of trace amounts of water in the mobile phase is another favorable aspect of the hydride-based stationary phases in contrast to most other materials currently in use.

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