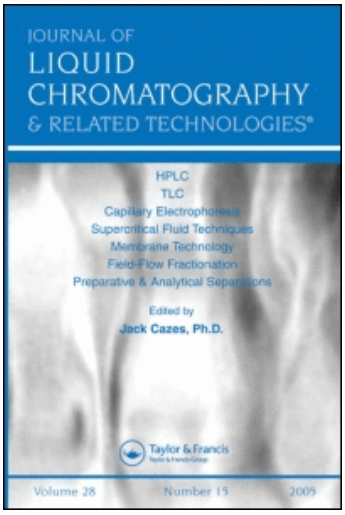

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THE EFFECT OF ALKYL CHAIN LENGTH AND CARBON LOADING IN SILICA BASED REVERSED PHASE COLUMNS ON THE SEPARATION OF BASIC COMPOUNDS

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ABSTRACT

A comparative study of the short range effect of run time (mobile phase volume - pH 2.0) on column parameters (k', α, R_s and peak asymmetry) of three different reversed phase columns was undertaken. The columns evaluated were C-18 nucleosil (low density carbon loading), C-18 ultracarb (high density carbon loading), and C-30 (medium density carbon loading). After an average run time of eleven hours using a buffered mobile phase at pH 2.0 and basic solutes, the results showed that the C-30 and C-18 high carbon loading columns gave better peak asymmetry than the C-18 nucleosil column, while there was no appreciable change in α or R_s with run time using the three test columns. A noticeable change in retention time between the three columns was observed.

INTRODUCTION

Recently we witnessed a continuous development in column technology. This includes the emergence of new stationary supports (e.g., polymeric, coated silica, etc.) and newly derivatized silica. Good reviews on chromatographic columns were recently published (1,2). Among the columns introduced are the high carbon density and very long C-30, alkyl chain columns. For

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basic compounds which are often used in the pharmaceutical industry, regular reversed phases cause tailing, low plate numbers, varying retention times, and undergo dramatic change in properties with use. The degree of irreproducibility in retention and plate number is a function of the number of remaining silanol groups on the silica surface after derivatization. This number increases as the alkyl chain length increases due to steric hindrance. To overcome this problem, special synthesis procedures and/or exhaustive endcapping are employed. Another method is the addition to the mobile phase of a competing base in order to moderate these effects. This paper describes some of the relative changes in resolution, selectivity, peak asymmetry and the number of theoretical plates as a function of column use. The goal of this study was not to measure stability of bonded RP columns with time, since this has been evaluated (3,4) but to observe the changes in basic chromatographic factors that affect the quality of separation and reliability of the method. Three reversed phase columns were selected for this study, a C-18, a C-30 and a high density C-18 carbon column.

EXPERIMENTAL

Materials:

Silica based C-18 nucleosil and high carbon density Ultracarb C-18 columns were a gift from PHENOMENEX (Ranco Palos Verdes,California). Triacontyl=Daltosil 100 (C-30) was purchased from SERVA BIOCHEMICALS (Westbury,New York). The column dimentions, silica particle diameter, pore size and carbon density are summerized in table 1. The solutes studied R-(+)-1-(1-Naphthyl)ethylamine (NA),proporanolol(P) and N-methylephedrine(NE) were purchased from Aldrich and were used without any further purification. Acetonitrile was glass-distilled UV grade. Water was deionized glass distilled. The potassium phosphate monobasic and triethylamine were purchased from Fisher and Aldrich, respectively.

Table 1. Column parameters

Column type	Column dimen. (mm)	Particle dp (μm)	Average pore diameter (A°)	Carbon load (%)	Surface area (m^2/g)
C-18 Nucleosil	150*4.6	5	100	14%	350
C-18 Ultracarb	150*4.6	5	60	31%	550
C-30 Triacontyl	250*4.6	4	100	24.6%	300

Instrumental:

A Hewlett-Packard model 1090 liquid chromatograph equipped with a photodiode array detector, an automatic injector, a strip chart recorder, a Hewlett-Packard Model 3392A integrator and a Hewlett-Packard Model 85 computer/controller, and built in oven was used.

