Researchers have designed a new polar-linked, reversed-phase column for separating polar, ionizable, and especially highly basic compounds with excellent column efficiency and peak shapes. The column combines an ultra-pure, low-acidity porous silica support with a unique stationary phase that has a polar, embedded amide group linking the sterically protecting disopropyl groups with a C14 n-alkyl functionality. This new column demonstrates stability in low-pH mobile phases because of its resistance to hydrolysis and stationary-phase loss. Dense triple-endcapping minimizes unwanted silanol interactions and allows excellent peak shapes and stability at intermediate pH. The column can be used with totally aqueous mobile phases without the phase collapse typical of alkyl stationary phases.

We performed the diisopropyl-C14-amide bonding and triple-endcapping procedures for separating polar compounds. We have developed a new silica-based, embedded polar group stationary phase that uses sterically protecting groups. This structure greatly enhances the stability of the bonded phase in low-pH applications by significantly decreasing the loss of stationary phase through hydrolysis (9,13,14). To enhance performance and stability in intermediate-pH applications, this stationary phase is densely triple-endcapped. This feature results in a packing material that resists dissolution of the silica support, which is the degradation process usually responsible for failure of bonded-phase columns in intermediate- and high-pH applications (11,15). This new packing material is based on Hewlett-Packard’s Zorbax Rx-Sil ultrapure, low-acidity Type B porous silica support (Wilmington, Delaware), which forms the basis for a large family of widely used, high performance reversed-phase columns (16). The combination of this silica support with the new alkyl-amide stationary phase results in a robust column packing that exhibits superior characteristics for separating difficult polar and ionizable compounds.

**EXPERIMENTAL**

**Apparatus and reagents:** We performed chromatographic tests using HP 1050, HP 1090, and HP 1100 chromatographs (Hewlett-Packard). We calculated plate numbers using the half-peak-height method described by equation 2.8a in reference 17. Peak tailing values were determined at 5% of the peak height using the conventional approach (17). Samples were injected with a Rheodyne model 7125 sampling valve (Rohnert Park, California).

The 15 cm × 0.46 cm columns were prepared at the Hewlett-Packard Newport Site using a conventional slurry-packing method (18). The porous silica support used in this work is a Type B silica formed by aggregating ultrapure silica sols (16). Type B silicas are the newer highly purified, less acidic chromatographic supports that provide superior separations, especially for ionizable compounds (19). The physical and surface properties of this silica have been reported elsewhere (16).

We performed the disopropyl-C14-amide (alkyl-amide) polar-linked stationary-phase bonding and triple-endcapping procedures...
Stationary-phase characteristics: Figure 1 depicts the sterically protected alkyl–amide stationary phase of the new column. The 10% carbon content for this packing typically were 10% for this support. The other experimental columns used in this study also were prepared at the Newport site. Columns of the endcapped alkyl–amide packing are now available from Hewlett-Packard under the trade name, Zorbax Bonus-RP.

We obtained HPLC-grade solvents from EM Science (Gibbstown, New Jersey). Test solutes from Sigma Chemical Co. (St. Louis, Missouri) were used as received.

RESULTS AND DISCUSSION
Low-pH studies: Previous studies with sterically protecting bulky groups on the silane silicon atom showed that stationary-phase stability at low pH is greatly enhanced by the much-decreased hydrolysis rate of the silane-attaching siloxane bond (9,13,14). This characteristic also is found for the alkyl–amide packing of this study, because the diisopropyl groups of the proposed structure of Figure 1 afford the usual steric protection against low-pH hydrolysis.

Figure 3 shows that the rate of change for the alkyl–amide column when continuously purged with a methanol–0.1% trifluoroacetic acid mobile phase (approximately pH 1.9) at 60 °C is much less than that for a nonsterically protected double-endcapped dimethyl–C18–amide column prepared with the same silica support and by a comparable reaction. In Figure 3a, the diisopropyl–C14–amide column shows only a slight increase in retention factor (k) for the basic drug separations obtained with an HP model 1090 chromatograph with UV detection at 254 nm. The mobile phases were methanol–10 mM sodium citrate buffers. We chose the citrate buffer because of its wide buffering range (pH 2.1–6.4). We collected and analyzed data using HP ChemStation software (Hewlett-Packard). The captions for each figure provide the experimental details for each separation shown in this study.

