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Chromatographic separation of phenylpropanol enantiomers on a quinidine carbamate-type chiral stationary phase

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Abstract

The retention and the separation of the enantiomers of 1-phenylpropanol (1PP), 2-phenylpropanol (2PP), and 3-chloro-1-phenylpropanol (3CPP) on silica-bonded quinidine carbamate under normal phase HPLC conditions were investigated. A relatively high selectivity of the stationary phase for 3CPP and 1PP ($\alpha \approx 1.07 - 1.09$) was achieved with eluents containing ethyl acetate as the polar modifier. These mobile phases were examined in detail. Based on the set of chromatographic and thermodynamic data collected, conclusions regarding the mechanism of enantioselectivity and the structure of the selector chiral center are made.

Keywords: 1-Phenylpropanol; 3-Chloro-1-Phenylpropanol; 2-Phenylpropanol; Quinidine carbamate; Enantioseparation; HPLC; Compensation effect

1. Introduction

In a previous work [1] it was shown that the surface of the quinidine carbamate chiral stationary phase (CSP) was composed of adsorption sites of two different types, the enantioselective and the nonselective sites. The adsorption energy on the nonselective adsorption sites is relatively high ($\approx$ 30 kJ/mole) and comparable with the energy involved in polar interactions and H-bonding. The number of nonselective sites is 30–40 times larger than that of the selective sites, which explains the rather low value of the selectivity, α. The considerable excess of the density of nonselective sites over the selective ones on this high density bonded CSP suggests that part of the chiral moieties act as nonselective sites. A similar behavior seems to characterize arylcarbinols on many other CSPs, especially those arylcarbinols that carry the small phenyl group. For phenylcarbinols, high enantioseparation factors, $\alpha > 1.5$, cannot be reached on various Pirkle-type phases while analytes with a more favorable structure exhibit a far higher selectivity on the same CSPs [2–5]. This fact suggests that the same selector may play the role of a chiral site under certain conditions or toward certain substrates and behave as an achiral site otherwise. This observation can be related to the well-known fact that a change in the nature of the mobile phase or even in its composition can considerably affect the enantioselectivity of a CSP. In terms of the two-site model of CSPs, this means that changes in the mobile phase nature and/or composition may suppress the undesirable nonselective behavior of potentially enantioselective sites and/or create experimental conditions that makes these adsorption sites to exhibit an enhanced chiral activity. The investigation of this phenomenon is of great interest. The poorly resolved enantiomers of arylcarbinols could be good cases in point to study the effects of the adsorbate structure and the experimental conditions on the adsorption thermodynamics, on the ratio of the selective and nonselective sites, and to find the conditions that allow the maximization of this ratio.

Typical studies of heterogeneous adsorption in HPLC consist in measuring the adsorption isotherm in the widest possible range of concentrations, deriving the adsorption energy distribution, calculating the profiles of overloaded elution bands profile, and comparing these profiles to experimental ones. However, this work should be preceded

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by a comprehensive examination of the column behavior under linear chromatography conditions. In addition to the usual determination of the Henry constants, which are necessary for the design of any adsorption model, these experiments are needed to inform on the composition of the mobile phase that permits elution of the studied compounds with retention factors between 2 and 6 [6]. They will also answer the important questions of whether the adsorption is strong, mild or athermal and of how do temperature and pressure influence retention. The former answer is important because it allows avoidance of the possible consequences of column overheating during its percolation with concentrated solutions owing to adsorption heat release. Moreover, data on the adsorption thermodynamics in the linear region of the isotherm furnish information on the interactions of the analyte with the adsorbent surface without the complication introduced by the adsorbate-adsorbate interactions. The goal of this work was the exploration of the linear chromatography behavior of 3-chloro-1-phenyl-propanol (3CPP), 1- and 2-phenyl-1-propanol (1PP and 2PP, respectively) which were selected as test compounds. Nonlinear chromatography behavior will be discussed in a forthcoming contribution.

The enantioseparation of enantiomers of chiral alcohols, e.g., arylalkylcarbinols and particularly phenylalkylcarbinols, is uneasy due to the presence of a single functional group, the rigidity of their molecular structure, and the attachment of an aryl residue to the stereogenic center, which can make impossible or arduous the arrangement of the residue in a position suitable for the formation of the π–π interactions that are important for the chiral resolution on Pirkle-type phases [2,7]. As a consequence, examples of highly selective separations are rare. The use of a β-cyclodextrin derivative as a chiral selector does not lead to any significant enantioseparation [8,9]. Selectivity factors (α) of about 1.2 toward 3-chloro-1-phenyl-1-propanol (3CPP) [10] and 1-phenylethanol [11] have been achieved with CSPs based on cellulose derivatives. Numerous separations have been reported on Pirkle-type phases, but their selectivity toward phenylalkylcarbinols does not exceeds 1.5 [2–5].

The quest for cheap chiral sorbents has led investigators to using natural chincona alkaloids as chiral selectors [12,13]. The selectivity of different versions of such CSPs toward this class of alcohols is low and almost all studies report values less than 1.27 [13–17]. Using polarizable aprotic solvents (e.g., arenes, carbon tetrachloride) with or without the addition of a small concentration of 2-propanol, Nesterenko et al. achieved enantioselectivities factors of 1.4–1.5 for 2,2,2-trifluoro-1-(9-anthryl)-ethanol (TFAE) on a silica-bonded quinine column [18]. Further developments of the concept of chincona alkaloid-based CSPs involves chemical modifications of the natural alkaloids by means of the substitution of some functional groups. So introduced, carbamoylated chinchona alkaloids are new stereoselective selectors designed to separate chiral acids [19–21]. However, they were shown to be able to resolve binaphthols [20] and several arylcarbinols [1,22]. Although a reasonable explanation of the chiral recognition of N-derivatized aminoacids by these CSPs was proposed [23,24], the detailed mechanism of the stereoselective adsorption of chiral alcohols remains unclear [1]. At the same time, the information that is now available allows the following conclusions to be drawn:

