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Chiral separation of some amino alcohols by addition of helical nickel(II) chelate to the mobile phase used in reversed-phase high-performance liquid chromatography

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Abstract

A set of underivatized chiral primary and secondary amino alcohols, including ephedrine analogues, were separated into the respective enantiomers by reversed-phase high-performance liquid chromatography employing acetonitrile–water as the mobile phase containing newly synthesized neutral, square-planar, helically distorted nickel(II) chelate. The four chelates studied, derived from condensation of optically active tetradentate Schiff base ligands with nickel(II) acetate, were differentiated according to the alkyl substituent on the chiral centres of the parent molecule. The influence of changes in the mobile phase concentration of each chelate or acetonitrile and its flow-rate on the observed enantiomeric discrimination of solutes was investigated. Based on the three-point interaction chiral recognition model, possible structures for the associates between solutes and the chelate responsible for the enantiomeric separation were suggested. The separation efficiency obtained with the developed HPLC system was compared with the resolution of amino alcohols observed with a typical ligand-exchange HPLC system with a chiral stationary phase in the form N-2′-hydroxy-*n*-dodecyl-L-hydroxyproline and a mobile phase containing copper(II) ion. The content of pseudoephedrine enantiomers in some representative samples of its oral dosage forms (tablets, syrups, elixirs) was determined using the developed RP-HPLC method.

1. Introduction

The amino alcohol structure is present in many pharmaceuticals, *e.g.*, β-blocker and adrenergic drugs. The most pronounced differences in pharmacokinetics and bindings between enantiomers of such drugs were observed in aged Wistar rats [1]. Consequently, stereoselective liquid chromatographic methods enabling the reliable and reproducible enantiomeric determinations of amino alcohols still attract much attention. Using reversed-phase high-performance liquid chromatography (RP-HPLC) with an octadecylsilane column a variety of amino alcohols could be resolved in the form of diastereoisomers prepared by a suitable precolumn derivatization procedure [2,3] or by introducing the complexing agent, *e.g.*, esters of tartaric acid [4,5] or N-benzyloxycarbonylglycyl-L-proline [6] into the mobile phase. HPLC with a chiral stationary phase (CSP) offers some indirect [7–11] and direct [12–15] procedures for obtaining the enantioselective separation of amino alcohols. Especially with the ligand-exchange mode of HPLC (HPLEC), Yamazaki *et al.* [13,14] achieved an excellent direct separation of enantiomers of norephedrine, norpseudoephedrine and various phenolic amino alcohols on columns of octadecylsilica coated with N-*n*-dodecyl-L-hydroxyproline (DHP) and a mobile phase containing

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copper(II) diacetate. However, the separation of enantiomers of aliphatic amino alcohols was not achieved with this chromatographic system. Lindner and Hirschbock [16] reported the partial resolution of noradrenaline isomers with an HPLEC system prepared by dynamically coating a reversed-phase packing with copper(II) or nickel(II) chiral complexes of (*R*,*R*)-tartaric acid mono-*n*-octylamide. In the second mode of HPLEC employing a chiral mobile phase additive (CMA) in the form of copper(II)–L-proline chelates, Lam and Malikin [17] resolved the *o*-phthalaldehyde (OPA) derivatives of series of aromatic and aliphatic amino alcohols. In view of the recently reported [18–21] selective separation of enantiomeric and diastereoisomeric alkylamines by RP-HPLC applying helically distorted nickel(II) Schiff base chelates as chiral selectors in the mobile phase, the aim of this study was to confirm the usefulness of this system for the enantioselective separation of non-derivatized amino alcohols.

2. Experimental

2.1. Chemicals

The following amino alcohols were obtained from Sigma (St. Louis, MO, USA): (*R*,*S*)-2-amino-1-(3,4-dihydroxyphenyl)ethanol (arterenol, norepinephrine, noradrenaline), (*R*,*S*)-2-amino-1-phenylethanol (phenylethanolamine, β-hydroxyphenethylamine), (*R*)- and (*R*,*S*)-2-amino-2-phenylethanol (α-phenylglycinol), (*S*)-2-amino-3-phenyl-1-propanol (L-phenylalaninol) and (1*S*,2*S*)- and (1*R*,2*R*)-pseudoephedrine.

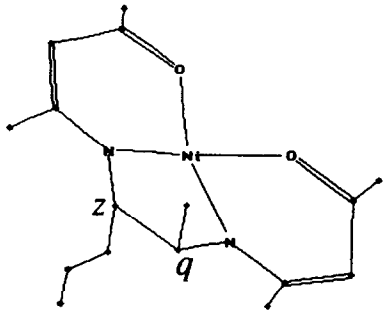

Fig. 1. Stereoscopic view of NiL[4] chelate (see Table 1) as an example of the structure of helical nickel(II) chelates used as CMA. The asymmetric carbon atoms are designated *z* and *q*. Some hydrogen atoms have been omitted for clarity.