Procedures:

The mobile phase was made of 0.02M KH_2PO_4 + 0.02% triethylammonium acetate (TEAA) + 20% acetonitrile (ACN) dissolved in 1 liter of water. The pH was adjusted to 2.00 with phosphoric acid. The test solutes were dissolved in the mobile phase solvent. The flow rate was kept constant at 1 ml/min for all the experiments, which were run at room temperature. The dead time (t_o), was estimated as the first negative peak in the chromatogram.

RESULTS AND DISCUSSION

Changes in k' and retention times as function of analysis time:

As mentioned earlier, the objective of this study was to find out what effect the continuous flow of mobile phase, i.e. analysis time, has on critical chromatographic parameters. We studied the short range effects rather than long term effects on column behavior. For this purpose, three reversed phase columns of varying degrees of alkyl chain length and carbon loading were selected. Basic solutes were chosen in order to evaluate what effect an acidic mobile phase (pH=2) has on retention, peak skewness, selectivity and resolution with analysis time, up to 10 hrs. of continuous flow of the mobile phase. Figure 1a shows the changes in retention expressed by capacity ratio of the solute NA as a function of analysis time. Each point represents a chromatographic run. The x-axis gives the continuous flow time of the mobile phase. As can be seen from the plot, there was no change in retention using the C-18 nucleosil (low carbon loading), while their was a moderate change in

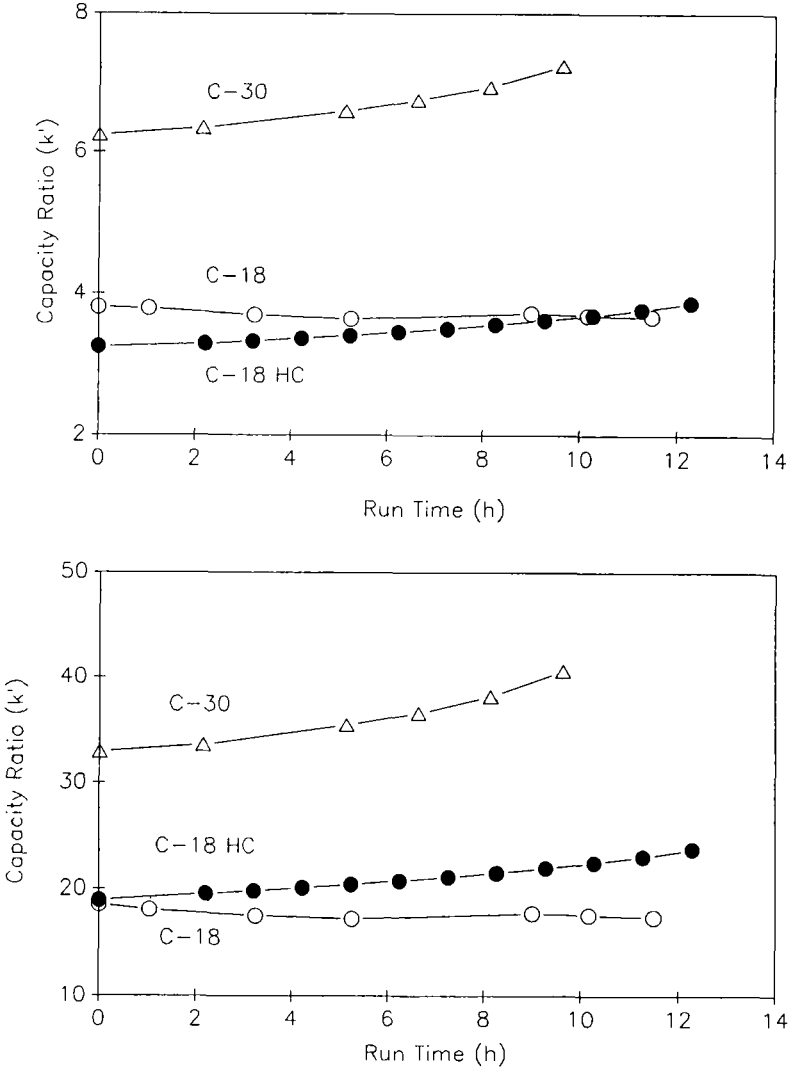

Figure 1. A plot of capacity factor versus run time for the solute NA (a) and the solute P (b), on the three test columns using a mobile phase of 0.02 M KH_2PO_4 +0.02% triethyammonium acetate +20% acetonitrile at a pH of 2.0, at a flow rate of 1 ml/min. Column parameters are listed in Table 1.

