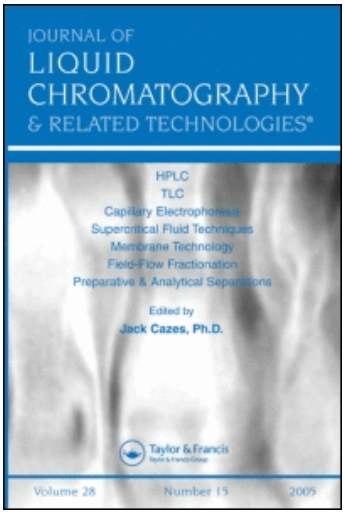

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATIONS USING SHORT COLUMNS PACKED WITH SPHERICAL AND IRREGULAR SHAPED ODS PARTICLES*

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ABSTRACT

In a comparative study of the separation of a mixture of polycyclic aromatic hydrocarbrons on 5 cm and 10 cm columns packed with 3μ and 5μ ODS spherical particles, the separations achieved on the 10 cm column were not significantly better than those on the 5 cm column. Although, columns packed with 3μ ODS spherical particles gave slightly better resolution than those packed with 5μ ODS spherical particles having the same physical properties, differences were observed when columns packed with ODS spherical particles were compared with columns packed with ODS irregular shaped particles of the same size.

The results show that separations on a 5μ or 3μ packed columns takes place at the first few centimeters.

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INTRODUCTION

The last decade witnessed the emergence of high performance liquid chromatography (HPLC) as a powerful and indispensable separation technique.

Initially, long and narrow columns were used (50 cm x 2 mm). As time passed the dimensions of the column changed. The length shrinked to 3-5 cm and the internal diameter to 4-6 mm. This is due to the change in particle size of the packing material (stationary phase) from 35μ to 3μ which resulted in high back pressure and greater efficiency. The higher efficiency of the small particles permitted the use of shorter columns.

The advantage of the shorter columns over 25-30 cm columns packed with 5μ or 10μ particles is the speed of analysis which results in savings of materials and time. Katz and Scott (1) described an HPLC system in which the analysis was completed in less than 1 minute. However, to achieve such rapid analyses, special equipment was required.

The objectives of this study are (a) to compare the separation of a five component mixture on 5 and 10 cm columns packed with 3 and 5μ spherical particles using standard HPLC equipment without modification, (b) to compare a 5 and 10 cm columns packed with 5μ spherical ODS particles and 10 and 25 cm columns packed with irregular ODS particles, and finally (c) to see where separation takes place on the column.

It must be noted that to compare columns, especially from different manufacturers, is not without pitfalls. However, care has been taken to pack the columns under the same conditions, and using materials, (reversed phase silica) from the same manufacturer with the same physical properties, (Table 1). The 3 and 5μ Spherisorb packings used in each of the 5 and 10 cm columns were from the same batch.

EXPERIMENTAL

Materials: Perylene, benzo(a)pyrene, benz(a)anthracene, methyl cholanthrene and coronene were received from the chemical carcinogens reference standard

TABLE 1

Physical Properties of 3 and 5μ Spherisorb ODS Packings*

Mean particle size (μ)	5	3
Size distribution (+1μ)	70%	95%
Pore size (nm)	8	8
Pore diameter (nm)	5.5-11	5.5-11
Surface area (m^2/g)	220	220
ODS capacity (m mol/g)	~ 0.3	~ 0.5

*Data supplied by Phase Sep., 255 Oser Avenue, Hauppauge, NY

repository, function of the Division of Cancer Cause and Prevention, NCI/NIH, Bethesda, MD 20205. Acetonitrile was glass distilled (Burdick & 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 of which two sets (50 mm x 4 mm and 100 mm x 4 mm) were each packed with 3 and 5μ Spherisorb ODS packings obtained from Phase Sep., (see Table 1 for packing physical properties). The third set (100 mm x 4 mm and 250 mm x 4 mm) of columns were packed with irregular shaped ODS materials having a mean diameter of 5μ, a surface area of $400 m^2/g$, a pore diameter of 80 A°, 10% total carbon and 95% silanization, obtained from Whatman, Inc.