(i) The relative C_8/C_9 configuration of the selector is predominantly responsible for the molecular recognition of chiral guest molecules [25,26].
(ii) The influence of the carbamate group on the enantioselectivity can be substantial, due to the formation of intermolecular hydrogen bonds involving amide and/or carbonyl group(s) [25,27].
(iii) The quinuclidine nitrogen atom may interact strongly with a selectand. Therefore, it is important that the geometry of the selectand molecule allows its approach of the quinuclidine moieties at distances that are close enough for these interactions [12].
(iv) A mixture of different conformers exists in solutions of carbamoylated chincona alkaloids. Their relative concentrations depend on several factors, e.g., the nature of the solvent and the nature and stereochemistry of the analyte associated with the selector molecule [23,24]. A similar situation is expected to take place for selector molecules that are bonded onto the surface of a support, e.g., porous silica particles.

As to the separation of the enantiomers of arylalkylcarbinols on unmodified quinine and quinidine based CSPs, it was ascertained that the steric hindrance of the alkyl group bound to the asymmetric carbon center of these analytes plays an important role in the degree of separation achieved [14,18].

2. Experimental

2.1. Apparatus

All the measurements made in this work were carried out using a HP 1100 liquid chromatograph (Agilent Technologies, Palo Alto, CA), equipped with an automatic injector, a column oven, a variable wavelength UV-detector with a high-pressure cell, and a data acquisition system using the HP Chemstation software. The flow rate was 0.99 ml/min, unless otherwise mentioned. Most retention data were acquired at 22 °C but the measurements made with solutions of ethylacetate in *n*-hexane as the mobile phase were performed at 0, 15, 22, 30 and 40 °C. Measurements made between 15 and 40 °C were carried out with the standard Agilent column oven. A ice-water bath was used to control the column temperature at 0 °C.

2.2. Chemicals and column

All solvents used to prepare the mobile phases were from Fisher Scientific (Fair Lawn, NJ, USA) and were HPLC

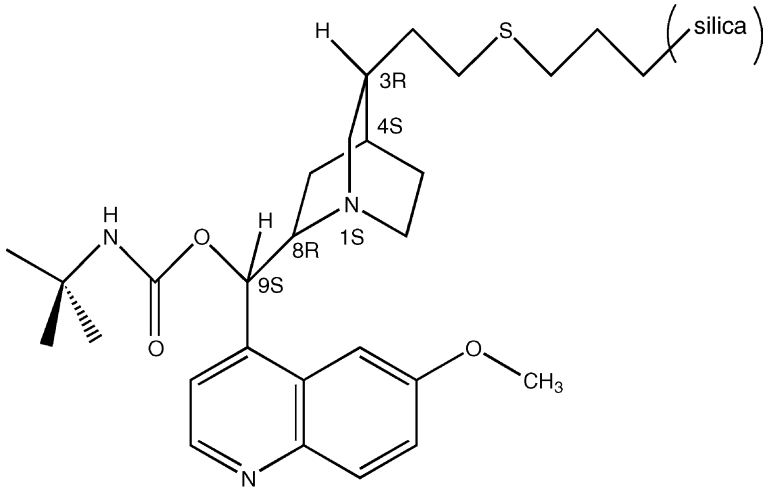

Fig. 1. Structure of the quinidine carbamate chiral selector.

grade, except chloroform, which was A.S.C. grade, and triethylamine, which was 99% from Acros Organics (Geel, Belgium). The enantiomers of 1-phenyl-1-propanol (1CP), 2-phenyl-1-propanol (2CP), 3-chloro-1-phenylpropanol (3CP), 1-phenyl-1-propylamine (1PPA), and 1,3,5-tri-tert-butylbenzene (TtBB) were from Aldrich (Milwaukee, WI, USA). Chloroform was prefiltrated through 0.45 μm type FH membranes (Millipore, Bedford, MA, USA); the other chemicals were used as supplied.

The column used in this work was a 150 mm × 4 mm *Chiris* Chiral AX:QD1 column, from Iris Technologies (Lawrence, KS, USA). It was packed with approximately 1.2 g of 5 μm silica particles on the surface of which quinidine carbamate (QD) was immobilized. The structure of this ligand is illustrated in Fig. 1. Before beginning the measurements with *n*-hexane–ethyl acetate mobile phases and unless otherwise mentioned, the column was flushed with a 2% triethylamine in isopropanol–*n*-hexane (90:10, v/v) solution for 0.5 h, then with the pure mobile phase for 2 h. That treatment was done in order to eliminate the vestigial protons that could still be bonded to the amine groups of the quinuclidine moieties.

2.3. Measurements of the hold-up volume

The hold-up volume of a chromatographic system is the sum of two contributions, the column hold-up volume, which is the volume in the column that is accessible to the mobile phase, and the extra-column volume, which is the volume of the connecting tubes and other parts between the point of injection of the sample and the detector. To determine the extra-column volume and be able to correct for, the column was replaced with a short connector having a negligibly small volume and a measurement of the hold-up volume of this system was made. The hold-up time was measured for every mobile phase used with TtBB as the tracer. This compound has long been considered as unretained on CSPs made of polysaccharide derivatives coated on silica [10,28]. However, we have found the retention time of TtBB on the CSP of interest here to decrease slightly as temperature increases. This proves a weak adsorption of the tracer. At the same time, the retention time of TtBB is shorter than that of toluene. To diminish the effect of TtBB own retention on the hold-up time value, the measurements with *n*-hexane–ethyl acetate solutions as the mobile phase were carried out at the highest temperature used, 40 °C. They were made at 22 °C with the other mobile phases.

