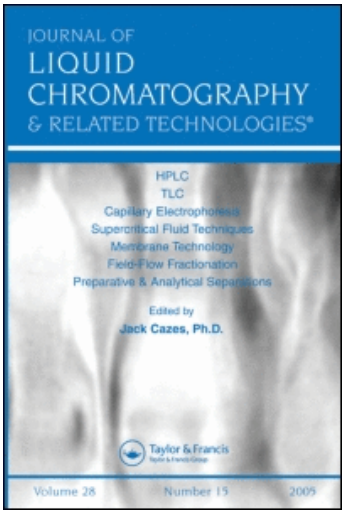

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Facile Liquid Chromatographic Separation of Positional Isomers with a γ-Cyclodextrin Bonded Phase Column

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FACILE LIQUID CHROMATOGRAPHIC SEPARATION OF POSITIONAL ISOMERS WITH A γ-CYCLODEXTRIN BONDED PHASE COLUMN

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ABSTRACT

Facile isocratic liquid chromatographic separation of positional isomers of substituted anilines is demonstrated using a γ-cyclodextrin(γ-CD) bonded phase column. It is found that normal phase separation is more effective than reversed-phase separation for these compounds. The effect of mobile phase composition on the retention of several substituted anilines is also examined. A minimum value of the capacity factor (k') for each compound is seen at a mobile phase composition of 60% 2-propanol in water and a rationalization of this unusual observation is proposed. The effect of cavity size of cyclodextrins on the retention of solutes is also discussed.

INTRODUCTION

It is now generally accepted that optimization of separation requires information on both physical properties of column, i.e. packing particle size, surface area, pore size, etc. and chemical properties of column, i.e. derivatization of packing material, as well as mobile phase eluotropic strength (1-3). Among those factors, the choice of column and mobile phase composition turns out to be the most important if others cannot be further improved. A number of reports have dealt with optimization by selecting the best combination of mobile phase solvents (4). However, for optimization

of separation, first-priority should be given to the choice of a column with great intrinsic separation power. Also, a "universal" column that can be used for both reversed-phase as well as normal phase separation would be advantageous for most chromatographers. In this regard, it is known that some bonded phase columns such as octadecylsilica, amine or cyano derivatized silica have shown some success. For example, the amine bonded phase column can be used in water/acetonitrile mixture to separate carbohydrates (5,6). It can also be used in a normal phase mode to separate a number of other complex mixtures such as phenoxyherbicides and substituted benzoic acids (7,8).

Recently we and others have reported the use of cyclodextrin (CD) bonded phase columns for the separation of a large number of optical, geometrical, and structural isomers (8-13). In particular, reversed-phase separations with β-cyclodextrin (β-CD) columns have been the most frequently exercised. On the other hand, the normal phase separations using CD columns cannot be overlooked (14). In this paper, we report the facile separation of a mixture of positional isomers of substituted anilines under either a normal or a reversed-phase mode using a γ-cyclodextrin bonded phase column (γ-CD).

EXPERIMENTAL SECTION

A Beckman Model 332 gradient liquid chromatographic system was used for the cyclodextrin bonded phase column separation. The cyclodextrin bonded phase columns (both β- and γ-CD) were obtained from Advanced Separation Technologies, Inc. The analytes, i.e. substituted anilines, were obtained mostly from Aldrich Chemical Co.. HPLC grade solvents were obtained from Fisher Chemical Co.. Chromatographic procedures were similar to those reported earlier (13-15). Column pre-equilibrium was always achieved before any separation was carried out. A flow rate of 1 ml/min. was set for all the chromatographic processes. A previously reported method was used to determine t_o values for the γ-CD column (16).

RESULTS AND DISCUSSION

The γ-CD bonded phases are prepared using 5μ silica according to a published method which is similar to that of β-CD bonded phases (17). In general, the first step involves the derivatization of non-nitrogen containing hydrocarbon epoxide functional groups on silica surface. The epoxide functional groups are then reacted with the cyclodextrin molecules to form the final bonded phases. Due to the difficulties in controlling the extent of the reactions described above, the resulting bonded phases always have mixed-surface coverage with unreacted silanol, diol (resulted from hydrolysis of unreacted epoxide functional groups), and cyclodextrin moieties. However, the major contribution on the retention of substrates is probably still due to the bonded cyclodextrin molecules because (1) there are not as many hydroxy groups on the diols and (2) the unreacted silanol groups are less accessible due to the presence of diol and bulky cyclodextrin groups. This can be further demonstrated by the excellent separation of substituted anilines and the different elution order for a number of compounds exerted by the γ-CD column as compared to the common silica column described below. None-the-less, the limited contribution from the silanol and diol groups toward the observed separation selectivity cannot be ruled out.

