Waters
Lab Highlights
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COLUMN COMPARISONS - CAVEAT EMPTOR

Recently, an article appeared in LC Magazine titled "Comparison of Six Different Reversed-Phase HPLC Columns Used to Separate Benzo[α]pyrene Metabolites (1). In this article, the authors seem to downgrade the performance of a Radial-PAK™ cartridge, but there is more to the comparison than at first meets the eye. The following C_{18} columns were included in the study, which involved the gradient separation of nine compounds: 1) a 10 cm X 4.6 mm 3μ Short-One® (Rainin), 2) a 25 cm X 4.6 mm 10μ HC ODS/Sil-X (Perkin Elmer), 3) a 10 cm X 5 mm Radial Compression Radial-PAK cartridge presumably 10μ RESOLVE™ C_{18}, and 4-6) three 25 cm X 4.6 mm 10μ 600RP columns (Alltech). The choices were arbitrary. Criteria for inclusion in the study ranged from attractive specifications to low price. The authors' conclusions regarding resolving power, speed, durability, and cost are listed in Table 1. Representative chromatograms are reproduced in Figures 1-6.

Space does not permit discussion of all the details of this paper, but there is much in this study to criticize. A 3μ packing is compared to several 10μ packings. It should hardly be surprising, then, that the 3μ column was found to have the sharpest peaks. The authors claim to have maximized the resolution obtainable on each column, but only methanol and water were employed as solvents, and gradients were constructed of linear segments only. Some columns were operated at controlled temperatures, and others at ambient (and variable) temperature. The major criterion for judgement of resolution appears to have been biased toward a column's ability to resolve the peaks labelled G and H; thus, Column 2, which shows a small shoulder for Compound G, is rated better than the Radial-PAK™ cartridge despite the fact that the Radial-PAK™ cartridge has superior resolution of close eluting peaks in every other instance (C and D, E and F, H and J).

If we look at the reported problems associated with the various columns we find that Column 1 cannot take the stress of gradient elution and is therefore short-lived; Column 2 has so-so resolution; and Columns 4-6, which should give similar results, in fact do not and also require 40 minute equilibrations between runs. Where does this leave the RCSS? Only with by far the shortest analysis and equilibration times and very reasonable resolution for a 10μ packing working with a difficult mixture and without any clear indication that the separation has in fact been optimized. Comparison studies can be valuable, but one should avoid making conclusions regarding the quality of a product unless the comparison is unbiased and adequately controlled. Regrettably, there appears to be both a degree of bias and a lack of consistent control in the present case.

1. Louis Swain and Paul Melius, LC 2 (9), 670 (1984).

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Peaks:
A = DMSO
B = BaP 9,10-diol
C = BaP 4,5-diol
D = BaP 7,8-diol
E = BaP 1,6-dione
F = BaP 3,6-dione
G = BaP 6,12-dione
H = 9-OH-BaP
I = 6-OH-BaP
J = 3-OH-BaP
K = BaP

TABLE 1: COMPARISON OF RESOLVING POWER, SPEED, DURABILITY, AND COST OF SIX HPLC COLUMNS

Category	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
Resolving power	1*	4	5	3	6	2
Speed**	2	3	1	4	4	4
Durability	4	1	3	2	2	2
Cost	1	1	2	3	3	3

* 1 was the highest rating; 6 was the lowest rating.
** Both the total elution and reequilibration times were taken into consideration in this category.

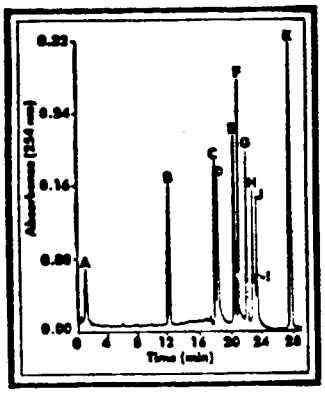


FIGURE 1: Chromatogram of a synthetic mixture of BaP metabolites using Column 1.

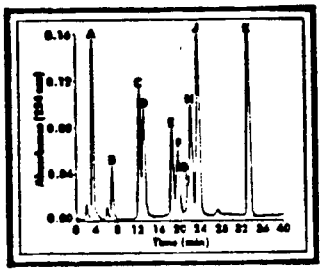
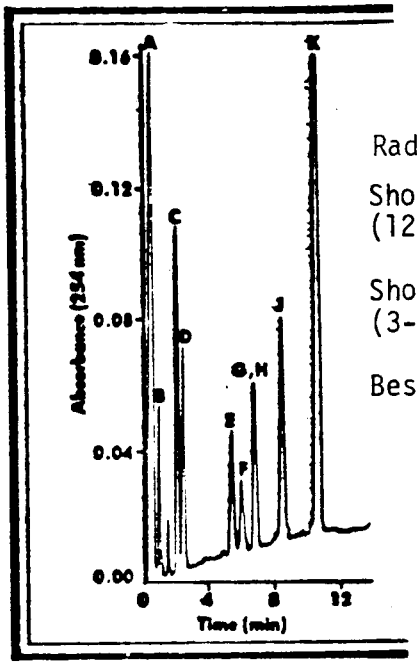


FIGURE 2: Chromatogram of a synthetic mixture of BaP metabolites using Column 2.



Radial Compression Separation System

Shortest Run Time
(12 minutes)

Shortest Re-equilibration Time
(3-4 minutes)

Best Resolution of:
Peaks C & D
Peaks E & F
Peaks H & J

FIGURE 3: Chromatogram of a synthetic mixture of BaP metabolites using Radial-PAK cartridge (Column 3).

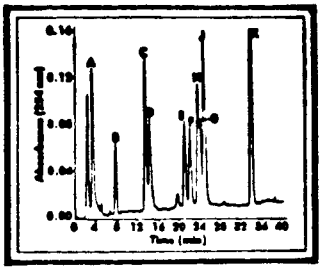


FIGURE 4: Chromatogram of a synthetic mixture of BaP metabolites using Column 4.

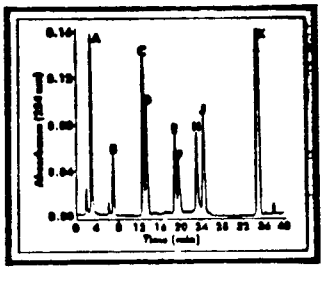


FIGURE 5: Chromatogram of a synthetic mixture of BaP metabolites using Column 5.

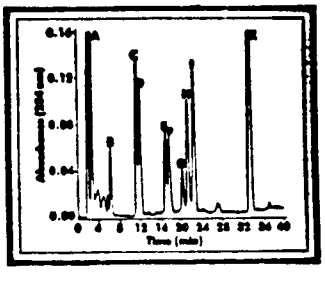


FIGURE 6: Chromatogram of a synthetic mixture of BaP metabolites separated using Column 6.