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Coupled column chromatography in chiral separations: systems employing β-cyclodextrin phases for chiral separation

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ABSTRACT

Two-dimensional chromatographic systems were evaluated which combine a β-cyclodextrin (β-CD)-containing column for the optical resolution of enantiomers with a non-chiral column for the preseparation of these enantiomers from other solutes or impurities. β-CD is used either as a chemically bonded stationary phase or as a mobile phase additive. This multi-column system allows the accurate determination of the enantiomeric composition even in complex samples and improves the significance and reliability of the results. The system was evaluated with respect to peak dilation caused by the carry-over, the maximum carry-over volume up to which severe adverse effects on the resolution can be avoided, and the implications with regard to sensitivity.

INTRODUCTION

The use of coupled columns in high-performance liquid chromatography (HPLC) has attracted strong interest since its introduction more than 15 years ago [1]. This development was facilitated by the technical progress with HPLC apparatus and stimulated by the high demands required for analytical methods in biological analysis. The more complex the samples became, the more the advantages of coupled column chromatography (CCC) with column switching became important.

In recent years, coupled column chromatography has been introduced also in chiral separations [2–10]. The well known features of CCC with respect to matrix separation and its unique power for dramatically enhancing the peak capacity become especially important and decisive in the context of chiral separation problems: first, because any chiral separation increases the number of peaks to be resolved additionally; second, because several chiral recognition principles suffer from strong peak dispersion and thus from low peak capacity; and third, because the determination of the enantiomeric excess often has to be performed at a high level of accuracy although the analyte concentration is at the trace level, and as many samples become complex at very low concentration levels. In these respects the enhancement of peak capacity is often required, because the peak capacity determines the degree of

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significance and reliability with which the presence of other compounds can be excluded.

On the other hand, significant simplifications can be adopted under certain conditions: for the determination of the enantiomeric excess the quantification of the enantiomeric peaks relative to each other is sufficient, and for this purpose one needs to transfer only parts of the peak of unresolved enantiomers, if no chiral preseparation has occurred in the first column [3].

Chiral systems operated with aqueous mobile phases are of great interest in CCC as they are most appropriate for samples of biological origin. It is no surprise, therefore, that chiral separation techniques using aqueous mobile phases were first introduced in CCC: chiral ligand-exchange systems [2], immobilized protein columns [4–6], and immobilized cyclodextrin columns [3,8]. Even the swollen microcrystalline cellulose triacetate system, which is usually operated at high concentrations of alcohols in the mobile phase, can readily be combined with reversed-phase columns [10].

The consecutive order in which the columns are combined can be varied (non-chiral–chiral or chiral–non-chiral), and one can find good arguments for both types. Obviously, the decision as to which combination is to be preferred depends on the problem to be solved and on the main constraints. Such constraints may be low analyte concentrations, low sample amounts, complex sample matrices or a high degree of optical purity to be monitored.

This paper examines the possibilities and limitations of column switching in a coupled system of a non-chiral alkylsilica column and a subsequent chiral column involving β-cyclodextrin (β-CD) phases either as a bonded chiral stationary phase (CSP) or as a chiral mobile phase. The criteria are compatibility of the mobile phases with respect to deconditioning of the second column, degree of peak compression or dilation obtained and the potential loss (or gain) in sensitivity. Perspectives with respect to sensitivity are also given for the reverse column order.

EXPERIMENTAL

Instrumentation

Chromatographic experiments were carried out with two high-pressure liquid chromatographic pumps [(1) Model L-6200 intelligent pump (Merck–Hitachi, Tokyo, Japan) and (2) Solvent Module 116, System Gold (Beckman, San Ramon, CA, USA)], a syringe-valve injector (Model 7161; Rheodyne, Cotati, CA, USA) equipped with a 20-μl loop, two switching valves (Model 7030; Rheodyne), and a UV detector (Model L-4000; Merck–Hitachi) connected to an integrator (Model D-2000 chromato-integrator; Merck–Hitachi).

Columns

The following columns were used:

(1) Non-chiral columns for non-chiral separations or for chiral separations by use of β-CD as chiral mobile phase additive (CMA): prepacked stainless-steel columns (250 mm × 4.0 mm I.D.) filled with chemically bonded octylsilica (OS) or octadecyl-silica (ODS) material, particle diameter 5 μm. (LiChrosorb RP-8 or RP-18; Merck, Darmstadt, Germany).

(2) Chiral column: prepacked column (250 mm × 4.0 mm I.D.) filled with Cyclobond I (chemically bonded β-CD) (Astec, USA), mean particle diameter 5 μm.

Reagents and samples

Organic solvents (LiChrosolv grade) were obtained from Merck. Water was distilled twice from a quartz apparatus and additionally purified using an Elgastat UHQ apparatus (Elga, High Wycombe Bucks., UK). Cyclodextrin was purchased from Merck. Alcohol–water mixtures were partially premixed and degassed in an ultrasonic bath. All eluent mixtures were filtered before use through a nylon 66 membrane filter with 0.45-μm pore diameter (Supelco, Bellefonte, PA, USA).

Standard solutions of the racemic test analytes were used for the evaluation of the peak compression effect. Solutions of less pure standard analytes were employed for demonstrating the separation power of the column-switching set-up.

