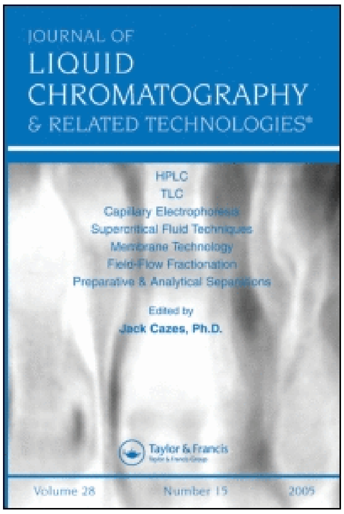

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High Performance Liquid Chromatography Separations Using Columns Packed with Spherical ODS Particles. III. Effect of Column Dimensions on the Resolution of a Complex Mixture

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS USING COLUMNS PACKED WITH SPHERICAL ODS PARTICLES - III. EFFECT OF COLUMN DIMENSIONS ON THE RESOLUTION OF A COMPLEX MIXTURE*

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ABSTRACT

Separations on short columns, (3 and 5 cm, packed with 3 μ ODS spherical materials) and somewhat larger ones (10 cm and 20 cm columns having 2.1 mm and 4.6 mm diameters packed with 5 μ ODS spherical materials) were compared using Aroclor 1254. With simple mixtures, the results showed that short columns can give separations comparable with those on longer columns when the percentage of the organic modifier in the mobile phase is adjusted. This was not so with more complex mixture. The results also showed that columns which have a comparable volume do not produce comparable separation. The longer column, 200 mm x 2.1 mm gave better resolution than the shorter 50 mm x 4 mm column. Also a shorter column, (100 mm x 4.6 mm), which had double the volume of a longer column. (200 mm x 2.1 mm), gave better resolution of the Aroclor 1254 test solution.

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INTRODUCTION

In a previous study (1) the separation of a mixture was compared on 5 cm and 10 cm columns packed with 3 μ and 5 μ spherisorb ODS spherical particles using standard high performance liquid chromatography (HPLC) equipment without modification. The results indicated that separations 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 a later study (2) the instrument was modified to accommodate the requirements of 3 cm, 5 cm, and 10 cm columns packed with 3 μ ODS spherical particles. The results showed that the 10 cm column gave much better resolution than the 3 cm and 5 cm columns under the same experimental conditions, i.e., mobile phase compositions and flow rate. The results also show that the 3 cm and 5 cm columns can give resolutions of simple mixtures, comparable with those on the 10 cm column if the composition of the mobile phase is adjusted to meet the requirement of longer solute residence time in the shorter column.

In this study, the separation of a complex mixture, Aroclor 1254, was compared on short 30 mm x 4 mm and 50 mm x 4 mm packed with 3 μ ODS particles, and 200 mm x 2.1 mm, 200 mm x 4.6 mm, 100 mm x 2.1 mm, and 100 mm x 4.6 mm packed with 5 μ spherical ODS material. The best resolution of the mixture was obtained when the 200 mm x 4.6 mm column was used. The effect of column length, diameter and volume is also discussed.

EXPERIMENTAL

Materials: Aroclor 1254 was received from Dr. Anderson, NCI-Frederick Cancer Research Facility. A solution of 4.9μg/μl Aroclor 1254 in acetonitrile was used. Acetonitrile (ACN) was glass distilled (Burdick and Jackson). Distilled deionized water was used.

Apparatus: A liquid chromatograph model 1090 equipped with a variable wave length detector, an oven, variable volume automatic injector and personal computer model HP-85 was used: results were printed on a HP-3390A reporting integrator. All these instruments were manufacctured by Hewlett-Packard. Both columns used (30 mm x 4 mm and 50 mm x 4 mm) were packed with 3 μ Spherisorb ODS packings obtained from Phase Separations, Inc. (see reference 1 for packing physical properties, and column packing procedure).

The other four columns (100 mm x 2.1 mm, 100 mm x 4.6 mm, 200 mm x 2.1 mm and 200 mm x 4.6 mm) were prepacked with 5 μ spherical C_{18} particles (Hewlett-Packard).

The experiments were run at 40°C, unless otherwise specified, using a mobile phase of ACN/H_2O.

