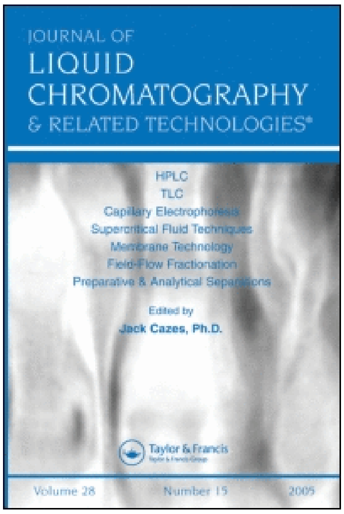

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High Performance Liquid Chromatography Separations Using Short Columns Packed with Spherical ODS Particles. II. Effect of Mobile Phase Composition on Resolution

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS USING SHORT COLUMNS PACKED WITH SPHERICAL ODS PARTICLES - II EFFECT OF MOBILE PHASE COMPOSITION ON RESOLUTION

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ABSTRACT

The effect of instrumental parameters, mobile phase composition, and flow rate on high performance liquid chromatography separations using 3 cm, 5 cm, and 10 cm columns packed with 3μ spherical ODS materials is discussed. The results indicate that when instrumental parameters were optimized, the 10 cm column gave better separation than the shorter columns under the same experimental conditions. However, when the mobile phase composition was adjusted so that the solute residence time was comparable in the three columns, short and long columns gave comparable results.

INTRODUCTION

In a previous study (1) a comparison was made of the separation of a mixture on 5 cm and 10 cm columns packed with 3μ and 5μ spherisorb ODS spherical particles using standard high performance liquid chromatography (HPLC) equipment without any modification. The results indicated that

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the separations achieved on the 10 cm column were not significantly better than those on 5 cm columns packed with supports of the same size and physical properties.

In the present study, which is an extension of the previous one, modifications were made on the instrument to accommodate the requirements of 3 cm, 5 cm, and 10 cm columns packed with 3μ ODS spherical particles. The results indicate that the 10 cm columns would give much better resolution than the 5 cm and 3 cm columns under the same experimental conditions, i.e., mobile phase compositions and flow rate. The results also show that the 5 cm and 3 cm columns can be made to give resolutions comparable to those of the 10 cm column if the composition of the mobile phase is adjusted to meet the requirements of solute residence time in the shorter columns.

EXPERIMENTAL

Materials: Perylene, benz(a)anthracene, and coronene were received from the Chemical Carcinogenesis Reference Standard Repository, function of the Division of Cancer Cause and Prevention, NCI/NIH, Bethesda, MD 20205. Acetonitrile (ACN) was glass distilled (Burdick and Jackson).

Apparatus: A modular HPLC system consisting of Laboratory Data Control (LDC) constametric I and II pumps attached to an LDC Gradient Master, a Chromatronix dual-channel UV absorbance detector (254 and 280 nm), a Rheodyne injector, and a strip-chart recorder operated at 0.2 in/min was used.

Three sets of columns were used (30 mm x 4 mm, 50 mm x 4 mm, and 100 mm x 4 mm) and were each packed with 3μ Spherisorb ODS packings obtained from Phase Separations, Inc. (see reference 1 for packing physical properties).

The experiments were run at room temperature using a mobile phase of acetonitrile/water (ACN/H_2O). One microliter of sample solution was injected. The mobile phase was degassed before use. The modifications made on the instrument to accommodate the requirements of the short columns were as follows: a 10μl solvent loop was used in place of the 100μl loop. The column was attached as closely as possible to the detector, and stainless steel tubing of the narrowest bore possible was used.

Column Packing: Supports were slurry packed into columns with acetone: ACN (1:1) at 8000 psi by the upward technique using a Haskel pneumatic pump.

RESULTS AND DISCUSSION

The effect of column length using 5μ and 3μ ODS spherical and irregular ODS materials on resolution was studied (1) using an unmodified HPLC system. Using 10 cm and 5 cm columns, the results indicated that it was possible to achieve the separation of a mixture without an appreciable loss of resolution. This was attributed to the fact that the instrument's parameters (such as sample loop, detector cell volume, internal diameter of tubing, distance between column and detector) that are acceptable when 25 to 30 cm columns are used are inadequate when used with the short columns. As a result, short and long columns did not give appreciably different results. In the present study, instrument parameters were optimized and the sample injected was scaled down from 10μl to 1μl. The results (Fig 1) show that the longer the column the better the resolution, in this case the 10 cm column is superior to the 3 cm column. If the flow rate was changed from 2 ml/min to 1 ml/min (Fig 2) and to 0.5 ml/min (Fig 3) resolution improved. However, the 3 cm column did not give baseline separation, which means that increasing solute residence time in the column by decreasing the flow rate is not the best possible approach, as will be discussed later.