(1*R*,2*S*)-Norephedrine was purchased from Serva (Heidelberg, Germany), (1*R*,2*S*)-ephedrine from Merck (Darmstadt, Germany) and (1*S*,2*R*)-ephedrine hydrochloride from Fluka (Buchs, Switzerland).

The four solid nickel(II) tetradentate Schiff base chelates (Fig. 1, Table 1) were synthesized, purified and characterized as described in previous reports [22–25]. (2*S*,4*R*,2′*RS*)-N-(2′-hydroxy-*n*-dodecyl)-4-hydroxyproline (HDHP) was synthesized according to the procedure described by Martens and co-workers [26,27].

2.2. Apparatus

A Pye LC3 XP chromatograph (Pye Unicam, Cambridge, UK) equipped with a multi-wavelength UV detector was applied. Separations were carried out on a Polsil ODS (ZOCh, Lublin, Poland) stainless-steel columns (25 × 0.4 cm I.D.) packed with octadecylsilica gel of particle size 7 μm.

Table 1
Nickel(II) Schiff base chelates used as the chiral mobile phase additives in HPCMA experiments

Abbreviation	Systematic name	Substituent	
		z	*q*
NiL[1]	*dl*-[4,4′-(Methylethane-1,2-diyldiimino)bis(pent-3-en-2-onato)]nickel(II)	H	CH_3
NiL[2]	*dl*-[4,4′-(1,2-Dimethylethane-1,2-diyldiimino)bis(pent-3-en-2-onato)]nickel(II)	CH_3	CH_3
NiL[3]	*dl*-[4,4′-(Butane-1,2-diyldiimino)bis(pent-3-en-2-onato)]nickel(II)	C_2H_5	H
NiL[4]	*dl*-[4,4′-(1-Methyl-2-propylethane-1,2-diyldiimino)bis(pent-3-en-2-onato)]nickel(II)	C_3H_7	CH_3

2.3. High-performance liquid chromatography with a chiral mobile phase additive (HPCMA)

For the preparation of the mobile phase, different amounts ($1 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $5 \cdot 10^{-4}$, $1 \cdot 10^{-3}$ and $1 \cdot 10^{-2}$ mol/l) of solid nickel(II) chelate (see Table 1) were carefully weighed and added to a 0.3 mol/l solution of sodium acetate, then ultrasonically degassed acetonitrile was added to the required volume (15, 20 or 25%, v/v). The pH of the final mobile phase was adjusted to 6.0 with acetate buffer. The mobile phase was then filtered and degassed under reduced pressure. The Polsil ODS column was equilibrated with 15–30 column volumes (4.20 ml) of the mobile phase prior to sample injection. The starting flow-rate was 1 ml/min. The column effluent was monitored with a UV absorbance detector operating at 254 nm. All measurements was made at 25°C. Capacity factors (k') of solutes were calculated as the means of four parallel measurements.

2.4. HPLEC measurements

The procedure for coating the column with Polsil ODS was adapted from Davankov *et al.* [28] with slight modification. The column (25 cm) was washed (flow-rate 0.5 ml/min) with 5 ml of a solution of 100 mg of HDHP in acetonitrile–water (20:80, v/v). Then 10 ml of coating mixture consisting of a concentrated solution of copper(II) acetate in acetonitrile–water (20:80, v/v) was delivered to the column. The column was equilibrated with the final mobile phase, acetonitrile–water (20:80, v/v) containing $2 \cdot 10^{-4}$ mol/l copper(II) acetate and adjusted to pH 6.0 with acetate buffer. The effluent from the column was monitored with a UV absorbance detector at 280 nm after acidification by adding 0.2 mol l^{-1} perchloric acid (1.0 ml/min) using a postcolumn mixing loop (30 cm × 0.5 mm I.D.).

2.5. Analysis of pharmaceuticals

Five products commercially available locally were used for the determination of the content of pseudoephedrine enantiomers using extraction procedures according to Peeran *et al.* [29] followed by the developed HPCMA method employing the NiL^4 chelate and the HPLEC method with HDHP chiral stationary phase.

2.6. Calculations

A standard statistical package was used for the calculation of precision and reproducibility parameters. The last-squares superimposition of nickel(II) chelates and solute enantiomers during association was made using the program PCMODEL (Serena Software, Bloomington, IN, USA).

