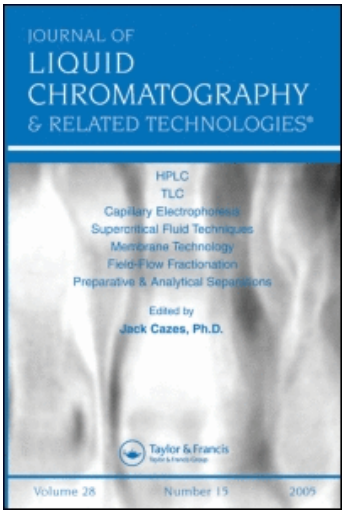

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A SEPARATION STUDY OF TRICYCLIC ANTIDEPRESSANT DRUGS BY HPLC WITH β-CYCLODEXTRIN BONDED STATIONARY PHASE

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ABSTRACT

A systematic study of the separation of a series of tricyclic antidepressant drugs (amitriptyline, nortriptyline, protriptyline, maprotiline, imipramine, chloripramine, doxepin-E and doxepin-Z) using reversed phase HPLC with β-cyclodextrin (β-CD) bonded stationary phase is presented. The effect of the organic modifier (methanol) content and pH value of the aqueous phase of the mobile phase on retention was examined and experimental equations of these dependences were derived.The separation is mainly due to the variations of the strength of the CD-solute complexes and this was proved by the good relationship found experimentally between corrected logk′ (referred to pure aqueous mobile phase) and the log of complex formation constants. Using the chromatographic response function optimization procedure a mobile phase of 50/50 methanol/buffer pH 6.5 was found as optimum for separations of analytical use. The elution order of the solutes was tried to be correlated to various structure differences.

INTRODUCTION

The formation of host-guest (inclusion) complexes has become a valuable tool in many areas of molecular recognition and separation (especially chiral recognition), including chromatography. Cyclodextrins (CDs) are cyclic oligomers of glucose units bonded through α-1,4-linkages. They can complex, via host-guest interaction, various moieties, such as organometallic (1) and organic compounds (2,3). Cyclodextrins have successfully and extensively been used, both as mobile phase additives (4,5) and as chiral bonded stationary phases in High Performance Liquid Chromatography (HPLC) (6-8).

Separation of solutes using cyclodextrins results mainly from the formation of inclusion complexes formed when solute molecules enter the cavity of the CDs. The ability of the solute to form an inclusion complex largely depends on the size, shape and chemical interactions between the solute molecule and the CDs. These interactions are van der Waals, hydrogen bonding and displacement of high-energy water molecules from the cavity. The hydrophobicity of the guest molecule also plays a key role in the stability of the complex and therefore its retention behaviour.

The degree and strength of complex formation, due to the physical orientation of the solute in the toroid structure and the symmetrical arrangement of hydrogen bonding sites of the cyclodextrin bonded stationary phases favour the retention of symmetrical structures in comparison to the traditional alkyl-bonded phases (9, 10).

Since the hydrolytically stable β-CD bonded phase was first developed for liquid chromatography in 1984 by D. Armstrong (11), its uses and the understanding of its behaviour have expanded tremendously (12). It has been used in pharmaceutical analysis in the reversed- (13, 14) and normal- (15) phase modes and in analytical (16, 17), preparative and microcolumn (18) applications.

The first goal of this work is to study the effect of various structural parameters on resolution of a series of structurally similar compounds, such as the tricyclic antidepressant drugs using β-CD bonded stationary phase.

Once a stationary phase is chosen, only the mobile phase composition and pH, at room temperature, significantly affect the separation. For this purpose an experimental mathematical relation was found to correlate the effects of pH and organic modifier on the relative retention of the studied series of tricyclic antidepressant drugs.

The use of tricyclic antidepressant drugs is becoming increasingly prevalent for the treatment of depression (19). The determination of drug levels in the plasma of clinical patients, may be useful in monitoring therapeutic effects as well as compliance and toxic effects. A series of drugs including amitriptyline (AMN), nortriptyline (NRN), protriptyline (PTN), maprotiline (MPN), imipramine (IMN), chloripramine (CMN), and doxepin (DXN) (a racemic mixture of DXN-E and DXN-Z) was used as a model series of structurally related compounds with a great pharmacological interest. It is well understood that the retention times, and therefore the capacity factors (k′), depend on the difference in the ability of the CDs to form inclusion complexes with a given series of solutes. Consequently, it may be useful to investigate a relationship between k′ and the formation constant values (K_f) of drugs. Furthermore, in this study the elution order of these drugs, in identical chromatographic conditions, was evaluated and discussed.

Finally the assessment of separation quality of this method, at different pH values and mobile phase compositions, was qualified using the chromatographic response function (CRF) developed by Berridge (20, 21).

EXPERIMENTAL

Materials

Pure substances of the seven drugs, in the form of hydrochloride salts, were provided by local manufacturers and were used without further purification. MeOH, AcN and water were of HPLC-grade and purchased from Tech-Line. Triethylamine and glacial acetic acid were of analytical reagent grade and purchased from Aldrich.