RESULTS AND DISCUSSION

High speed liquid chromatography (HSLC) is becoming increasingly popular. Generally in HSLC short columns (30-60 mm) with a diameter of 4-7 mm are used. In a previous study, (2) we have shown that when a simple mixture is used the 30 mm x 4 mm gives results similar to those obtained on 100 mm x 4 mm packed with 3 μ ODS material, if the organic modifier in the mobile phase is adjusted, lowering the percentage of the organic modifier for the shorter column to increase the solute residence time. The present study shows that, although this will hold for simple mixtures, it may not be the case when complex mixtures are being separated on shorter columns. Note that the longer columns are packed with different material than the short columns (3 cm and 5 cm), and this may affect the results. The shorter columns are packed with 3 μ ODS materials which is, theoretically, more efficient.

Figure 1 shows the separation of Aroclor 1254, using the 3 cm and 5 cm columns. The 5 cm column gave better separation than the 3 cm

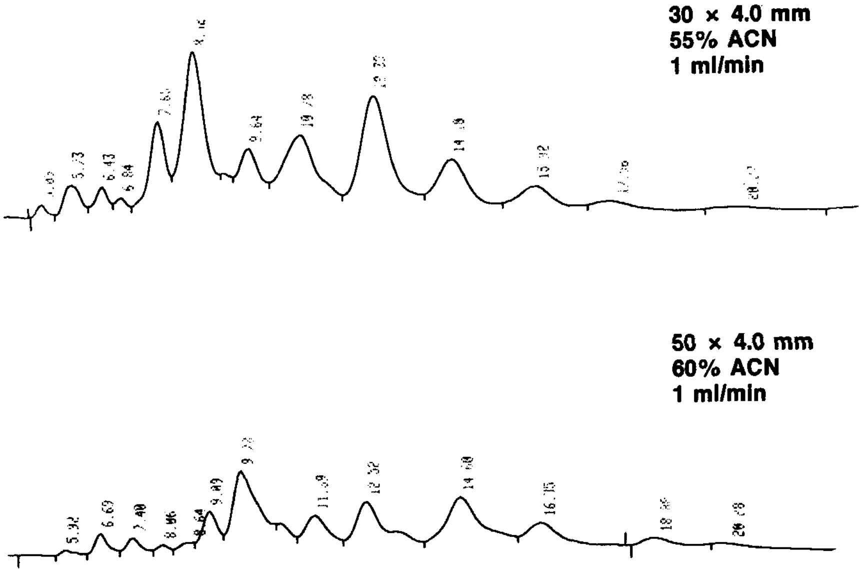

Figure 1. Separation of Aroclor 1254 on 30 mm x 4.0 mm and 50 mm x 4.0 mm columns packed with 3 μ spherisorb ODS material using a mobile phase of 55% and 60% ACN/H_2O respectively at a flow rate of 1 ml/min. Detection was carried out at 230 nm. Sample solution injected was 0.2 ul.

column although the mobile phase was adjusted to produce comparable residence time in the shorter column.

Figure 2 shows that the separation of Aroclor 1254 was improved when the 100 mm x 4.6 mm column was used rather tnan the 100 mm x 2.1 mm column. Note that the narrower column gave better sensitivity.

Figure 3 compares the separation of Aroclor 1254 using a 200 mm x 2.1 mm and 200 mm x 4.6 mm columns using 70% ACN/H_2O. The results show, as in figure 2, that, although both columns are of the same length, the wider column gave better separation. A comparison of the separation of Aroclor 1254 on the six columns tested clearly indicats the superiority of the