Based on a previous study (2) in which the mobile phase was adjusted to meet the hydrophobic properties of the columns, and our knowledge of reversed phase HPLC, we decided to increase the residence time of the solute mixture in the column by changing the composition of the mobile

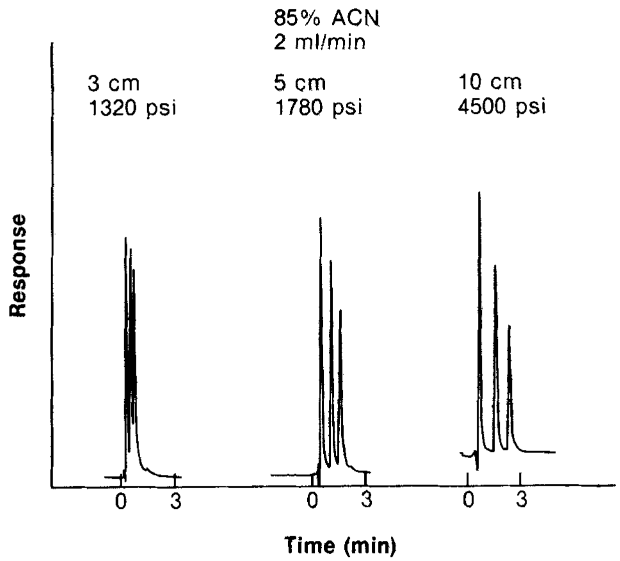

Figure 1. Separation of a test mixture on 3 cm, 5 cm, and 10 cm long columns packed with 3μ Spherisorb ODS material using a mobile phase of 85% acetonitrile/water at a flow rate of 2 ml/min.

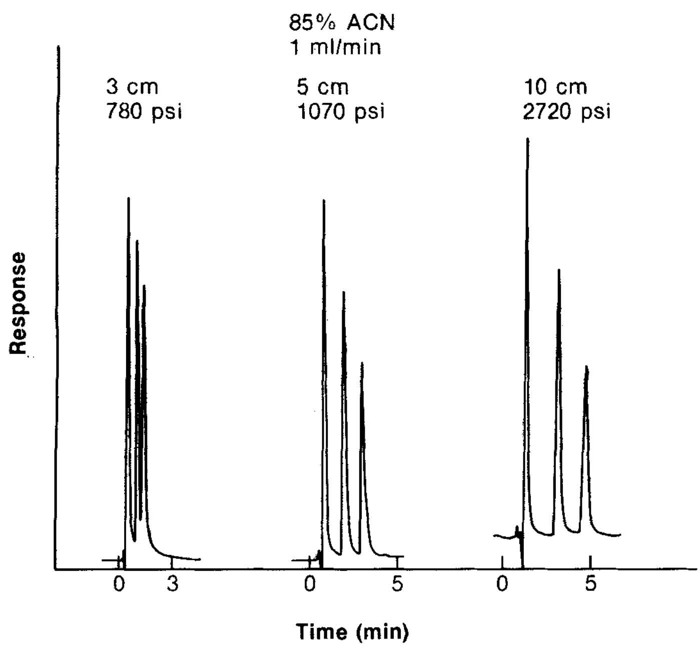

Figure 2. Same as Figure 1, except a flow rate of 1 ml/min.

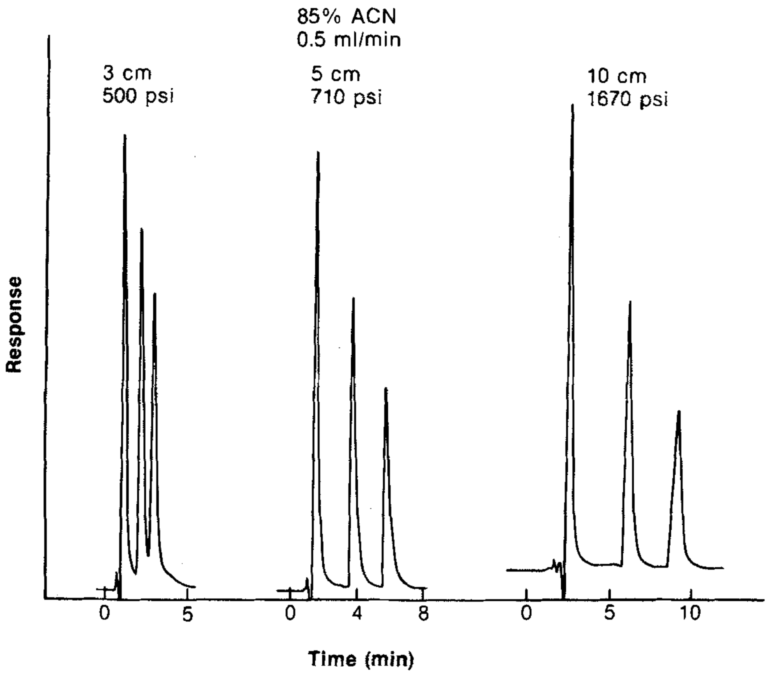

Figure 3. Same as Figure 1, except a flow rate of 0.5 ml/min.