Chromatographic procedure

A schematic diagram of the instrumental set-up is given in Fig. 1. A general chromatographic elution and column switching protocol is described in Table I for the CCC system with an alkylsilica column and a subsequent β-CD-containing phase

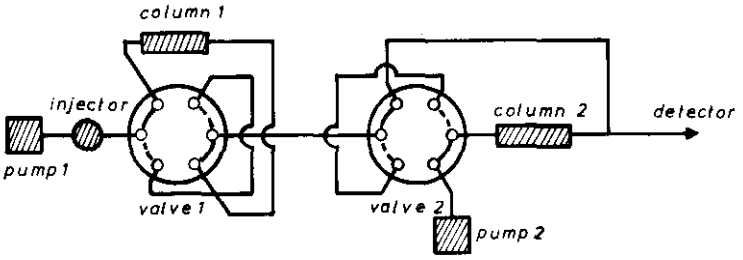


Fig. 1. Schematic diagram of the CCC system. Column 1 = alkylsilica (OS or ODS); column 2 = β-CD-CSP or a second alkylsilica column. Valves: rotor position A = solid lines; rotor position B = broken lines.

TABLE I

GENERAL ELUTION AND COLUMN-SWITCHING PROTOCOL

t_1 = Start of analyte transfer; t_2 = end of transfer; t_3 = end of the chromatogram at the RP column, change of eluent by changing the pump in operation; t_3–t_4 = elution of the chiral column. Rotor positions, A and B, of the switching valves refer to Fig. 1 (position A, solid lines; position B, broken lines). Column abbreviations: RP = reversed-phase (alkylsilica) column; β-CD = column with phase systems containing β-CD either as CSP or as CMA. Eluent 1 = eluent used for column 1 [*e.g.*, aqueous buffer with 40% (v/v) of methanol]; eluent 2 = eluent optimized for the separation at column 2 [*e.g.*, aqueous buffer with 20% (v/v) of methanol].

Time interval	Rotor position of valve		Columns in operation	Eluent	Comment
	1	2			
0–t_1	A	A	RP	1	Preseparation of the sample
t_1–t_2	A	B	RP + β-CD	1	Transfer of the racemic analyte
t_2–t_3	A	A	RP	1	Finishing of RP elution
t_3					Stopping of pump 1, starting of pump 2
t_3–t_4	A	A	β-CD	2	Chiral separation

system. Eluent 1 is delivered by pump 1 and eluent 2 by pump 2. By employing a second pump *ca.* 12–15 min (at a flow-rate of 0.5 ml/min) can be saved in one analysis cycle by avoiding repeated changing of the eluents. In principle, the same chromatograms can be obtained by using a single pump only and a capillary bypass according to the set-up described previously [10].

Ambient temperature was used for both columns, with UV detection at 254 nm. Flow-rates were 0.5 ml/min if not indicated otherwise.

RESULTS AND DISCUSSION

Retention characteristics in CCC systems as source of peak compression and dilation

Plots of logarithm of capacity factor (log k') *versus* alcohol concentration in the mobile phase are given in Figs. 2–4 for various analytes and different phase systems. The data in Fig. 2 were obtained with a simple octadecylsilica phase, those in Fig. 3 with the β-CD CSP and those in Fig. 4 with a system containing β-CD as CMA.

These data allow an approximate estimation of the main working range with respect to mobile phase composition (see broken lines). For the alkylsilica phase (column 1), this working range is determined mainly by the capacity factors of the analytes and lies between 30 and 60% (v/v) methanol (see Fig. 2) [corresponding to about 15–35% (v/v) ethanol]. The working range for the β-CD containing phases is mainly determined by the influence of the alcohol concentration on the enantioselectivity coefficients and lies near 20% (maximum 30%) (v/v) methanol for the CSP and near 20% (v/v) ethanol for the CMA system.

Assuming that the mobile phase composition employed is actually within these ranges, the capacity factor data given in the Figs. 2–4 allow an approximate estimation

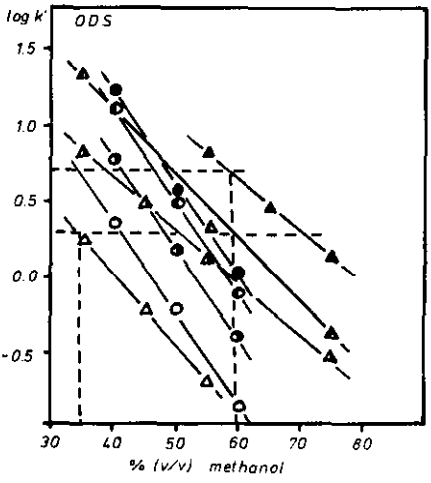
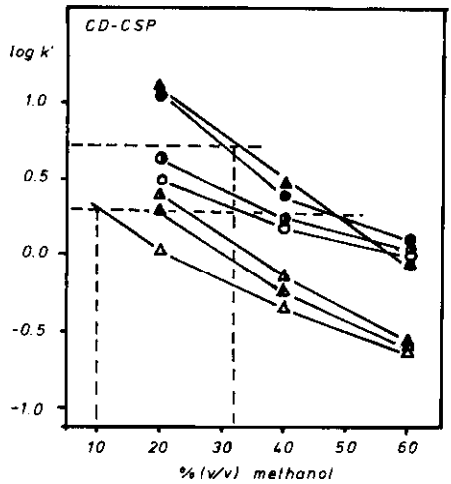


