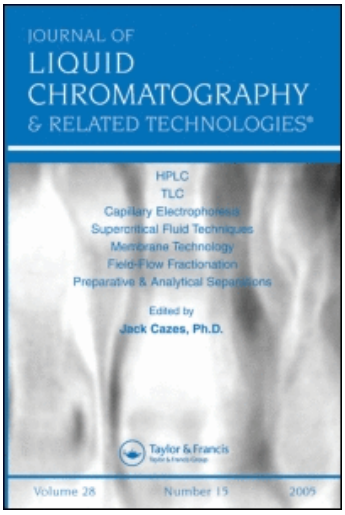

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Study of the Mechanism of Enantioseparation. IV. Study of Enantioseparation of Some Derivatives of Phenylcarbamic Acid Using π-Complex Stationary Phase in HPLC

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ABSTRACT

The Whelk-O 1 chiral stationary phase (CSP) is an useful column for the high performance liquid chromatographic (HPLC) resolution of enantiomers of 2-methoxy-1-[(4-methylpiperazino)methyl]ethyl esters of *N*-(2-,3-, and 4-alkoxyphenyl)carbamic acid (group of the local anaesthetic drugs) in the

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polar organic mode and the reversed mode. According to the results of enantiomeric separations in different mobile phases [containing organic modifiers (methanol, acetonitrile, water), with different additives of acetic acid and trimethylamine], it can be postulated that the piperazino part of the phenylcarbamic acid compound is essential because the separation of piperidino, pyrrolidino, and perhydroazepino esters of alkoxy phenylcarbamic acid were not observed. Also the influence of the environment near the analyte's stereogenic is important for enantioseparation. A comparison of the enantiomeric elution order and the configuration of the chiral selector shows that *R*-(−) enantiomers are more retained on the (*R*,*R*) Whelk-O 1 column and *S*-(+) enantiomers are more retained on the (*S*,*S*) Whelk-O 1 column.

Key Words: HPLC; Enantiomeric separation; (*R*,*R*) and (*S*,*S*) Whelk-O 1 chiral stationary phases; Alkoxysubstituted esters of phenylcarbamic acid; Study of mechanism.

INTRODUCTION

The direct separation of enantiomers on liquid chromatographic chiral stationary phases (CSPs) is known to be the most accurate and convenient means of solving many problems related to stereochemistry including the determination of enantiomeric composition of chiral compounds.[1] Consequently, significant efforts have been devoted to the development of effective CSPs for the liquid chromatographic direct separation of enantiomers.[2] Among others, the π-complex-type of CSPs have been known to be effective for the resolution of racemates containing π-electron accepting (π-acidic) or π-electron donating (π-basic) aromatic groups.[3] Research in this area has produced several useful CSPs that utilize π–π interactions.[4–6]

The first commercially available HPLC chiral stationary phase was introduced by Pirkle in 1981.[7] Whelk-O 1 is the most widely applicable of these types of stationary phases and is derived from 4-(3,5-dinitrobenzamido)tetrahydrophenanthrene, covalently bound to 5 μm 3-propyl silica. The Whelk-O 1 CSP contains both π-basic and π-acidic groups, which allows it to separate a wider variety of compounds.[2]

The "cleft-like" active site in the CSP is believed to be responsible for the ability of this CSP to separate enantiomers that possess certain structural features.[8] In general, these features include first, and foremost, a π-basic group (usually an aromatic system) in proximity to the stereogenic center. This group participates in a face to face π–π interaction with the π-acidic 3,5-dinitrobenzoyl moiety (DNB). Secondly, this CSP requires a hydrogen bond acceptor site in proximity to the stereogenic center to undergo hydrogen bonding by the relatively acidic DNB amide N—H. A final requirement is that the

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π-basic aryl group used in the face to face π–π interaction should be capable of entering the cleft of the CSP in such a way as to present the ring protons along one of its edges to the face of the naphthyl portion of the CSP. The mentioned considerations are consistent with prior chromatographic studies and with NMR studies employing the selector (or analogs thereof) used in CSP.[9–12]

Piperazino, pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acid form a group of potential drugs employing local anaesthesias.[13,14] The enantiomeric separation of derivatives of phenylcarbamic acid can be performed by means of different chromatographic techniques, including TLC[15] and HPLC.[16–20]

This paper deals with the HPLC enantioseparation of different esters of alkoxy phenylcarbamic acids by using the (*R*,*R*) Whelk-O 1 and (*S*,*S*) Whelk-O 1 chiral stationary phases. The influence of different parameters on enantioselectivity has been investigated. The effect of the mobile phase, the ratio of methanol, acetonitrile, and water, and addition of ionic organic modifiers (acetic acid, triethylamine) have been studied in order to examine the chiral separation mechanism.

