Selectivity Tuning of Serially Coupled (S,S) Whelk-O 1 and (R,R) Whelk-O 1 Columns in HPLC

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Abstract

The selectivity tuning of two columns coupled in series is investigated in chiral high-performance liquid chromatography. Two columns with reversal enantioselectivities [(R,R) Whelk-O 1 and (S,S) Whelk-O 1] are coupled in series via a T connector. Selectivity of such a column series is tuned by varying the mobile phase flows in the individual columns. The flow ratio necessary for the required selectivity is calculated on the basis of retention factors measured on the individual columns. The performance of this method for adjusting the required selectivity is studied by the separation of enantiomers of alkoxy substituted esters of phenylcarbamic acid. It is demonstrated that the change of the mobile phase flows in the individual columns enables change in the elution order of enantiomers.

Introduction

Because of the limited number of theoretical plates in high-performance liquid chromatography (HPLC), selectivity is the most important tool for achieving the required resolution. For a given pair of substances, selectivity is mainly determined by the nature of the phase system (1). The required selectivity of a stationary phase can, in principle, be changed discontinuously by selection of a proper column packing or continuously by: (*i*) tailor-made stationary phases (2), (*ii*) mixed stationary phases (3–6), (*iii*) coupling columns in series (7,8), or (*iv*) column-switching techniques (9). The last two methods have not yet been fully exploited for the chromatographic separation of enantiomers, even though this approach was successfully applied in achiral separations (10,11).

The aim of this paper is to show a procedure for selectivity tuning of two chiral columns [(R,R) Whelk-O 1 and (S,S) Whelk-O 1] coupled in series for the direct separation of enantiomers. This can be accomplished simply by changing the individual column mobile phase flow rates. The separation of a mixture of R-(+) and S-(–) enantiomers of 2-methoxy-1-(4-methylpiperazino) methylethyl *N*-(2-, 3-, and 4-alkoxyphenyl)carbamate acids (i.e., local anaesthetic drugs) was used to study this selectivity tuning procedure.

Theoretical

The schematic of the two-column system is shown in Figure 1A. The selectivity of such a coupled column system can be set somewhere between the selectivities of the individual columns by changing the flows of the mobile phase in the individual columns $F_{m,A}$ and $F_{m,B}$. The retention factor of a solute in the column series ($k_{i,AB}$) can be calculated from the following equation (12):

$$k'_{i,AB} = x_A \times k'_{i,A} + x_B \times k' \qquad \text{Eq. 1}$$

where $k'_{i,A}$ and $k'_{i,B}$ are the retention factors of this solute in the individual columns (A and B) and x_A, and x_B are weight factors

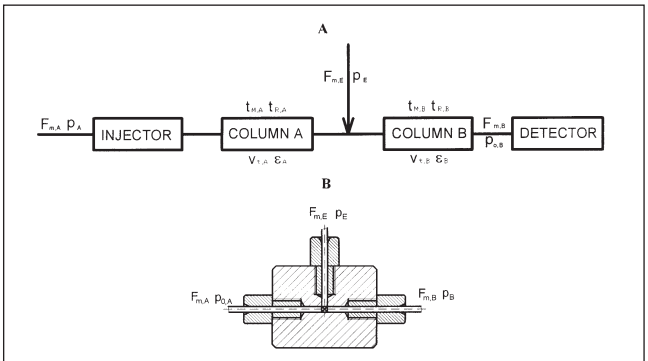

Figure 1. Schematics showing the two columns coupled in series (A) and the "T" connector (B): $F_{m,A}$ = flow of the mobile phase in the column A, $F_{m,E}$ = flow of the mobile phase put into the T connector, $F_{m,B}$ = flow of the mobile phase in the column B ($F_{m,B} = F_{m,A} + F_{m,E}$), p_A = inlet pressure in the column A, p_E = inlet pressure in the T connector, $p_{O,B}$ = outlet pressure in the column B, $t_{M,A}$, $t_{M,B}$ = dead time in column A and B, $t_{R,A}$, $t_{R,B}$ = retention time in column A and B, $V_{t,A}$, $V_{t,B}$ = total volume of column A and B, and A, B = porosity of column A and B.