Fig. 2. Capacity factor, k', as a function of the alcohol concentration in the mobile phase for the ODS column. Mobile phase, aqueous buffer (10 m*M* ammonium acetate, pH 7) with varying content of methanol; temperature, 30°C. The horizontal broken lines indicate k' values of 3 and 5, respectively, and the vertical broken lines confine the working range with respect to the alcohol concentration in the mobile phase. △ = Chlorthalidone; ◭ = hexobarbital; ◮ = oxazepam; ▲ = nomifensin; ○ = Dns-Thr; ◑ = Dns-Val; ◐ = Dns-Leu; ● = Dns-Phe.



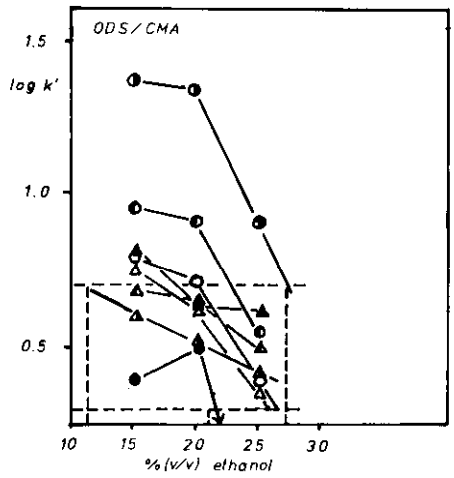


Fig. 3. Capacity factor, k', as a function of the alcohol concentration in the mobile phase for the β-CD-CSP column. Mobile phase, aqueous buffer (10 m*M* ammonium acetate, pH 7) with varying content of methanol; temperature, 30°C. Symbols as in Fig. 2.

Fig. 4. Capacity factor, k', as a function of the alcohol concentration in the mobile phase for the chiral phase system OS/β-CD-CMA. Mobile phase, aqueous buffer (25 m*M* ammonium acetate, pH 5.5–35 m*M* β-CD–1 *M* urea) with varying content of ethanol; temperature, 30°C. Symbols as in Fig. 2, except ◭ = ethylphenylhydantoin; ◐ = Dns-Ala; ● = Dns-Glu.

of the peak compression or peak dilation effects introduced by large carry-over volumes at the top of the second column. The thermodynamic peak compression effect for an analyte *i* caused by a step gradient is approximately calculated by the following equation [11–13]

$$pcf_i^{th} = \sigma_{v_i}(\text{before compr.})/\sigma_{v_i}(\text{after compr.}) = k'^{col.2}_{i_{co}}/k'^{col.2}_{i_{el.2}} \quad (1)$$

where pcf^{th} denotes the peak compression factor at the top of column 2, σ_v is the standard deviation of the peaks in volume units and the subscripts "co" and "el.2" denote the carry-over and the eluent employed for column 2, respectively.

Table II shows peak compression ($pcf > 1$) or dilation ($pcf < 1$) factors for various column combinations calculated according to eqn. 1. The given alcohol concentrations correspond to the actual working range. It is obvious from the $pcf < 1$ values that the column order alkylsilica–(β-CD-CSP) column is not very favourable from this point of view. The use of β-CD as CMA is superior in this respect, as the addition of β-CD to the mobile phase increases the solvation power of eluent 2, as indicated in Fig. 5. Peak compression factors near 1 or > 1 can thus be obtained in the optimum working range. The opposite situation is obtained with the reverse column order.

At least three observable effects are associated with deconditioning of the second column by the transfer of a carry-over with greater elution strength: peak broadening, distorsion of the peak shape (*cf.*, the computer simulation in ref. 14) and changes in (enantio)selectivity.

The degree of peak dilation given in Table II is found to be dependent on the

TABLE II

THEORETICAL PEAK COMPRESSION FACTORS, pcf^{th}, CALCULATED ACCORDING TO EQN. 1 FOR DIFFERENT COMPOSITIONS OF THE CARRY-OVER (ELUENT 1) AND ELUENT 2

Column sequence	Column I, ODS; column II, β-CD-CSP[a]					
Non-chiral–chiral	Analyte	Methanol in eluent 1 (%, v/v)[b]	pcf^{th}			
			Methanol in eluent 2 (%, v/v)[c]			
			10	20	30	40
	Dns-threonine	30		0.69[d]	1.00[d]	1.47
		40		0.47	0.68	1.00
	Hexobarbital	40		0.28[d]	0.49	
		50		0.18[d]	0.30	
		60		0.11	0.19	
	Chlorthalidone	20	0.55	1.00		
		30	0.36	0.66[d]		
		40	0.21	0.38		
	Column I, OS; column II, OS/β-CD-CMA[a]					
	Analyte	Ethanol in eluent 1 (%, v/v)[e]	pcf^{th}			
			Ethanol in eluent 2 (%, v/v)[f]			
			15	20	25	
	Dns-threonine	20	1.59	2.37[d]	4.09	
		25	0.62	0.93[d]	1.60	
		30	0.28	0.42	0.73	
	Hexobarbital	30	1.55	1.84[d]	2.31	
		40	0.53	0.63	0.79	
	Chlorthalidone	20	1.28	1.70[d]	3.28	
		25	0.48	0.64	1.23	
		30	0.20	0.27	0.53	
	Column I, β-CD-CSP; column II, ODS[a]					
Chiral–non-chiral	Analyte	Methanol in eluent 1 (%, v/v)[e]	pcf^{th}			
			Methanol in eluent 2 (%, v/v)[b]			
			20	30	40	50	60
	Dns-threonine	20		3.9	15.9	57.3	
		30		1.0	4.1	14.8	
		40		0.3	1.0	3.6	
	Hexobarbital	20			4.4	10.0	23.6
		30			2.0	4.6	10.8
	Chlorthalidone	10	3.0	8.5	25.3		
		20	1.0	2.8	8.4		