retention using the C-18 ultracarb column (high carbon loading) and the C-30 column. The same results were observed for the other two solutes P and NE; however, the relative change in retention and elution order differs from solute to solute, which is expected. For example as seen from figure 1a, NA eluted faster on the C-18 HC column, while P eluted faster on the C-18 column, Figure 1b. Also note that while the values of k' became closer with time for NA (Figure 1a), they diverged for P (Figure 1b) using the C-18 columns. Figures 2a,2b and 3a,3b show the chromatograms of the first and the last runs using the high density C-18 column and the C-30 column, respectively. It is clear that changes in retention with time have taken place. This change in retention as a function of analysis time can be related to the degree of interaction of the solutes with the isolated, non-hydrogen bridged highly acidic silanol groups (5). In this study, the degree of alkyl chain removal by the acidic mobile phase will determine the rate of increase in retention for the different solutes. Changes in retention using the C-18 column were also observed (results not shown).

Changes in selectivity and resolution as a function of analysis time:

Figure 4 shows changes in selectivity for the solute pair P and NE. No appreciable change in selectivity was observed with the analysis time using the C-18 nucleosil and the C-30 columns. For the C-18 ultracarb, there is a slow increase in the selectivity as a function of the analysis time. This behavior is attributed to the relative changes in the retention for the various solutes using the columns studied. Figure 5 gives the resolution of P and NA as a function of the analysis time. Again, there is no prominent change in resolution. Resolution depends on the relative changes of the retention between the two solutes of interest and on the relative changes in their peak widths. It seems that these two factors have changed in such a way that compensate for each other and as a result the values of resolution remained constant. Similar results were observed for the other test solutes.

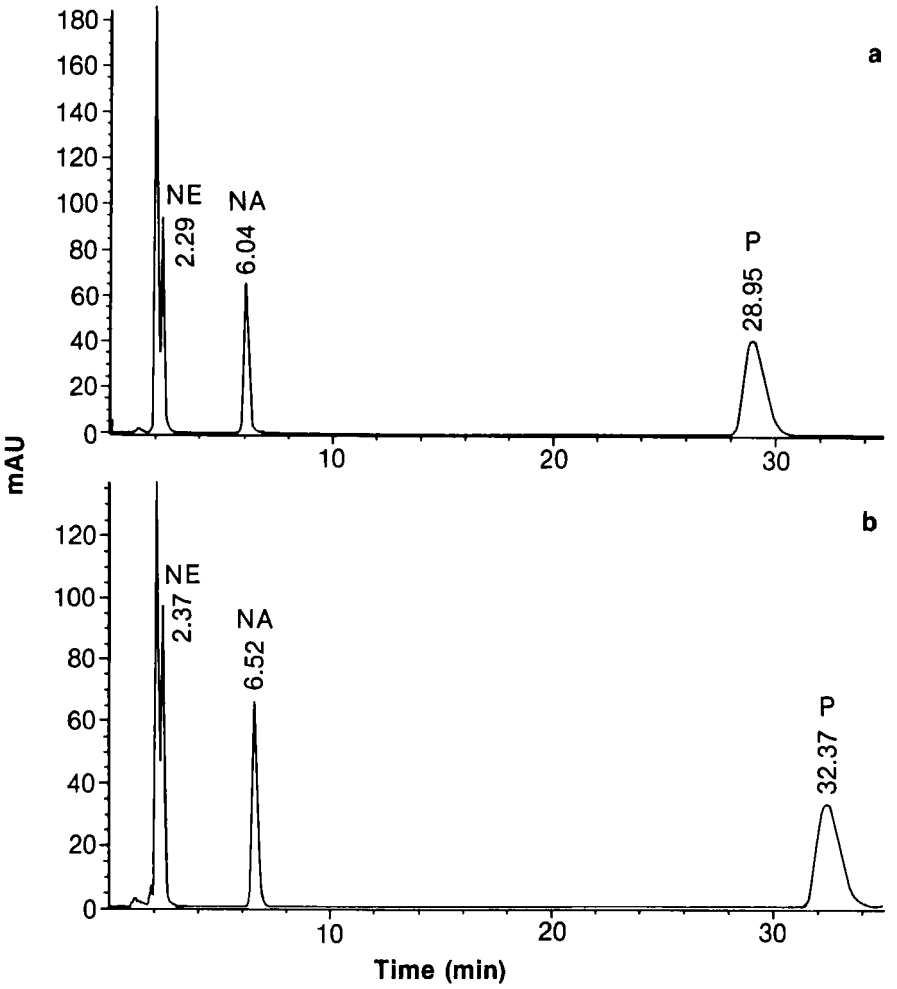

Figure 2. a. Chromatogram of the three test solutes using the high carbon density column for the first injection.

b. Chromatogram of the same solutes for the last injection. Experimental conditions as in Figure 1.