3. Results and discussion

3.1. HPCMA measurements

Some chiral, ionized, octahedral nickel(II) complexes, mainly *n*-alkylamide derivatives, have been utilized as chiral mobile phase additives in HPLEC systems [16,30], permitting the enantioselective separation of free or dansylated amino acids. However, Lochmuller and Hagac [31] showed that also neutral, square-planar nickel(II) chelates can be used as the mobile phase dopant for the selective separation of aromatic amines using RP-HPLC systems. Such excited nickel(II) chelates are differentiated from the previously mentioned ligand-exchange chelates by the fact that during the formation of unstable associates with resolved solutes the fundamental chelate structure remains unchanged, *i.e.*, no coordination bonds between the central nickel(II) ion and the parent ligand are broken or formed. Therefore, it has been suggested [31] that specific, electrostatic, induced dipole–dipole interactions between the square-planar, coordinately unsaturated nickel(II) chelate and the polar solute acted as the principle of the observed enhanced retention. One could expect that such interactions supported by the chiral environment of nickel(II) ion in such a defined chelate structure would lead to a potential chiral selector. This approach was applied in this study in the synthesis of the series of

chiral nickel(II) complexes (Fig. 1, Table 1) with optically active, tetradentate Schiff bases.

All four chelates studied contain two chiral carbon atoms (designated *z* and *q* in Fig. 1) localized in the ethylene bridge connecting both imine nitrogen atoms. This implies the formation of only *dl* isomers of NiL^1, NiL^3, NiL^4 chelates, respectively, or *dl* and *meso* isomers of NiL^2 chelate. The *meso* isomer was separated from the *dl*-NiL^2 chelate during the purification step using a silica gel with benzene–hexane (70:30, v/v) column chromatographic system and then its content was calculated as 0.15% from the results of gas chromatographic measurements [23]. The crystal structure of all *dl*-isomers of the synthesized complexes showed [23–25] an equatorial arrangement of the tetradentate ligands around the metal centre in a slightly distorted square-planar geometry. Of the two imino ketone ligand fragments, one deviates significantly from planarity whereas the other is planar, within experimental error. The distortion and deviation from planarity increased in parallel with the bulkiness of the alkyl *z* substituent in the molecule of the nickel(II) chelate. The determination of torsion angles indicated a stable axial position of the *z* substituent in the ethylene bridge which stabilized the λ conformation of the five-membered chelate ring and the Λ configuration of chiral carbon atoms in each kind of chelate studied. This phenomenon leads to the formation of a helical structure of the chelates studied and their chirooptical properties detected by circular dichroism spectra [25,32,33]. The six-membered chelate rings, contained significantly delocalized π bonds, are not perfectly coplanar. Such coplanarity increased with decrease in the molecular volume of the *z* substituent. The partial negative or positive charge is localized on the oxygen carbonyl atoms or central nickel(II) ion, respectively. However, the surface area of the chelates is mostly non-polar, *e.g.*, for the NiL^4 chelate the non-polar saturated surface area occupied *ca.* 212 $Å^2$ of the 283 $Å^2$ of the total surface area. As suggested on the basis of adsorption isotherm data [20], the more bulky *z* substituent and the greater hydrophobicity of the chelates in the series $NiL^1 < NiL^2 < NiL^3 < NiL^4$ increased the chelate concentration on the surface of the octadecylsilane stationary phase. NMR results confirmed [23,25,34,35] that *d*-isomeric species (with an axial position of the *z* alkyl substituent) predominates in mixtures of *dl*-isomers of the nickel(II) chelates studied.

The structure of the amino alcohols studied is presented in Table 2. The amino group is attached to the primary or secondary carbon atom as in noradrenaline and, phenylethanolamine or in the remaining solutes, respectively. Only one asymmetric carbon atom exists in an α- or β-position to the phenyl ring in compounds **1**, **2** and **4** or compound **3**, respectively. Two asymmetric carbon atoms with different environments are present in solutes **5**, **6** and **7**, which implies the formation of two pairs of enantiomers. In contrast to the other solutes, in compounds **1** and **2** the amine group is adjacent to the asymmetric carbon atom.

As can be seen from Table 3, the values of the capacity factors (k') and separation factors (α) determined for amino alcohols with one chiral centre increased with increasing bulkiness of the

Table 2
Structure of amino alcohols studied, $R^3NHCH(R^1)CH(R^2)OH$

No.	Compound	R^1	R^2	R^3
1	Noradrenaline	H	$C_6H_3(OH)_2$	H
2	Phenylethanolamine	H	C_6H_5	H
3	Phenylalaninol	$CH_2C_6H_5$	H	H
4	Phenylglycinol	C_6H_5	H	H
5	Ephedrine	CH_3	C_6H_5	CH_3
6	Pseudoephedrine	CH_3	C_6H_5	CH_3
7	Norephedrine	CH_3	C_6H_5	H

Table 3
HPLC separation of *R*- and *S*-enantiomers of amino alcohols containing one asymmetric atom with mobile phases modified by NiL^{1-4} chelates

