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Direct chromatographic resolution of carnitine and *O*-acylcarnitine enantiomers on a teicoplanin-bonded chiral stationary phase

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Abstract

R-(−)-Carnitine (vitamin B_T) plays an important role in human energy metabolism, by facilitating the transport of long-chained fatty acids across the mitochondrial membranes. Its (*S*)-enantiomer acts as a competitive inhibitor of carnitine acetyltransferase, causing depletion of the body *R*-(−)-carnitine stock. Consequently, the separation of carnitine enantiomers is very important both to study their biological activities and to control the enantiomeric purity of pharmaceutical formulations. In the present paper we describe an easy, fast and convenient procedure for the separation of the enantiomers of carnitine and *O*-acylcarnitines by enantioselective HPLC on a laboratory-made chiral column containing covalently bonded teicoplanin as selector. High enantioselectivity factors (α values ranging from 1.31 to 3.02) and short-time analyses characterize the analytical procedure; in addition, analytes are easily detected by evaporative light scattering with no need for preliminary derivatization. The effects of pH and ionic strength of the mobile phase and of the nature of the organic modifier on the enantioselective separations were also investigated.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Carnitine; Acylcarnitines; Teicoplanin; Vitamins

1. Introduction

Carnitine is a natural component of various animal and vegetal tissues, where it plays a fundamental role in the utilization of lipids [1]. In fact, it is the only carrier utilizable by long-chained fatty acids to cross the internal mithocondrial membrane prior to their metabolic β-oxidation. Indirectly, carnitine influences also the metabolism of glucides and protides: fatty acids oxidation reduces peripheric utilization of glucose, while allows the entry of acetyl residues from β-oxidation in the tricarboxylic acid cycle (Krebs cycle), therefore increasing cellular energetic availability.

This important function in fatty acid metabolism can be ascribed only to the (*R*)-enantiomer of carnitine, while the (*S*)-enantiomer has been found to have considerable toxic effects on several biochemical processes [2], due to competitive inhibition of

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carnitine acetyltransferase, thereby causing depletion of the body *R*-(−)-carnitine stock. Therefore, in pharmaceutical preparations used in the case of primary and secondary carnitine deficiencies [3] (acute and chronic myocardium ischaemia, myocardium sclerosis, angina pectoris, etc.), the use of the enantiopure (*R*)-enantiomer of carnitine and its esters is now mandatory.

At present, the industrial production of (*R*)-(−)-carnitine is performed by fractional crystallization of diastereoisomeric mixtures or stereoselective synthetic or microbiological processes. Enantioselective assays are needed to ascertain enantiomeric purity of these preparations, and could be useful also to investigate the different pharmacokinetic profile of (*R*)- and (*S*)-carnitine and its derivatives.

In the past, this problem was solved by nuclear magnetic resonance (NMR) spectroscopy with chiral shift reagents [4–6] or by using stereospecific enzymatic reactions [7–9]. Recently, high-performance liquid chromatographic methods have been successfully developed for the resolution of such a problem. They are based on either chiral stationary phases (CSPs) and pre-derivatization with chromophoric achiral reagents [10] or achiral stationary phases and pre-derivatization with enantiopure reagents [11–14]. Lastly, high-performance capillary electrophoresis (HPCE) has also been applied to the separation of (+)-FLEC [(+)-1-(9-fluorenylethyl) chloroformate] diastereomeric derivatives of (*R*)- and (*S*)-carnitine using simple phosphate buffer [11]; in addition the separation of enantiomeric FMOC (9-fluorenylmethyl chloroformate) derivatives was performed using acidic buffers and different cyclodextrins as chiral selectors [15].

However, to the best of our knowledge [16,17], high-performance chromatographic or electrophoretic methods for direct enantiomeric separation of carnitine and derivatives without preliminary pre- or post-column derivatization have never been reported in literature.

In this paper we describe an easy, fast and convenient procedure for the separation of the enantiomers of carnitine and *O*-acylcarnitines by enantioselective high-performance liquid chromatography (HPLC) on a laboratory-made CSP containing covalently bonded teicoplanin (TE) as selector.

TE is a macrocyclic glycopeptide antibiotic, produced by the growth of certain strains of *Actinoplanes teichomyceticus* (Fig. 1) [18]. It is applied in the treatment of severe hospital-acquired infections caused by Gram-positive bacteria [19]. It binds stereospecifically to the carboxy-terminal of D-Ala–D-Ala sequences of the muramylpentapeptide formed during the biosynthesis of peptidoglycan, a key component of the bacterial cell wall. More recently, glycopeptide antibiotics have been successfully used as chiral selectors to resolve the enantiomers of a variety of racemic compounds by means of thin-layer chromatography (TLC), HPLC and HPCE techniques [20]. Structurally, TE contains a heptapeptide aglycone that bears three sugar units. It is noteworthy that the peptide backbone contains a *cis* peptide bond, which is essential to keep the structure in its rigid macrocyclic form (Fig. 1). The aglycone moiety consists of four fused medium-size rings, which form a ''semi-rigid basket''. The basket contains seven aromatic rings, two of which have chloro-substituents and four have ionisable phenolic moieties. In the aglycone moiety, there are also a primary amine (the ''cationic site'') and a carboxylic acid group (the ''anionic site''). The three sugar units are monosaccharides, namely α-D-mannose, β-D-*N*-acetylglucosamine, β-D-*N*-acylglucosamine. Five main components of TE have been identified, differing from each others only in the nature of the hydrocarbon chain of the *N*-acylglucosamine moiety. The chemical structure of the prevalent component of TE glycopeptide complex (A_2-2,>85%) is reported in Fig. 1; it contains a nine carbon atoms apolar chain, conferring on the structure a hydrophobicity considerably higher than that of other related glycopeptides such as vancomycin and ristocetin [21].