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(often referred to as relative retentivities A and B) (12), which determine the contribution of individual column selectivity to the overall series column selectivity. These last items can be calculated from the following equations (12):

$$x_A = (\varepsilon_A \times V_{t,A} \times F_{m,B})/(\varepsilon_A \times V_{t,A} \times F_{m,B} + \varepsilon_B \times V_{t,B} \times F_{m,A}) \quad \text{Eq. 2}$$

and

$$x_B = (\varepsilon_B \times V_{t,B} \times F_{m,A})/(\varepsilon_A \times V_{t,A} \times F_{m,B} + \varepsilon_B \times V_{t,B} \times F_{m,A}) \quad \text{Eq. 3}$$

where ε is an overall column porosity, V_t is a total column volume, and F_m is the mobile phase flow.

Because physical parameters (ε and V_t) of (R,R) Whelk-O 1 and (S,S) Whelk-O 1 columns used in this study were expected to be equal, equations 2 and 3 were simplified as follow:

$$x_A = (F_{m,B})/(F_{m,B} + F_{m,A}) \quad \text{Eq. 4}$$

and

$$x_B = (F_{m,B})/(F_{m,B} + F_{m,A}) \quad \text{Eq. 5}$$

From equations 4 and 5 it follows that:

$$x_A + x_B = 1 \quad \text{Eq. 6}$$

Combination of equations 1 and 6 leads to the formula:

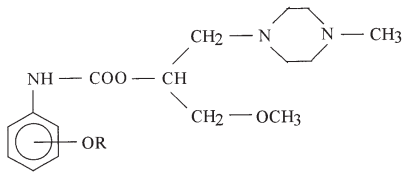
$$k'_{i,AB} = k'_{i,A} + x_B(k'_{i,B} - k'_{i,A}) \quad \text{Eq. 7}$$

and substituting equation 5 into equation 7:

$$k'_{i,AB} = k'_{i,A} + (F_{m,A})/F_{j,A} + F_{m,B}) \times (k'_{i,B} - k'_{i,A}) \quad \text{Eq. 8}$$

Equation 8 shows that the retention factor of an analyte on columns coupled in series ($k'_{i,AB}$) (*a*) depends on the retention factors in the individual columns ($k'_{i,B}$ and $k'_{i,A}$) and (*b*) the column series can be tuned by controlling the mobile phase flow in the individual columns ($F_{m,A}$ and $F_{m,B}$) (12). This treatment assumes that the porosity, E, and the total column volumes are equal.

Table I. Schematic Showing the Structures of the Alkoxy Substituted Derivatives of Phenylcarbamic Acid Esters



2-Position		3-Position		4-Position	
Analyte no.	R	Analyte no.	R	Analyte no.	R
1	C_4H_9	5	C_4H_9	9	C_3H_7
2	C_5H_{11}	6	C_5H_{11}	10	C_4H_9
3	C_6H_{13}	7	C_6H_{13}	11	C_5H_{11}
4	C_7H_{15}	8	C_7H_{15}	12	C_6H_{13}
				13	

Table II. Retention Factors (k') and Selectivity Factors (α) of Enantiomers of 4-Alkoxy Substituted Derivatives of Esters of Phenylcarbamic Acid Obtained for Single (R,R) Whelk-O 1 and (S,S) Whelk-O 1 Columns