2.4. Reproducibility and precision

The experiments made with all the mobile phases except those containing ethyl acetate were evaluative and no special care was taken to estimate their reproducibility nor to maintain a high precision. Only duplicate measurements were carried out in every sample and the average value was used to calculate the retention time. In contrast, the data measured with the *n*-hexane–ethyl acetate mobile phases that are used comprehensively in the discussions below were acquired with thoroughness. Every measurement was repeated at least six times. The confidence intervals of the retention times (at a 95% confidence level) were smaller than 0.012 min. The reproducibility of the hold-up time was less than 0.002 min. The systematic error resulting from the retention of the tracer is unknown and is not included in this estimate. Based on the definition of the retention factor (k'), its random error is evaluated to be smaller than 0.02 for the entire set of reported data. The absolute error on the separation factor (α) was less than 0.002.

3. Results and discussion

The influence of the nature and the composition of the mobile phase on the retention of the different analytes was measured using small samples, in order to avoid the effects

of a nonlinear behavior of the adsorption isotherm which would complicate the interpretation of the retention data. Measurements of the retention factors k' were performed under linear conditions and gave values that were independent of the sample size, a condition that was carefully checked. Carrying out measurements of retention factors at different temperatures allowed the determination of the thermodynamic characteristics of adsorption, using van't Hoff equation

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \beta \quad (1)$$

where ΔH° and ΔS° are the standard molar enthalpy and molar entropy of transfer of the solute from the mobile phase to the stationary phase, respectively, and β is the phase ratio. We will call ΔH° and ΔS° the adsorption heat and the adsorption entropy, respectively, for the sake of simplicity.

3.1. Influence of the mobile phase on the retention and the selectivity of 1-phenylpropanols

The search for the most appropriate mobile phase was carried out in both the reverse and the normal phase modes for 3CPP and 1PP. The results of the experiments carried out are reported in Table 1. Some examples of separations of the racemic mixture are given in Fig. 2. The first eluted enantiomer was always the (*R*)-enantiomer, whatever the composition of the mobile phase used. The data in Table 1 show that water containing mobile phases do not provide a sufficient selectivity, in contrast with *n*-hexane-based eluents. These data show that aprotic polar mobile phase modifiers provide higher separation factors than alcohols. Even the addition of a very small concentration of methanol (0.3–0.5%) to a *n*-hexane–ethyl acetate solution leads to a marked decrease of the selectivity. A logical explanation of this result would be the H-bonding of alcohol molecules to the active sites on the CSP that are responsible for the enantioseparation. This bonding would result in the blocking of these sites. The addition of the moderately polar dichloromethane or chloroform does not lead to values of α higher than 1.05 and the modification of these binary mobile phase with a more polar component (acetonitrile, ethyl acetate, or 2-propanol) does not increase the selectivity above 1.06. The highest values of the enantioselectivity were obtained with acetone (3CPP) and ethyl acetate (3CPP and 1PP) as polar modifiers. Thus, the presence of a carbonyl group in the structure of a modifier molecule makes it preferable for the separation of the enantiomers of 3CPP. It seems that the oxygen atom plays an important role in the interactions between the modifier molecules and the chiral center or part of the chiral center but that these interactions do not block that center. It is noteworthy that increasing the ethyl acetate content of the eluent more than three times does not affect the selectivity of the column with respect to 3CPP. Solutions of *n*-hexane and ethyl acetate were considered as the more worthy of further study.

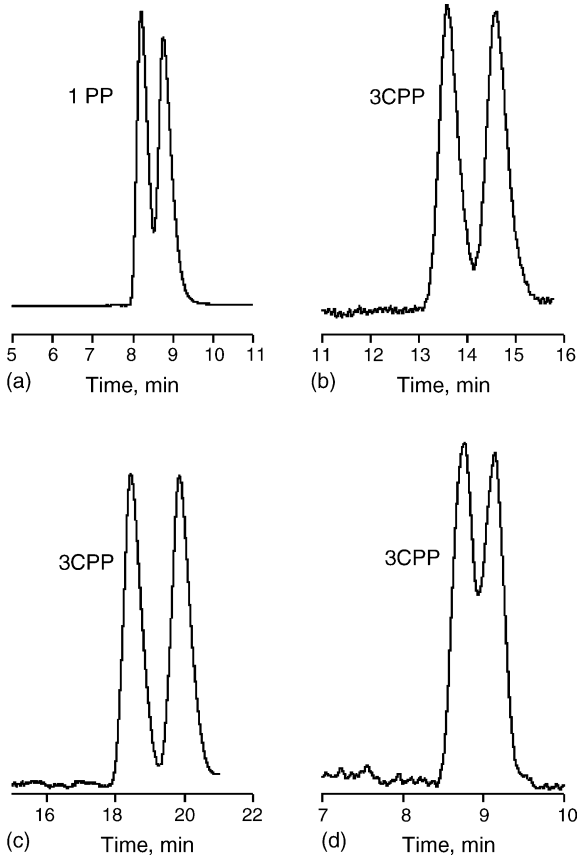

Fig. 2. Enantioseparation of the racemate of 3CPP and 1PP. Mobile phases: (a) and (b) *n*-hexane–ethyl acetate (95:5), (c) *n*-hexane–acetone (98:2), and (d) *n*-hexane–acetone (95:5). $t = 22\,^\circ$C. Racemates concentration, 1 mg/ml; sample volume, 2 μl.

