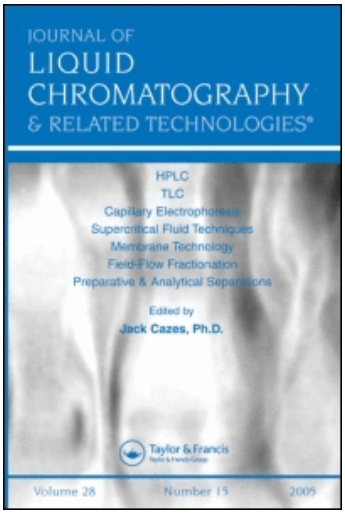

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Separation of Mycotoxins, Polycyclic Aromatic Hydrocarbons, Quinones, and Heterocyclic Compounds on Cyclodextrin Bonded Phases: An Alternative LC Packing

Daniel W. Armstrong[a]; Ala Alak[a]; Wade Demond[a]; Willie L. Hinze[b]; Terrence E. Riehl[b]
[a] Department of Chemistry, Texas Tech University Lubbock, Texas [b] Department of Chemistry, Wake Forest University Winston-Salem, North Carolina

To cite this Article Armstrong, Daniel W. , Alak, Ala , Demond, Wade , Hinze, Willie L. and Riehl, Terrence E.(1985) 'Separation of Mycotoxins, Polycyclic Aromatic Hydrocarbons, Quinones, and Heterocyclic Compounds on Cyclodextrin Bonded Phases: An Alternative LC Packing', Journal of Liquid Chromatography & Related Technologies, 8: 2, 261 — 269
To link to this Article: DOI: 10.1080/01483918508067076
URL: http://dx.doi.org/10.1080/01483918508067076

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(2), 261-269 (1985)

SEPARATION OF MYCOTOXINS, POLYCYCLIC AROMATIC HYDROCARBONS, QUINONES, AND HETEROCYCLIC COMPOUNDS ON CYCLODEXTRIN BONDED PHASES: AN ALTERNATIVE LC PACKING

Daniel W. Armstrong*[1], Ala Alak[1], Wade DeMond[1], Willie L. Hinze[2], and Terrence E. Riehl[2]

[1]*Department of Chemistry*
Texas Tech University
Lubbock, Texas 79409
[2]*Department of Chemistry*
Wake Forest University
Winston-Salem, North Carolina

ABSTRACT

β-Cyclodextrin and γ-cyclodextrin chiral bonded phases were previously shown to be useful in the separation of enantiomers, diastereomers and structural isomers. In this work it is demonstrated that these stationary phases are also useful in more routine separations. As such, they provide an alternative to the popular reverse phase packings. Because the selectivity of cyclodextrin packings is often unique they can be used to compliment conventional columns, particularly when separating complex mixtures where peak overlap is a problem. The separation of several important classes of compounds is used to demonstrate the general utility of this packing.

0148-3919/85/0802-0261$3.50/0

INTRODUCTION

Cyclodextrin bonded phase LC columns have been shown to be useful in separating enantiomers (1-4), diastereomers (2,5) and structural isomers (2,5-7). It has also been suggested that cyclodextrin columns might prove useful as an alternative to reverse phase columns in a variety of more conventional separations (1,2). The selectivity of cyclodextrin columns is often different from that of conventional reverse phase columns because the separation mechanism is based on inclusion complex formation (1-10). As a result, there is the possibility that one can resolve specific peaks from a complex mixture that are not easily resolved on more conventional packings. In this work the separation of several mycotoxins, polycyclic aromatic hydrocarbons (PAH's), quinones and heterocyclic compounds is examined. Emphasis is placed on the β-cyclodextrin packing (which seems to be the optimum size) although the PAH's are also separated on the γ-cyclodextrin packing.