No.	Compound	NiL^1			NiL^2			NiL^3			NiL^4		
		k'_S	k'_R	α	k'_S	k'_R	α	k'_S	k'_R	α	k'_S	k'_R	α
1	Noradrenaline	6.48	6.63	1.02	7.01	7.36	1.05	7.63	8.69	1.14	8.15	10.61	1.30
2	Phenylethanolamine	7.19	7.36	1.03	7.73	8.04	1.04	8.31	9.55	1.15	9.01	12.82	1.42
3	Phenylalaninol	8.03	8.17	1.01	8.41	8.83	1.05	9.21	10.50	1.14	10.32	15.10	1.46
4	Phenylglycinol	8.43	8.51	1.02	8.72	9.24	1.06	9.75	10.81	1.14	11.31	17.19	1.52

For experimental conditions, see Fig. 2.

nickel(II) chelate employed as the chiral mobile phase additive. In all proposed chromatographic systems the most polar noradrenaline molecules were least retained (see Fig. 2). However, in spite of the type of nickel(II) chelate the *S*-enantiomers of solutes were eluted first. Contrary to the retention observed by Yamazaki *et al.* [13,14], in the HPLEC system a satisfactory separation of phenylalaninol enantiomers was achieved in the developed HPCMA mode, especially using a mobile phase containing the NiL^4 chelate. Noradrenaline isomers, containing two phenolic hydroxy groups, were also sufficiently separated ($\alpha = 1.3$) by introducing the NiL^4 chelate into the acetonitrile–water (20:80) (pH 6) mobile phase. This result is opposite to the separation of noradrenaline isomers reported by Lindner and Hirschbock [16] in the HPLEC mode.

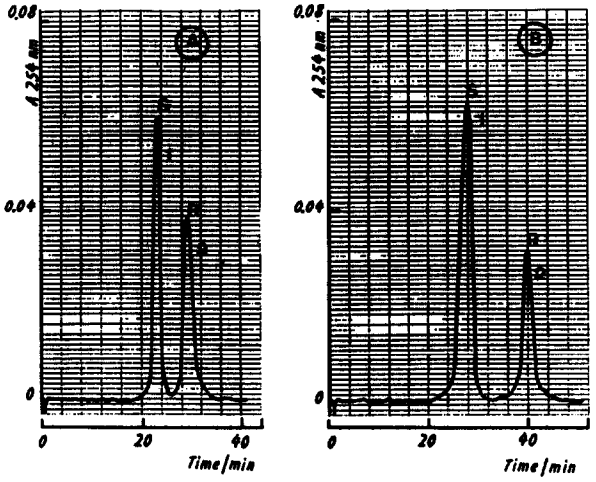

Fig. 2. Enantiomeric HPCMA separation of noradrenaline and phenylalaninol isomers of RP-HPLC with acetonitrile–water (20:80) (pH 6.0) as the mobile phase containing NiL^4 chelate ($1 \cdot 10^{-3}$ mol/l). Flow-rate, 1.2 ml/min; temperature 25°C; column, 25 × 0.4 m I.D.; column packing, Polsil ODS, 7 μm; UV detection at 254 nm. Peaks: (A) 1 = (*S*)-noradrenaline and 2 = (*R*)-noradrenaline; (B) 1 = (*S*)-phenylalaninol and 2 = (*R*)-phenylalaninol.

The observed chiral recognition of amino alcohols with one stereogenic centre can be explained using the "three-point-interaction" rule of Dalgliesh [36]. The formation of unstable associates with 1:1 stoichiometry between different square-planar nickel(II) tetradentate Schiff chelates and ammonium ion or alkylamines in polar and non-polar solutions was confirmed on the basis of reliable spectrophotometric results [25,32].

Molecular models (see Fig. 3) of the NiL^4–(*R*)-noradrenaline associate indicate that chiral recognition occurs mainly by specific attractive interactions between the negatively charged amine nitrogen atom and the positively charged, coordinated nickel(II) ion. The difference in stabilities of the enantiospecific associates is due to additional hydrogen bond formation between the hydroxyl group of the solute and the oxygen carbonyl atom in the chelate molecule. Tertiary lateral weak $\pi-\pi$ interactions between the phenolic ring of the solute and one of the six-membered chelate rings are also necessary for chiral discrimination in the developed HPCMA chromatographic system. The non-planar position of the solute in relation to the chelate ring system is

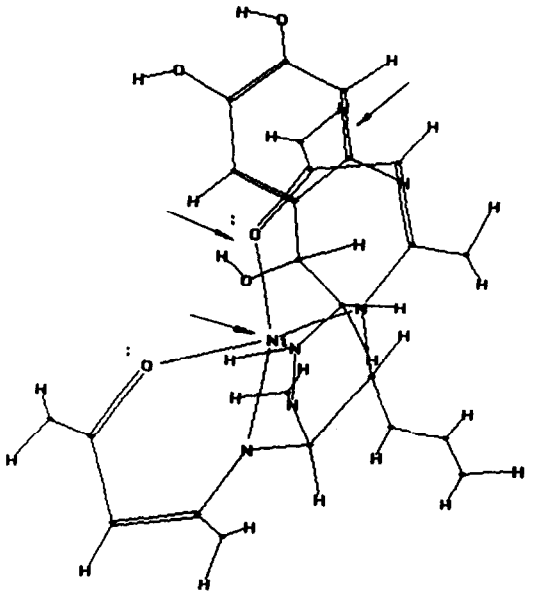

Fig. 3. Stereoscopic image of proposed structure of the NiL^4–(*R*)-noradrenaline associate formed in the developed HPCMA chromatographic system. Arrows indicate positions of the possible interaction sites.

