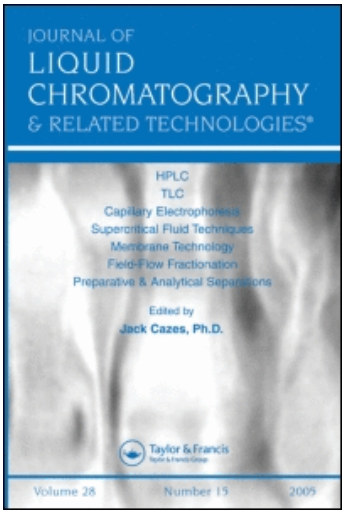

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MIXED PACKINGS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: II. MIXED PACKINGS VS.MIXED LIGANDS

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ABSTRACT

The use of mixed packing of different selectivities for the separation of antidepressants and anticonvulsants was studied. The results show that columns packed with mixed ligand supports (C_8 and cation exchange), gave the better resolution and peak shapes than the physically mixed C_8/cation exchange and serially connected columns. Also, the retention times obtained on the mixed ligands column, the physically mixed supports column and the two columns in series were different in each case.

INTRODUCTION

Mixed supports in high performance liquid chromatography (HPLC) can be divided into two main groups: (a) physically mixed supports and (b) chemically mixed bonded ligands. A physically mixed support column is made of two different selectivity materials, for example C_8 derivatized silica and β-cyclodextrin derivatized silica mixed in a predetermined ratio and packed into the same column. Conversely, a mixed ligand column is one where both ligands are derivatized to the same silica at a predetermined ratio.

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In a previous study (1) a comparison of physically mixed reversed phase C_{18} and β-cyclodextrin materials versus two serially coupled C_{18} and β-cyclodextrin columns was undertaken. The study focused on evaluating: (a) the utility of such mixed packings; (b) the effect of each experimental column on the resolution of components; and, (c) whether the observed retention times on the physically mixed packed columns were linear and predictable. In this study the above objectives are extended in comparing physically mixed columns and binary ligands attached to the same silica particle. Four columns packed with the following materials were used for this study: a reversed phase n-alkyl chain (C_8); a weak cation exchange (carboxymethyl); a physically mixed (1:1) C_8/cation exchange; and a (1:1) mixed ligand C_8/cation exchange.

EXPERIMENTAL

Materials

The anticonvulsants and antidepressants used in this study were a gift from Alltech Associates, Inc. (Deerfield, IL). The methanol and acetonitrile used were purchased from Burdick and Jackson (Muskegon, MI) (glass distilled, UV grade). Water was glass distilled and deionized. Potassium phosphate and phosphoric acid were purchased from Fisher Scientific (Fair Lawn, NJ).

The four columns in this study were custom packed and supplied free of charge by Alltech Associates, Inc., with each column having the dimensions 150x4.6 mm. The base silica used in the columns was from the same lot having the following properties: 5 μ spherical with a pore size of 100A°, a pore volume of 0.9 ml/g, and a surface area of 310 m^2/g. The carbon loading was 8.3% for the C_8, 8% for the cation exchange, and 11.2% for the C_8/cation exchange mixed ligand support. This support had a carbon loading ratio of 1.14:1 of C_8 to cation exchange.

Apparatus

A Hewlett-Packard Model 1090M equipped with a photodiode array detector and an auto injector was used. Ten microliters of solution was injected and monitored at 254 nm (anticonvulsants) or 215 nm (antidepressants). The mobile phase composition employed in the analysis is specified in the figure legends. All mobile phases were filtered and degassed before use and maintained under helium throughout the experiments. The pH of the phosphate buffer was adjusted to the required acidity with dilute phosphoric acid.

RESULTS AND DISCUSSION

The objectives of the present study were to evaluate the utility of mixed support columns in HPLC and to compare the retention times obtained using a serially-coupled support, a physically mixed support, and a mixed ligand support. Figure 1 shows the chromatogram obtained when 10 µl of the solution of the three antidepressants (Dexepin, Desipramine and Protriptyline) was injected on the cation exchange column. It is clear that no separation was achieved using this column. On the other hand, injecting the same solution on the C_8 column resolved the three antidepressants, as shown in figure 2. However, severe tailing of the peaks was observed with the retention time of the first peak (Doxepin) being 25 minutes and the last peak (protriptyline) 49.5 min. When the physically (1:1) mixed column was used (figure 3a) the three components were resolved with the first and last components eluting at 9 and 15 minutes, respectively. In comparison with the C_8 column, the retention times were lower while the tailing was diminished. Increasing the flow rate from 1 ml/min (figure 3a) to 1.6 ml/min (figure 3b) resulted in decreasing the peak widths while still maintaining resolution. The best results were obtained when the antidepressant mixture was analyzed using the mixed ligand (C_8/cation exchange) column (figure 4). The peaks were sharp, the resolution was excellent, retention times were short and tailing was eliminated. The worst