Instrumentation

The chromatographic system used was a Waters liquid chromatograph (model 590) equipped with an injector (Rheodyne 7125) fitted with a 25-µl sample loop. A β-cyclodextrin column (25 cm × 4.6 mm ID) was employed for all the experiments in this study. The column was a product from Advanced Separation Technologies (Whippany, NJ, USA) under the commercial name Cyclobond I. The detector was a variable-wavelength UV spectrophotometer (Waters Lambda Max model 481) equipped with a 8 µl flow cell. The chromatograms were recorded using a recorder (BBC Goerz Metrawatt, model SE 120).

pH readings were obtained using a Metrohm Herisau pH-meter (model 654).

Methods

Buffers were prepared by making a 1% (w/v) solution of triethylamine in HPLC-grade water and dropwise addition of glacial acetic acid until the desired pH value was obtained.

All separations were done at room temperature (about 25 °C). The compounds were detected at a wavelength of 250 nm. The mobile phases consisting of triethylammonium acetate buffer and the appropriate amount of the organic modifier, were freshly prepared, filtered and degassed under vacuum using a Millipore system.

The stock standard solutions of all drugs (1.00 mg/ml) were accurately prepared by dissolving an appropriate amount of the compounds in HPLC-grade water and were kept in amber bottles in the refrigerator and renewed every week. Working standards (5.0 µg/ml for AMN, NRN, and DXN, 3.4 µg/ml for PTN, IMN and CMN, and 10.0 µg/ml for MPN) were prepared fresh every day in mobile phase. Typically a volume of 25 µl of each solution was injected.

To obtain comparable results, experiments were performed in identical chromatographic conditions i.e., a flow rate of 1.2 ml/min and a sensitivity of 0.02 AUFS. The void volume of the column was determined by injecting each time 25 µl of pure methanol.

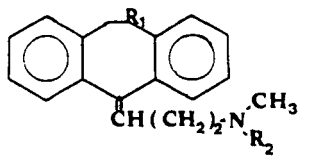
To evaluate the reproducibility of the retention times each run was triplicated.

RESULTS AND DISCUSSION

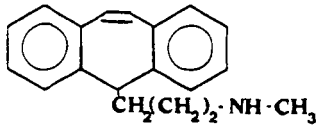
The drugs used are listed in figure 1. They are all tricyclic antidepressant drugs and their determination in biological samples can be accomplished using a variety of analytical techniques (22, 23). In this study, apart from the development of a new analytical technique involving complexation mechanism, the main goal was to investigate in details the selectivity of the CD-bonded stationary phase to achieve reversed-phase separations of closely relative compounds.

All drugs, except of maprotiline, are derivatives of dibenzo-[a,d][1,4]-cycloheptadiene or of 5H-dibenzo-[b,f]-azepine. The similarity of structure and molecular size of these compounds as well as their basicity makes the analytical problem of separation very challenging.

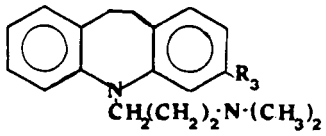
The retention time of a given solute using CD-bonded phase is a function of many factors which effect the formation constant of the complex (K_f). The two most important of these factors, i.e., the mobile phase composition and pH value were investigated in this study at room temperature.



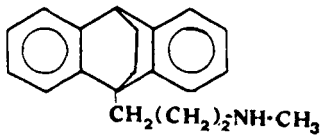
R_1	R_2	
-C-	$-CH_3$	(1) AMN
-C-	-H	(2) NRN
-O-	$-CH_3$	(3) DXN



(4) PTN



R_3	
-H-	(5) IMN
-Cl-	(6) CMN



(7) MPN

Figure 1. Stuctures of the tricyclic antidepressant drugs studied.

Effect of Organic Modifier Content on Retention

Controlling the mobile phase composition is one of the most powerful and ease means of adjusting both retention and selectivity in LC. Many studies have recently showed the effect of the organic modifier on retention. It is important to mention that in this type of separations, involving complexation, the organic modifier competes with guest molecules for the hydrophobic cavity of the CDs. Thus, an increase of the concentration of the organic modifier decreases the interaction between the molecule and the cavity, and results to a lower degree of retention (24).

There are quite a few articles dealing with the dependence of the capacity ratio on the modifier concen-

tration when reversed-phase mode is used. The exact dependence is a function still not clear and experimental linear or quadratic relationships are often used (25, 26).

In this study, the effect of methanol content on retention was investigated at different pH values, within the column pH limitations 3.5-7.5. In order to succeed an acceptable analysis time of the mixture, retention times of 3.5 min for the first eluted solute and 25 min for the last one were chosen as limits for the optimization of the mobile phase composition.

In all cases for this series of drugs the relationship between logk′ and %MeOH content of the mobile phase was a linear function:

$$\log k' = a - b\,(\%\mathrm{MeOH}) \qquad (1)$$

a and b being coefficients, the values of which depend on the solute and pH. Figure 2 shows the variation of the logk′ value on the %MeOH content in the range of 50-80% at pH 6.5 for the seven solutes as a function of methanol concentration in the mobile phase. (The pH values refer to the pH of the aqueous content of the mobile phase). The plots represent the best linear fit of the data. As shown a linear relationship exist between logk′ and the methanol content of the mobile phase. The experimental equations of this effect for all the pH studied are summarized in Table 1 along with the correlation coefficients varied in the range 0.980 - 0.999. The negative slope values, in all cases, are in agreement with the theoretical concept suggesting that an increase of the concentration of the organic modifier will decrease the interaction between the solute and the CD-cavity, and consequently decrease the retention.