Table I lists the capacity factors (k') for a number of substituted anilines as a function of mobile phase composition. Because of the fact that the γ-CD column can be used under both normal and reversed-phase mobile phase conditions, data are obtained in a systematic fashion starting from a 2-propanol/heptane mobile phase combination through 100% 2-propanol to a 2-propanol/water combination. It is interesting to observe that lowest k' values for these compounds do not occur at 100% 2-propanol but at 60% 2-propanol in water (Figure 1). Similar phenomenon is also observed when a β-CD column is used. Tentative rationalization of this fact can be provided by the reason that the 2-propanol forms rather strong inclusion

Table I. Capacity factors (k') of substituted anilines at various mobile phase compositions using a γ-CD bonded phase column.[a]

	% 2-Propanol in heptane					2-Propanol	% 2-Propanol in water				
	7.5%	20%	40%	60%	80%	100%	80%	60%	40%	20%	10%
N,N'-dimethylaniline	0.13	0.13	0.13	0.12	0.11	0.11	0.10	0.08	0.17	0.86	1.49
p-Methylaniline	1.56	0.92	0.55	0.38	0.38	0.35	0.10	0.08	0.11	0.45	0.74
Aniline	1.72	0.97	0.56	0.37	0.37	0.33	0.11	0.09	0.12	0.30	0.42
e-Nitroaniline	2.68	1.07	0.47	0.24	0.24	0.15	0.09	0.08	0.14	0.83	1.45
p-Chloroaniline	2.68	1.28	0.67	0.41	0.41	0.33	0.09	0.08	0.11	0.6	1.11
p-Methoxyaniline	3.73	1.98	1.07	0.72	0.72	0.62	0.14	0.11	0.15	0.48	0.72
m-Nitroaniline	6.23	2.09	0.84	0.37	0.37	0.21	0.10	0.08	0.12	0.51	0.78
p-Nitroaniline	18.6	4.33	1.28	0.44	0.44	0.22	0.11	0.09	0.14	0.60	1.01