TABLE II (*continued*)

Column I, OS/β-CD-CMA; column II, OS[a]					
Analyte	Ethanol in eluent 1 (%, v/v)[f]	*pcf*[b]			
		Ethanol in eluent 2 (%, v/v)[e]			
		20	25	30	40
Dns-threonine	15	0.6	1.6	3.5	
	20	0.4	1.1	2.4	
	25	0.2	0.6	1.4	
Hexobarbital	15			0.6	1.9
	20			0.5	1.6
	25			0.4	1.3
Chlorthalidone	15	0.8	2.1	4.9	
	20	0.6	1.6	3.7	
	25	0.3	0.8	1.9	

[a] Column symbols: OS = octylsilica; ODS = octadecylsilica; β-CD-CSP = chiral stationary phase using immobilized β-cyclodextrin; OS/β-CD-CMA = octylsilica stationary phase, β-cyclodextrin employed as chiral mobile phase additive.

[b] Eluent composition: aqueous buffer solution (10 m*M* ammonium acetate, pH 7)–methanol; content of methanol as indicated.

[c] Eluent composition: aqueous buffer solution (10 m*M* ammonium acetate, pH 7)–methanol; content of methanol as indicated.

[d] Indicates the data obtained in the preferred working range of the eluents with respect to the alcohol concentration (working ranges are illustrated in Figs. 2–4).

[e] Eluent composition: aqueous buffer solution (25 m*M* ammonium acetate, pH 5.5)–ethanol; content of ethanol as indicated.

[f] Eluent composition: aqueous buffer solution (25 m*M* ammonium acetate, pH 5.5–35 m*M* β-CD–1 *M* urea)–ethanol; content of ethanol as indicated.

chemical structure of the analyte. The *pcf* values for hexobarbital are smaller than those for chlorthalidone and Dns-threonine in the optimum working range. The situation is changed when employing β-CD as CMA. In this instance peak dilation is smallest for hexobarbital.

The observable degree of peak broadening and distortion depends on the volume of the carry-over. Experimental data illustrating the influence of the carry-over volume on the σ_v values at the end of column 2 and the stereoselectivity data in this column are given in Table III. It can be seen that the enantioselectivity coefficient, α, is unaffected by the carry-over in all instances. The decrease in resolution observed in the chromatograms is always due to peak dilation (with CSP) or the absence of significant peak compression (with CMA). The effect of the carry-over volume on peak width and resolution is illustrated by the chromatograms in Fig. 6 for the β-CD-CSP and in Fig. 7 for the β-CD-CMA system.

The data in Table III allow the approximate evaluation of a critical carry-over volume, below which no significant adverse influence on the resolution is observed. For the β-CD-CSP system and the analytes hexobarbital and chlorthalidone this critical limit in the carry-over volume is near 50 μl and for Dns-threonine near 100 μl.

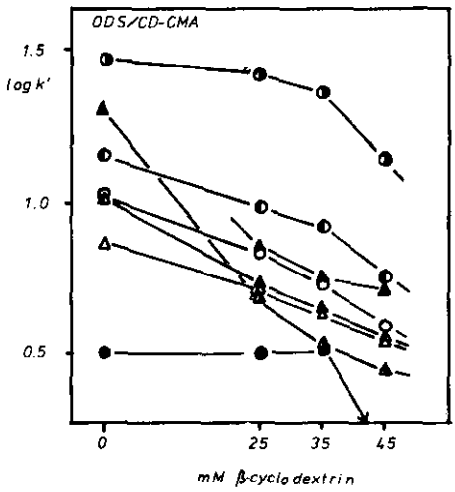


Fig. 5. Capacity factor, k', as a function of the concentration of β-CD in the mobile phase. Column, ODS; mobile phase, aqueous buffer (25 mM ammonium acetate, pH 5.5)–20% (v/v) ethanol, varying content of β-CD and urea (25 mM β-CD, no urea; 35 mM β-CD 1.0 M urea; 45 mM β-CD 1.7 M urea, respectively); temperature, 30°C. Symbols as in Fig. 4.

Above these values of the carry-over volume the apparent plate height values, H^{app}, increase dramatically. The critical carry-over volume is seen to depend on two parameters: first, the difference in the elution power of eluents 1 and 2, and second, the structure of the analytes. This influence is not yet well understood. Probably differences in the orientation of analytes during the inclusion in the β-CD ring play a major role.