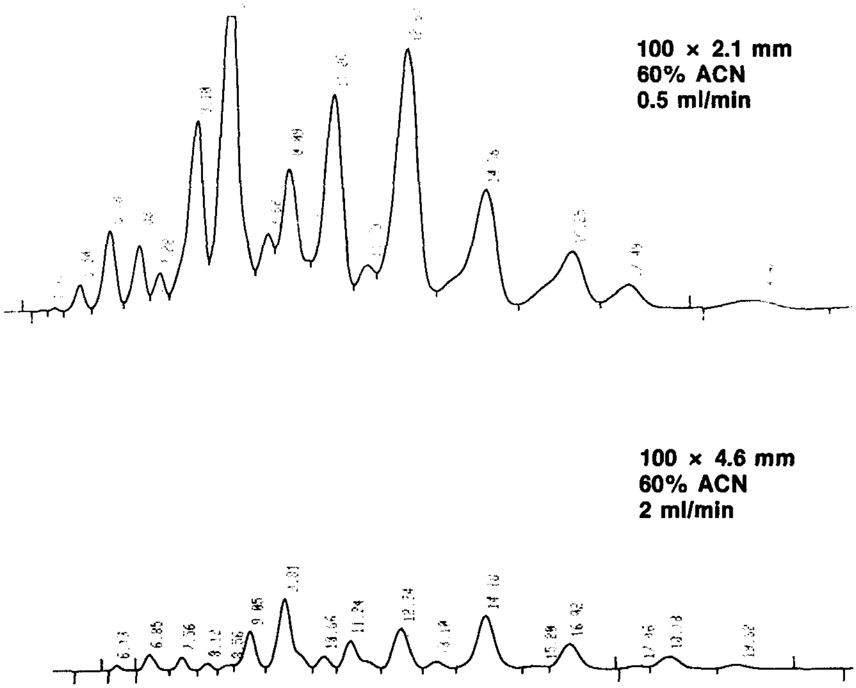

Figure 2. Separation of Aroclor 1254 on 100 mm x 2.1 mm and 100 mm x 4.6 mm columns packed with 5 μ spherical ODS material using a mobile phase of 60% ACN/H_2O at a flow rate of 0.5 ml/min and 2 ml/min respectively. Other conditions as in figure 1.

200 mm x 4.6 mm column over the others under the same conditions. However, when the organic modifier was adjusted (Figure 4), both 200 mm columns gave comparable results.

To find out the effect of column volume on separation, the 200 mm x 2.1 mm and 100 mm x 4.6 mm, which has double the volume, were packed with the same material and compared under the same mobile phase composition and flow rate (Figure 5a and 5b). The results show that the elution times were longer using the 100 mm x 4.6 mm column. As a result, the resolution

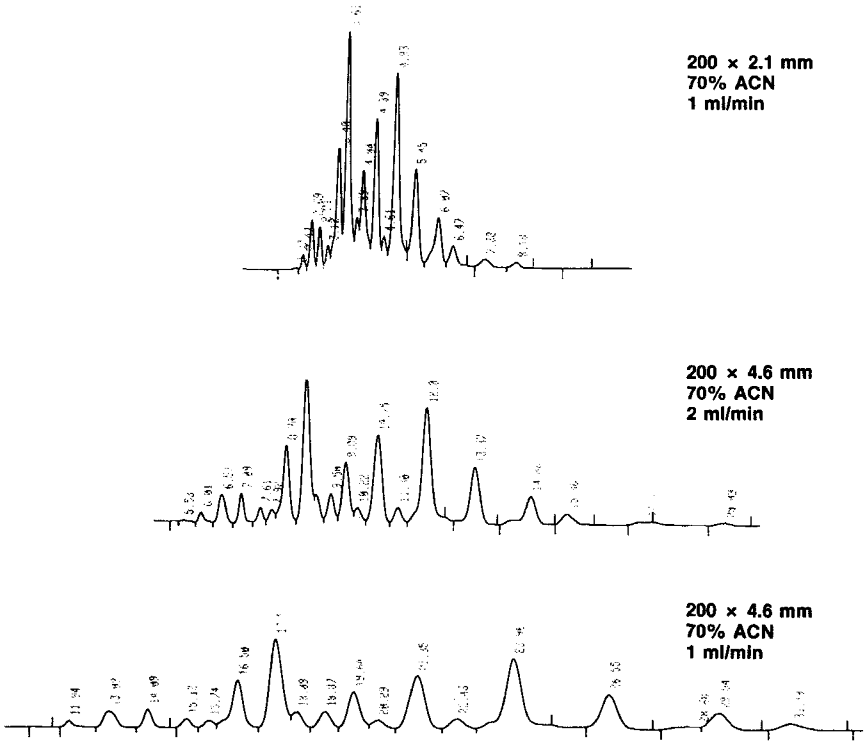

Figure 3. Same as in figure 2 except 200 mm x 2.1 mm and 200 mm x 4.6 mm columns using 70% ACN/H_2O.

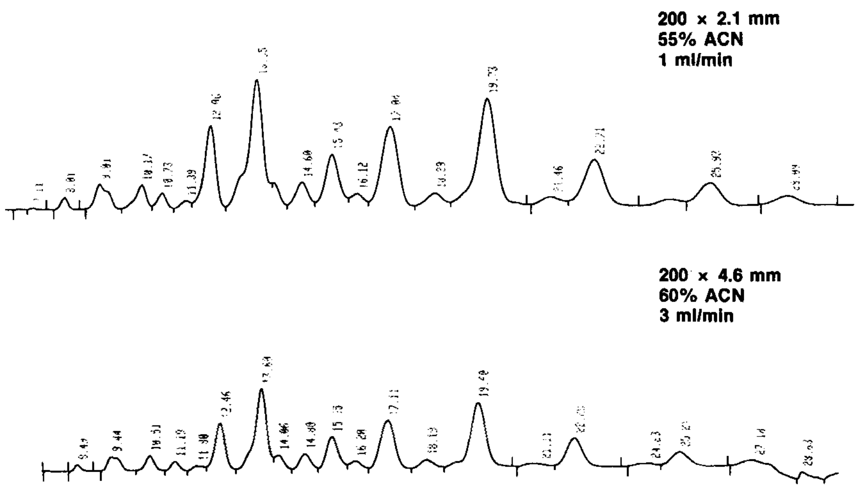

Figure 4. Same as in figure 3, but different percentage of organic modifier and flow rate.

