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Enantio- and chemo-selective HPLC separations by chiral–achiral tandem-columns approach: the combination of CHIROBIOTIC TAG™ and SCX columns for the analysis of propionyl carnitine and related impurities

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Dedicated to Professor Luciano Caglioti and Professor Domenico Misiti on the occasion of their 70th birthday.

Abstract

We describe a new tandem-columns chiral–achiral HPLC arrangement by using a chiral column (CHIROBIOTIC TAG™) connected in series with an achiral column (Spherisorb S5 SCX), based on a strong cationic exchange mechanism; this approach is very useful for the analysis of chiral molecules, containing cationic groups in their structures. We used this special combination to develop an easy and convenient procedure for the enantio- and chemo-selective dosage of propionyl L-carnitine (**1**) and relative impurities (**2**–**6**), which allowed for the simultaneous separation and quantitation within 30 min. Under the best chromatographic conditions (acetonitrile–10 mM sodium dihydrogen phosphate 65:35, v/v (pH_a 6.80) as the mobile phase and UV detection at 205 nm], all the individual peaks were well separated. The applicability of the method, fully validated, was demonstrated by the analysis of a pharmaceutical batch of propionyl L-carnitine, where we found the following contents: 98.5% for **1** (drug substance); 0.1_5% for **3**; 0.1% for **5** and 0.2% for **6**. The enantiomeric excess (e.e.%) measured for the drug substance was 98.9%. Finally, a single mixed-bed column, packed with a binary mixture of the chiral and achiral phases, in a 1:1 ratio, gave similar chromatographic results as the tandem-columns approach, and thus, offered an easy alternative solution to the separation of the considered mixture.

Keywords: Tandem-columns chiral–achiral HPLC; CHIROBIOTIC TAG™ column; Propionyl carnitine; Mixed-bed columns

1. Introduction

In preliminary experiments performed [1], we observed that an alternative approach to solve difficult analytical problems arising from low levels of enantio- and/or diastereoselectivity was the use of two or more different columns connected in series. In particular, in case of analytes with more than one stereogenic element, the necessary overall selectivity could be accomplished by combining chiral (enantio-selective) and achiral (chemo-selective) HPLC stationary phases. The latter can be a conventional packing like unmodified silica [2,3], the "racemic version" of the same chiral phase [4], or a reversed-phase derivatized silica. Three tandem-columns arrangements have been already reported in the literature for the direct resolution of an antifungal agent [5], for the complete separation of phenylthio-carbamoylated amino acids [6] and for the plasma levels determination of verapamil and its metabolite [7].

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In the present paper, a new tandem-columns chiral–achiral HPLC arrangement was performed, by using a chiral column (CHIROBIOTIC TAG^{TM}) connected in series with an achiral column (Spherisorb S5 SCX), based on a strong cationic exchange mechanism, for the simultaneous separation and quantitation of propionyl L-carnitine and all the related impurities. Propionyl carnitine is one of the various carnitine short-chain acyl esters with even more important pharmaceutical properties and applications in therapy. Its biological activity can be attributed only to the L-enantiomer, which improves muscle metabolism and function, since it stimulates energy production by increasing citric acid and cycle flux (anaplerotic effect), and promotes oxidative utilization of glucose by stimulating the activity of pyruvate dehydrogenase. In patients with peripheral vascular diseases it improves walking distance and quality of life [8], while in patients with congestive heart failure it reduces ventricular size and increases exercise capacity [9–11].

Enantio-selective high-performance liquid chromatographic (E-HPLC) methods developed for the resolution of carnitine enantiomers are based either on chiral stationary phases (CSPs) and preliminary derivatization with chromophoric achiral reagents [12], or achiral stationary phases and pre-derivatization with enantiopure reagents [13–15]. The direct chromatographic resolution of carnitine and its *O*-acyl derivatives on a teicoplanin bonded CSP has been recently reported, without any pre- or post-column derivatization [16].