The CSP used in this work was prepared by covalently linking the glycopeptide antibiotic TE to an aminopropyl-functionalized silica gel, via a bifunctional aliphatic isocyanate, according to a novel and efficient ''one-pot'' synthetic strategy [22]. The separations were carried out in hydro–organic eluent systems with or without the addition of ammonium acetate buffers. To overcome UV detection difficulties related to the absence of chromofores in the analytes, alternative detection systems like evaporative light scattering or polarimetry have been used [23,24].

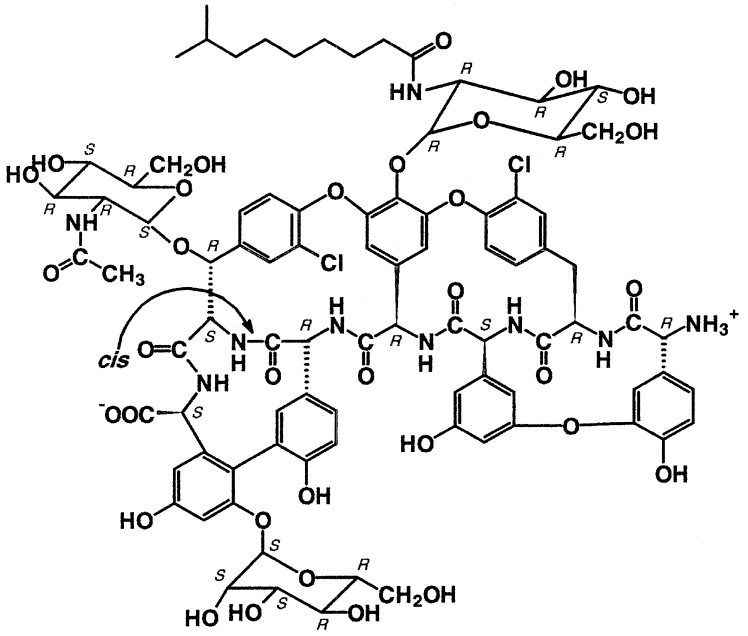


Fig. 1. Chemical structure of the prevalent teicoplanin component, A_2-2.

2. Experimental

2.1. Apparatus

Analytical liquid chromatography was performed on a Waters chromatograph equipped with a Rheodyne Model 7725i 20-μl injector and two Model M510 solvent-delivery systems. Different detectors were used, including a Model M490 programmable multi-wavelength detector (Waters Chromatography, Milford, MA, USA), a Model Sedex-55 evaporative light scattering detection (ELSD) system (S.E.D.E.R.E., France) and a Model OR-990 chiroptical detector (Jasco Europe, Italy). Chromatographic data were collected and processed using the Millenium 2010 Chromatography Manager software (Waters Chromatography).

2.2. Chemicals and reagents

LiChrosorb Si 100 silica-gel (5 μm particle size, 300 m^2/g) and HPLC-grade solvents were purchased from Merck (Darmstadt, Germany); (3-aminopropyl)-triethoxysilane, dry toluene, dry pyridine, 1,6-diisocyanatohexane and water for HPLC were from Fluka (Sigma–Aldrich, Buchs, Switzerland); ammonium acetate and acetic acid were purchased from J.T. Baker (Division of Mallinckrodt Baker, Phillipsburg, NJ, USA); teicoplanin was kindly provided by the Lepetit Research Center (Gerenzano, Italy); racemic and enantiomerically pure carnitine and derivatives were supplied from Sigma–Tau (Pomezia, Italy) and used as received, without further purification.

2.3. Preparation of the teicoplanin chiral stationary phase (TE-CSP)

All reactions were carried out in a laboratory-made modified Rotavapor-M rotary-evaporator apparatus (Büchi, Labortechnik, Flawil, Switzerland), in which the reaction flask is fitted with solvent condenser, solvent collector, argon inlet and allows syringe addition of reactant solutions and isolation of the CSP by filtration under an inert atmosphere. Stirring was obtained by spinning the flask around its axis.

Silica gel and teicoplanin were vacuum-dried before use (0.1 mbar, 1 h, at 150°C and 70°C, respectively). Chemical purity of teicoplanin samples

was checked by HPLC on a 250×4 mm I.D. ODS Hypersil column [mobile phase, A: 0.1 *M* ammonium acetate; B: 0.1 *M* ammonium acetate–acetonitrile (20:80); linear gradient from 10% B to 50% B in 20 min, to 75% B in 15 min, to 100% B in 5 min. Flow-rate 1.00 ml/min, T=25°C, UV detection at 254 nm]: the main teicoplanin peak area (A_2-2) was always greater than 85% of the total by relative area. All reactions were carried out under an argon atmosphere.