EXPERIMENTAL

Materials. β-Cyclodextrin columns (4.6 x 100 mm and 4.6 x 250 mm) and γ-cyclodextrin columns (4.6 x 100 mm) were obtained from Advanced Separation Technologies, Inc. HPLC-grade methanol and water were obtained from Burdick and Jackson, polycyclic aromatic hydrocarbon standards were from Supelco, mycotoxin standards were from Sigma, and the quinones and heterocyclic compounds were from Aldrich.

Methods: All separations were done at room temperature (20°C) using a Shimadzu Model LC-4A liquid chromatograph with a variable wavelength detector containing a 13 µl flow cell. All samples were dissolved in methanol prior to injection. Exact separation conditions are given with each chromatogram.

RESULTS AND DISCUSSION

The separation of mycotoxins is an important problem from both a military (e.g., yellow rain) and agricultural (e.g., fungal growth on grain) point of view (11-14). Stahr and co-workers have done a considerable amount of work on the chromatographic separation of these compounds (12-14). Many mycotoxins are also effectively separated by LC using cyclodextrin packings. Figure 1 shows the gradient LC separation of T-2 tetraol, verrucurol, T-2 triol, HT-2 toxin, diacetoxyscirpenol, and T-2 toxin on a 10 cm β-cyclodextrin column. All components were baseline resolved. The only possible difficulty with this separation is that ultraviolet detection must be at a fairly short wavelength (206 nm). This results in a slightly increasing baseline when solvent gradients are used, unless one employes a computer baseline correction as in Figure 1.

The reverse phase LC separation of polycyclic aromatic hydrocarbons (PAH's) is well documented (15-19). It has been noted that there can be considerable differences in selectivity

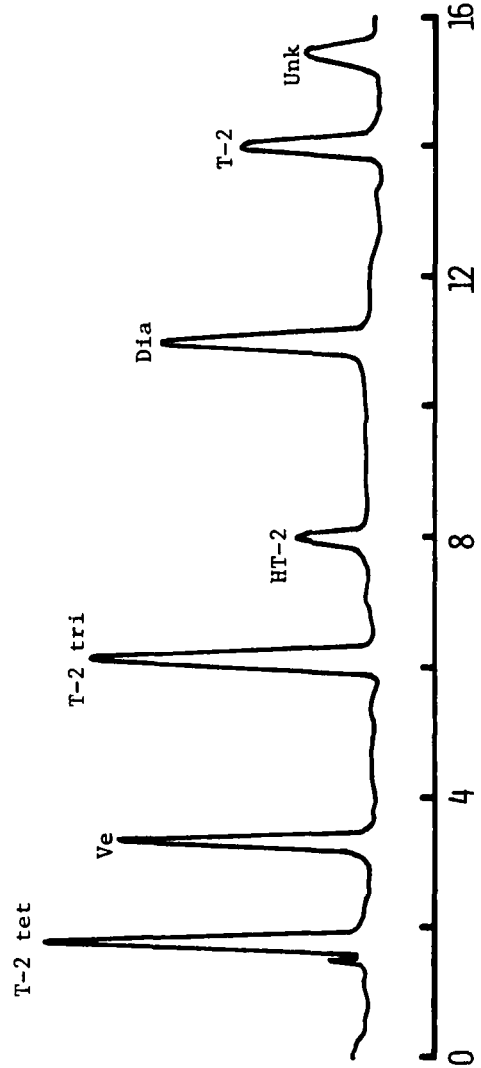

Figure 1. Chromatogram showing the separation of the mycotoxins (from left to right): (1) T-2 tetraol, (2) verrucurol, (3) T-2 triol, (4) HT-2 toxin, (5) diacetoxyscirpenol, (6) T-2 toxin, and (7) Unk = an unknown peak. The separation was done on a 10 cm β-cyclodextrin column with a gradient from 5% methanol (aq) to 25% methanol (aq) in 20 min. The flow rate was 1 ml/min and the detection wavelength was 206 nm. Computer leveling of the baseline was used.