Effect of pH on Retention

pH value of the aqueous content of the mobile phase affects retention and selectivity in LC as long as the

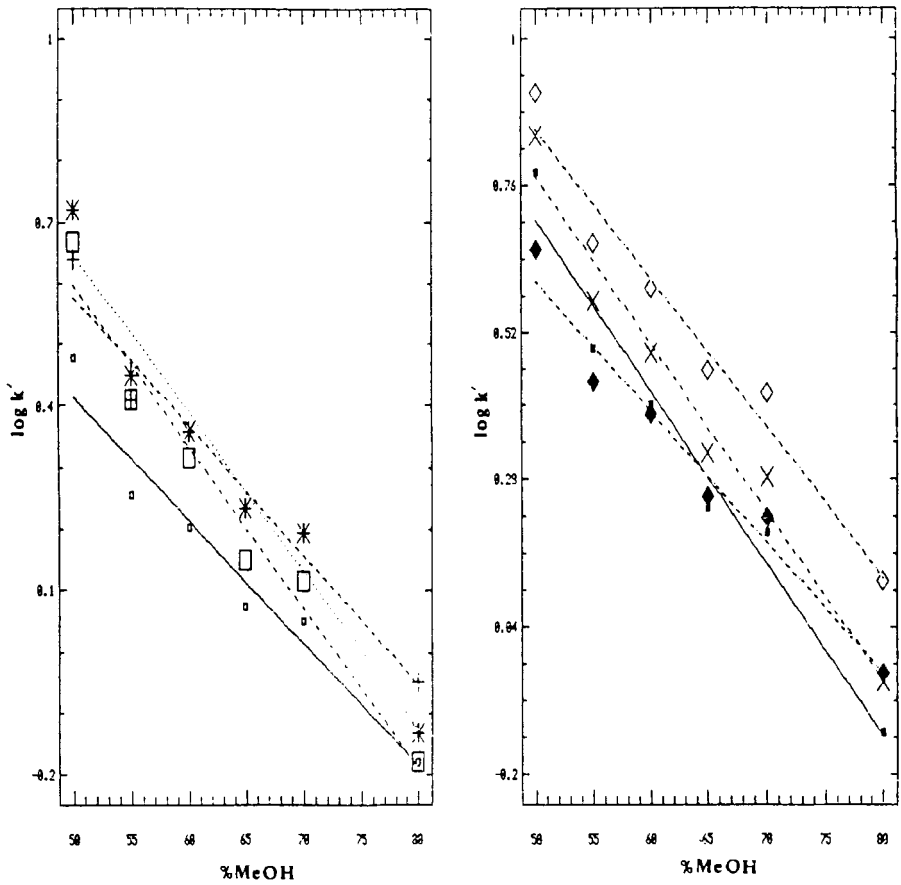


Figure 2. Dependence of retention (logk') on the methanol content of mobile phase at pH 6.5 for the seven antidepressant drugs: (— •) maprotyline, (— •) protriptyline, (- - +) imipramine, (····· *) chloripramine, (- - □) nortriptyline, (···· X) amitriptyline, (--- ◇) doxepin (E-isomer), (--- ♦) doxepin (Z-isomer).

TABLE 1.

Experimental Relationships of logk′ of the Drugs vs %MeOH Content of the Mobile Phase at Various pH (logk′ = a + b(%MeOH), n = number of points).