Analyte no.	(R,R) Whelk-O 1			(S,S) Whelk-O 1		
	k'_i	k'_j	α	k'_i	k'_j	α
2-Position						
1	2.64	2.80	1.06	3.31	3.67	1.11
2	2.84	3.00	1.06	3.70	4.13	1.12
3	2.94	3.12	1.06	3.96	4.41	1.11
4	3.03	3.24	1.07	4.51	5.06	1.12
3-Position						
5	1.99	2.18	1.10	2.22	2.58	1.16
6	2.09	2.29	1.10	2.38	2.74	1.15
7	2.14	2.37	1.11	2.50	2.88	1.15
8	2.26	2.47	1.09	2.69	3.09	1.15
4-Position						
9	2.38	2.68	1.12	2.93	3.49	1.19
10	2.58	2.91	1.13	3.32	3.96	1.19
11	2.74	3.10	1.13	3.65	4.38	1.20
12	2.92	3.31	1.13	5.03	4.17	1.21
13	3.07	3.49	1.14	5.55	4.59	1.21

* For $n = 3$, $k'_i = \pm 0.12$, $k'_j = \pm 0.15$, and $\alpha = \pm 0.05$.

Experimental

Materials

Column A, (R,R) Whelk-O 1 CSP (25-cm × 4.6-mm i.d. length), was obtained from Regis Technologies (Morton Grove, IL). Column B, (S,S) Whelk-O 1 CSP (25-cm × 4.6-mm i.d. length), was obtained from Regis Technologies.

The analytes separated in this study (2-methoxy-1-[(4-methylpiperazino)methyl]ethyl *N*-(2-, 3-, and 4-alkoxyphenyl) carbamate acid) were prepared according to Cizmárik et al. (13) (Table I). HPLC-grade solvents (methanol) were obtained from Merck (Darmstadt, Germany). Triethylamine and acetic acid were obtained from Lachema (Brno, Czech Republic).

Equipment

HPLC using single and coupled columns in series was performed on a Hewlett Packard (HP series 1100) HPLC chromatographic system (Palo Alto, CA), which consisted of a quaternary solvent pump, a Rheodyne Model 7724 injector fitted with a 20-µL sample loop (Rohnert Park, CA), and a photodiode array detector (DAD). Two chiral stationary phases [(R,R) Whelk-O 1 and (S,S) Whelk-O 1] were coupled in series in both sequences (AB or BA) according to the schematic shown in Figure 1A. The outlet of the first column was coupled to the inlet of the second column directly through a T-piece connector (Figure 1B).

Methods

Chromatographic separations were carried out at different mobile phase flow rates (total flow of the mobile phase was measured at the exit of the chromatographic system using a calibrated pipette) at ambient temperature. A DAD at 240 nm was used. The analytes were dissolved in methanol (concentration 1 mg/mL). Mobile phases were prepared by mixing methanol–water (90:10, v/v) with 17.5 mmol/L acetic acid and 14.36 mmol/L triethylamine.

Results and Discussion

Separation of enantiomers of alkoxysubstituted esters of phenylcarbamic acid on individual columns

In a recent study, we have shown that the separation enantiomers of esters of phenylcarbamic acid by HPLC on (R,R) Whelk-O 1 and (S,S) Whelk-O 1 columns vary with the position of alkoxy substituents on the phenyl group. The best enantiomer separation was obtained for 4- and the worst for 2-alkoxy-substituted derivatives. The elution order of all enantiomers was R-(–) and then S-(+) for the (S,S) Whelk-O 1 and S-(+) and then R-(–) for the (R,R) Whelk-O 1 (14). The reversed-phase mode was used.