[a] The relative error is within 1%. Flow rate = 1 ml/min. 25°C

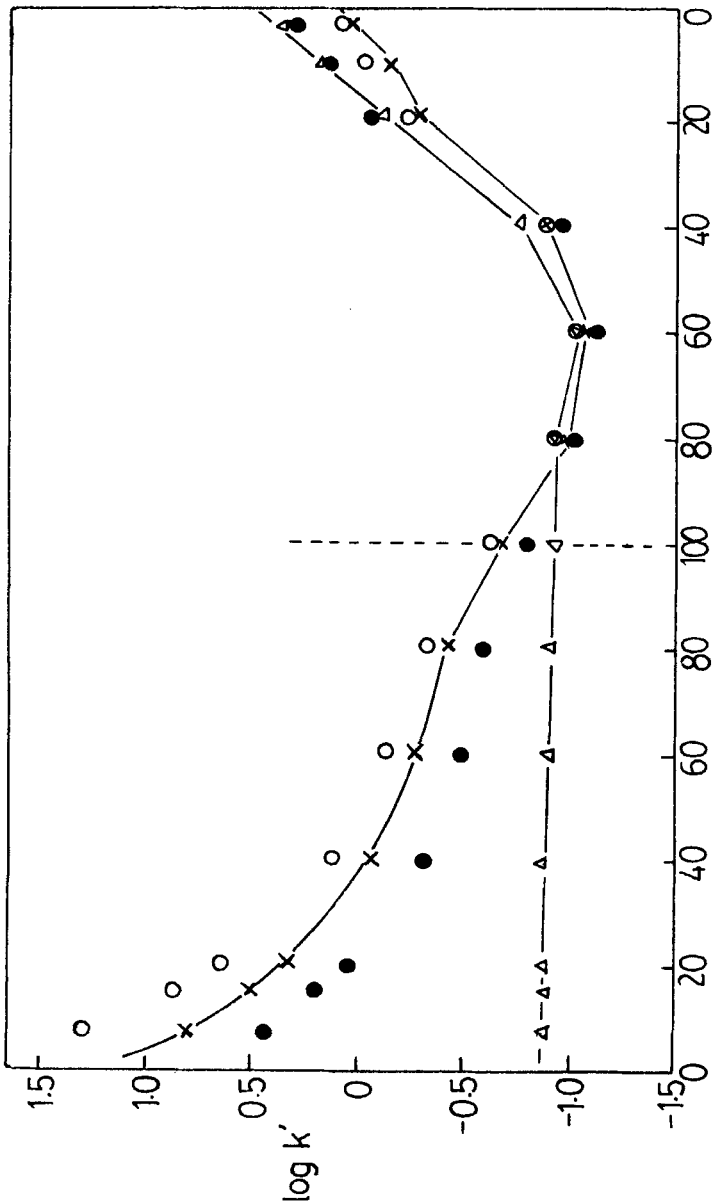

FIGURE 1. Plot of log k' values vs. mobile phase composition for N,N-dimethylaniline(Δ) and nitroaniline isomers; ortho(•); meta-(x); and para-(o); using a γ-CD bonded phase column.

complex with the cyclodextrin molecule and the large amount of 2-propanol can prevent significant inclusion of the substituted aniline. In the normal phase separation mode, the H-bonding between substrate and cyclodextrin seems to be the major interaction. Note that the inclusion process, is not significant in normal phase chromatography as evidenced by the fact that chiral resolution is ineffective. When water is introduced, the hydroxyl groups on cyclodextrin molecules are masked to a even greater extent. Thus, without appreciable H-bonding and inclusion complex formation with the bonded cyclodextrin molecules, substituted anilines shows the lowest k' values at an aqueous mobile phase containing 60% 2-propanol. Further increase of water content would facilitate the inclusion process, therefore increases the k' values.

Some effects of the mobile phase and the size of cyclodextrin cavity can also be seen from the data. For example, for nitroanilines, the retention times are o < m < p at all normal phase separations for both β-CD and γ-CD columns (14). The order changes to m < o < p for β-CD column but m < p < o for γ-CD column when in reversed-phase mode with water content greater than 60%. This indicates that p-nitroaniline forms a strong β-CD inclusion complex in a hydrophilic environment due to good size fit. However, it is unable to do so with γ-CD molecule apparently because of the larger cavity size of γ-CD.

Figure 2 is the chromatogram for the normal phase separation of sixteen substituted anilines using the γ-CD column under simple isocratic condition. A reversed-phase separation chromatogram is also shown in Figure 3. By comparison, it is concluded that the normal phase separation is superior in the present case. The peak half-widths are always broader for the reversed-phase retention presumably due to the slow kinetic event involved in the inclusion process. On the other hand, from a practical point of view, it is recommended that for normal phase separations using CD columns, the concentration of strong organic modifier (e.g. alcohol)

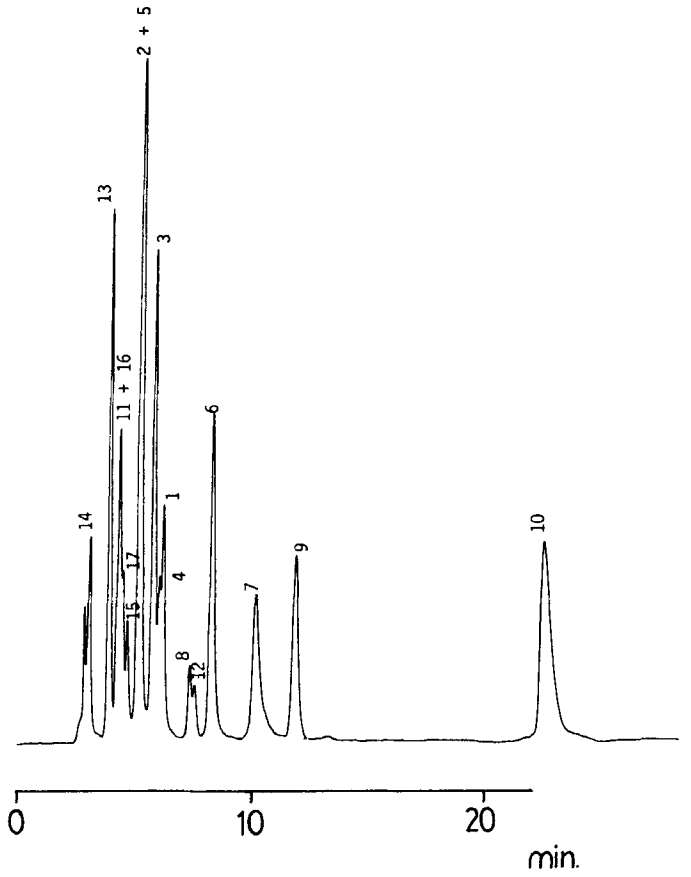

FIGURE 2. Normal phase separation (2-propanol:heptane = 7.5:92.5, v/v) of several substituted anilines using a γ-CD bonded phase column. Flow rate = 1 ml/min. 25°C.