A slurry of 5.0 g of silica (LiChrosorb Si 100, 5 μm) in 120 ml of toluene was heated to reflux, and residual water was azeotropically removed. After cooling to room temperature, (3-aminopropyl)triethoxysilane was added (2.5 ml, 11 mmol) and the mixture was heated to reflux for 4 h. After cooling to room temperature, modified silica was isolated by filtration, washed with 50-ml portions of toluene, methanol and dichloromethane and dried at reduced pressure (90°C, 0.1 mbar, 1 h). Anal. found: C, 3.96; H, 0.84; N, 1.01, corresponding to 785 μmol of aminopropyl groups per gram of silica (2.65 μmol/m^2) (based on nitrogen).

1,6-Diisocyanatohexane (2.5 ml, 15 mmol) was added with a syringe to an ice-bath cooled slurry of (3-aminopropyl)silica gel in dry toluene (3.0 g in 50 ml). The ice-bath was removed and the mixture was heated at 70°C for 2 h and, after cooling to room temperature, the liquid phase was removed by suction filtration through an immersion sintered PTFE filter under an argon atmosphere. The intermediate monoureidic silica was freed from excess 1,6-diisocyanatohexane by addition of 10 ml of dry toluene and removal of the liquid phase by suction filtration (two times). A suspension of teicoplanin in dry pyridine (1.0 g, 0.53 mmol in 100 ml) was added to the activated silica and the mixture was heated to 70°C for 12 h, with continuous stirring. Disappearance of the main teicoplanin peak from the reaction liquid phase was checked by HPLC as described above. After cooling to room temperature, the TE-CSP (LiChrosorb Si 100-TE-CSP) was isolated by filtration and washed with 50-ml portions of pyridine, water, methanol, acetonitrile and dichloromethane, and dried under reduced pressure (70°C, 0.1 mbar, 2 h). Anal. found C, 16.43; H, 1.51; N, 3.80, corresponding to ~165 μmol of teicoplanin per gram of silica (0.55 μmol/m^2) (based on carbon).

2.4. Column packing and efficiency test

A stainless steel column (250×4.6 mm I.D.) was packed with LiChrosorb Si 100-TE-CSP using a slurry packing procedure slightly modified with respect to that already described [25]: grafted silica (3.30 g) was dispersed in 60 ml of $CHCl_3$–acetone (1:1, v/v) containing 15% of acetic acid and treated ultrasonically for 5 min. The slurry obtained was packed with a Haskel DSTV-122 pump, using methanol as pressurizing agent (700 bar, 20 min).

Column efficiency was evaluated using *n*-hexane–$CHCl_3$ (90:10, v/v; EtOH stabilized ~0.25%) as eluent, delivered at a flow-rate of 1.0 ml/min at 25°C. The number of theoretical plates for acetophenone (k'=11.34) was N/m>40 000.

2.5. Chromatographic procedures

Racemic and enantiomerically pure carnitine and its derivatives were eluted with hydro–organic mobile phases consisting of an organic solvent (MeOH, EtOH, CH_3CN) and aqueous solutions of ammonium acetate (0.025–0.050 *M*) delivered at a flow-rate of 1.0 ml/min at 25°C. The final pH of the mobile phase [the apparent pH (pH_a) of the mixed organic–aqueous eluents] was measured with a Metrohm Model 632 pH meter (Metrohm, Heriscan, Switzerland). Acetic acid or NH_4OH were used to adjust the pH to the desired value. Samples of inner salts or hydrochloride salts of carnitine and carnitine derivatives were dissolved in the eluent or in water–MeOH mixtures and the resulting solutions were filtered through a 0.45-μm filter (injection volume: 10 μl).

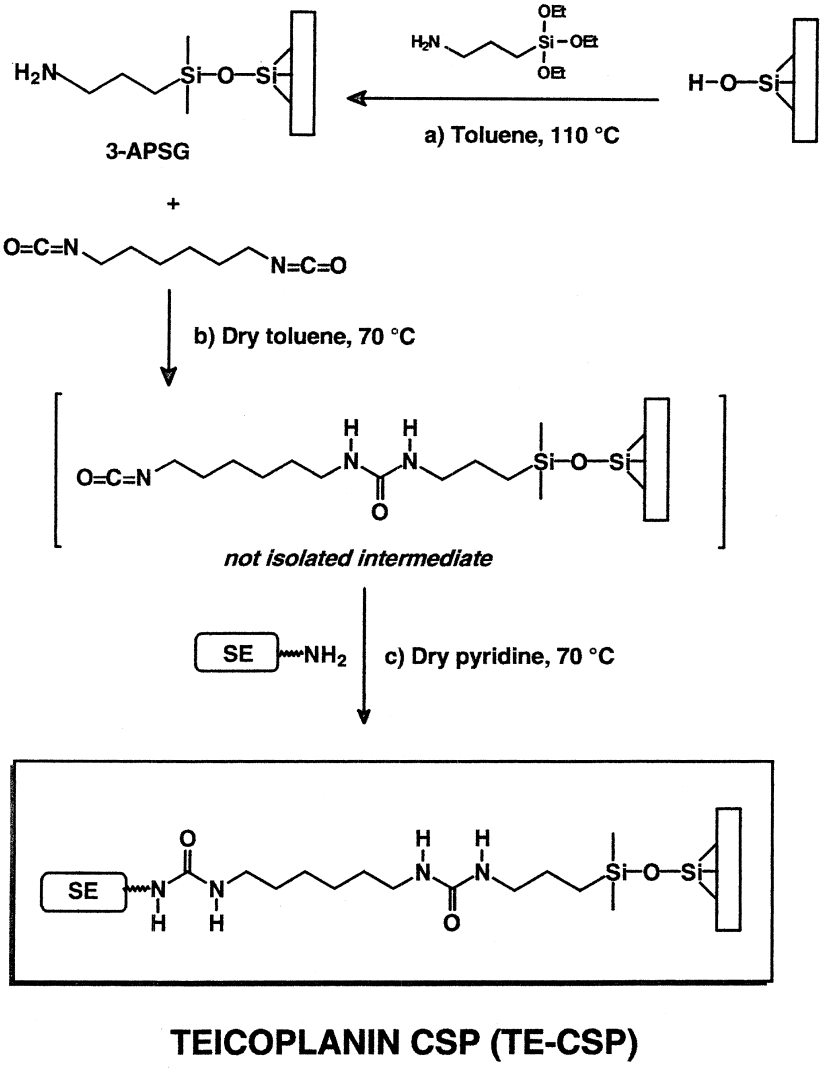
ELSD was performed at 57°C, 2.0 bar (air) and gain=7. With ammonium acetate buffers in the eluent, the ELSD evaporation tube temperature must be greater than 50°C to ensure a complete removal of the buffer itself. On the other hand, the pressure of the nebulization gas had little effect on the detector response in the range 0.5–2.5 bar.