EXPERIMENTAL

Materials

Analytical (25 cm × 4.6 mm I.D. length) (*S*,*S*) Whelk-O 1 CSP (No. 786101) and (*R*,*R*) Whelk-O 1 CSP were obtained from Regis Technologies (Morton Grove, IL). The analytes used in this study, 2-methoxy-1-[(4-methylpiperazino)-methyl]ethyl esters of *N*-(2-,3-, and 4-alkoxyphenyl) carbamic acid (Table 1) and 2-methoxy-[(pyrrolidino)methyl]ethyl ester of *N*-(2-buthoxyphenyl) carbamic acid, 2-methoxy-[(piperidino)methyl]ethyl ester of *N*-(2-buthoxyphenyl) carbamic acid, and 2-buthoxy-[(perhydroazepino) methyl]ethyl ester of *N*-(2-buthoxyphenyl) carbamic acid (Table 2), were prepared. All HPLC grade solvents (methanol and acetonitrile) were obtained from Merck (Germany). Triethylamine and acetic acid were obtained from Lachema (Czech Republic).

Apparatus

The HPLC chromatographic system Hewlett Packard (series 1100) consisted of a quaternary pump, a Rheodyne Model 7724 injector fitted with 20 μL sample loop, and a photodiode array detector.

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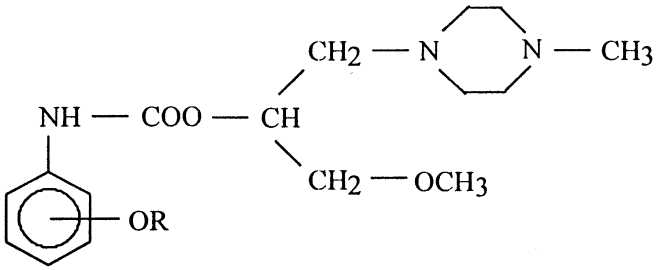
Table 1. Chemical structures of the piperazino esters of alkoxy phenylcarbamic acid that were examined.



2-Position		3-Position		4-Position	
Analyte nr.	R	Analyte nr.	R	Analyte nr.	R
				9	C_3H_7
1	C_4H_9	5	C_4H_9	10	C_4H_9
2	C_5H_{11}	6	C_5H_{11}	11	C_5H_{11}
3	C_6H_{13}	7	C_6H_{13}	12	C_6H_{13}
4	C_7H_{15}	8	C_7H_{15}	13	C_7H_{15}

Methods

Chromatographic analysis was carried out at a flow rate 0.7 mL min^{-1} at ambient temperature. DAD at 240 nm was used. The analytes were dissolved in methanol (concentration 1 mg mL^{-1}). Mobile phases were prepared by mixing methanol (acetonitrile), water, acetic acid, and triethylamine in different ratios. Compositions of the mobile phases are listed in the appropriate tables and figures.

For the measurement of optical rotation, the polarimeter Polar LμP (Na lamp, $\lambda = 589$ nm) (IBZ Messtechnik) was used. After the separation, the fractions of enantiomers (analytes were injected one hundred times into a chromatographic system) were collected to measure their optical properties. The enantiomers were preconcentrated by evaporation under a stream of nitrogen.

RESULTS AND DISCUSSION

If specific chiral analytes (such as derivates of phenylcarbamic acid with different alkoxy substitution in the 2-, 3-, and 4-positions, Tables 1 and 2) have a sufficient number of functional groups capable of strongly interacting with the

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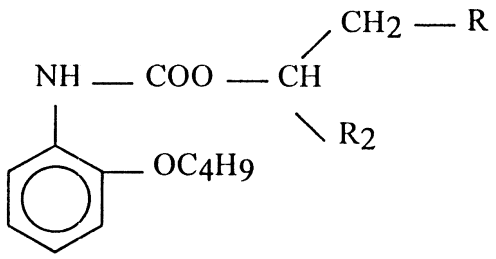
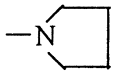
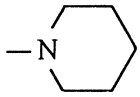
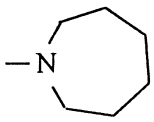
Table 2. Chemical structures of derivatives of the pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acid.



Analyte nr.	R_1	R_2
14		$-CH_2-OCH_3$
15		$-CH_2-OCH_3$
16		$-CH_2-OC_4H_9$

stationary phase, then the best mobile phase choice often consists of polar organic solvents. This mobile phase system utilizes methanol or acetonitrile, while acid (acetic acid) and base (triethylamine) additives are added in small quantities.

No enantiomeric separations and very high retention times were observed for piperazino esters of alkoxy phenylcarbamic acid when the mobile phase consisted of methanol, methanol with 17.5 mmol L^{-1} acetic acid, and methanol/water (90/10, v/v or 80/20, v/v) with 17.5 mmol L^{-1} acetic acid (retention factors were about 20 and more).