We measured diffuse reflectance infrared spectra using a Nicolet Avatar model 380 FTIR spectrometer (Madison, Wisconsin). We made no special attempt to maintain the dryness of the samples used in these tests.

Responsive FTIR spectra of alkyl–amide packing (solid line) and starting silica (dashed line).

Figure 1: Structure of the diisopropyl–alkyl–amide stationary phase.
amitriptyline, a strongly basic drug (pK_a ~ 9.5). Retention values increase steeply with aging for the comparable dimethyl–C18–amide column. Presumably, the k value increase is the result of a loss in silane stationary phase by hydrolysis with subsequent formation of silanol groups that bind the basic drug. Values of toluene k for the dimethyl–C18–amide column show a decrease of approximately 40% for the period of testing. Figure 3b shows that the amitriptyline plate height for the diisopropyl–C14–amide column is essentially unchanged (within experimental error) after purging with approximately 37,000 column volumes, when the test arbitrarily was terminated. This result equates to almost four months of 8-h/day operation. In contrast, the comparable nonsterically protected dimethyl–C18–amide column showed significant degradation in column efficiency for both amitriptyline and toluene after purging with approximately 2000 column volumes under these conditions.

Low-pH separations with the diisopropyl–C14–amide column produce excellent peak shapes and column efficiencies for both acidic and basic compounds. Figure 4 shows the separation of a mixture of acidic fruit acids of interest to the food industry using a methanol–trifluoroacetic acid mobile phase. This column also exhibits useful characteristics for basic compounds, as illustrated in Figure 5 for the separation of basic cardiac drugs using a methanol–sodium dihydrogen phosphate (pH 3.0) mobile phase. In both cases, column efficiency and peak shapes generally are superior to those found for columns with simple alkyl stationary phases (for example, C18).

Intermediate-pH studies: Columns with polar-linked groups often are used in the pH 4–8 range for separating polar or ionizable compounds. Under some separating conditions, particularly at intermediate pH, columns with conventional alkyl-bonded phases can show less than desirable peak shapes and column efficiencies for some compounds. In these instances, O’Gara and co-workers (2) speculated that the superior performance of polar-linked group columns may be the result of the association of the embedded polar groups with unreacted silanol groups on the surface of the silica support. An alternative postulation is that the linked-polar group reduces the hydrophobicity of the stationary phase near the silica support surface, allowing water molecules to approach and deactivate unreacted silanol groups. These effects would minimize unwanted interactions that cause poorer column performance, and they would result in better peak shapes and column efficiency for polar and ionizable compounds.

Compared with conventional alkyl-bonded-phase columns, the alkyl–amide column of our study usually produces superior peak shapes and column efficiencies for difficult ionizable compounds when operated with intermediate-pH mobile phases. Figure 6 shows the separation of a mixture of highly basic drugs on our alkyl–amide column, an endcapped alkyl–C18 column, and a nonendcapped alkyl–C8 column, all of which were prepared with the same silica support. Solute retention is lower on the alkyl–amide column, and the peak shapes and column efficiency clearly are superior under the same pH 6 conditions.

Figure 7 illustrates the improved performance of the alkyl–amide stationary phase compared with conventional alkyl phases. In this figure the separation of the strongly basic drug amitriptyline (pK_a = 9.4) is performed on three columns — an endcapped alkyl–amide column, an endcapped dimethyl–C18 column, and a nonendcapped C8 column — at pH 5. At this pH, amitriptyline should be largely protonated. All columns were prepared with the same silica support. Two important features arise from this comparison. First, the alkyl–amide column is less retentive, as in Figure 6, presumably because of the reduced hydrophobicity caused by the polar group in the stationary phase. However, another reason may be the greater interaction of the solute with surface silanol groups for the two pure alkyl columns. The second feature of this comparison is the broader, tailing peaks for the alkyl columns; the nonendcapped column shows the largest effect. Clearly, the alkyl–amide column provides superior performance under these separation conditions.