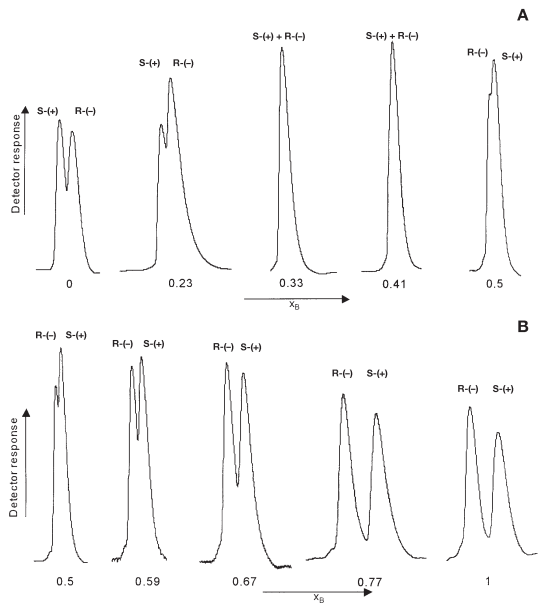


Figure 2. Separation of 4-butyloxy derivatives of esters of phenylcarbamic acid (analyte no. 10) by reversed-phase chromatography on the (R,R) Whelk-O 1 (A) and (S,S) Whelk-O 1 (B) column series at varied values of the weight factors: (A) $x_B = 0$ for individual column A, A coupled to B, $x_B < 0.23–0.5 >$; (B) B coupled to A, $x_B < 0.5–0.77 >$; and (C) $x_B = 1$ for individual column B. For other conditions see the Experimental section.

Table II lists the retention (k') and selectivity (α) factors determined for the enantiomers of esters of phenylcarbamic acid on individual (R,R) Whelk-O 1 and (S,S) Whelk-O 1 columns in this study. Comparison of this data shows that both the retention, as well as selectivity factors of the enantiomers are somewhat higher on the (S,S) Whelk-O 1 than on the (R,R) Whelk-O 1 column. This difference could be connected with the fact that there was extensive use of the (R,R) Whelk-O 1 column prior to this series of experiments. Also, there are always small column-to-column differences for most chiral stationary phases.

Separation of enantiomers of esters of alkoxysubstituted phenylcarbamic acid on columns coupled in series

The separation of enantiomers of 2-, 3-, and 4-alkoxyderivatives of esters of phenoxy carbamic acid was studied coupling the (R,R) Whelk-O 1 (column A) and (S,S) Whelk-O 1 (column B) both in the AB and BA series. The use of different column orders allowed us to change the weight factor (x_B) in the interval 0.23–0.77 (from 0.23 to 0.5 for the AB and from 0.5 to 0.77 for the BA column series, respectively). Figure 2 demonstrates that changing the weight factor within this interval does not influence the peak shapes of enantiomers substantially). Comparison of the measured and calculated retention factors according to equation 1 in Table III shows reasonable agreement of these data both for the AB and BA column order. The data in this table shows that changing the weight factor induces a dramatic retention change for the enantiomers. The dependence of enantiomeric retention on the weight factor is illustrated in Figure 2

Table III. Calculated ($k'_{i,AB,c}$) and Measured ($k'_{i,AB,m}$) Retention Factors on (R,R) Whelk-O 1 and (S,S) Whelk-O 1 Columns Coupled in Series at Different Weight Factors (x_B) for 4-Alkoxy-Substituted Esters of Phenylcarbamic Acid*

		4-Position of alkoxy derivatives of phenylcarbamic acid									
x_B	$k'_{i,AB}$	9 S-(+)	9 R-(–)	10 S-(+)	10 R-(–)	11 S-(+)	11 R-(–)	12 S-(+)	12 R-(–)	13 S-(+)	13 R-(–)
0.23	*m*	2.71	2.81	2.93	3.04	3.16	3.26	3.30	3.35	3.50	3.60
	c	2.64	2.74	2.90	3.00	3.12	3.23	3.40	3.50	3.64	3.74
0.33	*m*	2.80	2.80	3.03	3.03	3.25	3.25	3.53	3.53	3.75	3.75
	c	2.75	2.76	3.03	3.04	3.28	3.28	3.61	3.59	3.89	3.85
0.41	*m*	2.83	2.83	3.07	3.07	3.30	3.30	3.62	3.62	3.90	3.90
	c	2.83	2.78	3.14	3.08	3.41	3.33	3.79	3.66	4.09	3.94
0.50	*m*	2.94	2.87	3.36	3.29	3.51	3.40	3.84	3.75	4.18	4.07
	c	2.94	2.81	3.27	3.12	3.56	3.38	3.98	3.74	4.31	4.04
0.50	*m*	3.01	2.90	3.45	3.30	3.364	3.45	4.05	3.85	4.40	4.18
	c	2.94	2.81	3.27	3.12	3.56	3.38	3.98	3.74	4.31	4.04
0.59	*m*	3.13	2.92	3.57	3.32	3.75	3.48	4.26	3.92	4.58	4.23
	c	3.03	2.83	3.39	3.15	3.71	3.42	4.16	3.82	4.53	4.14
0.67	*m*	3.20	2.95	3.69	3.33	3.83	3.55	4.40	3.99	4.82	4.35
	c	3.12	2.84	3.50	3.18	3.77	3.49	4.33	3.89	4.73	4.23
0.77	*m*	3.36	3.00	3.69	3.28	3.95	3.50	4.58	4.04	5.10	4.49
	c	3.23	2.87	3.64	3.23	4.00	3.52	4.54	3.97	4.98	4.34