For the β-CD-CMA system similar limits for the carry-over volume are obtained (50 μl for chlorthalidone and Dns-threonine and about 100 μl for hexobarbital). Hexobarbital, most strongly affected by high carry-over volumes with β-CD-CSPs, is the least affected in the β-CD-CMA system, which is the opposite of the situation with Dns-threonine.

Sensitivity obtainable with the column order non-chiral alkylsilica–chiral β-CD system

The sensitivities achievable by a particular column configuration can become a main factor in the selection of the consecutive order of columns. Three factors affect the obtainable sensitivity: (i) the percentage of analyte mass transferred between the columns, (ii) the degree of peak compression or dilation during transfer and (iii) the dispersion characteristics of the last column. These criteria will be referred to in evaluating the potential of different column configurations.

In the mentioned consecutive column order, peak dilation and distortion are often obtained as discussed before in detail. These adverse effects on the peak profile can be avoided by reducing the carry-over volume, if a consequent decrease in sensitivity can be accepted.

First, we consider this decrease in sensitivity by restricting the carry-over volume. To assess an approximate value for this loss, we assume a plate number of 9000 for the first column (alkylsilica, 250 mm × 4 mm I.D.) and a typical k' value of 4.

TABLE III

VOLUME STANDARD DEVIATION, σ_v, AT THE END OF COLUMN II, THE CORRESPONDING APPARENT THEORETICAL PLATE HEIGHT, H^{app}, AND THE ENANTIOSELECTIVITY COEFFICIENTS, α, AS A FUNCTION OF THE CARRY-OVER VOLUME

Column I, OS; column II, β-CD-CSP[a]

Analyte	Methanol in eluent (%, v/v)		Carry-over volume (μl)	σ_v (μl)	H^{app}[c]	α
	1[b]	2[b]				
Hexobarbital	40	20	50[d]	95	26	1.12
			100	125	(50)	1.12
Chlorthalidone	20	20	20	60	30	1.17
			40	65	34	1.17
	30	20	40	70	40	1.17
			100[d]	77	43	1.17
			200	127	(118)	1.17
	40	20	50[d]	80	44	1.18
			100	147	(150)	1.18
Dns-threonine	40	20	50	150	26	1.06
			100[d]	160	27	1.06

Column I, OS; column II, OS/β-CD-CMA[a]

Analyte	Methanol in eluent (%, v/v)		Carry-over volume (μl)	σ_v (μl)	H^{app}[c]	α
	1[e]	2[f]				
Hexobarbital	40	20	20	78	27	1.12
			50	60	29	1.12
			100[d]	70	(39)	1.12
			200	120	(90)	1.12
Chlorthalidone	30	20	20	75	30	1.21
			50[d]	80	35	1.21
			100	120	(66)	1.20
			200	220		1.21
Dns-threonine	30	20	20	95	29	1.19
			50	90	34	1.19
			100[d]	110	(62)	1.18
			200	170		1.19

[a] Column symbols as in Table II.

[b] Aqueous buffer solutions (10 m*M* ammonium acetate, pH 5.5)–methanol; content of methanol as indicated.

[c] Parentheses indicate H^{app} values strongly affected by the carry-over volume.

[d] Indicates values near the critical carry-over limit, above which the peak profile or resolution is significantly affected.

[e] Aqueous buffer solutions (25 m*M* ammonium acetate, pH 5.5)–ethanol; content of ethanol as indicated.

[f] Aqueous buffer solution (25 m*M* ammonium acetate, pH 5.5–35 m*M* β-CD–1 *M* urea)–ethanol; content of ethanol as indicated.

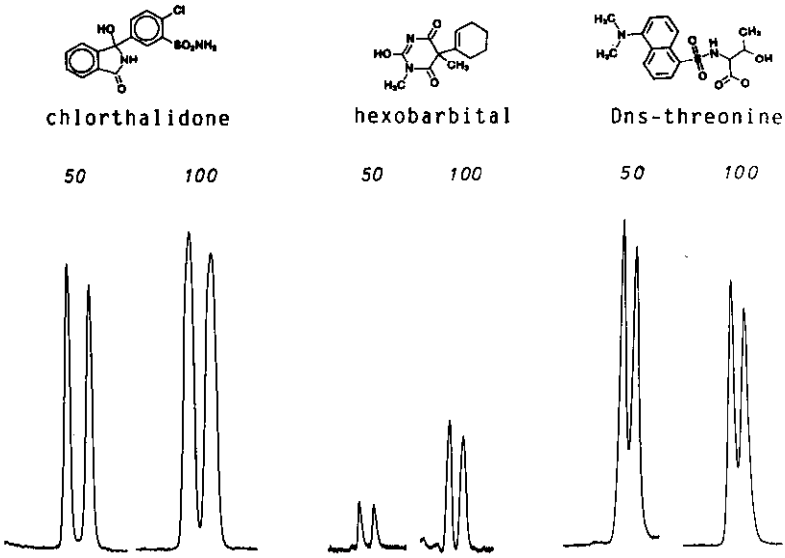


Fig. 6. Effect of the carry-over volume on the enantiomeric separations of the racemic analytes chlorthalidone, hexobarbital and Dns-threonine. CCC system: column 1, octylsilica; column 2, β-CD-CSP. Each pair of peaks represents the two separated enantiomers of the analytes indicated above. The peak pairs are small sections of the total chromatograms. Carry-over volumes: 50 or 100 μl as indicated. Mobile phases: aqueous buffer (0.1 *M* ammonium acetate, pH 5.5)–methanol (60:40, v/v) in eluent 1 and (80:20, v/v) in eluent 2.