Separation of Enantiomers in the Polar Organic Mode

Influence of Concentration of Triethylamine in the Mobile Phase

The influence of the amount of triethylamine in the mobile phase on the values of the retention factors for the piperazino esters of alkoxyphenylcarbamic acid with alkoxy substitution (C_4 and C_7) in the 2-, 3- and 4-positions

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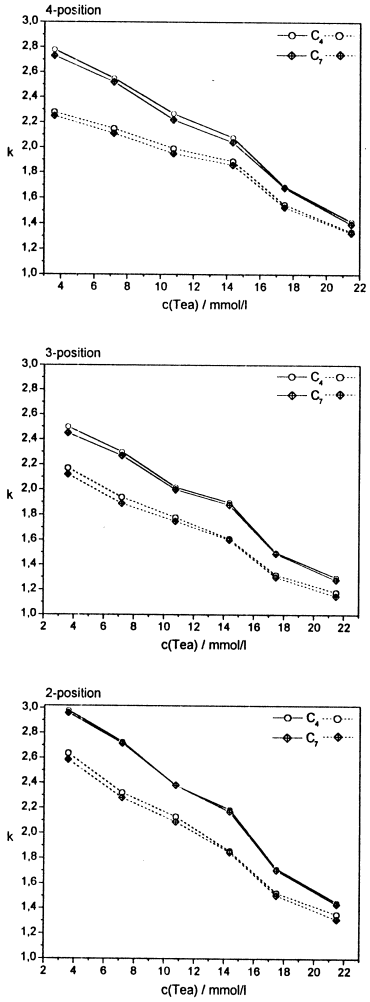

Figure 1. The influence of the concentration of triethylamine in the mobile phase on the retention factors of the *S*-(+) enantiomers of the 2-, 3-, and 4-alkoxy substituted piperazino esters of carbamic acid with the number of carbon atoms in —OR (C_4 and C_7). Chiral stationary phase: (*R*,*R*) Whelk-O 1; mobile phase: solid line, methanol containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}); dashed line, methanol containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}) at the constant ionic strength of the mobile phase ($I = 24.5$ mmol L^{-1}).

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is shown in Fig. 1. Note, that there are similar trends for compounds of all alkoxy-chain lengths (i.e., C_3, C_5, and C_6), and substitution dependencies have similar tendency for 2-, 3-, and 4-position of patterns (i.e., the 2-, 3-, and 4-positions). It is evident, that an increase of the concentration of triethylamine in the mobile phase in the range 3.59–21.50 mmol L^{-1} decreased the retention of three enantiomers. In addition, the influence of base concentration on the enantiomeric resolution was significant [concentration of acetic acid was constant (17.5 mmol L^{-1})] (Table 3, R_{ij}[a]). The retention of these compounds is not dependent on the number of carbons in the alkoxy chain in this chromatography mode. On the other hand, the enantioresolution of these analytes is dependent on the position of the alkoxy chain on the aromatic ring of these molecules (with the 4-substituted compounds being best resolved, $R_{ij} = 0.8$–1.2) and the least enantioresolution obtained for 2-alkoxysubstitued esters of phenylcarbamic acid, $R_{ij} \geq 0.4$–0.8 (Table 3, R_{ij}[a]).

The enantioseparation of pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acids (one atom of nitrogen in the ring) were not observed using the above-mentioned chromatographic conditions. The retention factors decreased with increasing content of triethylamine in the mobile phase from 1.98 to 0.36 for pyrrolidino enantiomers, 1.42–0.21 for piperidino enantiomers, and 0.98–0.14 for the perhydroazepino ester of alkoxy phenylcarbamic acid.

Influence of Concentration of Triethylamine in the Mobile Phase at Constant Ionic Strength

The influence of different amounts of triethylamine (at a constant concentration of acetic acid) in the mobile phase with a constant ionic strength ($I = 24.5$ mmol L^{-1}, as adjusted with lithium chloride) on the values of retention factors of analytes is illustrated in Fig. 1 [see dashed line; the dependencies have similar trends for 2-, 3-, and 4-substituted alkoxy chains (C_3, C_5, and C_6 carbon numbers) for 2-, 3-, and 4-piperazino esters of alkoxy phenylcarbamic acid]. The values of the retention factors (Fig. 1) and the resolutions (Table 3, R_{ij}[b]) of these enantiomers, using mobile phases with different concentrations of triethylamine at constant ionic strength, decrease in comparison with the results achieved in the mobile phase without lithium chloride. The content of lithium chloride in the mobile phase has a negative influence on the enantioseparation (the added salt decreases the interaction of these analytes with the stationary phase).

Pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acids were not separated in the constant ionic strength mobile phase and the values of retention factors were zero.

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Table 3. The influence of the concentration of triethylamine on the resolution values of piperazino esters of alkoxy phenylcarbamic acid. Chiral stationary phase: (*R*,*R*) Whelk-O 1.