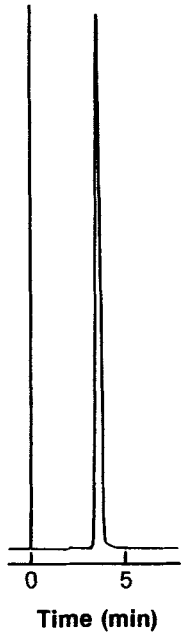

Figure 1. A chromatogram of antidepressants injected on a cation exchange column (150 x 4.6 mm) using a mobile phase of 30% acetonitrile/0.008 M potassium phosphate buffer, pH 3.75 at a flow rate of 1 ml/min.

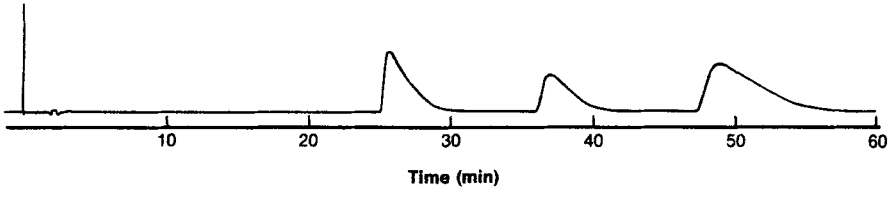

Figure 2. Same as Figure 1, except reversed phase C_8 column was used.

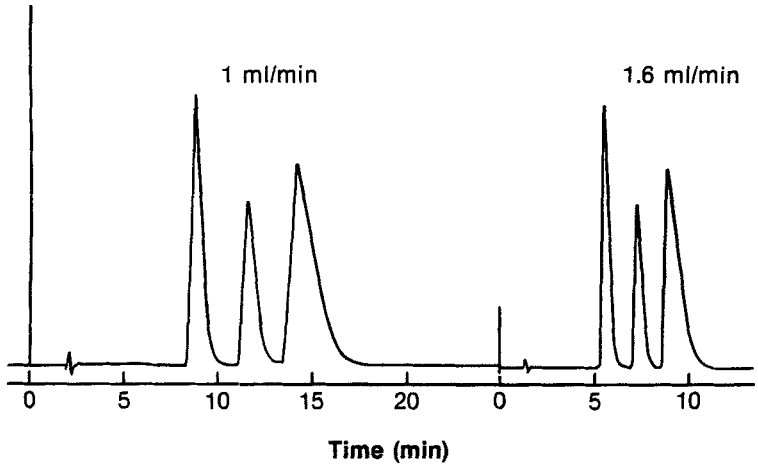

Figure 3. Same as Figure 1, except a mixed packings C_8/cation exchange column was used at a flow rate of 1 ml/min (left) and 1.6 ml/min (right).

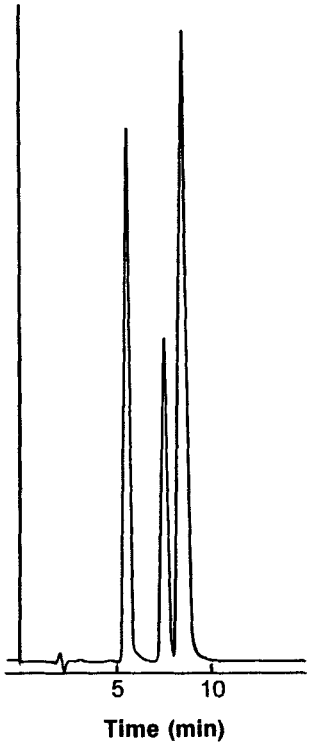

Figure 4. Same as Figure 1, except a mixed ligands C_8/cation exchange column was used.