The experiments were run at room temperature using a mobile phase of 75% acetonitrile/water at a flow rate of 1 ml/min. unless specified. Ten μl

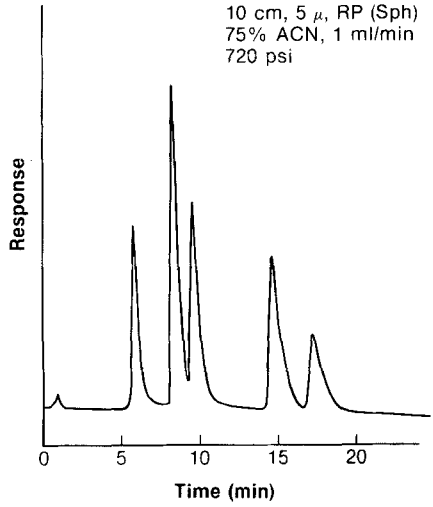

Figure 1. Separation of perylene, benzo(a)pyrene, benz(a)anthracene, methyl cholanthrene and coronene of a 10 cm long column packed with 5μ Spherisorb ODS. 75% acetonitrile/water was used as the mobile phase.

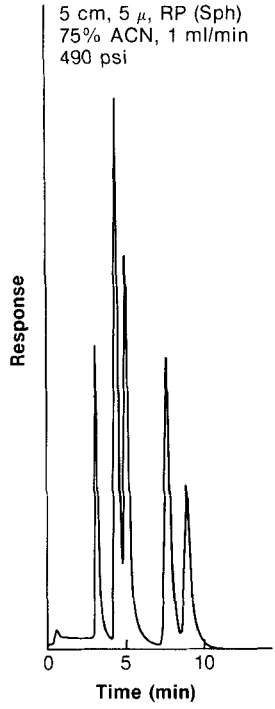

Figure 2. Same as Figure 1, but 5 cm column.

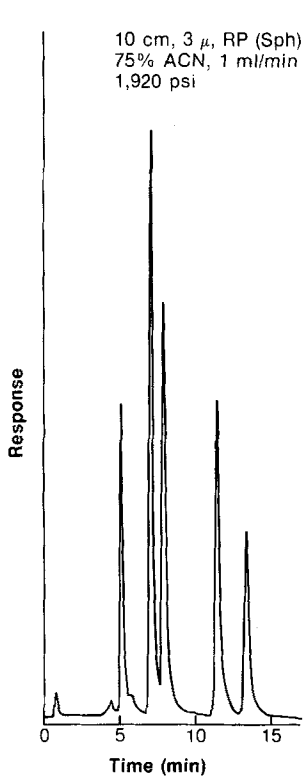

Figure 3. Same as Figure 1, but 10 cm column packed with 3μ Spherisorb ODS.

sample solutions was injected. The mobile phase was degassed before use. No modifications of any kind were made on the instrument to accommodate the requirements of the short columns.

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

RESULTS AND DISCUSSION

The objective of this study was to examine the effect and possibility of using short columns with standard HPLC equipment. The results (Figures

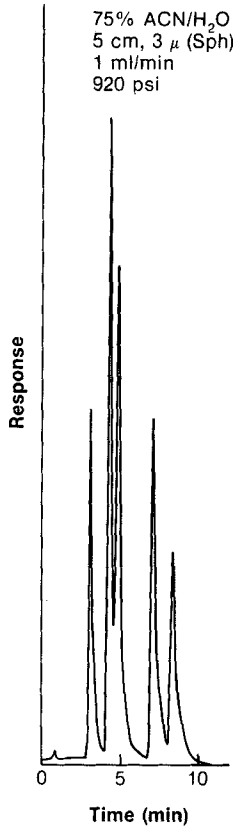

Figure 4. Same as figure 3, but 5 cm cloumn.

1-4) indicate that, other than peak broadening, due to the 100 μl injector loop and the long tube connections to the column and the detector, the short columns can be adapted to standard instruments with a small loss of resolution which may not affect the results drastically.

Figures 1 and 2 show the chromatograms from 10 and 5 cm columns packed with 5μ ODS Spherisorb material. It is clear, from the figures, that in both columns the mixture was separated into its five components. However, the peaks were broader with the 10 cm column. When back pressure, peak heights and retention times are compared, the result favor the shorter