The correlation between the retention factor of (*R*)-3CPP and the polarity of the eluents measured in the Snyder's scale [29] is shown in Fig. 3. This graph does not include the aqueous solutions used. In the case of 1PP the same pattern was obtained (data not shown). The values of the polarity of a

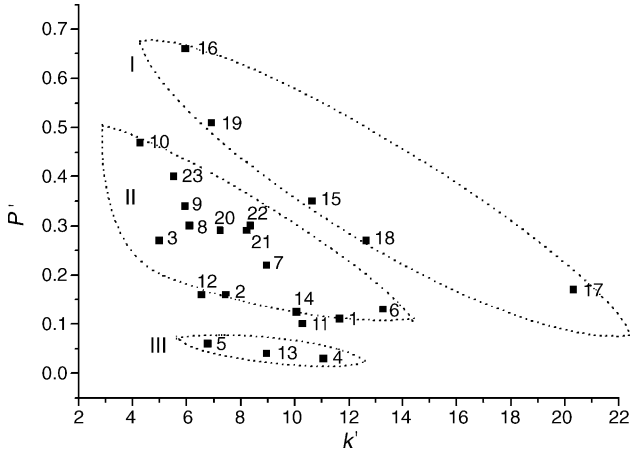

Fig. 3. Correlation between the polarity of the mobile phase (P') and the retention factor. The number by a point corresponds to the number in Table 1. See also explanations in the text.

Table 1
Influence of the mobile phase composition on the retention and separation of the enantiomers of 1PP and 3CPP

No.	Mobile phase	Composition	1PP		3CPP	
			k' [a]	α	k' [a]	α
1	*n*-Hexane + acetone	98:2			11.65	1.09
2		97:3			7.45	1.08
3		95:5	2.97	1.06	4.99	1.05
4	*n*-Hexane + methanol	99.5:0.5			11.05	1.03
5		99:1			6.77	1.01
6	*n*-Hexane + ethyl acetate	97:3	6.68	1.02	13.28	1.07
7		95:5	5.19	1.08	8.95	1.07
8		93:7	3.48	1.08	6.11	1.07
9		92:8			5.94	1.08
10		89:11			4.28	1.08
11	*n*-Hexane + ethyl acetate + methanol	97.9:1.8:0.3	5.23	1.06	10.29	1.05
12		96.5:3:0.5	3.83	1.05	6.55	1.04
13	*n*-Hexane + 2-propanol	99:1	4.22	1.06	8.95	1.04
14	*n*-Hexane + acetonitrile	98:2			10.06	1.06
15	*n*-Hexane + chloroform	92:8	4.49	1.06	10.64	1.05
16		85:15			5.96	1.04
17	*n*-Hexane + dichloromethane	95:5			20.32	1.05
18		92:8			12.65	1.05
19		85:15			6.91	1.05
20	*n*-Hexane + dichloromethane + 2-propanol	91.5:8:0.5	3.33	1.05	7.22	1.05
21	*n*-Hexane + dichloromethane + ethyl acetate	92.3:4.8:2.9	4.52	1.06	8.22	1.06
22	*n*-Hexane + dichloromethane + acetonitrile	92.5:6:1.5	4.03	1.06	8.35	1.06
23		90:8:2	2.82	1.05	5.52	1.06
	Water + acetonitrile [b]	75:25			5.73	1.01
	Water + methanol [c]	50:50			3.13	1.02

[a] The first eluted enantiomer.
[b] Flow rate 0.6 ml/min.
[c] Flow rate 0.4 ml/min.

mixed solvent was derived from the equation

$$P' = \phi_1 P'_1 + \phi_2 P'_2 + \phi_3 P'_3 \quad (2)$$

where P'_i and ϕ_i are the polarity and the volume fraction of component *i* in the mixture. The polarities of the pure solvents are listed in Table 2. The experimental data in Fig. 3 are clustered in three regions corresponding to the chlorinated hydrocarbon modifiers (I), the aprotic polar modifiers (II), and the alcohol modifiers (III). The mixtures of group II allow the achievement of a higher enantioselectivity than those of groups I and III. The addition to a binary solvent of a third component results in a shift of the representing point toward the region to which that third component belongs (see points # 11, 12, and 20–23). Group II has its own substructure. The mobile phases containing acetone and acetonitrile are at the bottom of the region, those containing *n*-hexane-ethyl acetate are higher in the region. Both acetone and acetonitrile have a weak acidity, in contrast to ethyl acetate which has no acidity, in accordance with the solvatochromic scale of Kamlet

Table 2
Polarity in the Snyder's scale and solvatochomic parameters of solvents

Solvent	P'	π^*	*a*	*b*
n-Hexane	0	−0.04		
Acetone	5.4	0.71	0.08	0.48
Acetonitrile	6.2	0.75	0.19	0.31
Ethyl acetate	4.3	0.55	0	0.45
Methanol	6.6	0.60	0.93	0.62
2-Propanol	4.3	0.48	0.76	0.95
Dichloromethane	3.4	0.82	0.30	0
Chloroform	4.4	0.58	0.44	0

π^*: Index of solvent dipolarity/polarizability; *a*: hydrogen-bonding donating acidity; *b*: hydrogen-bonding accepting basicity.

et al. [30] (Table 2). On the other hand, chlorinated hydrocarbons which have higher values of the hydrogen-bonding donor parameter are at the very top of the group II region in the diagram. This shows the importance of another property of the modifier, its hydrogen-bonding acceptor basicity. The higher the basicity of a modifier in a group, the lower its position in the diagram. In summary, the mobile phases containing a polar modifier that has a low basicity and a small or zero acidity give the highest values of the enantioselectivity of 1PP and 3CPP.

3.2. Influences of a pre-treatment of the column by a base and of the humidity of the solvent on the retention data

The QD selector can be protonated on the quinuclidin nitrogen. The protonated and unprotonated forms exhibit different sorption properties. Commercial columns are not supposed to be protonated but it is possible that a few QD-groups are protonated. Moreover, even in the normal-phase mode, quinuclidine groups can be protonated during their use, by reaction with water or with some acidic contaminants contained in the mobile phase. For this reason, the properties of the column may change with time. Another factor affecting the reproducibility of the retention data is the humidity of the solvent. Deep dehydration of a solvent is a tedious procedure and, in common analytical practice, chromatographers rarely control minor water concentrations in non-polar eluents nor do they saturate the non-polar components of a mobile phase with water, in order to achieve data reproducibility. If no effort is made to control its water content, the mobile phase used is in equilibrium with the atmospheric humidity, hence its water content fluctuates slowly, depending on the local weather.