3. Results and discussion

3.1. Synthesis of the chiral stationary phase

The structure of the teicoplanin-CSP (TE-CSP)

and the procedure for its preparation are reported in Fig. 2. The macrocyclic antibiotic was covalently bonded to the silica matrix in three steps: (a) introduction of 3-aminopropyl groups on the silica surface by standard silanization with (3-aminopropyl)triethoxysilane; (b) activation of the aminopropylated silica with 1,6-diisocyanatohexane to give the monoureidic–monoisocyanate intermediate. In this step, the large excess (7:1) of difunctional isocyanate prevents the formation of bridged aminopropyl chains from a single diisocyanate molecule reacting with two amino groups; (c) surface-linking of teicoplanin via addition of its free amino group (the better nucleophile in the molecule) to the



SE = SELECTOR = Teicoplanin complex (A_2-2 > 85 %)

Fig. 2. Synthetic pathway for the preparation of teicoplanin chiral stationary phase (TE-CSP).

pendant isocyanate groups. However, additional linkage, in which carbamate groups are formed between the glycopeptide alcoholic or phenolic hydroxyls and surface-linked isocyanate groups, may be present in the final material. Since the activated monoisocyanate silica is highly reactive (exposure to moisture causes a conversion of the isocyanate to amino groups), the last two steps (b and c) were carried out ''one-pot'' under an inert atmosphere (argon).

In the resulting CSP, the teicoplanin macrocyclic is tethered to the aminopropyl silica via stable ureidic bonds and a six-carbon aliphatic spacer. Elemental analysis of the final stationary phase gave a macrocycle loading of ~165 μmol per gram of silica.

3.2. Retention and enantioselectivity

After packing in a stainless steel column, the TE-CSP was evaluated in the enantiomeric resolution of carnitine and carnitine derivatives (Fig. 3).

Retention and enantioselectivity data, collected using hydro–organic mobile phases, are summarized in Table 1. For comparison purposes we used, whenever possible, the same mobile phase composition (i.e., retention and enantioselectivity are not optimized).

The order of elution of the enantiomers from the teicoplanin CSP was determined by chromatography of samples enriched in one enantiomer of known configuration. Elution order is invariant, with the (*S*) enantiomer preferentially more retained by the immobilised teicoplanin. The signs of optical rotation are correlated with elution order and, for compounds **1–4** and **6–8**, with configuration: the second eluted enantiomers always show peaks with a positive sign. Given the small structural variations presented by the carnitine derivatives **5** and **9**, we believe that their (*S*) enantiomers are also more strongly retained by the TE-CSP.

Within *O*-acyl derivatives of carnitine, retention of both enantiomers decreases with the number of carbon atoms in the acyl moiety (k_1' for compounds **2–6** drops from 3.05 to 0.89 and k_2' from 7.02 to 2.69, going from acetyl- to palmitoylcarnitine) whereas enantioselectivity approximately shows the opposite trend, i.e., it increases with the carbon number of the ester alkyl chain (α changes from 2.30 to about 3.0 going from acetyl to longer chain carnitine esters). However, carbon count is not the only factor governing enantioselectivity: branching in the chain, in fact, causes a small drop in the α value (**4**) due to a larger loss in retention for the second eluted enantiomer. The *O*-alkyl derivative of carnitine (**9**) shows an enantioselectivity much smaller than the corresponding *O*-acyl derivative with the closest chain length (**5**): in this case it is a larger increase in retention of the first eluted enantiomer that gives rise to lower enantioselectivity. Carnitine itself, its *O*-methanesulfonyl derivative (**8**) and the carnitine analogue with a primary amino group in place of the alcoholic group (**7**, emeriamine) all show less enantioselectivity than the *O*-acyl derivatives.

The effect of structural variation at the carboxyl terminus of carnitine was studied for esters, amide or nitrile C1-terminated carnitines (compounds **10–13**): all of them are retained (k' between 1.02 and 3.71) but not resolved by TE-CSP, suggesting that a free carboxylate group on the analytes plays a key role in the enantioselective recognition process.