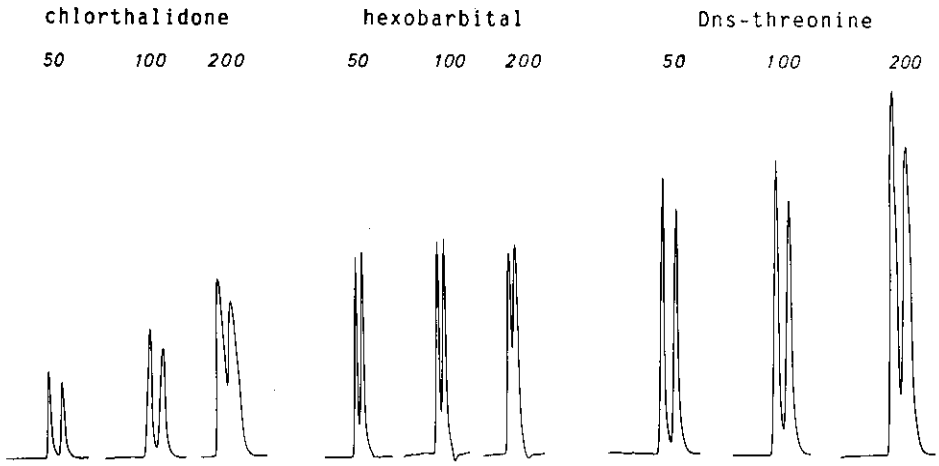


Fig. 7. Effect of the carry-over volume on the enantiomeric separations of the racemic analytes chlorthalidone, hexobarbital and Dns-threonine. CCC system: column 1, octadecylsilica; column 2, octylsilica with β-CD employed as chiral mobile phase additive. Each pair of peaks represents the two separated enantiomers of the analytes indicated above. The peak pairs are small sections of the total chromatograms. Carry-over volumes: 50, 100 or 200 μl as indicated. Eluent 1: aqueous buffer (0.1 *M* ammonium acetate, pH 5.5)–ethanol (60:40, v/v) for hexobarbital and Dns-threonine, (70:30, v/v) for chlorthalidone. Eluent 2: aqueous buffer (25 m*M* ammonium acetate, pH 5.5–35 m*M* β-CD–1 *M* urea)–ethanol (80:20, v/v).

A peak standard deviation at the end of column 1 of 126 μl is evaluated from these data. Assuming a maximum carry-over volume (up to which no influence on the enantiomeric resolution is practically observed) of about 100 μl, and transfer cuts symmetrical to the peak maximum of a Gaussian-type peak, one obtains a transfer yield of about 31% of the analyte mass, and thus a decrease in sensitivity of about 70%. This value may serve as an approximate guideline. It can be confirmed by calculating individual values of the sensitivity decrease for the single analytes, using the particular values of the maximum carry-over volume in Table III and experimentally obtained k' and plate-height data. These individual sensitivity decrease values are given in Table IV; most of them lie between 60 and 70%.

A means of reducing the decrease in analyte mass at constant carry-over volume is to lower the inner diameter of the first column. Such a reduction, however, lowers the sample volume which can be injected without affecting the resolution in the first column. Working with limited concentrations of analytes, the reduction in sample volume also reduces the injected analyte mass, and the overall sensitivity of the method

TABLE IV

ESTIMATED DECREASE IN SENSITIVITY ASSOCIATED WITH A RESTRICTION OF THE CARRY-OVER VOLUME

Values for the maximum carry-over-volume, V_{co}^{max} (μl), are approximate values obtained from the data in Table III. The standard deviation data for the analyte peak at the end of column 1, $\sigma_v^{col.1}$ (μl), were obtained by direct measurement. The percentage of mass transferred was evaluated by assuming a Gaussian distribution and transfer symmetric to the peak maximum.

Column I, OS; column II, β-CD-CSP[a]

Analyte	Methanol in eluent (%, v/v)		V_{co}^{max}	$\sigma_v^{col.1}$	$\frac{V_{co}^{max}}{\sigma_v^{col.1}}$	Mass transferred (%)	Decrease in sensitivity (%)
	1[b]	2[c]					
Hexobarbital	40	20	100	130	0.77	30	70
Dns-threonine	40	20	100	100	1.00	38	62
Chlorthalidone	30	20	150	100	1.50	54	46
	40	20	80	80	1.00	38	62

Column I, ODS; column II, OS/β-CD-CMA[a]

Analyte	Methanol in eluent (%, v/v)		V_{co}^{max}	$\sigma_v^{col.1}$	$\frac{V_{co}^{max}}{\sigma_v^{col.1}}$	Mass transferred (%)	Decrease in sensitivity (%)
	1[d]	2[e]					
Hexobarbital	40	20	100	110	0.91	35	65
Dns-threonine	30	20	100	130	0.77	30	70
Chlorthalidone	30	20	100	80	1.25	48	52