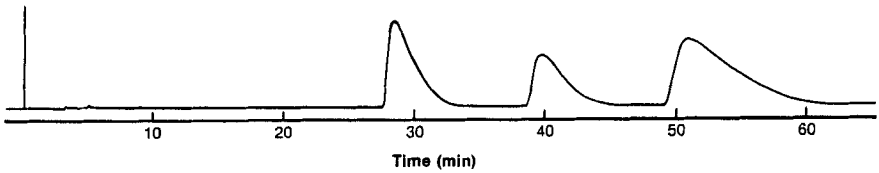

Figure 5. Same as Figure 1, except C_8 and cation exchange columns in series were used.

results were obtained when the C_8 and cation exchange columns were coupled in series (figure 5). The analysis time was long with the peaks broad and badly tailing. The column of choice, then, for the separation of this antidepressant mixture, under the present experimental conditions is the mixed ligand column.

The effect of mixed packing columns on retention

As stated earlier one of the objectives of this study was to see what effect the mixed packings have on retention. Table 1 lists the retention times obtained for the antidepressants using the C_8, cation exchange, C_8/cation exchange columns in series, the C_8/cation exchange physically mixed and mixed ligand columns. The results show that the retention times obtained using two columns in series is equal to the sum of the retention times obtained using the individual columns. Also, as have been previously observed (1,2) the order of the columns in series is not important and does not affect the resulting retention times (results not shown). Table 1 shows very interesting results which agree with our previous findings (1) in which the retention times obtained on the physically mixed and mixed ligand columns are not equal to half the retention times obtained on the two columns in series (half the retention times of the two columns in series are used because all the columns have the same dimensions). The retention times of doxepin were 14.5 min., 9.0 min., and 6.0 min. when the columns in

TABLE 1

Comparison of retention times obtained for the antidepressants on the four columns in this study using a mobile phase of 30% acetonitrile/0.008M KH_2PO_4, pH 3.75

Compound	Columns t_R (min)				
	CX	C_8	C_8/CX[1]	C_8-CX[2]	C_8.CX[3]
doxepin	3.9	25.5	29.0	9.0	6.0
desipramine	3.9	37.0	40.0	12.0	7.5
protriptyline	3.9	48.0	51.0	14.5	8.7

1. columns in series C_8/cation exchange.
2. physically mixed C_8/cation exchange.
3. mixed ligands C_8/cation exchange.

series, physically mixed and mixed ligand columns were used, respectively. The same is true for the other two compounds. This shows a difference in retention due to the mixing of the particles, becoming more pronounced when the two different ligands are on the same silica.

The longest retention times were obtained when the columns were coupled in series. However, when they are physically mixed (each ligand on a separate silica) in one column, a reduction of 38% in retention is observed. Further decrease in retention (33%) is observed using the mixed ligand column. This clearly indicates, in this case, that the closer the C_8 and carboxymethyl groups are to each other, the less the retention. The 1.14:1 ratio of carbon loading of the C_8 to carboxymethyl does not account for the