Figure 8 shows the greatly reduced interaction of surface silanols when using the alkyl–amide column for protonated or partially protonated basic compounds at intermediate pH. Peak-tailing factors for a series of basic compounds remain essentially constant throughout...
the pH 3–6 range for a methanol–citric acid buffer mobile phase. In contrast, peak-tailing values for an endcapped C18 column and a nonendcapped C8 column generally are higher and increase with pH increases. These results suggest less interaction of the alkyl–amide column for these protonated basic compounds with increasingly ionized silanol groups as the pH increases. We found that the alkyl–amide column produced better peak shapes and column efficiencies for polar ionizable compounds in the intermediate pH range where ionizable solutes and unreacted silanol groups on the silica support often are at least partially ionized.

Previous studies have shown that column packings that were exhaustively endcapped show superior stability in intermediate- and high-pH mobile phases (10, 20). This effect apparently is the result of increased protection of the silica support surface from dissolution, the typical cause of silica-based column failure at intermediate and high pH. To test the stability of the diisopropyl-C14-amide column at intermediate pH, we continuously purged a column of this material with 40:60 (v/v) acetonitrile–25 mM sodium phosphate buffer (pH 7.0) at ambient temperature.

Periodically, we tested this column chromatographically, with the results shown in Figure 9. For both toluene (Figure 9a) and amitriptyline (Figure 9b), plate height values show little change for the diisopropyl-C14-amide column after purging with more than 36,000 column volumes, after which the experiment was terminated arbitrarily. This result suggests that the column underwent little change after the use equivalent of more than three months of 8-h workdays. We believe that the exhaustive endcapping method used in preparing the diisopropyl-C14-amide column provided superior protection for the silica support from dissolution by the pH 7 mobile phase.

Retention values for the basic solute amitriptyline increased approximately 35% after the purging study, indicating the formation of more acidic silanol groups on the silica support surface caused by slow silica hydrolysis.

The alkyl–amide column can be operated routinely at intermediate pH for separating a wide range of polar and ionizable compounds. Figure 10 shows the separation of a mixture of benzodiazepine drugs using a pH 4.6 buffer with good peak shapes and column efficiencies for all solutes. Bromazepam (pKₐ = 2.9, 11.0), clobazam (pKₐ unavailable), lorazepam (pKₐ = 1.3, 11.5), and diazepam (pKₐ = 3.3) compose this mixture, which has a wide range of protonated (ionized) and nonionized structures at the separation pH.

Figure 11 shows the separation of cephalosporin drugs at pH 7.0, again with superior peak shapes and column efficiency. These compounds have pKₐ values ranging from 2.2 to 7.3, and some compounds, such as cefalexin, have multiple pKₐ values.

As a result of the linked polar amide group of the alkyl–amide structure, this stationary phase is readily wetted by totally aqueous mobile phases. Consequently, analysts can separate compounds that require little or no organic modifiers for proper retention. Figure 12 shows an example of this effect in which a mixture of nucleic acid bases and related compounds were separated with a simple aqueous sodium acetate mobile phase. Studies showed no change in retention and column efficiency properties for this column when the mobile phase was adjusted from partially organic to totally aqueous mobile phases and back again; equilibration also is very rapid. These results indicate that this stationary phase does not collapse when used with low-organic-content or totally aqueous mobile phases.
CONCLUSION
A silica-based column with a sterically protected, triple-endcapped C14 stationary phase with a linked polar amide group showed significant promise for separating a wide range of polar and ionizable compounds with good peak shapes and column efficiency. Because of the presence of protecting diisopropyl groups on the silane silicon atom, this column demonstrates outstanding stability at low pH. The column also exhibits excellent stability with intermediate-pH mobile phases because of the exhaustive endcapping that inhibits degradation of the column, typically caused by dissolution of the silica support. Based on previous experiences, monofunctional attachment of the silane-based stationary phase should provide good preparation reproducibility. The excellent kinetic properties (column efficiency and peak shape) we found for the bidentate stationary phase is typical of those of monofunctional structures.

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REFERENCES

FIGURE 6: Separation of basic drugs at pH 6 for (a) endcapped diisopropyl–C14–amide, (b) endcapped C18, and (c) nonendcapped C8 columns. Column dimensions: 150 mm x 4.6 mm; mobile phase: 62:38 (v/v) methanol–0.01 M citrate buffer (pH 6.0); flow rate: 1.0 mL/min; temperature: 30 °C; detection: UV absorbance at 254 nm. Peaks: 1 = uracil, 2 = propranolol, 3 = nortriptyline, 4 = doxepin, 5 = amitriptyline, 6 = trimipramine.