[a] Column symbols as in Table II.
[b] Aqueous buffer (10 mM ammonium acetate, pH 5.5)–methanol, content of methanol as indicated.
[c] Aqueous buffer (10 mM ammonium acetate, pH 5.5)–methanol, content of methanol as indicated.
[d] Aqueous buffer (25 mM ammonium acetate, pH 5.5)–ethanol, content of ethanol as indicated.
[e] Aqueous buffer (25 mM ammonium acetate, pH 5.5–35 mM β-CD–1 M urea)–ethanol, content of ethanol as indicated.

is not improved in this instance. This drawback can be compensated for in principle via an enrichment step in the first column, applying a step gradient after sample injection, as has often been described for trace analysis.

Considering, finally, the efficiency of the two columns, it is noteworthy that the plate numbers employing a β-CD system are lower than those of alkylsilica columns, but not drastically: *ca.* 8000 plates in a 250-mm β-CD-CSP column and about 7000 in a column with the β-CD-CMA system. These data are approximate and average values obtained for the mentioned analytes under the experimental conditions described in the tables, *i.e.*, flow-rate 0.5 ml/min and ambient temperature.

TABLE V

THERMODYNAMIC PEAK COMPRESSION FACTORS, pcf^{th}, IN COLUMN 2 AND MAXIMUM EXPLOITABLE PEAK COMPRESSION EFFECT, pcf^{max}, AS A FUNCTION OF COLUMN DIAMETER FOR THE COLUMN ORDER CHIRAL–NON-CHIRAL

pcf^{th} calculated according to eqn. 1 and pcf^{max} according to eqn. 2, assuming $R_s^{(1+2)} = 0.90 R_s^{(2)}$ (*i.e.*, the resolution in column 2 is affected by the peak profile of the transferred peak by not more than 10%). Column 1 I.D. = 4 mm, column 2 I.D. as specified under pcf^{max}.

Column I, β-CD-CSP; column II, OS[a]

Analyte	Methanol in eluent (%, v/v)		$\sigma_v^{col.1}$	$\sigma_v^{col.2}$	pcf^{th}	pcf^{max}			
						4 mm I.D.	3 mm I.D.	2 mm I.D.	1 mm I.D.
	1[b]	2[c]							
Hexobarbital	20	40	100	130	4.3	1.6	2.8	6.4	25
Dns-threonine	20	40	150	100	5.6	3.1	5.5	12	50
Chlorthalidone	20	30	60	100	2.0	1.2	2.2	5.0	20
	20	40	60	80	4.2	1.6	2.8	6.2	25

Column I, OS/β-CD-CMA; column II, ODS

Analyte	Ethanol in eluent (%, v/v)		$\sigma_v^{col.1}$	$\sigma_v^{col.2}$	pcf^{th}	pcf^{max}			
						4 mm I.D.	3 mm I.D.	2 mm I.D.	1 mm I.D.
	1[d]	2[e]							
Hexobarbital	20	30	70	230	3.2	0.6	1.1	2.5	10
	20	40	70	110	10.3	1.3	2.3	5.3	21
Dns-threonine	20	30	100	130	3.4	1.6	2.8	6.4	25
	20	40	100	70	16.6	3.0	5.2	12	47
Chlorthalidone	20	30	70	80	3.1	1.8	3.2	7.2	29
	20	40	70	60	14.4	2.4	4.3	9.6	39

[a] Column symbols as in Table II.
[b] Aqueous buffer (0.1 *M* ammonium acetate, pH 5.5)–methanol (80:20, v/v).
[c] Aqueous buffer (0.1 *M* ammonium acetate, pH 5.5)–methanol; content of methanol as indicated.
[d] Aqueous buffer (25 m*M* ammonium acetate, pH 5.5 35 m*M* β-CD–1 *M* urea)–ethanol (80:20, v/v).
[e] Aqueous buffer (25 m*M* ammonium acetate, pH 5.5)–ethanol; content of ethanol as indicated.

Perspectives for the column order chiral–non-chiral

The data given in Table II for the chiral–non-chiral column order show that strong peak compression is obtained for the β-CD-CSP column when choosing the working range shown in the Figs. 2 and 3. However, the whole peak compression effect at the top of column 2 cannot be exploited because of the peak dispersion taking place in the second column. The maximum exploitable peak compression factor, pcf^{max}, is calculated according to the following equation [10]

$$pcf^{max} = \frac{\sigma_v^{col.1}}{\sigma_v^{col.2}}\left[\left(\frac{R_s^{(2)}}{R_s^{(1+2)}}\right)^2 - 1\right]^{-1/2} \quad (2)$$

where $R_s^{(2)}$ is the chromatographic resolution obtained by use of column 2 and $R_s^{(1+2)}$ is that obtained by the use of a combination of both columns.

One can see from this equation that the peak compression at the top of column 2 has no practical (*i.e.* < 10%) effect on the resolution and peak height up to a carry-over volume equal to approximately half the volume standard deviation produced by column 2. One has to keep in mind that with this consecutive column order the complete peak has to be transferred, and that the carry-over volume therefore has to be at least six times $\sigma_v^{col.1}$.