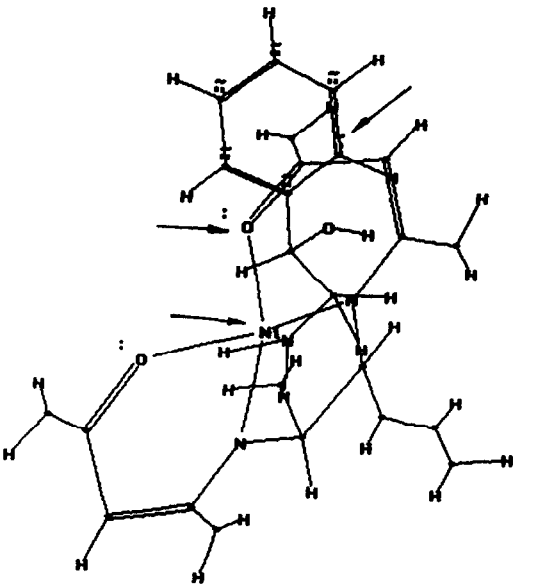

Fig. 4. Stereoscopic image of proposed structure of the NiL^4–(*S*)-phenylethanolamine associate formed in the developed HPCMA chromatographic system. Arrows indicate positions of the possible interaction sites.

forced by the antiperiplanar conformation of the *q* and *z* substituents in the chelate molecule. This should lead to a situation where an electron-deficient coordinated nickel(II) ion can be reached only by a non-hindered amine group attached or adjacent to the chiral centre of the solute. As was pointed out by Wainer *et al.* [9], the mutual relationship between the intensity of such a number of competing intermolecular interactions determines the final stereoselective associate stability, the observed retention order and the overall chromatographic enantioselectivity. Hence the recognition mechanism is changed from "three-point" like for (*R*)-noradrenaline to "two-point" as for (*S*)-phenylglycinol. The *S*-entantiomers are less retained because hydrogen bond formation at the second interaction point is two difficult (see Fig. 4). The binding affinities of the amine solutes to the coordinated nickel(II) ion increased if the amine and phenyl group were linked to the chiral carbon atom (negative inductive effect, $-I$), as is seen from the enhanced retention of phenylglycinol compared with phenylalaninol and phenylethanolamine.

The influence of the nickel(II) chelate structure on the chiral separation of amino alcohols with two stereogenic centres in the proposed HPCMA system is shown in Tables 4 and 5. Stronger retentions of all ephedrine analogues were observed for mobile phases containing the more bulky NiL^4 chelate (see Fig. 5). The norephedrine isomers, containing a primary amine group, were less retained in all instances. The best separation of the four stereoisomers was obtained with a mobile phase modified by the NiL^4 chelate (see Table 5). However, the α values for the most pronounced enantiomer pair, *R,S–S,R* of ephedrine and norephedrine or *R,R–S,S* of pseudoephedrine, were relatively low, even for the more effective selectand in the form of the NiL^4 chelate. However, it should be noted that the reported separation is more efficient than that obtained previously in an HPLEC system [13,14,16] when ephedrine isomers were not resolved. The observed retention order of

Table 4
Capacity factors, k', of isomers of amino alcohols containing two chiral centres in HPLC systems employing mobile phases modified by NiL^{1-4} chelates

No.	Compound	*R,S*				*S,R*				*S,S*				*R,R*			
		NiL^1	Nil^2	NiL^3	NiL^4	NiL^1	Nil^2	NiL^3	NiL^4	NiL^1	Nil^2	NiL^3	NiL^4	NiL^1	Nil^2	NiL^3	NiL^4
1	Ephedrine	20.7	21.0	21.3	21.6	21.4	21.8	22.4	22.7	22.8	23.1	23.7	24.8	23.8	24.3	25.7	27.3
2	Pseudoephedrine	22.3	22.8	23.6	24.1	23.7	24.1	24.7	25.8	25.6	26.7	27.3	28.4	26.1	28.9	30.1	32.3
3	Norephedrine	19.1	19.4	19.8	20.0	19.7	20.1	20.3	22.0	20.6	20.9	21.0	23.5	21.4	21.7	22.3	27.0

For experimental conditions, see Fig. 5.