Peak identification:

1. Aniline
2. o-toluidine
3. m-toluidine
4. p-toluidine
5. o-methoxyaniline
6. m-methoxyaniline
7. p-methoxyaniline
8. o-nitroaniline
9. m-nitroaniline
10. p-nitroaniline
11. o-chloroaniline
12. p-chloroaniline
13. N-methylaniline
14. N,N-dimethylaniline
15. 2,5-dimethylaniline
16. 2,6-dimethylaniline
17. o-ethylaniline

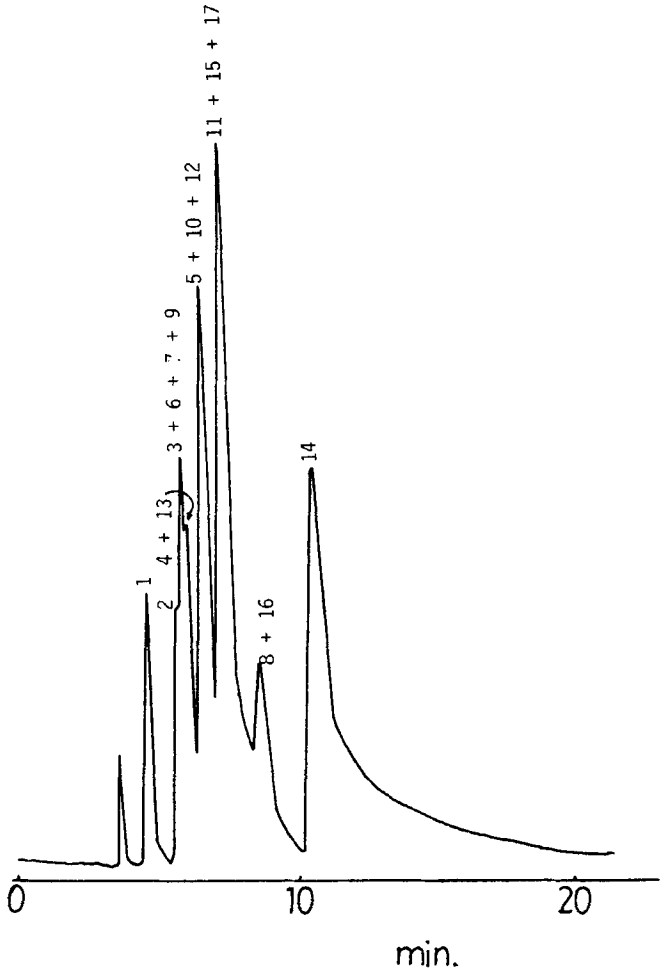

FIGURE 3. Reversed-phase separation (2-propanol: water = 3:97, v/v) of several substituted anilines using a γ-CD bonded phase column. Flow rate = 1 ml/min. 25°C
Peak identification is the same as Figure 2.

should be kept low to maximize the substrate-bonded phase interaction for optimum resolution. While for reversed-phase separations, the water content should be high to facilitate the inclusion complex formation for better results.

Finally, it is of interest to compare the separation of substituted anilines using both β-CD and γ-CD columns with that using Partisil PXS ODS column. In general, other than the differences in retention order of a few positional isomers, the separation power of γ-CD is similar to that of the β-CD column in the normal phase chromatography, which in turn is better than that of the Partisil PXS ODS column. This is possibly due to the larger number of hydroxyl groups available for interaction on both β-CD and γ-CD columns as compared to the number of silanol groups on Partisil PXS ODS column. On the other hand, in reversed-phase chromatography, the β-CD column is better than both γ-CD and Partisil PXS ODS columns presumably because of the good size fit for analyte/β-CD inclusion complex formation. Although the cavity of γ-CD molecule provides a reversed-phase environment which is more or less similar to the environment of the Partisil PXS ODS column, the larger γ-CD cavity size does not generate as satisfactory a separation as compared to the β-CD column.

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