	Concentration of triethylamine (mmol L^{-1})											
	3.59		7.18		10.77		14.36		17.50		21.50	
Analyte nr.	R_{ij}^a	R_{ij}^b	R_{ij}^a	R_{ij}^b	R_{ij}^a	R_{ij}^b	R_{ij}^a	R_{ij}^b	R_{ij}^a	R_{ij}^b	R_{ij}^a	R_{ij}^b
2-Position												
1	>0.4	>0.4	0.7	0.5	0.7	0.5	0.8	0.5	0.7	0.5	0.5	>0.4
2	>0.4	>0.4	0.7	0.5	0.7	0.4	0.8	0.5	0.6	0.5	0.5	>0.4
3	>0.4	>0.4	0.7	0.4	0.7	0.5	0.8	0.5	0.7	0.5	0.5	>0.4
4	>0.4	>0.4	0.7	0.5	0.6	0.5	0.8	0.6	0.7	0.5	0.6	>0.4
3-Position												
5	>0.4	>0.4	0.8	0.6	0.9	0.6	0.9	0.8	0.9	0.7	0.7	>0.4
6	>0.4	>0.4	0.7	0.6	0.9	0.7	1.0	0.8	0.9	0.6	0.6	>0.4
7	>0.4	>0.4	0.7	0.6	0.9	0.6	0.9	0.7	0.8	0.6	0.6	>0.4
8	>0.4	>0.4	0.7	0.6	0.9	0.6	1.0	0.8	0.9	0.7	0.6	>0.4
4-Position												
9	0.6	>0.4	0.8	0.6	0.9	0.8	1.2	1.1	1.2	1.1	0.8	0.6
10	0.6	>0.4	0.8	0.7	1.0	0.8	1.2	0.9	1.1	1.0	0.9	0.7
11	0.5	0.5	0.8	0.6	1.0	0.7	1.1	1.0	1.0	1.0	0.8	0.6
12	0.5	0.6	0.8	0.7	1.0	0.8	1.1	1.0	1.1	0.9	0.8	0.6
13	0.5	0.5	0.9	0.6	0.9	0.7	1.2	0.9	1.1	0.9	0.8	0.6

Note: For $n = 3$; $R_{ij} = \pm 0.1$.

[a]Methanol containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

[b]Methanol containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}) at the constant ionic strength of the mobile phase ($I = 24.5$ mmol L^{-1}).

Influence of the Content of Acetonitrile in the Mobile Phase

The influence of the ratio of acetonitrile and methanol in the mobile phase at a constant concentration of acetic acid (17.5 mmol L^{-1}) and with different concentrations of triethylamine (3.59–21.5 mmol L^{-1}) on the values of retention factors of piperazino esters of alkoxy phenylcarbamic acid with alkoxy substitution (C_4 and C_7) in 2-, 3-, and 4-position is shown in Fig. 2 (see solid line). The same dependences were found for compounds of all alkoxy-chain lengths (i.e., C_3, C_5, and C_6), and substitution (i.e., the 2-, 3-, and 4-positions). The values of the resolution factors are listed in Table 4. Increasing the acetonitrile content in the range of 0–20% resulted in decreasing retention factors and resolutions (Fig. 1, solid line and Fig. 2) for the analytes (Tables 3 and 4). No separations were observed for the 2-piperazino esters of alkoxy phenylcarbamic acid when the mobile phase consisted of methanol/acetonitrile (80/20, v/v) and any concentration of triethylamine. A comparison of the retention factors listed in Fig. 2 shows that they are not dependent on the length of the analytes, alkoxy chain, but are dependent on the substitution position of alkoxy chain of the piperazino esters of the alkoxy phenylcarbamic acid on the aromatic ring.

The separation of enantiomers of compounds with one ring nitrogen (pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acid) were not observed with these mobile phases containing methanol/acetonitrile (90/10, v/v), 17.5 mmol L^{-1} acetic acid, and different concentrations of triethylamine (3.59–21.5 mmol L^{-1}). The retention factors decreased with increasing content of triethylamine in the range of 1.06–0.21 for pyrrolidino enantiomers, 1.00–0.15 for piperidino enantiomers, and 0.87–0.11 for perhydroazepino esters of alkoxy phenylcarbamic acid. The values of the retention factors were zero if the mobile phase consisted of methanol/acetonitrile (80/20, v/v), acetic acid (17.5 mmol L^{-1}), and triethylamine (3.59–21.5 mmol L^{-1}).

Separation of Enantiomers in the Reversed Mode

Influence of Concentration of Triethylamine in the Mobile Phase

The influence of the base concentration in the mobile phase on the resolution of piperazino esters of alkoxy phenylcarbamic acid was also studied (Table 5). Mobile phases consisted of methanol/water (90/10 and 80/20, v/v), 17.5 mmol L^{-1} acetic acid and different concentrations of base. The highest values of resolution were achieved in the mobile phase that contained of 17.5 mmol L^{-1} acetic acid and 14.36 mmol L^{-1} of triethylamine. Plots of retention factors vs. the amount of base in the mobile phase (Fig. 3) gave

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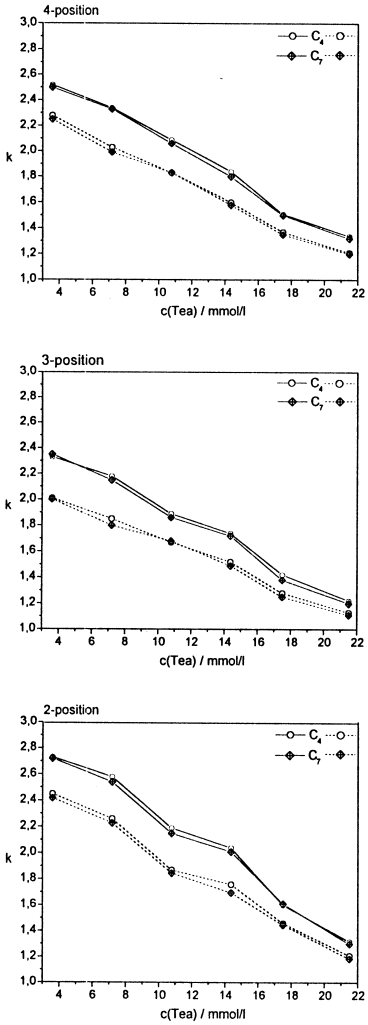