Table 5
Selectivity of separation of isomers of amino alcohols containing two chiral centres in HPLC systems employing mobile phases modified by NiL^{1-4} chelates

Isomer pair	Ephedrine				Pseudoephedrine				Norephedrine			
	NiL^1	NiL^2	NiL^3	NiL^4	NiL^1	NiL^2	NiL^3	NiL^4	NiL^1	NiL^2	NiL^3	NiL^4
R,R–R,S	1.14	1.16	1.21	1.26	1.17	1.27	1.28	1.34	1.12	1.12	1.13	1.35
R,R–S,R	1.11	1.11	1.13	1.21	1.10	1.20	1.22	1.25	1.09	1.08	1.10	1.23
R,R–S,S	1.04	1.05	1.08	1.10	1.02	1.08	1.10	1.14	1.04	1.04	1.06	1.15
R,S–S,R	1.03	1.03	1.06	1.03	1.06	1.06	1.06	1.07	1.03	1.04	1.04	1.10
R,S–S,S	1.10	1.27	1.11	1.15	1.15	1.17	1.16	1.18	1.08	1.08	1.06	1.17
S,R–S,S	1.06	1.06	1.04	1.11	1.08	1.08	1.11	1.11	1.04	1.04	1.10	1.07

For experimental conditions see Fig. 5.

diastereoisomers ($RS < SR < SS < RR$) is consistent with the proposed chiral discrimination mechanism in which "three-point" associates are formed (see Fig. 6) by strongly retained *R,R*-enantiomers and nickel(II) chelates.

As shown in Fig. 7, an increasing concentration of nickel(II) chelate in the mobile phase usually slightly increased the observed values of the separation factors. The most effective are changes in concentration of the NiL^4 chelate.

Increasing the concentration of organic modifier decreased the enantiomeric separation, as illustrated in Fig. 8 for the phenylglycinol, phenylalanine, pseudoephedrine and norephedrine isomers.

Increasing the flow-rate of the mobile phase had virtually no effect on the observed enantioselectivity, as is shown in Fig. 9, probably owing to the minor effect on the values of the reduced plate height of the column as suggested by Rizzi [37] for another reversed-phase HPLC system employing chiral mobile phase additives.

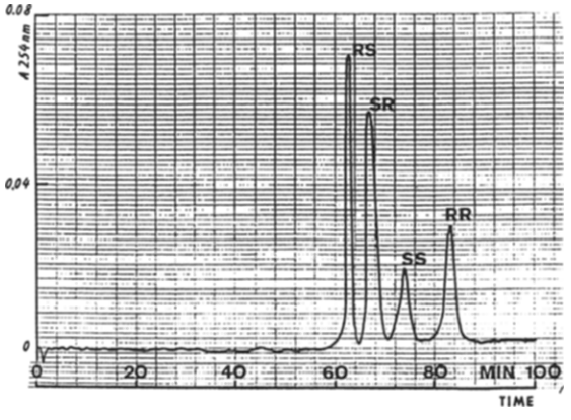

Fig. 5. Diastereomeric HPCMA separation of pseudoephedrine isomers by RP-HPLC with water–acetonitrile (20:80) (pH 6.0) as the mobile phase containing NiL^4 chelate ($1 \cdot 10^{-3}$ mol/l). For other experimental conditions, see Fig. 2.

3.2. HPLEC measurements

The results of the enantiomeric separation of amino alcohols obtained with the developed HPCMA system using helical nickel(II) chelates were compared with those for an HPLEC system

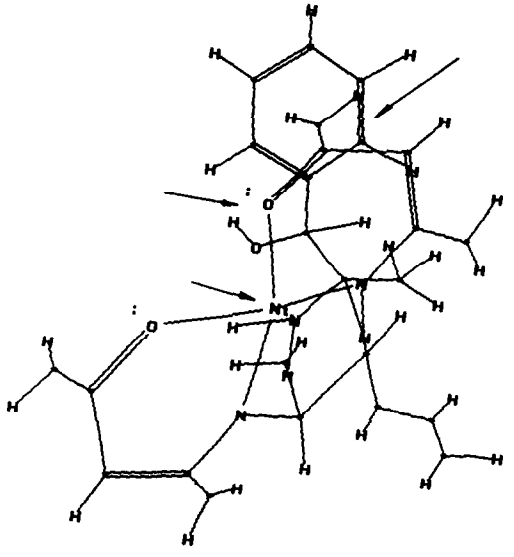

Fig. 6. Stereoscopic image of proposed structure of the NiL^4–(*R,R*)-norephedrine associate formed in the developed HPCMA chromatographic system. Arrows indicate positions of the possible interaction sites.