The picture that emerges from these data can be summarized as follows: (1) a free carboxyl group is necessary for enantioselectivity; (2) enantioselectivity is higher with *O*-acyl than with *O*-mesyl, *O*-alkyl and free OH and NH_2 groups on the stereogenic center; (3) enantioselectivity and retention are modulated by H-bonding between the carboxylate of the analytes and H-bond donor sites of the teicoplanin binding pocket; the carbonyl oxygen of acylated carnitines also plays a role in the enantiorecognition process.

The mechanism of molecular recognition of the enantiomers of carnitine and its derivatives is consistent with the recently reported binding mode of the acetate anion [26–29] and of simple dipeptides to vancomycin and ristocetin A, two antibiotics structurally related to teicoplanin [30].

3.3. Retention and enantioselectivity as a function of pH, ionic strength and organic modifier

The effect of mobile phase pH on retention and enantioselectivity was studied for *O*-propionyl- and *O*-palmitoylcarnitines at constant ionic strength of

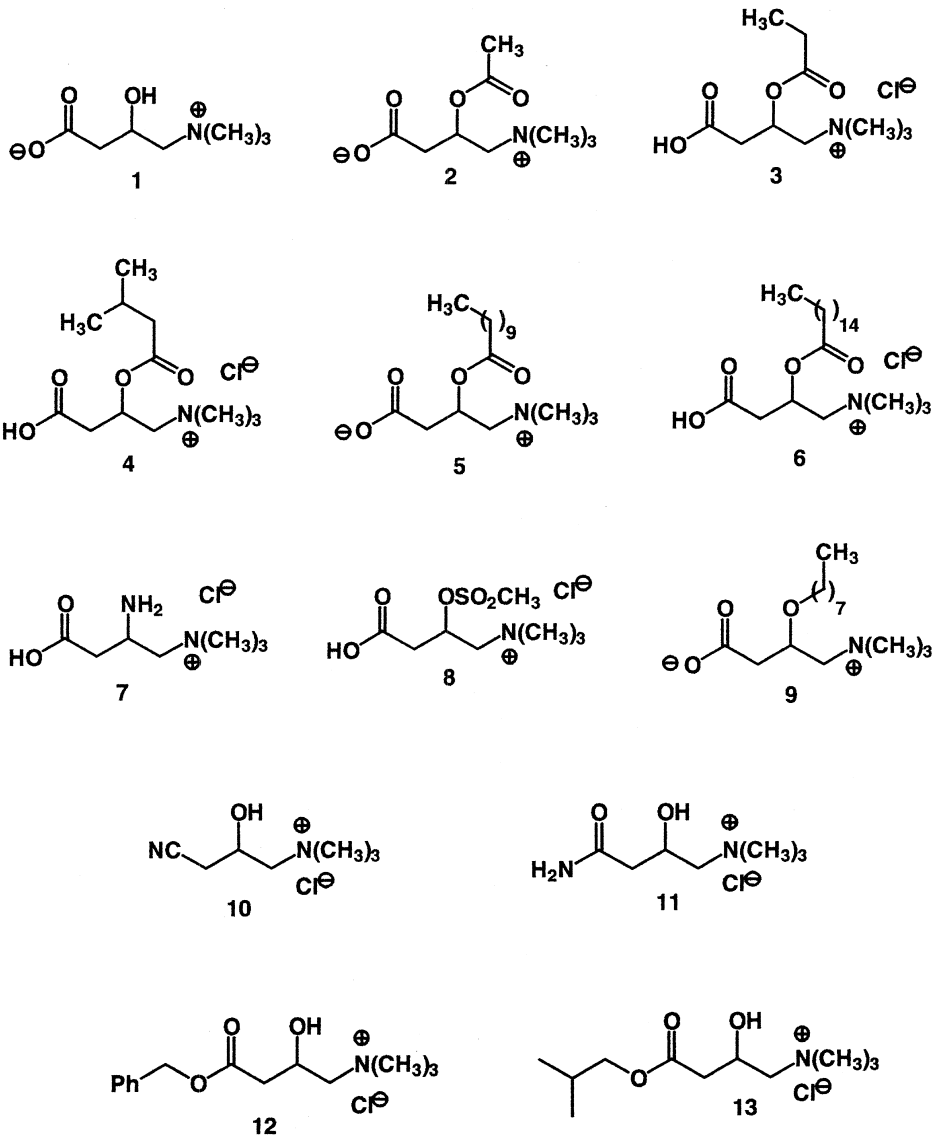


Fig. 3. Chemical structure of carnitine (**1**) and carnitine derivatives (**2–13**) analyzed on TE-CSP.

the mobile phase (25 m*M* acetate concentration). As shown in Fig. 4, the first and second eluted enantiomers of the analytes respond differently to pH changes. Retention of the first eluted enantiomers decreases with raising pH, with a drop in retention between pH 6.5 and 7.0; retention of the second eluted enantiomers, on the other hand, raises from pH 5.7 to 6.5 and then smoothly decreases at higher pH values. As a result, for both *O*-propionyl- (**3**) and *O*-palmitoyl- (**6**) carnitines enantioselectivity increases with pH, the α values changing from 1.71 and 1.70 at pH 5.7 to 2.13 and 2.27 at pH 8.1 for **3** and **6**, respectively.