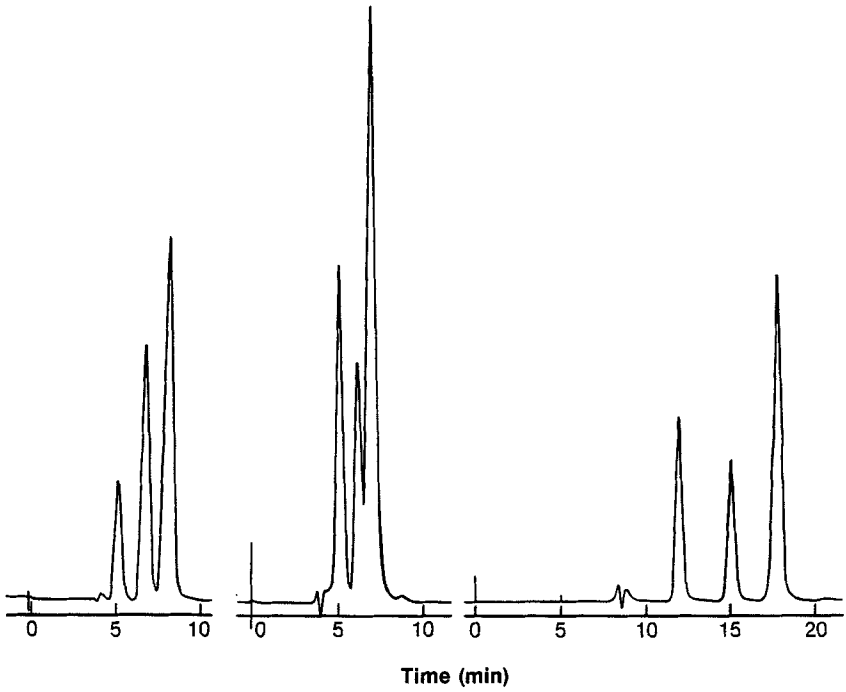

Figure 6. Chromatograms of a mixture of three anticonvulsants injected onto a mixed ligand C_8/cation exchange (left) mixed phases C_8/cation exchange (middle) and C_8/cation exchange columns in series (right) using a mobile phase of 44% methanol/0.05 M phosphate buffer, pH 4.0 at a flow rate of 1 ml/min.

33% decrease in retention. Experiments are underway to find a reasonable explanation for this phenomenon.

Separation of Anticonvulsants

Figure 6 shows the separation of anticonvulsants using a mobile phase of 44% methanol in 0.05 M phosphate buffer, pH 4.0 at a flow rate of 1 ml/min. The figure shows the chromatograms using a mixed ligand column, a mixed phase column and a serial C_8 and cation exchange column. Under the same experimental conditions the mixed ligand column gave baseline separations as did the serially coupled column. The physically mixed phase

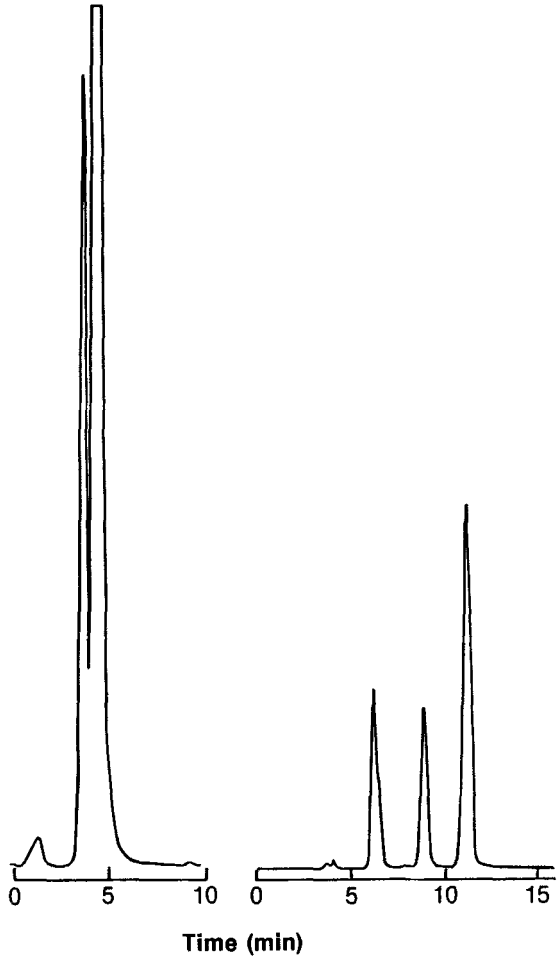

Figure 7. Chromatograms of anticonvulsants using a cation exchange column (left) and a reversed phase C_8 column. The mobile phase used is the same as in Figure 6.

column, however, did not baseline resolve the three solutes. This may be due to the ratio of carbon loading of the C_8 group to carboxymethyl group in the mixed ligand column. Figure 7 shows the chromatograms obtained using the cation exchange column and the C_8 column using the same mobile phase as that used in the previous experiment (figure 6). These two chromatograms show that the separation is carried out mostly by the C_8 group and not by the cation exchange group.

Comparison of the retention times in figure 6 shows a reduction of 10% in retention between the C_8/cation columns in series and the physically mixed phase column, while there was no appreciable difference in retention between the mixed phase and mixed ligand columns. It is felt that the differences and lack of agreement of retention times obtained using the mixed phase, mixed ligands and serial columns is greatest when the difference in retention on the individual columns is large, as in the case of antidepressants. This phenomenon was observed before (1).

CONCLUSION

The results of this study show the mixed ligand column to give better or comparable separations than the mixed C_8/cation phases column. Also, in most cases shorter retention times were observed using the mixed ligand column. It was interesting to see the effective use of two ligands on the same silica, in which the cation moiety was practically ineffective. However, this was the column which gave the faster analysis with the needed resolution. The retention times using the mixed phase column were less than those obtained using the C_8/cation columns in series, a result which was observed earlier (1).

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