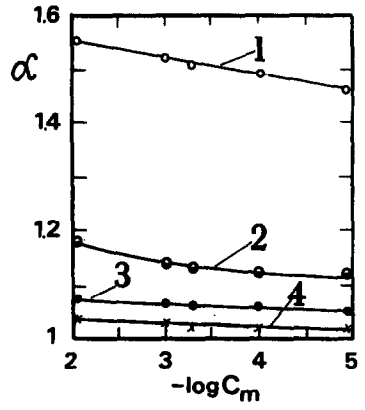

Fig. 7. Influence of the concentration of nickel(II) chelates (log C_m) in acetonitrile–water (20:8, v/v) mobile phase (pH 6) at a flow-rate of 1.3 ml/min on the separation factors (α) of phenylglycinol enantiomers: 1 = NiL^4; 2 = NiL^3; 3 = NiL^2; 4 = NiL^1.

an employing chiral stationary phase in the form of N-2′-hydroxy-*n*-dodecyl-L-hydroxyproline (HDHP) and a mobile phase containing copper(II) ions. This dynamically based chiral stationary phase was used by Busker and coworkers [26,38] for the HPLC separation of penicillamine enantiomers. Gunther [39] reviewed numerous applications of HDHP as a component of hydrocarbonaceous silica gel layers designated Chiralplate for thin-layer chromatographic enantioselective separations. The presence of an additional hydroxyl group in the alkyl side-chain of HDHP forms the second

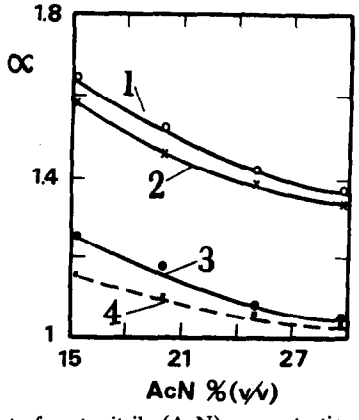

Fig. 8. Effect of acetonitrile (AcN) concentration in acetonitrile–water mobile phase (pH 6; concentration of NiL^4 chelate = $1 \cdot 10^{-3}$ mol/l; flow-rate 1.2 ml/min) on separation factors (α) of amino alcohol enantiomers: 1 = (*S–R*)-phenylglycinol; 2 = (*S–R*)-phenylalaninol; 3 = (*SS–RR*)-pseudoephedrine; 4 = (*RS–SR*)-norephedrine.

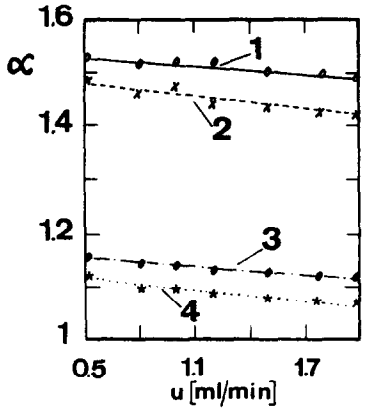

Fig. 9. Effect of flow-rate (u) of mobile phase [acetonitrile–water (20:80) (pH 6), concentration of NiL^4 chelate = $1 \cdot 10^{-3}$ mol/l) on separation factors (α) of amino alcohol enantiomers: 1 = (*S–R*)-phenylglycinol; 2 = (*S–R*)-phenylalaninol; 3 = (*SS–RR*)-pseudoephedrine; 4 = (*RS–SR*)-norephedrine.

stereogenic centre near the parent chiral hydroxyproline molecule. Both factors differentiate HDHP from N-*n*-dodecyl-L-hydroxypropline (DHP) and its N-alkyl derivatives applied previously as chiral stationary phases in the HPLC of amino acids and amino alcohol mixtures [13,14,28]. Considering the chiral recognition model developed by Davankov *et al.* [28], one would expect the hydroxyl substituent in HDHP to act as a supporting interactive site influencing the enatioselectivity of polar solute separations.

In Table 6 and Fig. 10 the observed retention of amino alcohols on the column packed with HDHP is illustrated. The *S*-enantiomers of noradrenaline and phenylglycinol were eluted first. Such a retention order of enantiomers is reversed for analogous solutes on the DHP column [14]. Phenylalaninol enantiomers were resolved using HDHP and acetonitrile–water (20:80) (pH 6) as the mobile phase containing $2 \cdot 10^{-4}$ mol l^{-1} copper(II) acetate. This result is also contrary to the previously reported retention of phenylalaninol on DHP phase using acetate buffer (pH 6) with an 8 mmol/l concentration of copper(II) acetate [14]. However, the retention orders of noradrenaline, phenylethanolamine and phenylglycinol on both the HDHP and DHP stationary phases under the applied experimental conditions were identical. Moreover, on HDHP shorter retention times of these solutes were obtained. Comparing the

Table 6
Capacity factors, k', and selectivity, α, of enantiomeric separations of amino alcohols in HPLEC experiments