Figure 2. The influence of the concentration of triethylamine in the mobile phase on the retention factors of the *S*-(+) enantiomers of the 2-, 3-, and 4-alkoxy substituted piperazino esters of carbamic acid with the number of carbon atoms in —OR (C_4 and C_7). Chiral stationary phase: (*R*,*R*) Whelk-O 1; mobile phase: solid line, methanol/acetonitrile (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}); dashed line, methanol/acetonitrile (80/20, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

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Table 4. The influence of the concentration of triethylamine on the resolution values of piperazino esters of alkoxy phenylcarbamic acid. Chiral stationary phase: (*R*,*R*) Whelk-O 1.

	Concentration of triethylamine (mmol L^{-1})											
	3.59		7.18		10.77		14.36		17.50		21.50	
Analyte nr.	R_{ij}^{a}	R_{ij}^{b}	R_{ij}^{a}	R_{ij}^{b}	R_{ij}^{a}	R_{ij}^{b}	R_{ij}^{a}	R_{ij}^{b}	R_{ij}^{a}	R_{ij}^{b}	R_{ij}^{a}	R_{ij}^{b}
2-Position												
1	>0.4	>0.4	0.6	>0.4	0.5	>0.4	0.6	>0.4	0.5	>0.4	>0.4	>0.4
2	>0.4	>0.4	0.6	>0.4	0.6	>0.4	0.7	>0.4	0.5	>0.4	>0.4	>0.4
3	>0.4	>0.4	0.6	>0.4	0.5	>0.4	0.6	>0.4	0.4	>0.4	>0.4	>0.4
4	>0.4	>0.4	0.5	>0.4	0.6	>0.4	0.6	>0.4	0.4	>0.4	>0.4	>0.4
3-Position												
5	0.6	>0.4	0.5	0.5	0.6	0.5	0.8	0.4	0.5	>0.4	0.4	>0.4
6	0.6	>0.4	0.6	0.4	0.6	0.5	0.7	0.4	0.6	>0.4	0.5	>0.4
7	0.5	>0.4	0.6	0.5	0.6	0.6	0.8	0.5	0.5	>0.4	0.5	>0.4
8	0.5	>0.4	0.6	0.5	0.7	0.6	0.7	0.4	0.6	>0.4	0.4	>0.4
4-Position												
9	0.7	>0.4	0.8	0.6	0.9	0.6	1.0	0.6	0.8	0.6	0.5	>0.4
10	0.6	>0.4	0.7	0.5	0.8	0.6	0.9	0.5	0.7	0.5	0.6	>0.4
11	0.6	>0.4	0.8	0.6	0.8	0.7	0.9	0.6	0.7	0.6	0.6	>0.4
12	0.7	>0.4	0.8	0.6	0.9	0.7	1.0	0.5	0.8	0.5	0.5	>0.4
13	0.6	>0.4	0.8	0.6	0.8	0.7	1.0	0.5	0.7	0.5	0.6	>0.4

Note: For $n = 3$; $R_{ij} = \pm 0.1$.

[a]Methanol/acetonitrile (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

[b]Methanol/acetonitrile (80/20, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

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Table 5. The influence of the concentration of triethylamine on the resolution values of piperazino esters of alkoxy phenylcarbamic acid. Chiral stationary phase: (*R*,*R*) Whelk-O 1.

	Concentration of triethylamine (mmol L^{-1})											
	3.59		7.18		10.77		14.36		17.50		21.50	
Analyte nr.	R_{ij}[a]	R_{ij}[b]	R_{ij}[a]	R_{ij}[b]	R_{ij}[a]	R_{ij}[b]	R_{ij}[a]	R_{ij}[b]	R_{ij}[a]	R_{ij}[b]	R_{ij}[a]	R_{ij}[b]
2-Position												
1	>0.4	>0.4	0.6	>0.4	0.7	0.5	0.9	0.8	0.9	0.8	0.7	0.6
2	>0.4	>0.4	0.5	>0.4	0.7	0.5	0.9	0.8	0.8	0.8	0.7	0.5
3	>0.4	>0.4	0.5	>0.4	0.7	0.6	0.9	0.8	0.9	0.9	0.7	0.5
4	>0.4	>0.4	0.6	>0.4	0.8	0.6	0.8	0.8	0.9	0.9	0.8	0.6
3-Position												
5	0.5	>0.4	0.9	0.5	0.9	0.6	1.0	0.9	1.0	0.9	0.8	0.7
6	0.5	>0.4	0.8	0.5	0.9	0.6	1.1	1.0	1.0	0.9	0.8	0.6
7	0.6	>0.4	0.8	0.6	1.0	0.7	1.0	1.0	1.0	0.9	0.8	0.6
8	0.6	>0.4	0.8	0.5	0.9	0.6	1.1	1.0	1.0	0.9	0.9	0.6
4-Position												
9	0.9	0.5	1.1	0.7	1.1	0.9	1.2	0.9	1.1	0.9	0.8	0.6
10	0.9	0.5	1.1	0.7	1.1	0.9	1.3	0.9	1.2	0.9	0.8	0.7
11	1.0	0.6	1.1	0.7	1.1	0.9	1.3	1.0	1.1	0.8	0.9	0.6
12	1.0	0.6	1.2	0.7	1.2	0.9	1.2	1.0	1.1	0.9	0.8	0.6
13	1.1	0.6	1.2	0.7	1.2	0.9	1.2	0.9	1.2	0.9	0.9	0.6