To evaluate the influence of some of these factors on the retention of analytes after the column has been used for a long time with a water-methanol mixture, we measured the retention factor, the enantioselectivity, and the thermodynamic functions of retention of 3CPP under different sets of experimental conditions. We measured them in a 5% ethyl acetate solution in *n*-hexane, before and after conditioning the column with a triethylamine solution, and after addition to the eluent of 0.02% of water. The data are summarized in Tables 3 and 4. They show that the deprotonation of the stationary phase does not affect much the adsorption of 3CPP whereas the addition of a small amount of water to the mobile phase has a notable effect. After treatment of the column by the triethylamine solution, the retention of both enantiomers and their separation factor increased slightly. The addition of a small amount of water to the eluent resulted in a decrease of the retention factors (10%) and of the selectivity (0.2–1%). It is interesting to note that, in contrast with what happens in the other cases, the separation factor in a humid mobile phase is practically independent of the temperature. This suggests that water interacts strongly with a fraction of the enantioselective sites, making enantiorecognition by these centers more difficult and that this interaction is so strong that it is independent of the temperature in the range investigated. The leveling of the difference between the thermodynamic quantities of adsorption of the enantiomers for the wet eluent (Table 4) supports this suggestion.

Table 3
Retention and selectivity of the 3CPP enantiomers in *n*-hexane/ethyl acetate mobile phases

t (°C)	k'_R	k'_S	α
5% Ethyl acetate, before triethylamine pre-treatment ([a] $\beta = 0.456$)			
15	10.50	11.33	1.08
22	8.97	9.62	1.07
30	7.79	8.32	1.07
40	6.43	6.81	1.06
5% Ethyl acetate, after triethylamine pre-treatment ($\beta = 0.456$)			
15	10.64	11.52	1.08
22	9.61	10.37	1.08
30	8.13	8.72	1.07
40	6.73	7.18	1.07
5% Ethyl acetate + 0.02% H_2O ($\beta = 0.453$)			
15	9.23	9.86	1.07
22	8.10	8.65	1.07
30	7.13	7.61	1.07
40	6.05	6.45	1.07
8% Ethyl acetate ($\beta = 0.482$)			
15	6.82	7.37	1.08
22	6.02	6.49	1.08
30	5.24	5.61	1.07
40	4.54	4.81	1.06
11% Ethyl acetate ($\beta = 0.489$)			
15	4.81	5.22	1.09
22	4.30	4.64	1.08
30	3.77	4.04	1.07
40	3.24	3.45	1.06

[a] β: Phase ratio.

The heats of adsorption, $(-\Delta H^\circ)$, are slightly lower on the deprotonated column while the retention of the 3CPP

Table 4
Thermodynamic characteristics of the adsorption of the enantiomers of 3CPP from *n*-hexane/ethyl acetate mixtures

	R-3CPP	S-3CPP
5% Ethyl acetate, before triethylamine pre-treatment ($\beta = 0.456$)		
ΔH (kJ/mole)	−14.5	−15.1
ΔS (J/mole K)	−24	−26
5% Ethyl acetate, after triethylamine pre-treatment ($\beta = 0.456$)		
ΔH (kJ/mole)	−14.0	−14.4
ΔS (J/mole K)	−22	−23
5% Ethyl acetate + 0.02% H_2O ($\beta = 0.453$)		
ΔH (kJ/mole)	−12.6	−12.7
ΔS (J/mole K)	−19	−18
8% Ethyl acetate ($\beta = 0.482$)		
ΔH (kJ/mole)	−12.3	−12.9
ΔS (J/mole K)	−21	−22
11% Ethyl acetate ($\beta = 0.489$)		
ΔH (kJ/mole)	−11.9	−12.4
ΔS (J/mole K)	−22	−23

enantiomers is lower on the column before than after the treatment with triethylamine, due to the impact of the entropy term. The heat of adsorption becomes smaller for the wet mobile phase. This phenomenon is well known in surface chemistry; it is called the "poisoning of the surface". Water is preferentially adsorbed on the most active adsorption sites, preventing them from interacting with the substrate.

3.3. Influence of the concentration of ethyl acetate on the retention and enantioselectivity

The concentration of the polar modifier is an important factor in the optimization of NPLC separations. Its influence on the retention of 3CPP is studied here. The concentration of ethyl acetate hardly affects the enantioselectivity in the range investigated (Table 3). In the same time, the retention factor and the heat of adsorption decrease with increasing ethyl acetate content of the mobile phase. The enthalpy of adsorption at the solid/liquid interface is a complex quantity that includes the contributions of the heats of adsorption of (1) the eluent and (2) the eluate, (3) the heat of solvation of the eluate by the eluent, and (4) the dilution heat, since the replacement of the eluent molecules adsorbed on the surface by the analyte molecules lead to a local change in the composition of the bulk solution. Thus, the correlation observed between the heat of adsorption and the concentration of ethyl acetate may be caused by the decrease of the net adsorption heat of a 3CPP enantiomer when the polar modifier content increases, by the increase of the solvation heat, or by a combination of both. The former could mean a blocking of the strongest adsorption sites by the modifier molecules. The latter would result from a greater stabilization of the analyte in the liquid phase.