No.	Compound	First-eluting	k_1' [a]	k_2' [b]	α
1	Noradrenaline	*S*	1.10	1.65	1.50
2	Phenylethanolamine	*S*	7.15	15.02	2.10
3	Phenylglycinol	*S*	10.71	18.21	1.71
4	Phenylalaninol	*S*	11.73	16.32	1.39
5	Norephedrine	*S,R*	12.30	23.34	1.89
6	Ephedrine	*S,R*	15.12	27.18	1.78
7	Pseudoephedrine	*R,R*	18.31	31.14	1.70

For experimental conditions, see Fig. 10.
[a] Capacity factor of first-eluting enantiomer.
[b] Capacity factor of second-eluting enantiomer.

results obtained on the HDHP with amino alcohol retentions achieved with the developed HPCMA system, the shorter retention and slightly greater values of the separation factors α (especially for phenylethanolamine enantiomers) are notable when the former HPLC system is applied (see Tables 4–6). Examining the retentions of ephedrine-like solutes (**5–7**) one can state that *S,R*-enantiomers were eluted first on both HDHP and DHP (see results in refs. 13 and 14). However, on HDHP the amino alcohols are less retained compared with the retentions reported on DHP [14] and with the developed HPCMA system. The selectivity of the prepared HPCMA system is greater, leading to separations of all enantiomers pairs (see Tables 5 and 6). Comparing the α values for *SR–RS* enantiomers of ephedrine calculated for the HDHP column with those obtained with the proposed HPCMA system, the former method was found to be more selective for the separation.

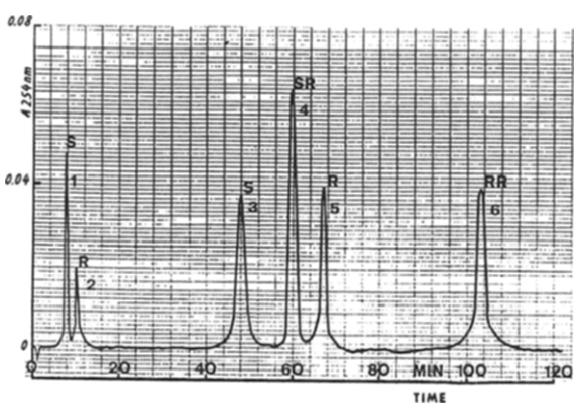

Fig. 10. Separation of noradrenaline, phenylalanine and ephedrine isomers of the HPLEC method employing an HDHP-coated Polsil ODS, 7 μl, column (25 × 0.4 cm I.D.) with acetonitrile–water (20:80) as mobile phase (pH 6.0) containing $2 \cdot 10^{-4}$ mol/l copper(II) acetate; flow-rate, 1.0 ml/min; column temperature 25°C; UV detection at 280 nm. Peaks; 1 = (*S*)-noradrenaline; 2 = (*R*)-noradrenaline; 3 = (*S*)-phenylalaninol; 4 = (*S,R*)-ephedrine; 5 = (*R*)-phenylalaninol; 6 = (*R,S*)-ephedrine.

3.3. *Analysis of pharmaceuticals*

The HPCMA separation method reported here was applied to determination of the content of pseudoephedrine enantiomers in samples of its oral dosage forms. Three different pseudoephedrine samples were analysed by both the HPCMA and HPLEC methods with four replicates. The results in Table 7 indicate that the methods have comparable repeatability and random error, as indicated by lower calculated values of the *F*-test compared with the critical value appropriate at the 0.95 probability level [40]. The methods do not differ significantly in accuracy, as verified by the values of *t* being lower than their critical value at the 0.99 probability level [40]. The correlation coefficient between the HPCMA and HPLEC results is near 0.97. However, the proposed HPCMA method offers a slightly lower relative error.

Table 7
Determination of pseudoephedrine enantiomers in pharmaceuticals

Formulation	Isomer	Method	Mean concentration[a], x (%)[b]	Sample S.D. (%)	F-Test[c,d]	t-test[c,e]
Tablet	*S,S*	HPCMA	43.1	0.181	1.90	1.10
		HPLEC	42.8	0.231		
	R,R	HPCMA	56.9	0.109	1.80	1.30
		HPLEC	57.2	0.156		
Syrup	*S,S*	HPCMA	45.1	0.123	2.10	1.80
		HPLEC	44.6	0.143		
	R,R	HPCMA	54.9	0.111	2.05	1.90
		HPLEC	55.4	0.120		
Elixir	*S,S*	HPCMA	40.1	0.125	1.10	1.05
		HPLEC	39.2	0.140		
	R,R	HPCMA	59.9	0.132	1.30	1.56
		HPLEC	60.8	0.142		

[a] Mean of four determinations.
[b] Expressed as a percentage of the claimed content.
[c] F- and t-tests were calculated for comparison of x values obtained in HPCMA and HPLEC measurements.
[d] Theoretical F ($P = 0.95$, $f_1 = f_2 = 3$) = 9.28.
[e] Theoretical t ($P = 0.99$, $f = 6$) = 3.71.

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