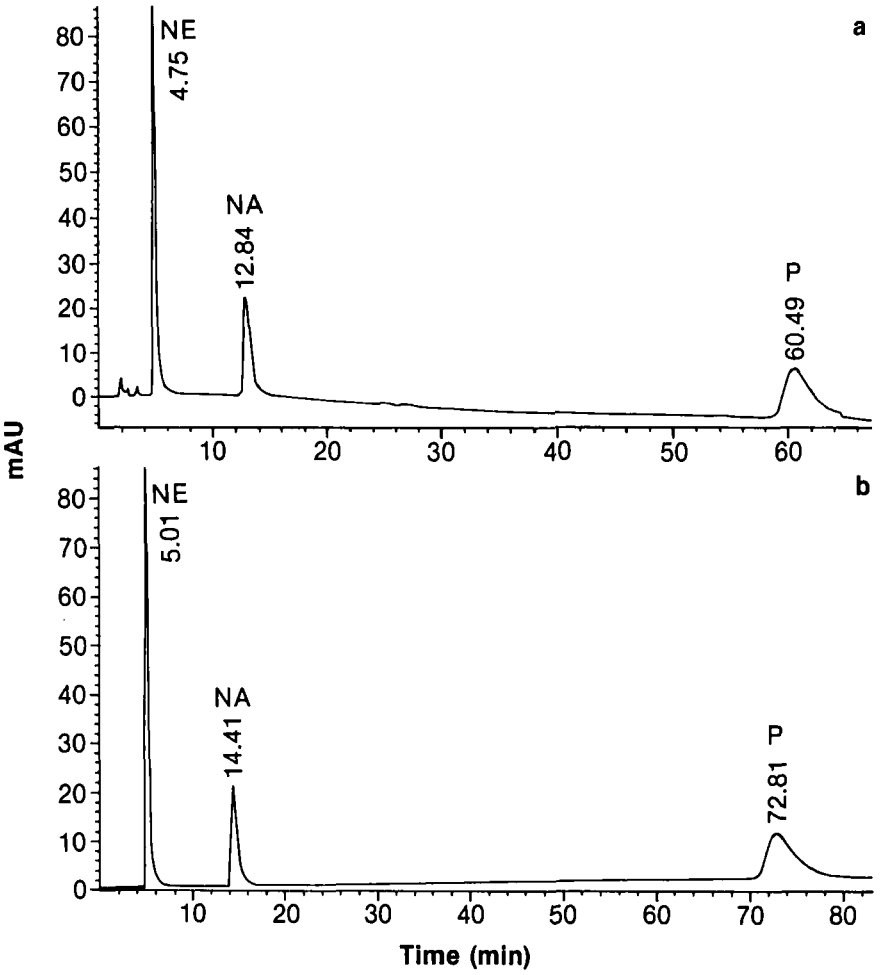

Figure 3. a. Chromatogram of the three test solutes using C-30 column for the first injection.

b. Chromatogram of the same solutes for the last injection. Experimental conditions as in Fiugure 1.