with different types and/or lots of reverse phase packing material (15-19). Overlapping peaks can be a significant problem in separations involving complex mixtures of PAH's. Consequently two or more columns of different selectivity are sometimes used to completely resolve all components of a mixture. Both β- and γ-cyclodextrin packings effectively separate a variety of PAHs (Figure 2). Not only is the selectivity different from that of traditional reverse phase columns, but isomers of compounds such as benzopyrene and dibenzanthracene are easily separated as well (Figure 2A). In addition, there are considerable selectivity differences for some compounds on the β-cyclodextrin packing as compared to the larger γ-cyclodextrin packing. For example, the selectivity factor (α) for acenaphthene (relative to benzo(a)pyrene) goes from 1.3 on the β-cyclodextrin column to 0.45 on the γ-cyclodextrin column (Figure 2).

A particularly interesting separation involves a series of structurally related compounds. Fluorene, carbazole, dibenzothiophene, dibenzofuran and biphenyl differ only in the type or presence of a heteroatom between the two aromatic rings All are easily resolved on a 25 cm β-cyclodextrin column (Figure 3). Figure 4 illustrates the separation of a series of quinones on the same column.

In conclusion, it has been shown that the effectiveness of cyclodextrin bonded phase packings is not limited to the separation of enantiomers (as are many other chiral stationary