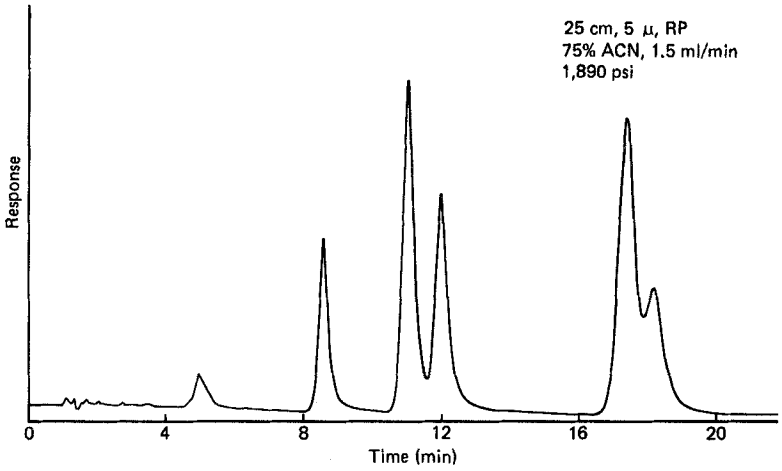

Figure 5. Same as Figure 1, but 25 cm column packed with 5μ irregular shape ODS.

column by approximately 2 to 1. It was quite surprising that the 5 cm column performed as well as the 10 cm column. This was also true when the same test mixture was injected into the 10 cm and 5 cm columns packed with 3μ ODS Spherisorb material (Figures 3 and 4). However, the peak heights differential, i.e., sensitivity, observed with 5μ packed columns (Figures 1 and 2) was absent here.

When the results obtained using the 3μ and 5μ packed columns were compared, no significant differences in separation was observed. The differences observed were in retention times and back pressures. Otherwise each set of columns separated the five components mixture. The chromatographer can probably double the flow rate, using the 10 cm column packed with 5μ particles, without any loss of resolution, but shortening the retention times.

The results with the 10 and 25 cm columns packed with irregular 5μ ODS materials (Figures 5 and 6) were different from those using 3 and 5μ Spherisorb packings (Figures 1-4). The 25 cm column (Figure 5) did not give significantly improved resolution when the results are compared with those with the 10 cm column (Figure 6).

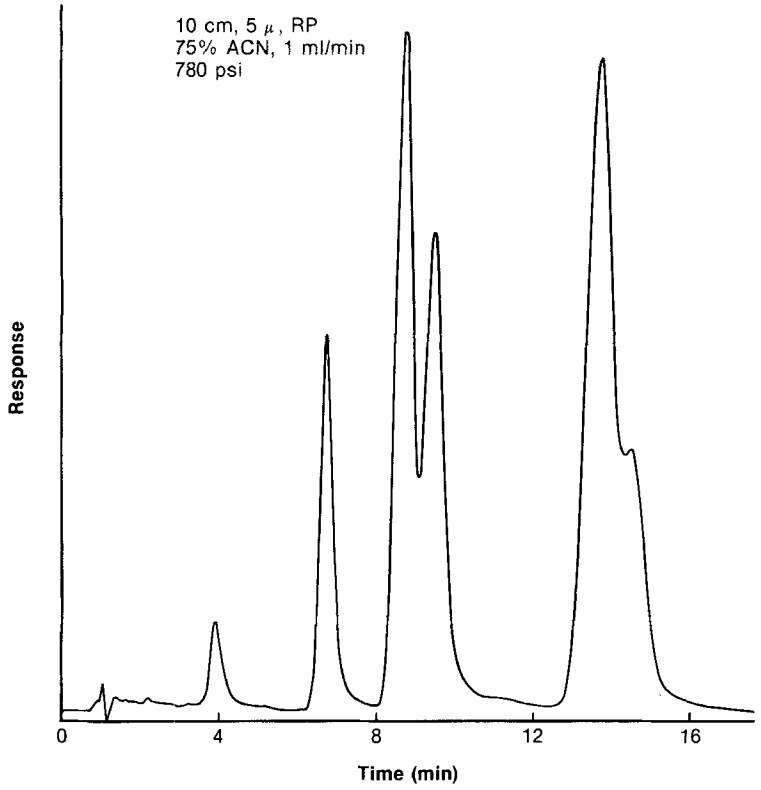

Figure 6. Same as Figure 5, but 10 cm column.

CONCLUSION

The results showed that when the physical properties of the particles are closely related, the differences in the results with 3 and 5μ particles or 5 and 10 cm columns is minimal. The results also showed that there was a difference, under our experimental conditions, between columns packed with spherical and irregular particles. This is because both particles were manufactured under different conditions by different manufacturers. This may be due to carbon loading, particle diameter and particle size distribution and end capping.

It is also clear that separations take place in the first few centimeters of a column, and increasing the length of the column does not improve the resolution significantly, although this will increase the retention time and back pressure. This phenomenon has been reported by Regnier (2) for large molecules.

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