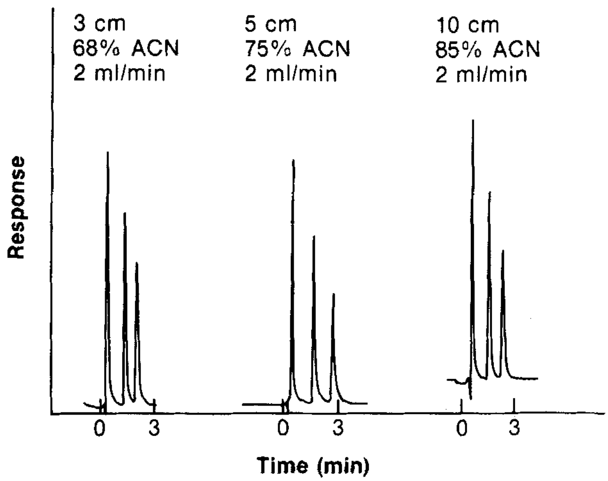

Figure 4. Separation of the test mixture on 3 cm, 5 cm, and 10 cm columns packed with 3μ Spherisorb ODS material using different ratios of acetonitrile/water at a flow rate of 2 ml/min.

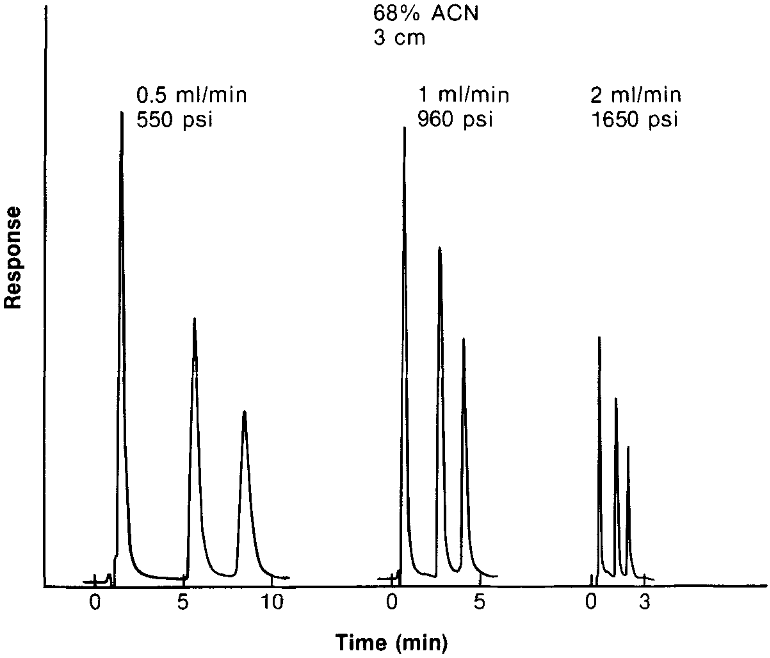

Figure 5. Separation of the test mixture on 3 cm column using a mobile phase of 68% acetonitrile/water and a mobile phase flow rate of 0.5, 1.0, and 2.0 ml/min.

phase. This was achieved by making the binary mobile phase richer in water as the column length decreased. Fig 4 shows that the same resolution of the test mixture was obtained on columns of the three different lengths when the mobile phase composition was changed. The 3 cm column and a 68% ACN/H_2O mobile phase gave the same separation as the 5 cm column with 75% ACN/H_2O and the 10 cm column with 85% ACN/H_2O at a flow rate of 2 ml/min. The separation was achieved in each case in less than 3 minutes. Fig 5 shows that when an optimum mobile phase is found, decreasing the flow rate will give better separation.

Mobile phases of the same composition, e.g., ACN/H_2O, methanol/water, etc., that contain a higher percentage of water result in higher back

TABLE 1

Effect of Column Length on Back Pressure
at a Mobile Phase Flow Rate of 2 ml/min

Column Length (cm)	Mobile Phase (% ACN)	Back Pressure (psi)
3	68	1650
5	75	1960
10	85	4500

pressure. Since the mobile phase had to be adjusted so that it contained a higher percentage of water as the column length decreased, it was necessary to compare the back pressure resulting from the use of each of the columns under optimum mobile phase conditions. Table 1 shows that the 3 cm column with a mobile phase of 68% ACN/H_2O gave a much lower back pressure than the 10 cm column with a mobile phase of 85% ACN/ H_2O and the 5 cm column with 75% ACN/H_2O.

CONCLUSION

This study shows that improving the instrumental parameters will lead to better results when columns of different lengths packed with the same material are used: the longer the column, the better the resolution. A 3 cm column can be made to give the same results as a 10 cm column, packed with the same material, if the residence times of the solutes in the column are comparable. This is achieved by decreasing the percentage of organic modifiers as the column length decreases. Although slowing the flow rate from 2 ml/min to 0.5 ml/min improves the resolution, it may not result in baseline separation.

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