* For $n = 3$: $k'_{i,AB,c} = \pm 0.12$; $k'_{i,AB,m} = \pm 0.15$. For details see the Experimental section.

for enantiomers of 4-butyloxy derivatives of esters of phenylcarbamic acid (analyte no. 10) and in Figure 3 for enantiomers of 2-alkoxy (Figure 3A), 3-alkoxy (Figure 3B), and 4-alkoxy (Figure 3C) esters of phenylcarbamic acids. It has been expected that selectivity of column series AB and BA would be equal; Figure 2, however, shows differences in the AB (Figure 2A) and BA (Figure 2B) column series selectivity at a weight factor x_B = 0.5. This unexpected phenomenon could be related with the different porosity of the individual columns or errors in the mobile phase flow rate measurements (or both) through individual columns.

Figure 4 shows the dependence of the retention factors ($k_{i,AB}$) on the weight factor (xB) for enantiomers of 2- (bottom figure), 3- (middle), and 4- (upper) alkoxyderivates of esters of phenylcarbamic acid separated on the column series AB or BA. Intersections of $k'_{i,AB}$ straight lines with the perpendicular lines show the differences in selectivity between both column series. The perpendiculars in Figure 4 were constructed from the retention factors ($k'_{i,AB}$) determined for enantiomers of the esters of alkoxy substituted acid of phenylcarbamic acid in the column series AB (left perpendicular) and in the column series BA (right perpendicular) with the constant flow in both columns. The Figure 4 dependences were constructed using the retention factors of the corresponding enantiomers obtained in individual columns, as it follows from equation 1 for x_B = 0 and x_B = 1, respectively.

The dependence of the selectivity factor (α) on the weight factor (k'_B) for enantiomers of 2-, 3-, and 4-alkoxy derivatives (C3–C7) of esters of phenylcarbamic acid is demonstrated in Figure 5. From this figure, it follows that a change of weight factor in the range 0.23–0.77 will induce a change in the selectivity factors in the region of 0.96–1.13 for 4-alkoxy derivatives, 0.96–1.09 for 3-alkoxy derivatives, and 0.98–1.08 for the 2-alkoxy derivative analytes. Moreover, this figure illustrates that the change in flow rate allows one to change the elution order of enantiomers of all the enantiomeric analytes in this study.