The effect of mobile phase ionic strength on retention and enantioselectivity was studied for carnitine and its *O*-acetyl, *O*-propionyl and *O*-palmitoyl derivatives, at pH=7.0 in the concentration range 0.015–0.050 *M* of ammonium acetate added to

Table 1
Chromatographic data for the resolution of carnitine and carnitine derivatives on teicoplanin-CSP (TE-CSP)[a]

Compound	k_1'[b]	k_2'[b]	R_s	α[c]	Eluent	Config.[d]
1	2.45	3.22	1.03	1.31	A	(—)-(*R*)
2	3.05	7.02	3.03	2.30	A	(—)-(*R*)
3	2.09	5.96	3.63	2.85	A	(—)-(*R*)
4	1.42	3.57	3.83	2.51	A	(—)-(*R*)
5	1.07	3.17	3.60	2.96	A	(−)
6	0.89	2.69	3.63	3.02	A	(−)-(*R*)
7	0.59	0.90	2.31	1.53	B	(−)-(*R*)
8	0.38	0.67	3.22	1.75	B	(−)-(*R*)
9	2.53	3.75	3.02	1.49	A	(−)
10	2.81	2.81	–	1.00	A	–
11	3.71	3.71	–	1.00	A	–
12	1.70	1.70	–	1.00	A	–
13	1.02	1.02	–	1.00	A	–

[a] Column: TE-CSP on LiChrosorb Si 100, 5 μm (250×4.6 mm I.D.). Eluent A: EtOH–water (90:10, v/v)+0.050 *M* NH_4OAc (pH: not adjusted). Eluent B: EtOH–MeOH–water (35:35:30, v/v/v)+0.050 *M* NH_4OAc (pH_a=7.54). Flow-rate: 1.00 ml/min. Pressure: 2000 p.s.i. (1 p.s.i.=6894.76 Pa). Temperature: 25°C. Detection: ELSD; gain: 7; sens.: ×1; *T* evaporator=57°C; *P*=2.0 bar (injected volume: 10 μl).
[b] Retention factors.
[c] Enantioselectivity factor.
[d] Absolute configuration of the first eluted enantiomer.

a MeOH–water mobile phase (Fig. 5). Retention drops as the buffer concentration increased: for carnitine, k_1' and k_2' were reduced of about 11 and 16% by doubling the buffer concentration (from 0.025 to 0.050 *M*). Enantioselectivity was almost insensitive to the buffer concentration for carnitine and acetylcarnitine, whereas it slightly decreases for propionyl- and palmitoylcarnitine as the buffer concentration increases.

The effect of mobile phase organic modifier on retention and enantioselectivity was studied for *O*-propionyl- and *O*-palmitoylcarnitine. 2-Propanol (IPA), ethanol, methanol, acetone and acetonitrile were evaluated using mobile phases consisting of organic modifier–0.050 *M* ammonium acetate (~90:10). Within alcoholic modifiers, retention changes in the following order IPA>ethanol> methanol for both enantiomers of **3** and **6**, while acetone and acetonitrile afforded retentions similar to ethanol and IPA, respectively. Retention of the enantiomers of **3** is much more sensitive to the nature of the organic modifier. The highest enantioselectivities (α values in the range 2.58–3.02) were observed with 2-propanol and ethanol, the latter having a beneficial effect on α especially for palmitoylcarnitine. A sizeable decrease in enantioselectivity was observed with methanol and acetone; with acetonitrile the loss in enantioselectivity was even larger, the α values dropping from 2.72 and 3.02 with ethanol to 1.43 and 1.51 for **3** and **6**, respectively.

3.4. Detection strategies

The analysis of carnitines has always represented a problem in the choice of appropriate detection systems, both for chromatographic and electrophoretic methods: chromophore groups being lacking, carnitines show very weak UV absorption in the same low wavelength UV absorption region (210–220 nm) of commonly used mobile phase additives, with consequent loss of detection sensitivity. On the other hand the use of refracting index detection, aside from sensitivity issues, prevents the application of gradient elution for the analysis of complex mixtures containing species with large retention differences.

Pre- or post-column derivatization of carnitine with UV absorbing or fluorescent reagents has been used to solve detection problems [10–14]; however most of these methods require a free hydroxyl group on the analytes and cannot be used for *O*-derivatized carnitines. In this work, carnitine and its derivatives were easily detected without preliminary derivatiza-

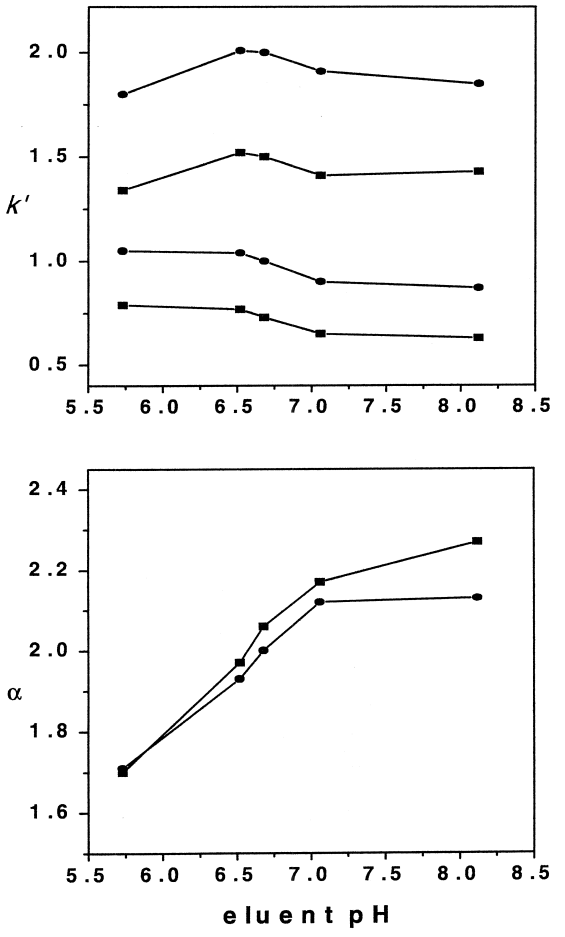


Fig. 4. Effect of mobile phase pH on retention [k' (k'_1 and k'_2), top graph] and enantioselectivity (α, bottom graph) for carnitine derivatives. Eluent: MeOH–water (90:10, v/v)+0.025 *M* CH_3COO^-; (■) palmitoylcarnitine (**6**); (●) propionylcarnitine (**3**).