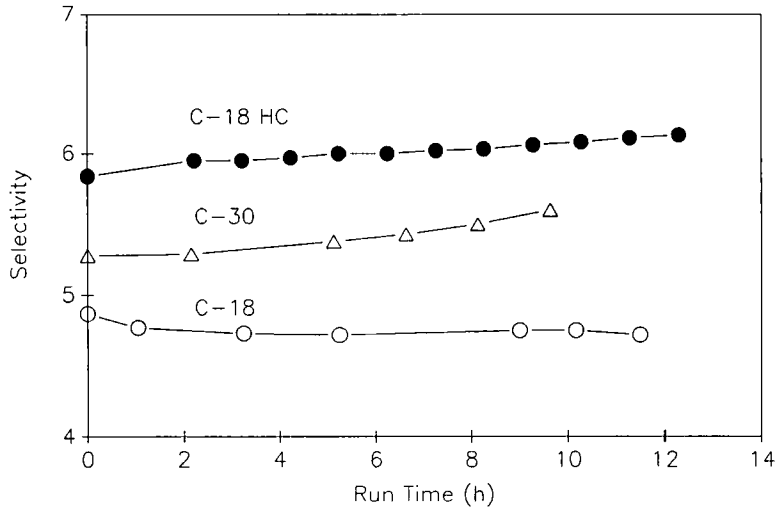

Figure 4. Change of the selectivity value (α) versus run time for the test solutes P and NA. Experimental conditions as in Figure 1.

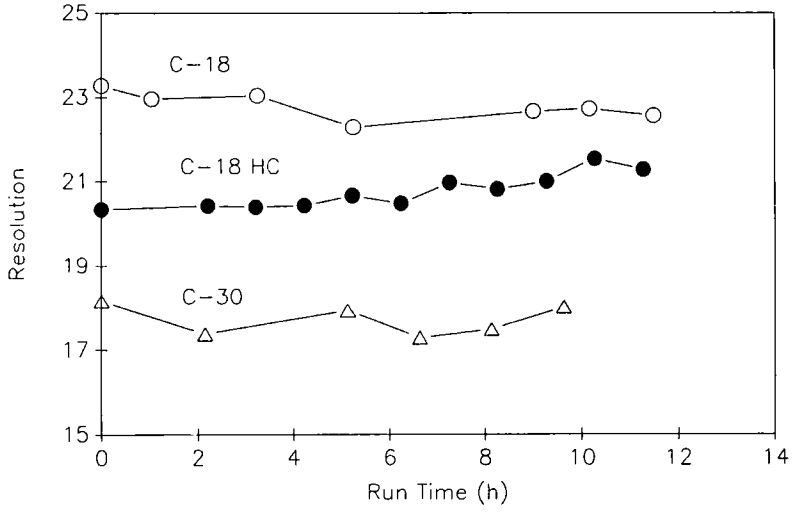

Figure 5. Change of the resolution value (R_s) versus run time for the test solutes P and NA. Experimental conditions as in Figure 1.

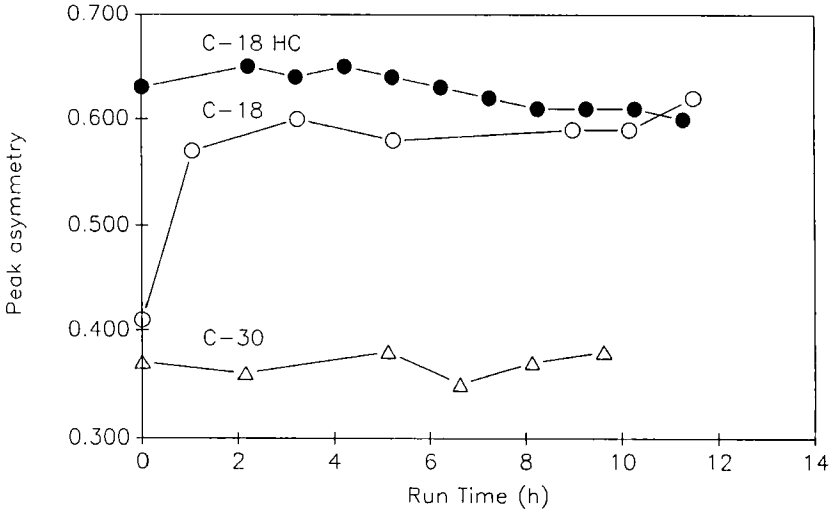

Figure 6. Change of peak asymmetry at h = 0.1 of the peak height for the test solute NA as a function of the run time. Experimental conditions as in Figure 1.

Changes in peak skewness (peak asymmetry) as function of the analysis time:

Figure 6 gives the change in peak asymmetry at 1/10 of the peak height for the solute NA. For the high density C-18 and the C-30 columns, there was no change in peak asymmetry, while the C-18 nucleosil column showed a slight increase until 3 hours of use then stabilized.

CONCLUSION

The results of this study show that although the columns were continuously used for only 11 hours, changes in peak asymmetry and retention times were observed. This means that in studies where retention times are important a test solution should be used, to check what influence the column use has on retention time and peak asymmetry.

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