A few authors [31,32] found that adsorbate-modifier interactions in the mobile phase result in deviations from linear behavior of the plot of $1/k'$ versus X_M, the mole fraction of the modifier in the mobile phase. In the case of 3CPP, this plot (Fig. 4) is practically linear (correlation coefficient $R = 0.99$). Yet, there are no doubts that solvation of 3CPP by ethyl acetate takes place. Preliminary measurements of the solubility of 3CPP showed that 3CPP is sparingly soluble in pure *n*-hexane at ambient temperature but dissolves notably after the addition of 3% (v/v) of ethyl acetate. Therefore, the linearity of the $1/k'$ versus X_M plot seems to mean that all the molecules of 3CPP in the bulk solution are solvated by ethyl acetate molecules. In other words, only solvated molecules of 3CPP undergo adsorption and their solvation is complete throughout the entire range of ethyl acetate content investigated. Under such circumstances, the adsorption model [32] (see Appendix A) still gives a linear dependence of the reciprocal retention factor on the modifier mole fraction. Thus, solvation does not affect the observed trend of the heat of adsorption and the explanation of the role of ethyl acetate by the competitive character of adsorption appears to remain the only possibility. Finally, it is worth noting that the retention of 3CPP with pure *n*-hexane as a mobile phase is very high,

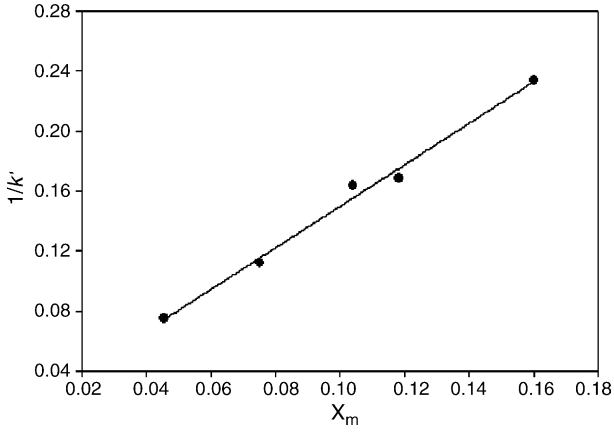

Fig. 4. Reciprocal of the retention factor as a function of the ethyl acetate mole fraction in the mobile phase. $t = 22\,^{\circ}C$. The solid line represents the linear fitting.

with a retention factor exceeding 100. This confirms that *n*-hexane plays the role of a mere inert "carrier" and of a diluent of the active component of the mobile phase, ethyl acetate.

3.4. Retention and enantioseparation with a 5% ethyl acetate–n-hexane mobile phase

A relatively satisfactory selectivity of the CSP for the enantiomers of the phenylpropanol compounds studied is obtained with solutions of *n*-hexane and ethyl acetate. The retention factors of 1PP, 2PP, and 3CPP at different temperatures are compared in Tables 3 and 5. It was not possible to separate the enantiomers of 1-phenylpropylamine (1PPA) with this mobile phase because the retention factors of both enantiomers are too small, $k' \sim 0.4$. The weak retention of 1PPA confirms the important contribution of the hydroxyl group in the adsorption. It does not contradict the assumption that the N-atom of the quinuclidine moiety interacts with the proton of the hydroxyl group in the enantioselective interaction of the QD carbamate selector with arylalkylcarbinols [1]. Indeed, the hydrogens of the amine group are much less acidic than the proton of the OH-group. Moreover, steric hindrance may also play a role since a primary amine group fills more space than a OH-group. This explains why the retention of 1PPA is much lower than that of 1PP or 3CPP.

The QD carbamate selector has another potential hydrogen-bond acceptor center, the oxygen atom of the carbamate group. This atom is supposed to be involved by

Table 5
Retention and selectivity of the enantiomers of two phenylcarbinols with *n*-hexane/ethyl acetate (95:5, v/v) mobile phase as a function of temperature

t (°C)	1PP			2PP		
	k'_R	k'_S	α	k'_R	k'_S	α
0				14.97	14.64	1.02
15	5.98	6.54	1.09	9.35	9.17	1.02
22	5.19	5.62	1.08	7.77	7.65	1.02
30	4.44	4.77	1.07	6.46	6.39	1.01
40	3.65	3.89	1.07	5.12	5.07	1.01

H-binding in the formation of a 1:1 adduct between the N-substituted amino-acids and the carbamoylated quinidine [19]. Calculations using the Gasteiger–Hückel model as implemented in Sybyl 6.9.1 software (Tripos Associates [33]) shows that the partial negative charge of this oxygen atom is even larger then that of the quinuclidine nitrogen. But, owing to its side chain position that suggests that it has a high steric accessibility (see data in ref. [19,23,24]), the ability of this C=O group to participate in an enantioselective interaction with a molecule having one functional group is doubtful since enantiodiscrimination requires some degree of steric hindrance [7]. Besides, the carbamate group being polar and having a proton bonded to an amine group, the interactions that it undergoes with ethyl acetate must be stronger than those of the quinuclidine moiety. Hence, the carbamate group is expected to be more strongly shielded by the solvent than the quinuclidine group. The proton of the NH_2 group of the carbamate group could be involved in enantioselective interactions with 3CPP, through H-binding with the chlorine atom (see item (ii) in Section 1). Even in this case, however, the key role in the enantioselectivity belongs to the interaction of the –C(OH)Ph group with the quinuclidine nitrogen and the close surroundings of the C_8–C_9 bond.

The reversal of the elution order of the enantiomers of 2PP compared to those of 1PP and 3CPP (Tables 3 and 5) shows that the mechanisms of enantioselectivity of these two groups of compounds are different. When the phenyl and the hydroxyl groups are attached to the C_1 atom of the propane backbone, making it chiral, the (*R*)-(+)-1PP enantiomer is first eluted. If the C_2 atom is the chiral center, the (*S*)-(−)-2PP enantiomer is first eluted. Such an inversion is not entirely surprising because different atoms are chiral in these two phenylpropanol isomers but the new elution order is not obvious owing to the Chan–Ingold–Prelog convention, since a sign of optical rotation is changed.