Note: For $n = 3$; $R_{ij} = \pm 0.1$.
[a]Methanol/water (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).
[b]Methanol/water (80/20, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

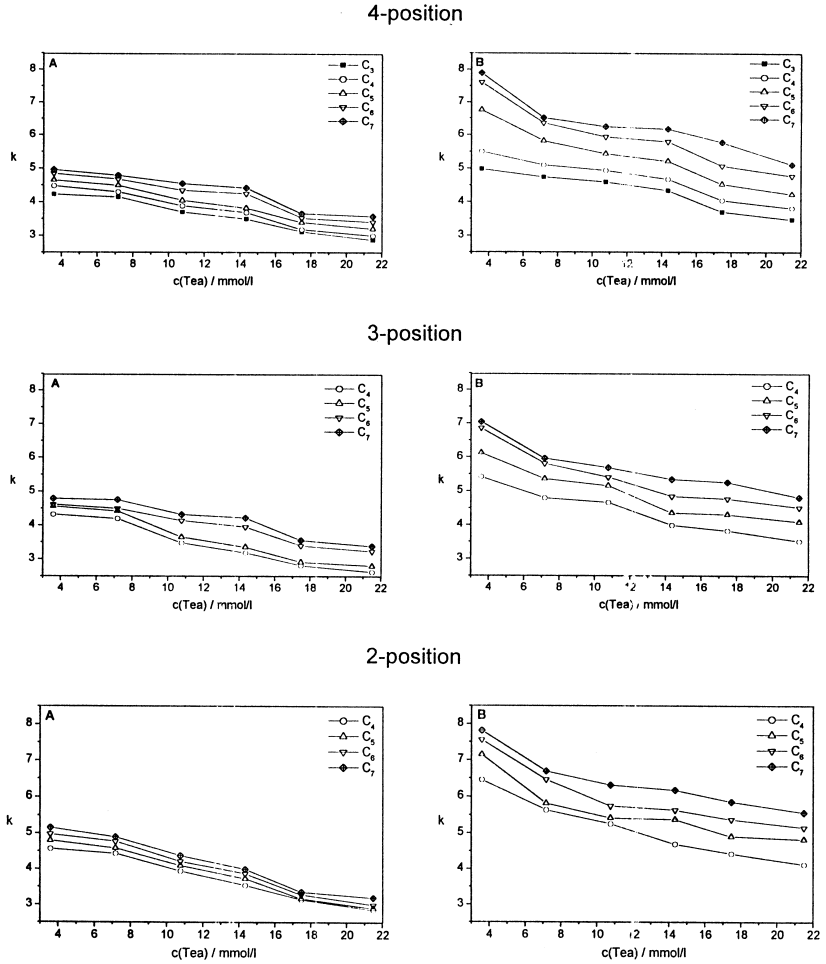

Figure 3. The influence of the concentration of triethylamine in the mobile phase on the retention factors of the *S*-(+) enantiomers of the 2-, 3-, and 4-alkoxy substituted piperazino esters of carbamic acid with the different number of carbon atoms in —OR (C_x). Chiral stationary phase: (*R*,*R*) Whelk-O 1; mobile phase: (A) methanol/water (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}); (B) methanol/water (80/20, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}).

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similar profiles for each of the different piperazino esters of alkoxy phenylcarbamic acid. An increase in the concentration of triethylamine in the mobile phase (in the range of 3.59–21.50 mmol L^{-1}) caused decreasing retention of these enantiomers.

The retention of these enantiomers increased both with the concentration of water in the mobile phase, as well as with the length of the alkoxy chain of the analyte, i.e., that is, classic reversed phase chromatography behavior (Fig. 3). A comparison of the resolution values for the enantiomeric compounds in this study is given in Tables 3–5. The best separation of the piperazino esters of alkoxyphenylcarbamic acid is observed when a mobile phase containing methanol/water (90/10, v/v), 17.5 mmol L^{-1} acetic acid, and 14.36 mmol L^{-1} triethyamine was used.

Enantiomers of pyrrolidino, piperidino, and perhydroazepino esters of alkoxy phenylcarbamic acids were not separated with mobile phases containing methanol/water (90/10 and 80/20, v/v), 17.5 mmol L^{-1} acetic acid and different concentrations of triethylamine (3.59–21.5 mmol L^{-1}). However, the retention factors of these compounds also increased with increasing amounts of water in the mobile phase, and decreased with increasing triethylamine in the mobile phase. The values of the retention factors were in range 2.14–0.91 (3.68–1.92) for pyrrolidino enantiomers, 2.64–0.84 (3.24–1.65) for piperidino enantiomers, and 2.12–0.74 (2.97–1.43) for the perhydroazepino esters of alkoxy phenylcarbamic acid in mobile phases of methanol/water 90/10, v/v (or 80/20, v/v), plus acetic acid (17.5 mmol L^{-1}), and triethylamine (in the range from 3.59 to 21.5 mmol L^{-1}).