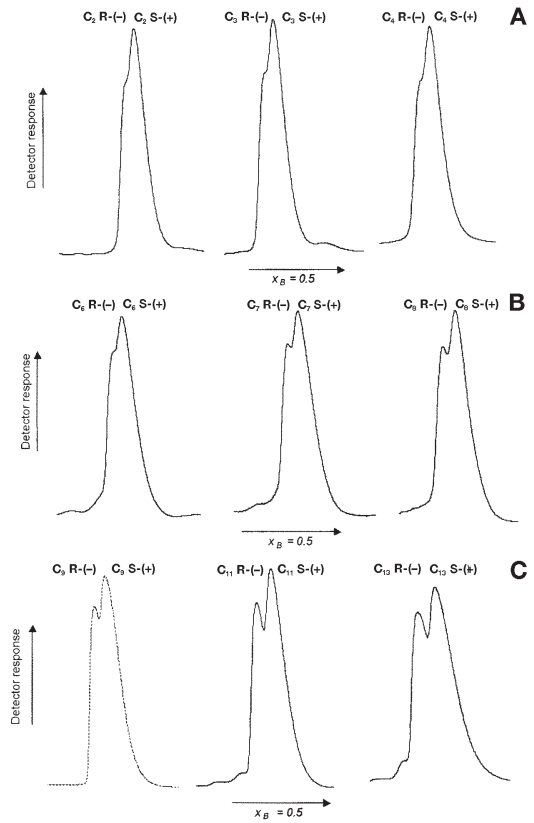


Figure 3. Separation of enantiomers of some alkoxysubstituted esters of phenylcarbamic acid on the AB column series: (A) separation of 2-alkoxy-substituted esters of phenylcarbamic acid, (B) separation of 3-alkoxysubstituted esters of phenylcarbamic acid, and (C) separation of 4-alkoxysubstituted esters of phenylcarbamic acid (the number of the peaks in these chromatograms corresponds to the numbering in Table I). For details see the Experimental section.

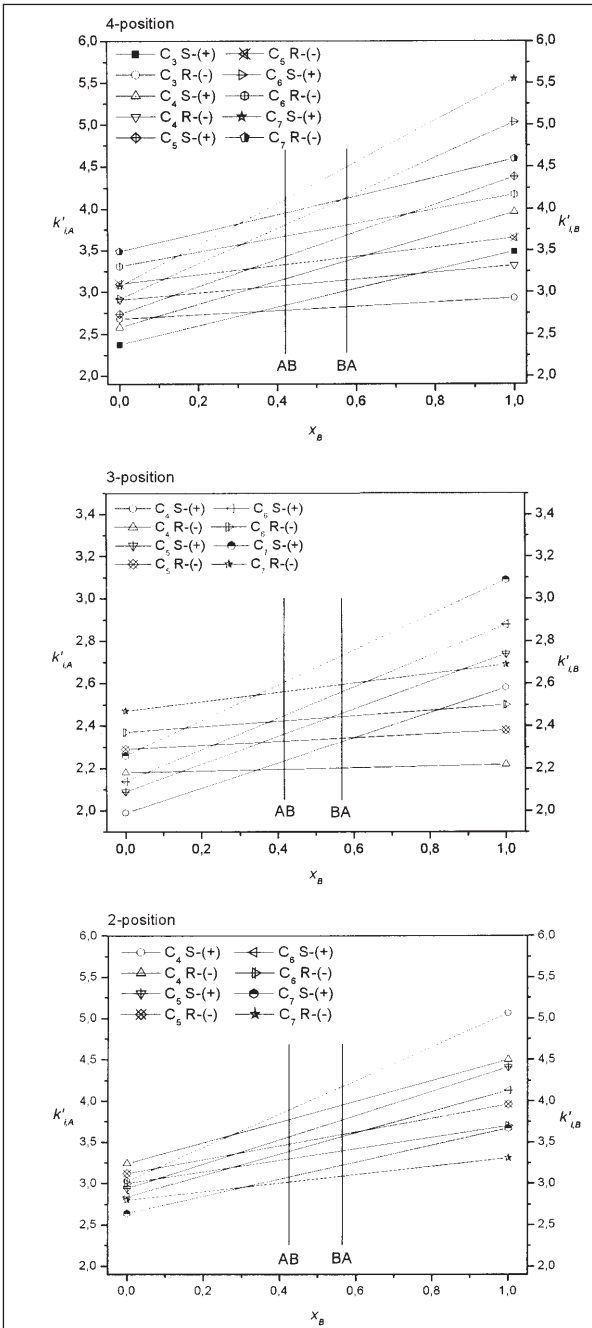


Figure 4. Dependence of retention factors ($k'_{i,AB}$) of enantiomers of 2, 3-, and 4-alkoxy-substituted derivatives of esters of phenylcarbamic acid on the weight factor (x_B). For details see the Experimental section.