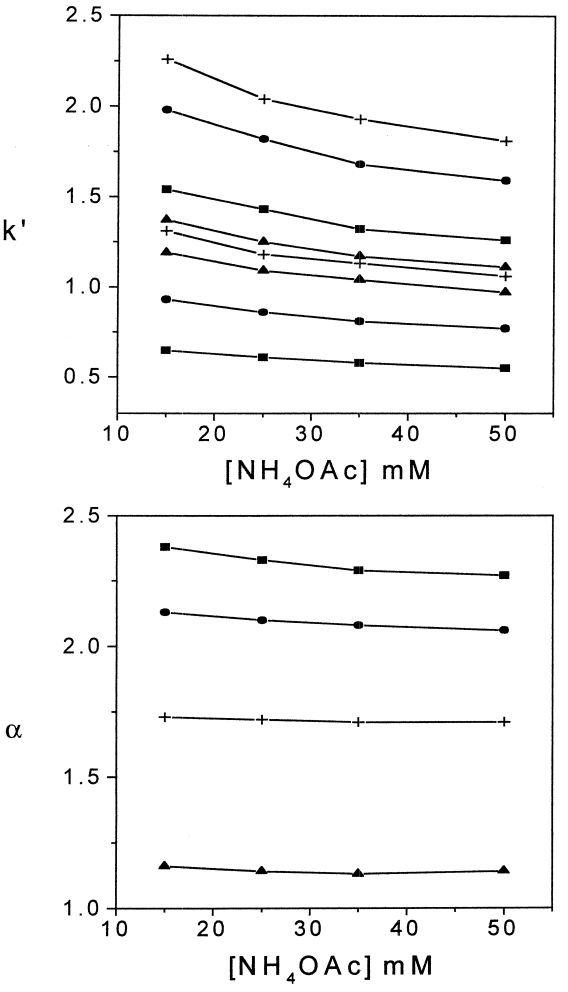


Fig. 5. Effect of mobile phase ionic strength on retention [k' (k'_1 and k'_2), top graph] and enantioselectivity (α, bottom graph) for carnitine derivatives. Eluent: MeOH–water (90:10, v/v); pH adjusted to 7.00 with acetic acid; (■) palmitoylcarnitine (**6**); (●) propionylcarnitine (**3**); (+) acetylcarnitine (**2**); (▲) carnitine (**1**).

tion using ELSD. All the examined compounds are non-volatile solids and gave optimal ELSD response under a variety of experimental conditions (eluents with and without buffers, flow-rates from 0.5 to 1.5 ml/min, different kind of organic modifiers and in variable proportions), with *S/N* ratios always much larger than UV detection. The limit of detection for a sample of racemic undecanoyl carnitine (10 μl, 0.02 mg/ml) was ~0.2 μg at a *S/N* ratio of 3. Since ELSD response is only marginally affected by changes in the eluent composition, we extensively used it during method development as well as in the analysis of multicomponent samples with gradient elution. Examples of enantioselective resolutions using ELSD are shown in Figs. 6 and 7.

In addition to ELSD, polarimetric detection can be used to circumvent the low UV detectability of carnitine samples. In spite of the low optical rotation values of carnitine and of its simple derivatives at the sodium D line ($[\alpha]_D = -31.5$ for *R*-(−)-carnitine·HCl; $c = 1.00$, H_2O) [11,12], multi-wavelength polarimetric detection (230–900 nm range) resulted in good *S/N* values (Fig. 8). While sensitivity of

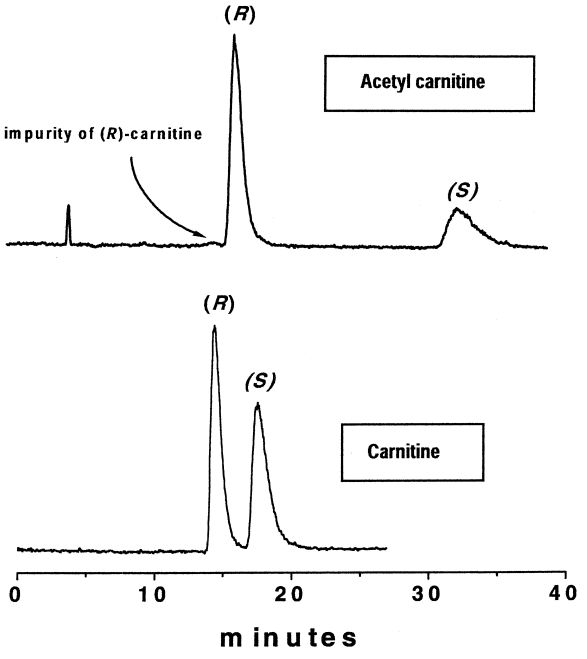


Fig. 6. Typical chromatograms obtained for the resolution of acetylcarnitine (top chromatogram) and carnitine (bottom chromatogram) enantiomers on TE-CSP. Experimental conditions as in Table 1.