Influence of Concentration of Triethylamine in the Mobile Phase at Constant Ion Strength

The influence of different amounts of triethylamine (when the concentration of acetic acid is held constant) in the mobile phase at constant ion strength ($I = 24.5$ mmol L^{-1}), on the values of retention factors is indicated in Fig. 4 for 2-, 3-, and 4-piperazino esters of alkoxyphenylcarbamic acid. The resolution values for piperazino enantiomers are listed in Table 6. The values of retention factors (Fig. 4) and resolutions (Table 6) were lower than the results achieved in mobile phase without lithium chloride [Fig. 3(A), Table 5, R_{ij}[a]].

The enantiomers listed in Table 2 were not separated in the constant ionic strength mobile phase ($I = 24.5$ mmol L^{-1}) as mentioned above. All retention factors had a value of zero.

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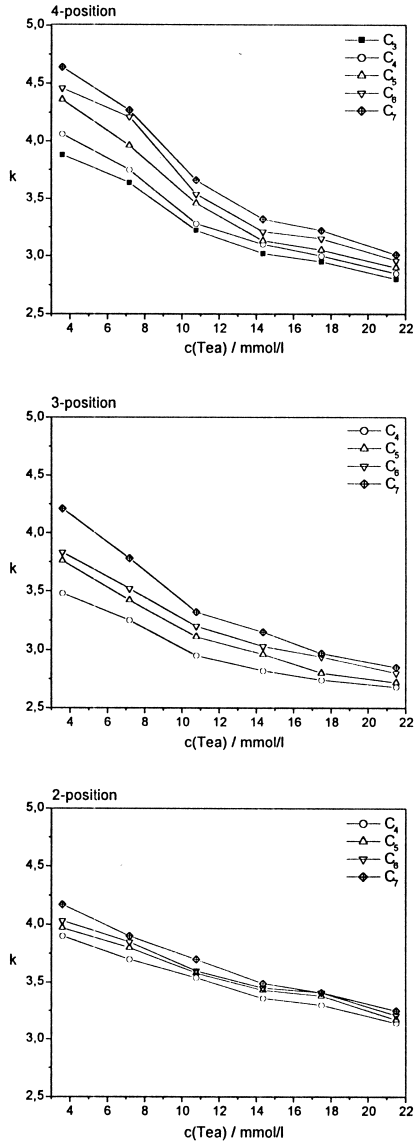

Figure 4. The influence of the concentration of triethylamine in the mobile phase on the retention factors of the *S*-(+) enantiomers of the 2-, 3-, and 4-alkoxy substituted piperazino esters of carbamic acid with the different number of carbon atoms in —OR (C_x). Chiral stationary phase: (*R*,*R*) Whelk-O 1; mobile phase: methanol/water (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}) at the constant ionic strength of the mobile phase ($I = 24.5$ mmol L^{-1}).

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Table 6. The influence of the concentration of triethylamine on the resolution values of piperazino esters of alkoxy phenylcarbamic acid. Chiral stationary phase: (*R*,*R*) Whelk-O 1.

	Concentration of triethylamine (mmol L^{-1})					
Analyte nr.	3.59	7.18	10.77	14.36	17.50	21.50
2-Position						
1	>0.4	>0.4	0.6	0.7	0.7	0.5
2	>0.4	>0.4	0.6	0.8	0.7	0.5
3	>0.4	>0.4	0.7	0.8	0.7	0.5
4	>0.4	>0.4	0.6	0.7	0.8	0.6
3-Position						
5	>0.4	0.6	0.7	0.9	0.9	0.7
6	>0.4	0.6	0.7	0.9	0.9	0.7
7	>0.4	0.6	0.8	1.0	0.9	0.8
8	>0.4	0.5	0.7	0.9	0.8	0.6
4-Position						
9	>0.4	0.7	0.9	1.0	1.0	0.8
10	>0.4	0.7	0.8	0.9	1.0	0.8
11	>0.4	0.8	0.9	0.9	0.9	0.7
12	>0.4	0.8	0.8	1.0	0.9	0.8
13	>0.4	0.7	0.8	1.0	0.9	0.7

Note: For $n = 3$; $R_{ij} = \pm 0.1$. Methanol/water (90/10, v/v) containing 17.5 mmol L^{-1} acetic acid and triethylamine (3.59, 7.18, 10.77, 14.36, 17.50, and 21.50 mmol L^{-1}) at the constant ionic strength of the mobile phase ($I = 24.5$ mmol L^{-1}).

Elution Order of the Enantiomers on (*R*,*R*) Whelk-O 1 and (*S*,*S*) Whelk-O 1 Chiral Columns

The separation of piperazino esters of 3-, 4-buthoxyphenyl carbamic acid by HPLC on the (*R*,*R*) Whelk-O 1 and (*S*,*S*) Whelk-O 1 chiral columns are shown in Figs. 5 and 6, respectively. In both cases, the best separation was achieved for compounds substituted in the 4-position, while the worst separations were for those with the 2-alkoxy chain substituents. The elution order of enantiomers was determined by measuring the optical rotation (see Experimental).