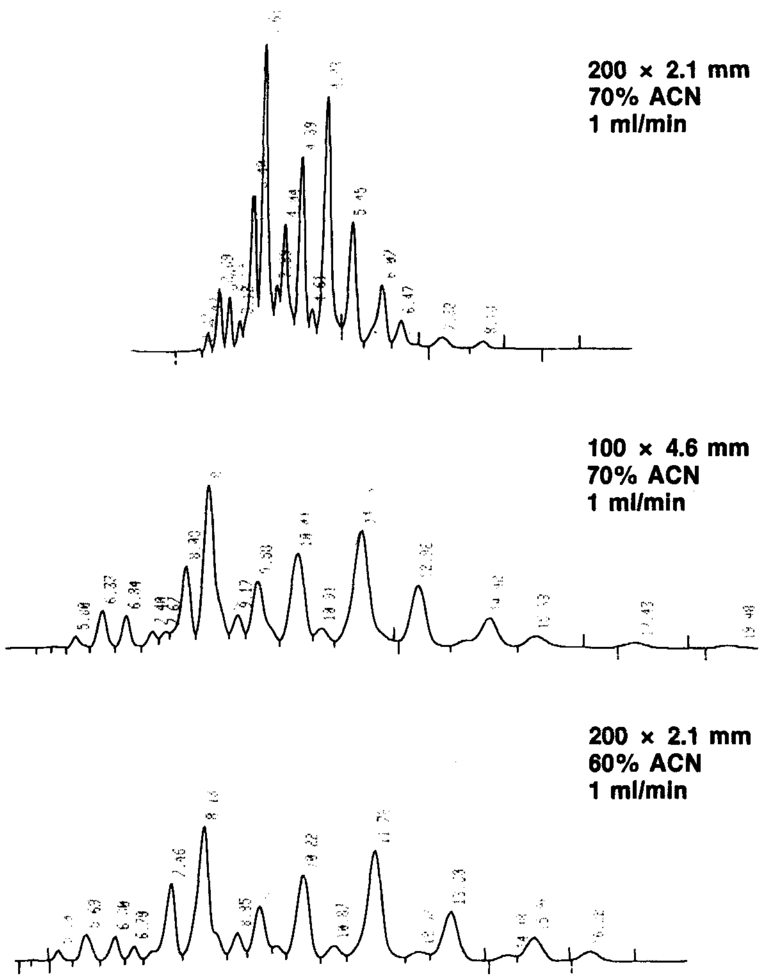

Figure 5. Comparison of the separation of Aroclor 1254 on 50 mm x 4.0 mm column packed with 3 μ spherisorb ODS material, 100 mm x 4.6 mm and 200 mm x 2.1 mm packed with spherical ODS material; (a) top, (b) middle, (c) bottom.

improved but the sensitivity did not. When the mobile phase was changed from 70% to 60% ACN for the 200 mm x 2.1 mm column (Figure 5c) the results were comparable with those obtained with the 100 mm x 4.6 mm and 70% ACN. Again, when the results obtained using the 50 mm x 4 mm column (Figure 1) are compared with those using the 200 mm x 2.1 mm column (Figure 5) both having comparable volume, the longer column gave better resolution of Aroclor 1254. It is clear, therefore, that not only is the column volume important, but also the length and the internal diameter. A detailed study of the relationship and effect of column volume, length and diameter on resolution is being conducted and the results will be published later.

CONCLUSION

The choice of column is governed by the type and size of sample (sensitivity) and its complexity. For small size samples HSLC will suffice, while for complex samples an efficient column will be needed, such as 200 x 4 mm, if the sample size and detection method permit.

Although short columns can be made to give comparable separations with those obtained on longer columns for simple mixtures, this was not the case when Aroclor 1254 was tested. Although the concentration of the modifier in the mobile phase was adjusted, there are limits to the level of the organic modifier in the mobile phase can be lowered in the attempt to achieve comparable results between short and long, wide and narrow columns packed with the same material. In the final analysis this is governed by (a) the solubility of solutes in the mobile phase; (b) instrument back pressure; and (c) sensitivity.

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