pH	Component	a	b	r	n	pH	Component	a	b	r	n
4.1	MPN	1.74	-0.040	0.991	6	6.0	MPN	1.61	0.025	0.997	5
	PTN	2.22	-0.041	0.996	6		PTN	2.40	-0.035	0.996	5
	IMN	1.69	-0.036	0.994	6		IMN	1.71	-0.025	0.997	5
	CMN	2.06	-0.043	0.991	6		CMN	2.05	-0.030	0.998	5
	NRN	2.25	-0.045	0.995	6		NRN	2.15	-0.032	0.998	5
	AMN	2.25	-0.042	0.997	6		AMN	2.27	-0.031	0.998	5
	DXN-E	2.16	-0.039	0.997	6		DXN-E	2.10	-0.027	0.998	5
	DXN-Z	1.78	-0.037	0.997	6						
4.5	MPN	1.67	-0.034	0.998	6	6.5	MPN	1.41	-0.020	0.978	6
	PTN	2.36	-0.041	0.998	6		PTN	2.10	-0.028	0.983	6
	IMN	1.70	-0.033	0.996	6		IMN	1.62	-0.021	0.981	6
	CMN	2.04	-0.038	0.995	6		CMN	1.93	-0.026	0.980	6
	NRN	2.24	-0.040	0.998	6		NRN	1.91	-0.026	0.984	6
	AMN	2.17	-0.037	0.992	6		AMN	2.14	-0.027	0.984	6
	DXN-E	1.98	-0.032	0.990	6		DXN-E	2.06	-0.024	0.983	6
	DXN-Z	1.67	-0.031	0.991	6		DXN-Z	1.66	-0.021	0.984	6
5.0	MPN	1.59	-0.032	0.997	6	7.0	MPN	1.42	-0.019	0.997	6
	PTN	2.28	-0.039	0.997	6		PTN	1.93	-0.025	0.999	6
	IMN	1.60	-0.029	0.996	6		IMN	1.62	-0.020	0.996	6
	CMN	1.94	-0.035	0.998	6		CMN	1.95	-0.025	0.998	6
	NRN	2.09	-0.037	0.996	6		NRN	1.76	-0.024	0.998	6
	AMN	2.18	-0.036	0.998	6		AMN	2.07	-0.026	0.998	6
	DXN-E	1.98	-0.032	0.996	6		DXN-E	2.06	-0.024	0.998	6
	DXN-Z	1.71	-0.031	0.997	6		DXN-Z	1.71	-0.022	0.998	6
5.5	MPN	1.49	-0.027	0.996	6	7.5	MPN	1.41	-0.020	0.995	6
	PTN	2.26	-0.037	0.997	6		PTN	2.01	-0.027	0.995	6
	IMN	1.57	-0.027	0.996	6		IMN	1.75	-0.023	0.998	6
	CMN	1.92	-0.032	0.990	6		CMN				
	NRN	2.07	-0.035	0.996	6		NRN	1.81	-0.025	0.993	6
	AMN	2.12	-0.033	0.996	6		AMN	2.06	-0.026	0.996	6
	DXN-E	1.91	-0.028	0.996	6		DXN-E	2.02	-0.024	0.998	6
	DXN-Z	1.97	-0.032	0.990	6		DXN-Z	1.74	-0.022	0.998	6

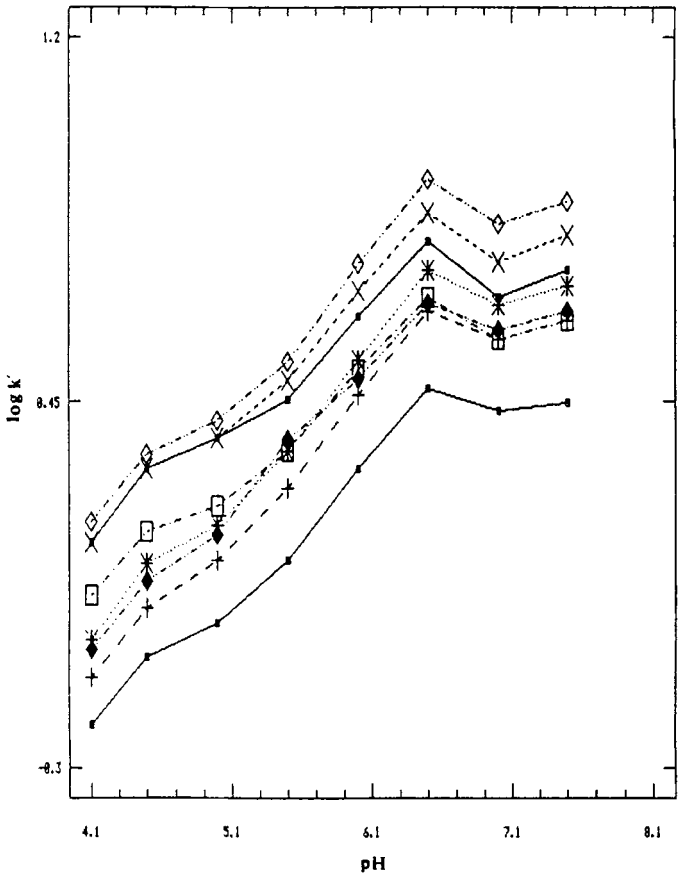


Figure 3. Dependence of retention (logk') on pH of the aqueous content of the mobile phase (buffer/methanol = 50/50) for the seven antidepressant (keys as in legend of figure 2).

separation concerns ionizable solutes (14). One of the aims of this work was to derive an experimental mathematical equation of the relative retention behaviour of the ionizable antidepressants in the same ratio aqueous -organic solvents at different pH values of the aqueous content of the mobile phase. Figure 3 shows the depende-

nce of logk′ values of the antidepressants on pH at the same ratio of buffer/methanol (50/50) in the mobile phase. As shown, an almost linear dependence of logk′ on pH exists in the pH range 4.1-6.5. The logk′ remains then constant at pH greater than 6.5. The linear fitting of all experimental data, obtained in various buffer/methanol ratios, on the equation

$$\log k' = c + d\,(pH) \qquad (2)$$

are summarized in Table 2. As shown the slope d remains statistically constant in each set of experiments and almost in all buffer/methanol ratios (mean value in all experiments: 3.40, range 2.78 ± 0.47 - 4.13 ± 0.81). In contrary, the value of the term c varies for each solute according to the pKa_w (pKa in aqueous solution) of each solute. For example, MPN (pKa_w = 10.5) showed the greatest value of c while DXN, (pKa_w = 8.0) the smallest one, in all sets of experiments. In almost all cases, the value of the term c decreases as methanol concentration increases in the mobile phase.