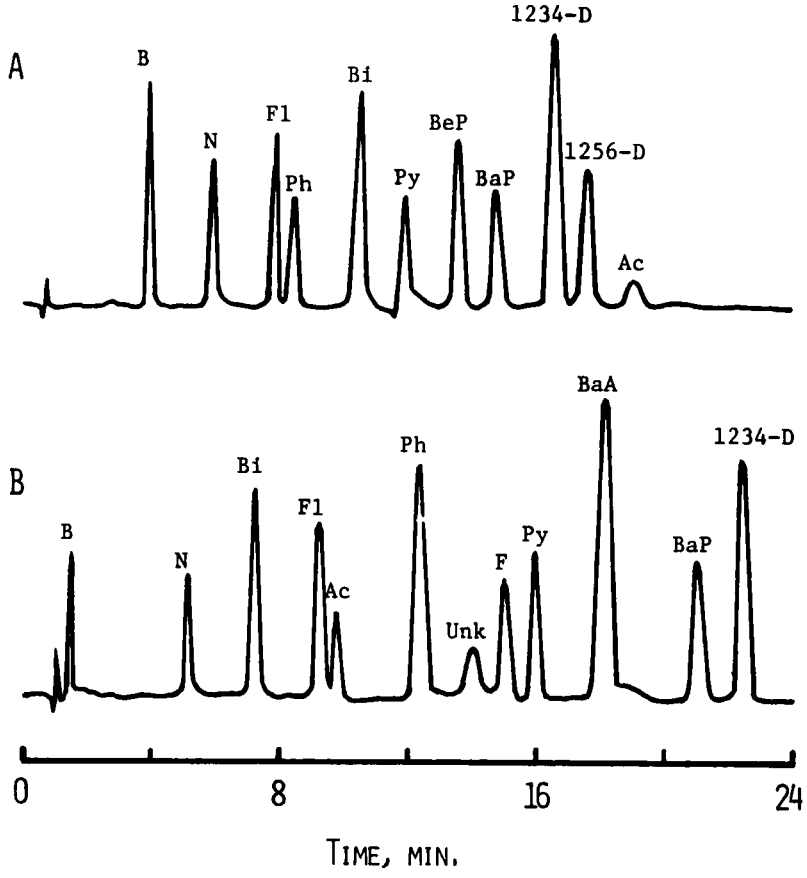

Figure 2. Chromatogram "A" shows (from left to right) the separation of: (1) benzene, (2) naphthalene, (3) fluorene, (4) phenanthrene, (5) biphenyl, (6) pyrene, (7) benzo(e)pyrene, (8) benzo(a)pyrene, (9) 1,2,3,4-dibenzanthracene, (10) 1,2,5,6-dibenzanthracene, and (11) acenaphthene on a 10 cm β-cyclodextrin column with a gradient going from 40% to 70% methanol (aq) in 25 min. The flow rate was 1.5 ml/min and the detection wavelength was 254 nm. Chromatogram "B" shows (from left to right) the separation of: (1) benzene, (2) naphthalene, (3) biphenyl, (4) fluorene (5) acenaphthene, (6) phenanthrene, (7) unknown peak, (8) fluoranthene, (9) pyrene, (10) benzo(a)anthracene (11) benzo(a)pyrene, and (12) 1,2,3,4-dibenzanthracene on a 10 cm γ-cyclodextrin column with a gradient going from 30% to 65% methanol (aq) in 25 min. Other conditions were as in chromatogram "A".

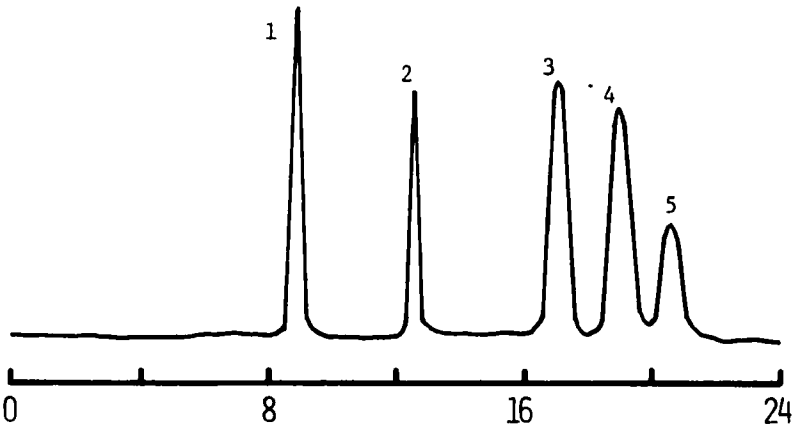

Figure 3. An LC chromatogram of (1) carbazole, (2) fluorene, (3) dibenzothiophene, (4) biphenyl, and (5) dibenzofuran on a 25 cm β-cyclodextrin column. This was an isocratic separation (50% methanol (aq)). The flow rate was 1.0 ml/min and the wavelength of detection was 250 nm.

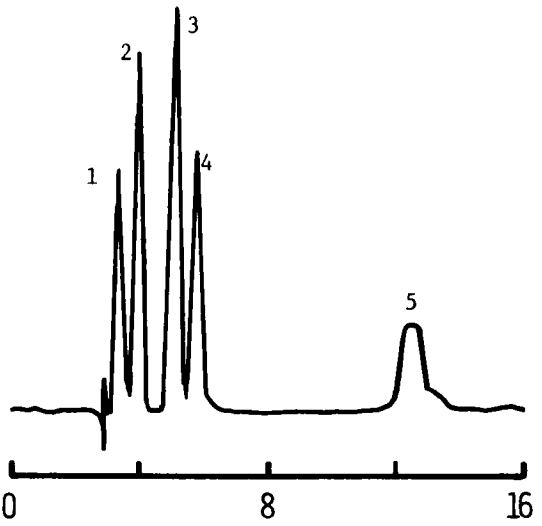

Figure 4. An LC chromatogram of (1) 1,4-benzoquinone, (2) 2,5-dimethyl-p-benzoquinone, (3) 1,2-naphthaquinone, (4) 1,4-naphthaquinone, and (5) anthraquinone. This isocratic separation was done on a 25 cm β-cyclodextrin column (45% methanol (aq)). The flow rate was 1.0 ml/min and the wavelength of detection was 254 nm.

phases) or to the separation of diastereomers and structural isomers. Indeed, it appears to be a generally useful packing in the "reverse phase mode" and its unusual selectivity makes it a useful complimentary column for those using traditional reverse phase packings in the separation of complex mixtures.