Conclusion

The selectivity of two HPLC chiral stationary phase-containing columns of opposite enantioselecity and coupled in series can be tuned by altering the flows of the mobile phases in the individual columns. The retention order of enantiomers of alkoxysubstituted esters of phenylcarbamic acid were changed by tuning the flows in the column series, which consisted of (R,R) Whelk-O 1 and (S,S) Whelk-O 1 columns. The described selectivity tuning may be used in analytical praxis for the determination of enantiomer purity where the configuration of the principal enantiomer may be changed. In these cases, minor enantiomers should elute prior to the main one.

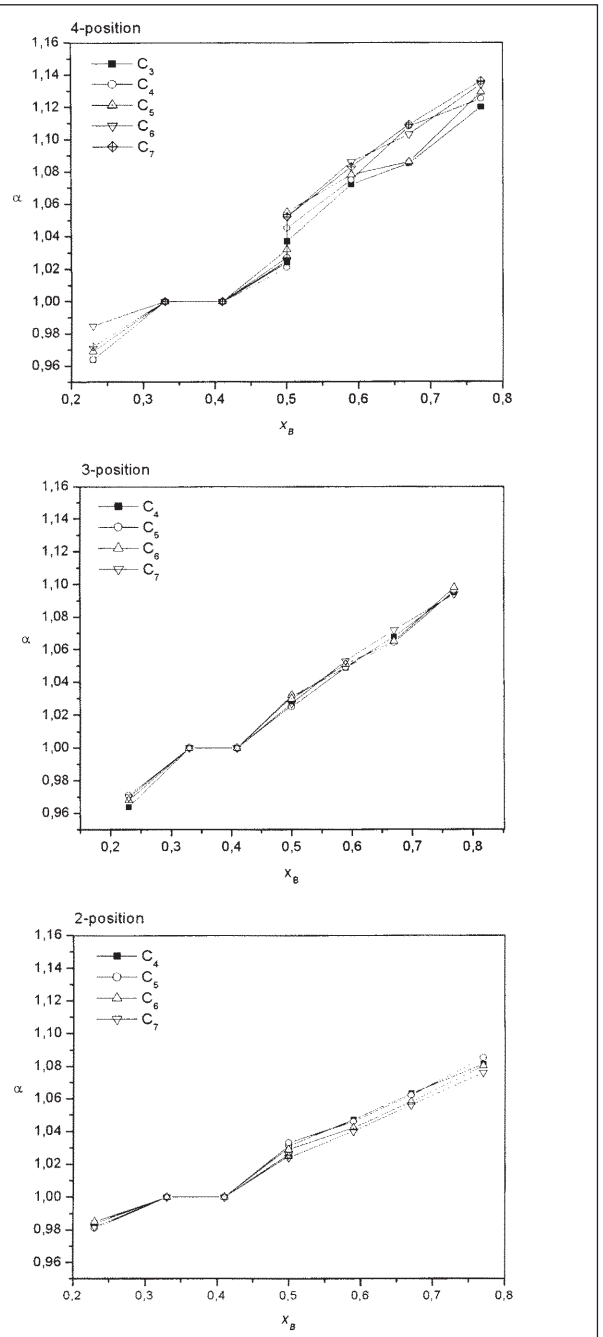


Figure 5. Dependence of selectivity factors (α) on weight factors (x_B) for 2-, 3-, and 4-alkoxy derivatives of esters of substituted phenylcarbamic acid. For details see the Experimental section.

Acknowledgments

The authors acknowledge the support of the Grant Agency of Slovak Republic (VEGA 1/9127/02) and the Agency for International Science and Technology Cooperation in Slovakia. DWA acknowledges the support of the National Institutes of Health (Grant NIH R01 GM 53825-08.

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Manuscript accepted January 13, 2004.