The selectivity of the enantioseparation of 2PP is markedly lower than that of 3CPP and 1PP. At the same time, the retention factor and the heat of adsorption (Tables 4 and 6) of 2PP are higher than those of 1PP and 3CPP. The latter result suggests that offsetting the phenyl group allows a stronger H-binding between the quinuqlidine nitrogen and the proton of the hydroxyl group. Calculation shows that the acidities of the hydroxyl protons of the two phenylpropanols are almost the same. The partial positive charges on the proton are 0.210 and 0.213 for 2PP and 1PP respectively. Therefore, only steric reasons may be responsible for the difference in their enantioseparation. The presence of the phenyl group at the C_1 atom is a steric factor making the interactions with the quinuclidine moiety and the region surrounding the C_8/C_9 atoms and involving the H-bond more sensitive toward the conformation of either enantiomer. In 2PP molecules, the steric influence of the phenyl group on the energy of the H-bond is no longer important. The entropy change resulting from the transfer of an analyte molecule from the mobile to the stationary phase also supports the formation of a less mobile, or less variant, adsorbed complex between 2PP and the surface of the CSP. The value of this entropy change for 2PP is about 10 J/mole more than for 1PP (Table 3). It is also possible that different parts of the selector moiety be responsible for the separation of the enantiomers of 1PP and 2PP. But, due to the chiral atoms C_8, C_9, and N_1 being close to each other, the hypothesis that there is only one chiral center which, due to its complex structure, operates in a different way with different phenylcarbinols is more probable. NMR studies in combination with molecular modeling could clarify this question [12,23,24,34].

Table 6
Thermodynamic characteristics of the adsorption of the enantiomers of 1PP, 2PP, and 3CPP from an *n*-hexane/ethyl acetate (95:5, v/v) solution

Compound	Enantiomer	ΔH (kJ/mole)	ΔS (J/mole)
1PP	R	−14.8	−30
	S	−15.5	−32
2PP	R	−19.0	−41
	S	−18.8	−40

Although the heat of adsorption from *n*-hexane–ethyl acetate solutions increases from 3CPP to 1PP to 2PP, the retention factor changes in the opposite order, increasing from 1PP to 2PP to 3CPP, due to the contribution of the entropy term ($T\Delta S$, see Table 3). The relative value of this term is between 47 and 63% of the enthalpy term at 22 °C. These rather high values show the great importance of the entropy factor in the retention. Note that the enthalpy (ΔH) and the entropy ($T\Delta S$) terms act in opposite directions, the enthalpy term promoting adsorption and the entropy term promoting desorption.

3.5. Thermodynamic aspects of the enantioselectivity

The selectivity of a separation is related to the difference in the Gibbs free energy of adsorption of the two compounds($\Delta\Delta G = \Delta G_S - \Delta G_R$) by means of the expression

$$-RT\ln(\alpha) = \Delta\Delta G \quad (3)$$

In turn the free energy difference is related to the changes in the enthalpy and entropy due to adsorption as

$$\Delta\Delta G = \Delta\Delta H - T\Delta\Delta S \quad (4)$$

The values of $\Delta\Delta G$ along with the values of the enthalpy and entropy terms in *n*-hexane–ethyl acetate mobile phases are summarized in Table 7. In all cases, except for the wet eluent, both the enthalpy and the entropy terms are negative, the entropy term ($T\Delta\Delta S$) being 50–80% of the enthalpy term. Thus, in virtue of Eq. (4), the value of $\Delta\Delta G$ is smaller than it could be, without the entropy contribution. In contrast, for the wet mobile phase, the enthalpy and the entropy contributions have opposite signs. It is easy to observe that $\Delta\Delta H$ correlates with $\Delta\Delta S$ (Fig. 5). The positive value of $\Delta\Delta S$ for the wet eluent is consistent with this plot. This phe-

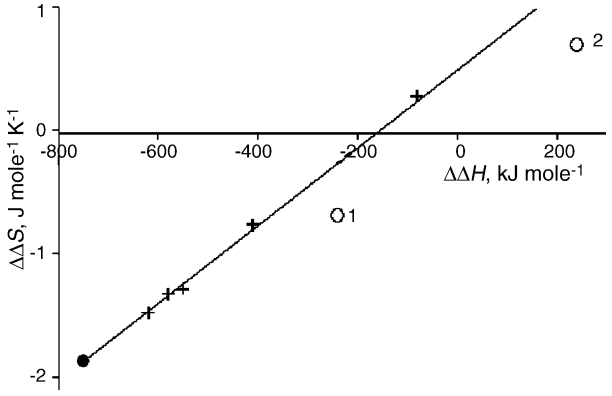

Fig. 5. Illustration of the compensation effect. Data from Table 7; (+), 3CPP; (●) 1PP, (◯) 2PP. To take into account the inversion of elution order in a case with 2PP a point with coordinates $(-\Delta\Delta H; -\Delta\Delta S)$ is also represented (2). Note that neither the given point nor the point 1 is on the compensation effect line.

Table 7
Comparison of the contributions of the enthalpy and entropy terms to the gibbs free energy difference ($T = 22\,^{\circ}C$)

Compound	$\Delta\Delta G$ (J/mole)	$\Delta\Delta H$ (J/mole)	$T\Delta\Delta S$[a] (J/mole)
5% Ethyl acetate, before triethylamine pre-treatment			
3CPP	−1701	−550	−380
5% Ethyl acetate, after triethylamine pre-treatment			
1PP	−200	−750	−550
2PP	40	240	200
3CPP	−180	−400	−220
5% Ethyl acetate + 0.02% H_2O			
3CPP	−160	−80	80
8% Ethyl acetate			
3CPP	−180	−620	−440
11% Ethyl acetate			
3CPP	−190	−580	−390

[a] $T\Delta\Delta S = \Delta\Delta H - \Delta\Delta G$.

nomenon is known as the "compensation effect" [35,36]. As seen in Fig. 5, the points for 3CPP and 1PP belong to one compensation effect line, which is not the same for 2PP. In accordance with [37,38], this observation supports the suggestion made earlier that the mechanisms of enantioselectivity for 3CPP and 1PP are similar whereas the separation of the enantiomers of 2PP takes place after a somewhat different mechanism.