ACKNOWLEDGEMENT

Support of this work by the Department of Energy (DE-AS05-84ER13159) is gratefully acknowledged.

REFERENCES

1. Armstrong, D. W., Chiral Stationary Phases for High Performance Liquid Chromatographic Separation of Enantiomers: A Mini-Review, J. Liq. Chromatogr., 7, Suppl.2, 353 (1984).

2. Armstrong, D. W., DeMond, W., Cyclodextrin Bonded Phases for the Liquid Chromatographic Separation of Optical, Geometrical and Structural Isomers, J. Chromatogr. Sci., 22, 411, (1984).

3. Hinze, W. L. Riehl, T. E., Armstrong, D. W., DeMond, W., Alak, A., Ward, T., Liquid Chromatographic Separation of Enantiomers Using a Chiral β-Cyclodextrin Bonded Phase and Conventional Aqueous-Organic Mobile Phases, Anal. Chem., in press (1984).

4. Armstrong, D. W., DeMond, W., Czech, B., Separation of Organo-Metallic Enantiomers by Liquid Chromatography, submitted (1984).

5. Armstrong, D. W., DeMond, W., Alak, A., Hinze, W. L., Riehl, T. E., Bui, K. H., LC Separation of Diastereomers and Structural Isomers on Cyclodextrin Bonded Phases, submitted (1984).

6. Fujimura, K., Ueda, T., Ando, T., Retention Behavior of Some Aromatic Compounds on Chemically Bonded Cyclodextrin Silica Stationary Phase in Liquid Chromatography, Anal. Chem., 55, 446 (1983).

7. Kawaguchi, Y., Tanaka, M., Nakae, M., Funazo, K., Shono, T., Chemically Bonded Cyclodextrin Stationary Phases for Liquid Chromatographic Separation of Aromatic Compounds, Anal. Chem., 55, 1852 (1984).

8. Bender, M. L., Komiyama, M., Cyclodextrin Chemistry, Springer-Verlag, Berlin, 1978.

9. Hinze, W. L., Applications of Cyclodextrins in Chromatographic Separations and Purification Methods, Sep. Purific. Methods, 10, 159 (1981).

10. Szejtli, J., Cyclodextrins and Their Inclusion Complexes, Adademiai Kiado, Budapest, 1982.

11. Ember, L. R., Yellow Rain, Chem. Eng. News, 62, 8 (1984).

12. Stahr, H. M., Domoto, M., Adv. Thin Layer Chromatogr. (Proc. Bienn. Symp.) 2nd 1980, Touchstone, J. C., Ed., Wiley, NY, 1980, p. 403.

13. Stahr, H. M., Lerdal, D., Pfeiffer, R., Analytical Procedures for Trichlothecene Micotoxins, Appl. Spec., 37, 396 (1983).

14. Stahr, H. M., Advances in the Application of TLC to Diagnostic Toxicology, J. Liq. Chromatogr., 6, 123 (1983).

15. Ogan, K., Katz, E., Retention Characteristics of Several Bonded-Phase Liquid Chromatographic Columns for Some Polycyclic Aromatic Hydrocarbons, J. Chromatogr., 188, 115 (1980).

16. Ogan, K., Katz, E., Selectivity Factors for Several PAH Pairs on C_{18} Bonded Phase Columns, J. Liq. Chromatogr., 3, 1151 (1980).

17. Wise, S. A., May, E. W., Effect of C_{18} Surface Coverage on Selectivity in Reversed-Phase Liquid Chromatography of Polycyclic Aromatic Hydrocarbons, Anal. Chem., 55, 1479 (1983).

18. May, W. E., Wise, S. A., Liquid Chromatographic Determination of Polycyclic Aromatic Hydrocarbons in Air Particulate Extracts, Anal. Chem., 56, 225 (1984).

19. Sander, L. C., Wise, S. A., Synthesis and Characterization of Polymeric C_{18} Stationary Phases for Liquid Chromatography, Anal. Chem., 56, 504 (1984).