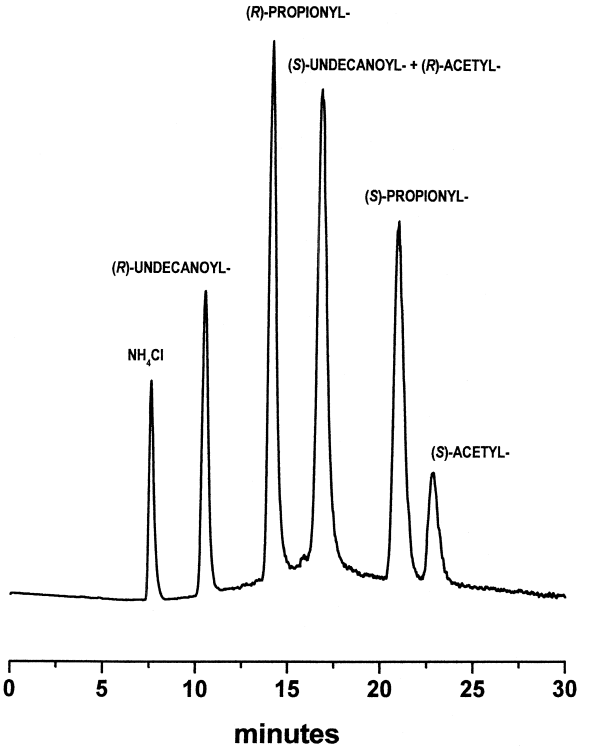


Fig. 7. Chemio- and enantioselective separations obtained on TE-CSP for a mixture of *O*-acylcarnitines (acetyl- **2**, propionyl- **3**, undecanoyl- **5**). Gradient elution: eluent A: EtOH+0.050 *M* NH_4OAc; eluent B: EtOH–MeOH–water (35:35:10, v/v/v)+ 0.050 *M* NH_4OAc; from 100% A to 15% A in 25 min (linear); 15% A for 5 min (isocratic). Other conditions as in Table 1.

polarimetric detector was not sufficient to perform enantiomeric trace analysis, the stereochemical information contained in the bisignate polarimetric response was useful in establishing elution order for those compounds not available as single enantiomers of known configuration.

4. Conclusions

The direct chromatographic resolution of carnitine and *O*-acylcarnitines was obtained for the first time on a TE-CSP, without any pre- or post-column derivatization. The glycopeptide antibiotic was covalently linked to aminopropyl silica gel via a bifunctional aliphatic isocyanate. This novel and efficient procedure affords a chiral stationary phase showing high chemical inertness and effective passivation of the underlying silica by the two ureidic functions.

Shielding of polar or ionizable sites on the silica matrix lowers unselective retention and results in high levels of enantioselectivity accompanied by symmetrical peak shapes for permanently charged carnitine and its derivatives. Retention and enantioselectivity of the analytes were not affected by small changes in mobile phase pH or ionic strength. Evaporative light scattering and optical rotation detections were used because of the low UV absorbing samples; finally, it is noteworthy that the utilization of such volatile mobile phases can also allow an easy connection with a mass spectrometer.

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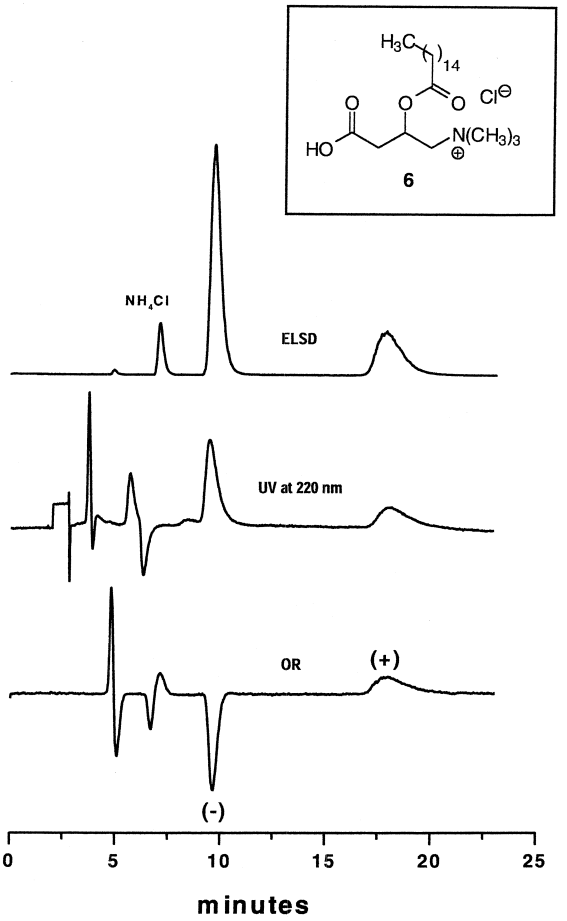

Fig. 8. Application of different detection systems in the enantioselective chromatography of racemic palmitoylcarnitine: evaporative light scattering detection (top trace); ultraviolet at 220 nm (middle trace) and optical rotation (response: STD; gain: ×10; range: ×32; bottom trace). Experimental conditions as in Table 1 for compound **6**.

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