4. Conclusion

The enantiomers of arylalkylcarbinols can be separated on silica-bonded quinidine carbamate under NPLC conditions with mobile phases made of *n*-hexane and a polar aprotic modifier. The best results ($\alpha = 1.07$–1.09) are obtained with solvents that combine a moderate proton-acceptor basicity and a low or zero proton-donor acidity. The presence in the molecule of the modifier of hydroxyl groups (hence a certain proton-donor acidity) decreases the separation factor, due to a blocking of chiral centers of the immobilized QD carbamate selectors. The study including the investigation of thermodynamic aspects of the separation allows the following conclusions

- The transfer of the phenyl group from the C_1 to the C_2 atom turns the order of elution of the enantiomers from the (+)-enantiomer being first (3CPP, 1PP) to the (−)-enantiomer (2PP) being first.
- The enantioselectivity is determined by the interactions between the hydroxyl group of the alcohol and the quinuclidine nitrogen of the chiral selector, the substitute of the chiral carbon atom playing a role of steric regulator of this interaction.
- The carbamate residue seems to be a center of nonselective adsorption, affecting the retention of both enantiomers but not the selectivity in the case of the phenylalkylcarbinols having only one functionality. It can be a third site in a three-point interaction, in accordance with Dalgliesh's concept [39], if the chiral alcohol studied has two functionalities, like 3CPP.
- The pre-treatment of the column with a deprotonizing reagent or with a mildly protonizing one affects but slightly the separation. Deprotonization tends to increase it slightly, suggesting that the separation of nonionogenic arylcarbinols is better done if the quinuclidine nitrogen is unprotonated.

Alternately, the enantioseparation of the enantiomers of 1PP and 2PP could be due to interactions of these molecules with different centers of the CSP. Clarification of this question needs additional measurements. Molecular modeling, NMR and X-ray data on the adducts of the investigated compounds with the quinidine carbamate selector could be useful.

The thermodynamic patterns of adsorption of 3CPP from *n*-hexane-ethyl acetate solutions are similar to those of simple competitive adsorption. The heat of adsorption decreases slightly with increasing concentration of the modifier and this trend is the same for both enantiomers. Minor additions of water has some influence on the adsorption thermodynamics, due to the shielding of active adsorption sites but the mechanism of adsorption remains fundamentally unchanged.

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Appendix A

The following description is adapted from the work of Lanin and Nikitin [32]. Consider a liquid/solid system, the liquid phase constituted from a sorbate, S, a main component of the solution, L, and a modifier (a compound more strongly adsorbed than L), M. Assume that the bulk solution and the surface solution are both ideal. By treating the partition of the sorbate between the liquid phase and the sorbent surface as a quasichemical reversible exchange reaction, and assuming the simplest case in which the areas occupied by the S, M, and L molecules in the surface layer are equal, we obtain the following equilibria for the sorbate

$$\mathrm{S_m} + \mathrm{L_s} \rightleftharpoons \mathrm{L_m} + \mathrm{S_s}$$

$$K_\mathrm{s} = \frac{X_\mathrm{s}^\mathrm{S} X_\mathrm{m}^\mathrm{L}}{X_\mathrm{m}^\mathrm{S} X_\mathrm{m}^\mathrm{L}} \tag{A.1}$$

and for the modifier

$$\mathrm{M_m} + \mathrm{L_s} \rightleftharpoons \mathrm{L_m} + \mathrm{M_s}$$

$$K_\mathrm{m} = \frac{X_\mathrm{s}^\mathrm{M} X_\mathrm{m}^\mathrm{L}}{X_\mathrm{m}^\mathrm{M} X_\mathrm{m}^\mathrm{L}} \tag{A.2}$$

where X is the mole fraction, the superscript designates the component (S, M, or L) and the subscript indicates the phase, stationary (s) or mobile (m), in which the mole fraction is considered.

In the stationary phase, we have

$$X_\mathrm{s}^\mathrm{M} + X_\mathrm{s}^\mathrm{S} + X_\mathrm{s}^\mathrm{L} = 1$$

For the bulk solution, one can write

$$X_\mathrm{m}^\mathrm{L} + X_\mathrm{m}^\mathrm{M} = 1$$

since $X_\mathrm{m}^\mathrm{S} \ll X_\mathrm{m}^\mathrm{M}$. Hence, taking into account that $k' = \beta(X_\mathrm{s}^\mathrm{S}/X_\mathrm{m}^\mathrm{S})$, we obtain

$$\frac{1}{k'} = \frac{1}{\beta K_\mathrm{s}} + \frac{K_\mathrm{m} - 1}{\beta K_\mathrm{s}} X_\mathrm{m}^\mathrm{M} \tag{A.3}$$

If all the sorbate molecules in the liquid phase are solvated, we can merely exchange the symbol S_m by the symbol S_m(solvated) in the respective equilibrium ($X_\mathrm{s}^\mathrm{S} = X_\mathrm{m}^{\mathrm{S(solvated)}}$) without changing the resulting equation in any way. It is also worth to note that, in the given model, the surface of sorbent is considered to be homogeneous whereas real surfaces are energetically heterogeneous. In the same time, when the concentration of a sorbate in a liquid phase is low (i.e., under linear conditions) it is possible to remain formally in framework of the described model with the adsorption coefficient being apparent coefficients, averaged over all types of adsorption sites.

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