Combined Effect of Organic Modifier Content and pH on Retention

As the experimental data showed a sufficient fitting on the equations (1) and (2), the combined effect of pH of the aqueous content of the mobile phase and the methanol concentration (%MeOH) on logk′ was further examined. The experimental data for each compound were fitted to the following equation using multiple regression analysis (intercorrelation does not exist between the two parameters):

$$\log k' = a'pH - b'\,(\%MeOH) + c' \qquad (3)$$

As shown in Table 3 a sufficient correlation ($r>0.94$) exists between logk′ and pH, and %MeOH. The values of a′

TABLE 2.

Dependence of logk′ of the Drugs on pH of the Aqueous Content of the Mobile Phase (logk′ = c + d(pH)).

Mobile Phase buffer/methanol	Component	c	d	r	n
35 / 65 (pH range 5.0-7.0)	MPN	6.31(±0.08)	3.05(±0.33)	0.983	5
	PTN	5.69(±0.08)	3.90(±0.41)	0.983	5
	IMN	5.84(±0.08)	3.14(±0.39)	0.977	5
	CMN	5.84(±0.08)	3.19(±0.40)	0.970	5
	NRN	6.03(±0.08)	3.50(±0.41)	0.980	5
	AMN	5.46(±0.10)	3.42(±0.45)	0.974	5
	DXN-E	5.04(±0.10)	3.38(±0.40)	0.968	5
	DXN-Z	5.00(±0.10)	2.78(±0.47)	0.967	5
40 / 60 (pH range 4.5-6.5)	MPN	5.86(±0.07)	2.94(±0.27)	0.987	5
	PTN	5.20(±0.13)	3.02(±0.60)	0.945	5
	IMN	5.41(±0.05)	2.95(±0.23)	0.991	5
	CMN	5.41(±0.06)	2.94(±0.23)	0.991	5
	NRN	5.44(±0.09)	3.13(±0.43)	0.972	5
	AMN	4.90(±0.10)	3.13(±0.41)	0.974	5
	DXN-E	4.70(±0.14)	2.90(±0.40)	0.973	5
	DXN-Z	5.40(±0.07)	3.04(±0.30)	0.990	5
45 / 55 (pH range 4.1-7.0)	MPN	5.62(±0.09)	3.14(±0.30)	0.976	6
	PTN	4.44(±0.17)	3.97(±0.41)	0.966	6
	IMN	5.15(±0.08)	3.16(±0.20)	0.983	6
	CMN	5.07(±0.09)	3.10(±0.32)	0.982	6
	NRN	4.90(±0.12)	3.74(±0.41)	0.969	6
	AMN	4.40(±0.11)	3.58(±0.32)	0.974	6
	DXN-E	4.10(±0.16)	3.51(±0.32)	0.976	6
	DXN-Z	5.00(±0.09)	3.34(±0.26)	0.984	6
50 / 50 (pH range 4.1-6.5)	MPN	4.89(±0.05)	3.54(±0.21)	0.992	6
	PTN	3.43(±0.16)	4.05(±0.30)	0.988	6
	IMN	4.49(±0.05)	3.23(±0.14)	0.996	6
	CMN	4.24(±0.07)	3.24(±0.17)	0.994	6
	NRN	3.90(±0.10)	4.00(±0.28)	0.990	6
	AMN	3.53(±0.13)	3.64(±0.25)	0.991	6
	DXN-E	3.45(±0.14)	3.45(±0.26)	0.990	6
	DXN-Z	4.28(±0.04)	3.41(±0.12)	0.998	6
55 / 45 (pH range 4.1-6.5)	MPN	4.33(±0.12)	3.69(±0.50)	0.971	5
	PTN	2.63(±0.48)	4.13(±0.81)	0.946	5
	IMN	3.98(±0.15)	3.39(±0.41)	0.978	5
	CMN	3.62(±0.21)	3.40(±0.40)	0.971	5
	NRN	3.16(±0.21)	4.08(±0.81)	0.945	5
	AMN	2.73(±0.40)	3.89(±0.62)	0.957	5
	DXN-E	2.77(±0.32)	3.70(±0.64)	0.958	5
	DXN-Z	3.80(±0.14)	3.33(±0.33)	0.985	5

TABLE 3.

Combined Effect of pH and %MeOH of Mobile Phase on Retention(logk′ = a′pH - b′(%MeOH) + c′, n = 47)

Maprotiline	logk′ = 0.24 pH - 0.027 (%MeOH) + 0.168	r = 0.944
Protriptyline	logk′ = 0.20 pH - 0.034 (%MeOH) + 1.057	r = 0.959
Imipramine	logk′ = 0.25 pH - 0.026 (%MeOH) + 0.259	r = 0.953
Chroripramine	logk′ = 0.25 pH - 0.032 (%MeOH) + 0.560	r = 0.957
Amitriptyline	logk′ = 0.21 pH - 0.032 (%MeOH) + 0.936	r = 0.959
Doxepin (E-isomer)	logk′ = 0.22 pH - 0.029 (%MeOH) + 0.775	r = 0.954
Doxepin (Z-isomer)	logk′ = 0.24 pH - 0.027 (%MeOH) + 0.352	r = 0.948

and b′ are statistically constant while that of c′ varies significantly.