The first eluted enantiomer for all tested analytes, rotates of the plane of polarised light (wavelength 589 nm) to the right *S*-(+), and the second eluted enantiomer shows the opposite rotation. A correlation of the enantiomeric elution order to the configuration of chiral selector shows that *R*-(−) enantiomers are more retained on the (*R*,*R*) Whelk-O 1 column and *S*-(+) enantiomers are more retained on the (*S*,*S*) Whelk-O 1 column.

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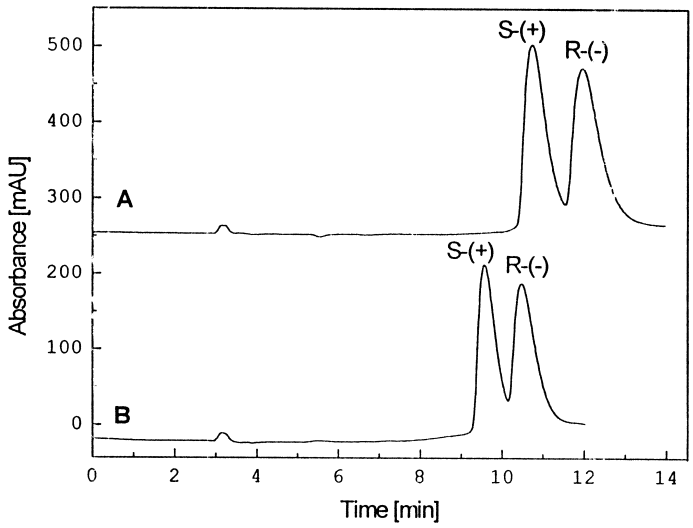

Figure 5. Chromatograms of enantioseparation of the piperazino ester of 4-buthoxyphenyl carbamic acid (A) and the piperazino ester of 3-buthoxyphenyl carbamic acid (B). Chiral stationary phase: (*R*,*R*) Whelk-O 1; mobile phase: methanol/water (90/10, v/v); 17.5 mmol L^{-1} acetic acid and 14.36 mmol L^{-1} triethylamine.

CONCLUSIONS

Whelk-O 1 chiral stationary phase can be used to separate enantiomers of piperazino esters of phenylcarbamic acid. The separation can be accomplished in the polar organic mode and reversed phase mode. According to the separation results of using different mobile phase modes, it can be postulated:

The piperazino part of the compounds (two nitrogen atoms in the ring) is very important because the separation of piperidino, pyrrolidino, and perhydroazepino (one nitrogen atom in the ring) enantiomers of phenylcarbamic acid were not observed.

The position of the alkoxy substituent of the phenylcarbamic acid derivative has an influence on chiral resolution in the polar organic and reversed phase mode. The length of the alkoxy chain was not of great importance, particularly in comparison to the position of the alkoxy-substituent on the aromatic ring. It is clear that the steric crowding of the stereogenic carbon affects enantioresolution (the lowest values of enantioresolution were measured for the 2-position of the alkoxy

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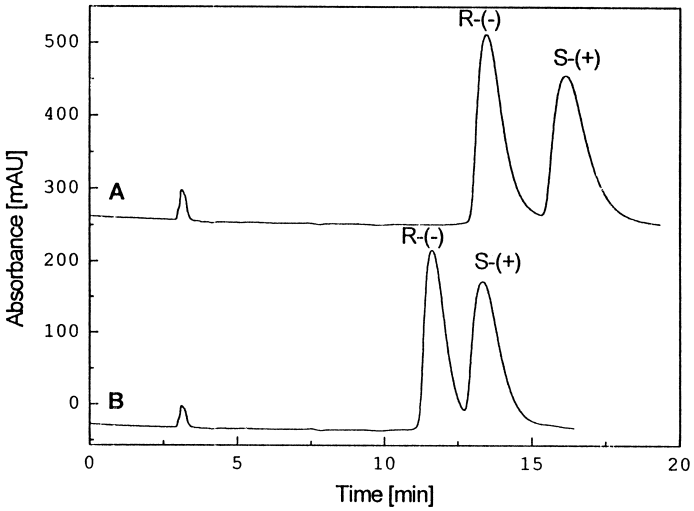

Figure 6. Chromatograms of enantioseparation of the piperazino ester of 4-buthoxyphenyl carbamic acid (A) and the piperazino ester of 3-buthoxyphenyl carbamic acid (B). Chiral stationary phase: (*S*,*S*) Whelk-O 1; mobile phase: see Fig. 5.

chain and the highest for the 4-position of alkoxy chain in the piperazino esters of alkoxy phenylcarbamic acid).

The order of the enantiomer elution was also determined. In the case of the (*R*,*R*) Whelk-O 1 CSP the first enantiomer eluted was the *S*-(+) form. Using (*S*,*S*) Whelk-O 1 CSP the order of enantiomer elution was the opposite. This indicates that the interaction of alkoxy substituted esters of phenylcarbamic acid with the same configuration chiral selector is stronger than of those with different configurations.

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