Table V shows the theoretical peak compression factors, pcf^{th}, calculated according to eqn. 1 and the maximum exploitable values, pcf^{max}, calculated according to eqn. 2 for the CCC systems (β-CD-CSP)–OS and (OS/β-CD-CMA)–ODS. It can be seen that the theoretical peak compression effect achievable cannot be fully exploited by columns of equal (4 mm) inner diameter. This can be done more completely by reducing the peak dispersion (in volume units) in the second column by reducing its inner diameter. The calculation shows[a] that columns of 2 mm I.D. are best suited to exploit fully the peak compression obtained at top of column 2. On the other hand, the full potential of a column of 1 mm I.D. cannot be completely used owing to the given peak compression conditions. Depending on the phase systems employed, peak compression factors between 5 to 10 can be obtained and can be exploited by use of columns with reduced inner diameter, allowing in this way an increase in sensitivity by the same factor.

CONCLUSIONS

Retention data for several chiral compounds on alkylsilica- and cyclodextrin-containing phase systems are used as a basis for the evaluation of approximate peak compression or peak dilation effects, which are obtained at the top of the second column when choosing typical eluent compositions. Both types of consecutive column order are considered.

Peak compression factors smaller than 1, meaning peak dilation, are obtained in

[a] In the calculations of pcf^{max}, the plate number is assumed to be independent of the column inner diameter. This assumption implies that extra-column contributions can be kept small even with very small diameter columns. This is not very realistic for columns of 1–2 mm I.D. When accounting for extra-column dispersion, the pcf^{max} values are expected to be smaller in such instances in comparison with those given in Table V.

most instances with the column sequence alkylsilica (β-CD-CSP). Less pronounced peak dilation or even small peak compression is obtained when using β-CD as CMA, owing to the additional solvation power of this mobile phase constituent.

The severity of the deconditioning effect observed depends on the volume of the carry-over. The volume up to which no adverse effect is observed is found experimentally to be between 50 and 100 μl, for both β-CD-CSP and β-CD-CMA systems. This result means that simple and straightforward column switching in the convenient column order alkylsilica–(β-CD) system can be successfully performed, when choosing sufficiently low carry-over volumes. Obviously, this method is restricted to instances where one can adopt the strategy of transferring only parts of the chiral analyte. The decrease in sensitivity associated with this strategy lies between 60 and 70% in most instances. A typical example for CCC with β-CD phases which illustrates the decrease in sensitivity is given in Fig. 8.

There are two alternative strategies, in principle, to overcome the adverse situation of higher elution power of the carry-over. First, the eluent dilution technique described and used by Lindner and co-workers [15,16], which reduces the elution strength of the carry-over by adding a weak eluent component, can be applied. This promising technique, however, needs an additional pump (a third pump if the analysis

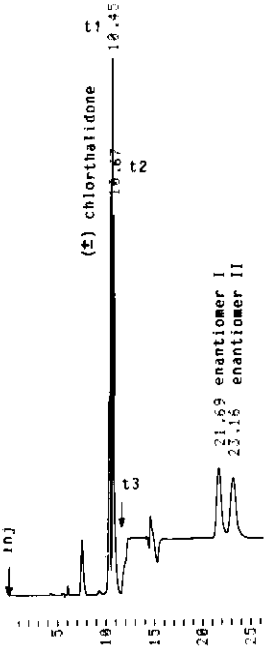


Fig. 8. Typical chromatogram obtained by CCC using octadecylsilica–octylsilica/β-CD-CMA columns. Analytes: enantiomers of chlorthalidone. Peaks (from the left): (±)-chlorthalidone with heart cut in the middle of the peak; first enantiomer of chlorthalidone eluted from column 2; and second enantiomer of chlorthalidone eluted from column 2. Chromatographic conditions: mobile phase, aqueous buffer (25 m*M* ammonium acetate, pH 5.5–35 m*M* β-CD–1 *M* urea) 30% (v/v) ethanol; temperature, 30°C; flow-rate, 0.5 ml/min. Times of switching events: t_1 = 10.40 min; t_2 = 10.60 min; t_3 = 11.50 min (for the definition of t_1, t_2 and t_3, see Table I). Bottom scale: time in minutes.

time should not be increased by repeated changes of eluents). This technique is being investigated further. Second, a small ion-exchange column can be inserted between the alkylsilica and the β-CD column for the retention of analytes which are charged at certain pH values. Peak compression can then be obtained by increasing the concentration of buffer ions, even if the alcohol concentration in the eluent is simultaneously reduced. Again, this set-up requires additional entities and is still being evaluated. We expect advantages especially for those chiral columns which cannot be used with higher concentrations of organic solvent components, such as some protein columns.

The reduction of the carry-over volume evaluated here is a possible means of using β-CD-containing phase systems within a multi-column set up of simple design.

For the reversed column order, β-CD alkylsilica, values between 2 and 5 are calculated for the potential peak compression factors at the top of column 2. The actual value depends on the analyte structure and the separation problem. The use of columns with reduced inner diameter is required in order to exploit the full potential of peak compression achievable in this way and to enhance the sensitivity of determination by the same factors as mentioned above.

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