All points representing a set of experimental data for a solute, can be presented as a plane surface having a slope in space. Figure 4 illustrates the three dimentional plots of equation (3) for maprotiline and protriptyline which show the smallest and greatest value of c′, respectively.

Relationship Between Retention and the Formation Constant of the CD-Solute Complexes

Chromatographic separations, using CD bonded phases, are mainly the result of variation of the stability of the inclusion complexes of the analytes with the CD (15). The elution time of a solute is a function of the stability of these complexes. Several intermolecular interactions are responsible for the formation of these complexes. These driving forces act synergistically and are related to the physicochemical properties of the guest molecule. Since the formation constant (K_f) of the complex is dependent on many factors, the CD stationary phase provides a high degree of selectivity. From this point of view it is useful to investigate a possible relationship between the logk′ value and the K_f of each

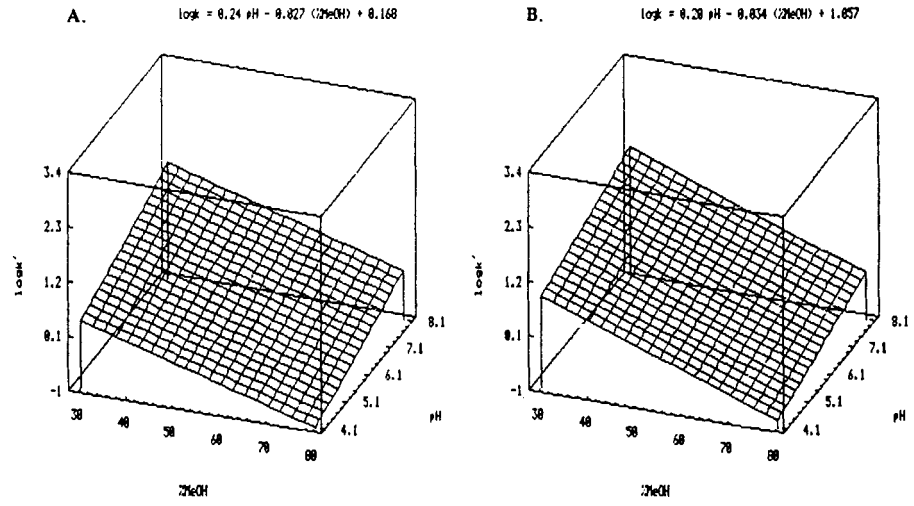


Figure 4. Three dimentional plots of eq. logk′ = a′pH - b′(%MeOH) + c′ for maprotyline (A) and protriptyline (B).

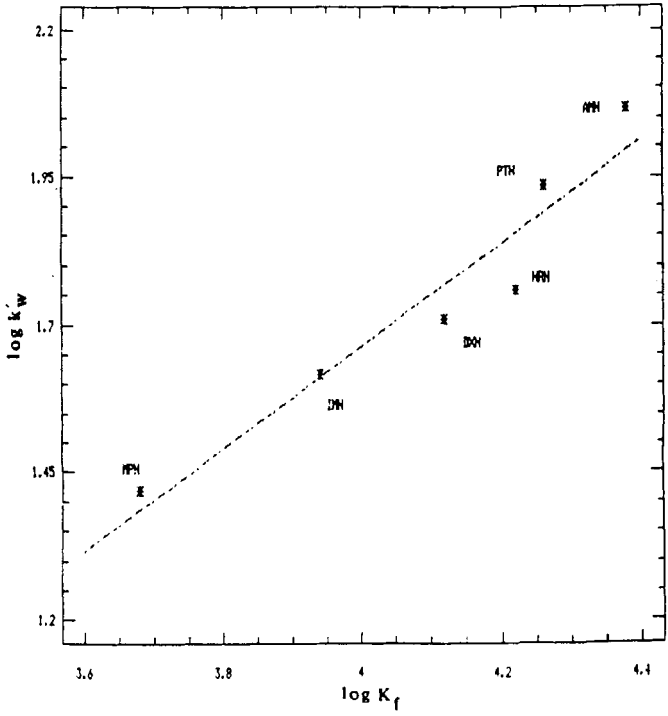


Figure 5. Dependence of the expected in pure water and pH 7.0 ($logk'_w$) on the stability constants of the drug-β-CD complexes at pH 7.0 ($logk'_w = 0.87\ K_f - 1.81$, $r = 0.960$).

drug. The values of the formation constants are available in the literature (27), and were determined in about neutral aqueous solutions using direct potentiometry with drug ion selective electrodes. Since k′ values were determined in a mobile phase which was a mixture of methanol and aqueous buffer, the correlation was unsatisfactory. However, the value of logk′ in pure aqueous phase ($\log k'_w$) can easily be determined by extrapolation (28,29), as the intercept of equation (1), i.e.

$$\log k' = a\,(\%MeOH) + \log k'_w \qquad (1a)$$

As shown in figure 5, a linear relationship exists between $\log k'_w$ at pH 7.0 and $\log K_f$ value of each drug described by the equation

$$\log k'_w = a'' + b''\log K_f \qquad (4)$$

This good correlation reveals that the main factor of the separation efficiency of CD-stationary phases is the complexation of the solutes. As expected, the correlation was not sufficient at pH different than 7.0 ($r<0.94$), since the K_f values used were determined in neutral solutions. A linear relationship also exists between the intercept of equation (3) (term c′), i.e., the logk′, in pure aqueous solution and zero pH (in $[H^+] = 1$ M) (figure 6). The equation is

$$\log k'_{w(0)} = -4.92 + 1.36\log K_f, \qquad r = 0.932 \qquad (5)$$

Optimization of the Chromatographic Separations

From preliminary experimental studies using acetonitrile or methanol as organic modifier it was found that the latter one gave more efficient separations.

In order to optimize the experimental parameters (methanol content and pH value of the aqueous constituent of the mobile phase) the chromatographic response

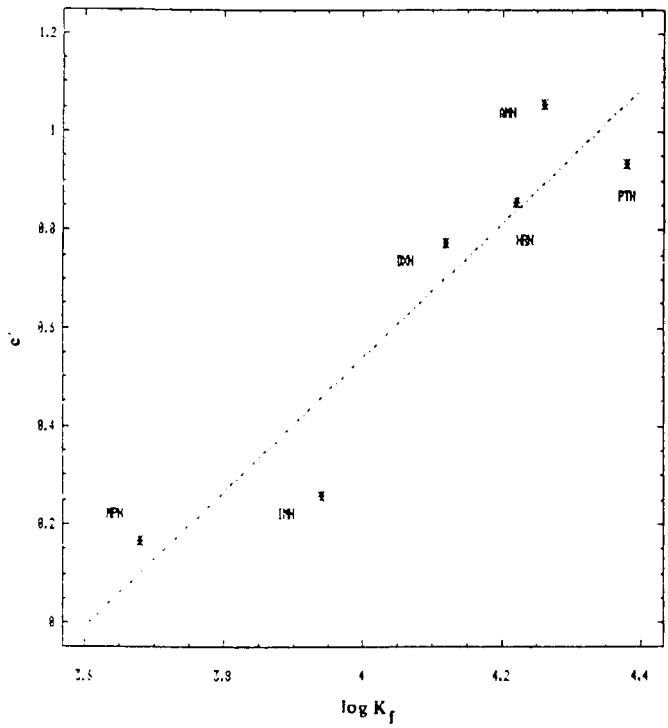


Figure 6. Dependence of the calculated logk' in pure aqueous solution and zero pH (intercept of equation 3) on the stability constants of drug-β-CD complexes.

function (CRF) developed by Berridge (20,21) was used

$$CRF = \sum_{i=1}^{n-1} R_i + n^a + b\,|T_A - T_L| - c(T_o - T_1) \qquad (6)$$

where R_i is the resolution between the i^{th} pair of adjacent peaks, n the number of peaks detected, T_A the maximum acceptable time, T_L the retention time of the last component, T_o the minimum acceptable time for the first peak and a, b, c weighting factors, usually in the range

0.5-2.0, selected by the analyst. In this application, values of 1, 0.5 and 1 were chosen for the factors a, b and c, respectively and 25 min and 3.5 min for T_A and T_o, respectively.

Figure 7 shows the CRF for all the combinations of pH - %MeOH investigated to separate all drugs. As shown CRF is maximum for mobile phase composition 50/50 MeOH/ buffer at pH 6.50. This mobile phase is proposed for an efficient analytical method for the separation /determination of a mixture of these drugs, as it is shown in figure 8. A simplex optimization procedure in which the two parameters are changed simultaneously can also be used (21).

Relation of Elution Order to Drug Structures

The formation of inclusion complexes depends on the physical orientation of the solute in the cavity, which depends on the structure, shape and size of the solute, as well as the displacement of water by the analyte in the cavity (30).

Hydrophobic interactions between solutes and the hydrophobic cavity of the CDs are predominantly responsible for the separation mechanism, by which the formation strength of inclusion complexes can be differentiated during the chromatographic process. Further, it is well known that the formation constants for polar and ionic compounds are usually smaller than those for non -polar and uncharged compounds. Consequently, non-polar and uncharged compounds are, in principle, more strongly retained by cyclodextrin than the polar and ionic compounds (15).

From this point of view, the elution order of the drugs studied was evaluated in regarding of the effects of different structural variations on resolution.

All components under study are derivatives of dibenzo[a,d] [1,4]-cycloeptadiene (stucture-type I) or of

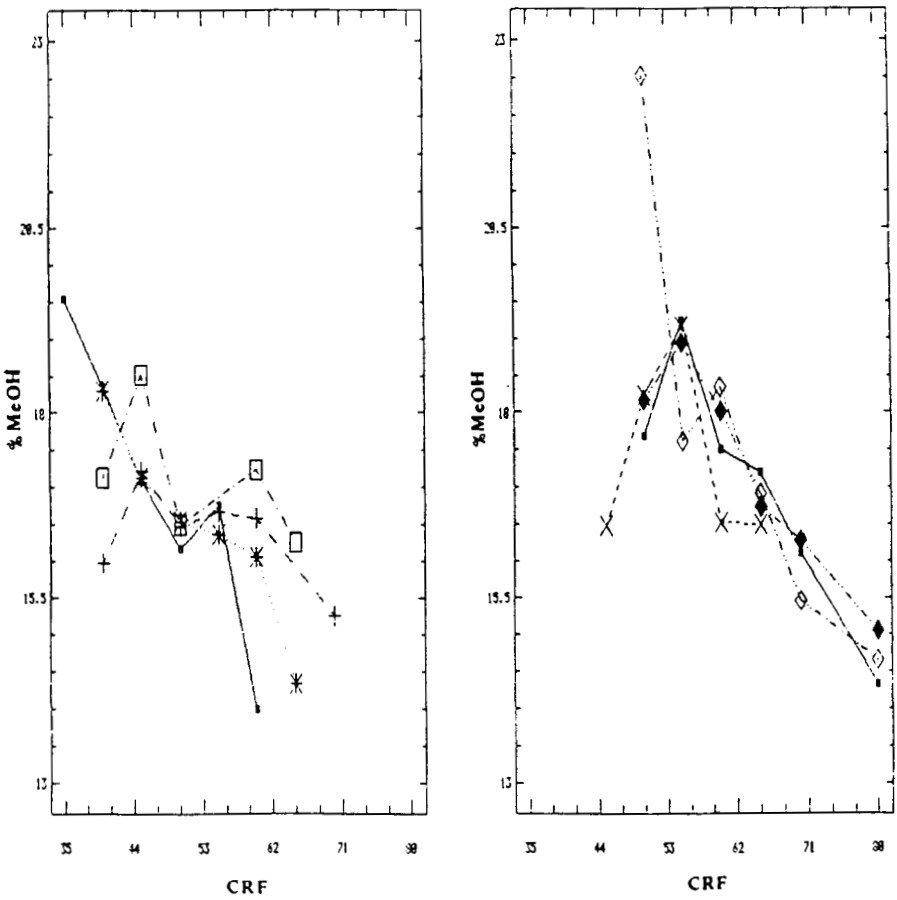


Figure 7. Chromatographic Response Factor (CRF) for the various combinations of pH-%MeOH:(— •) pH 4.1, (- - +) pH 4.5, (- - □) pH 5.5, (··· X) pH 6.0, (-·- ◊) pH 6.5, (··- ♦) pH 7.0, (— •) pH 7.5, (····· +) pH 5.0.

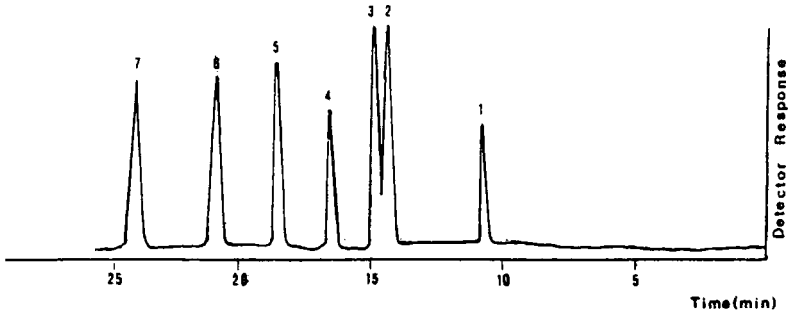


Figure 8. Chromatogram of the seven antidepressant drugs separated using the optimized mobile phase: 50/50 MeOH/buffer at pH 6.5. 1. Maprotiline, 2. Imipramine, 3. Nortriptyline, 4. Chloripramine, 5. Protriptyline, 6. Amitriptyline, 7. Doxepin (E-isomer).

5H-dibenz[b,f]-azepine (stucture-type II), and they are characterized by similar basicity and hydrophobicity, generally considered as non-polar compounds.

In the most of cases, nortriptyline was eluted before than amitriptyline. The replacement of -H by $-CH_3$ in the molecule of amitriptyline may cause alterations in the hydrophobicity and basicity of the solute and consequently an increase in retention time. On the other hand, the presence of a double bond in the cycloeptediene ring in the molecule of protriptyline makes the solute less flexible and so protriptyline was more retained than nortriptyline. Furthermore, the replacement of -C- by -O- in the molecule of doxepin causes different contributions to the retention. In all cases examined, the two isomers of doxepin (Z- and E-) were separated. Probably the presence of -O- permits hydrogen-bonding interactions between the molecule and the -OH groups at the rim of the CD-cavity, in the case of the E-isomer (last eluted). On the contrary in the case of the Z-isomer, the presence of -O- decreases the strength of hydrophobic interaction (first eluted).

The compounds of stucture type II (imipramine and chloripramine), which have azepine structure, differ from the compounds of stucture type I in the complex formation ability. It was observed that using mobile phases with low pH, they were less retained, and by increasing pH the retention increased too. The replacement of -H by -Cl in the molecule of chloripramine causes an increase in the retention time, in all cases with a successful resolution between the two components. This should be attributed to the greater affinity of CDs for the -Cl atoms (31).

Finally, an increased retention of maprotiline was expected in comparison to the other solutes, due to its high lipophilicity. However, in all chromatographic conditions investigated in this study, it was the first eluted. This may be attributed to the presence of a bulky chain giving rise to a stereochemical inhibition in the formation of inclusion complex with the CD. This is in accordance to the K_t value of maprotiline reported previously (22).

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