We finally packed a single mixed-bed column with a binary mixture of the chiral and achiral phases, in a 1:1 ratio; the column gave similar chromatographic results as the tandem-columns approach, and thus, offered an easy alternative solution to the separation of the considered mixture. Tandem- and/or mixed-bed columns have been shown to offer a convenient tool to manipulate stationary phase selectivity. In particular, proteins retention has been investigated on HPLC columns packed with mixtures of cation- and anion-exchangers [17,18]. To the best of our knowledge, the preparation and application of a mixed-bed chiral and achiral chromatographic column have never been reported in the literature.

2. Experimental

2.1. Apparatus and columns

The HPLC system consisted of a Waters 2690 chromatograph equipped with a Rheodyne Model 7725i 20-μl injector and a Model 2487 multi-wavelength ultra-violet (UV) detector. Chromatographic data were collected and processed using the Millennium 32 Chromatography Manager software (Waters Chromatography, Milford, MA, USA).

The columns used in the tandem-columns arrangement were the Spherisorb S5 SCX (250 mm × 4.6 mm i.d. and 150 mm × 4.6 mm i.d.), 5 μm particle size (Phase Separations Ltd., Deeside, Flintshire, UK), connected in series with the CHIROBIOTIC TAG^{TM} (250 mm × 4.6 mm i.d. and 150 mm × 4.6 mm i.d.), 5 μm particle size (Advanced Separation Technologies Inc., Whippany, NJ, USA).

2.2. Preparation of the mixed-bed column

The dry chiral stationary phase CHIROBIOTIC TAG^{TM}, 5 μm particle size (Advanced Separation Technologies Inc., Whippany, NJ, USA; 1.87 g) and the dry phase Spherisorb S5 SCX, 5 μm particle size (Phase Separations Ltd., Deeside, Flintshire, UK; 1.88 g) were mixed and suspended in a mixture of acetone–chloroform–acetic acid 42:42:16, v/v/v (30 ml). After 5 min of ultrasonication, the resulting slurry was packed in a 300 mm × 4.6 mm i.d. stainless steel column with an Haskel DSTV-122 pump, using methanol as pressurizing agent (700 bar for 20 min). The mixed-bed column efficiency was >45,000 plates per meter of column, checked with naphthalene ($k = 2.44$), using water–acetonitrile 65:35, v/v as eluent, at a flow-rate of 1.0 ml/min ($P = 1000$ psi at 25 °C) and UV detection at 254 nm.

2.3. Chemicals and reagents

HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany); distilled water was filtered using a MilliQ water purification system (Millipore, Watford, UK); analytical reagent grade sodium dihydrogen phosphate was purchased from Carlo Erba (Milan, Italy); HPLC-grade sodium hydroxide 50% aqueous solution and acetic acid were purchased from Baker (Division of Mallinckrodt Baker Inc., Phillipsburg, NJ, USA).

2.4. Reference substances and samples

Propionyl L-carnitine hydrochloride (**1**, assay: 99.4%), propionyl D-carnitine hydrochloride (**2**, assay: 99.6%), acetyl L-carnitine hydrochloride (**3**, assay: 98.7%), (*E*)-4-(trimethyl-ammonium)-2-butenoic acid hydrochloride or *trans*-crotonoylbetaine hydrochloride (**5**, assay: 98.9%), and L-carnitine hydrochloride (**6**, assay: 99.0%) samples were synthesized in the laboratories of Sigma-tau S.p.A., according to already published and patented procedures [19]. (*Z*)-4-(trimethylammonium)-2-butenoic acid hydrochloride or *cis*-crotonoylbetaine hydrochloride (**4**), an impurity of **5**, has been isolated in trace amount and identified by ^{1}H NMR. Bulk samples of **1** were a kind gift from Biosint S.p.A. (Latina, Italy). Percent assay values (checked by HPLC) are expressed as the hydrochloride salts.

2.5. Chromatographic procedures

HPLC separations were performed by using the CHIROBIOTIC TAG^{TM} column connected in series with the Spherisorb S5 SCX column, at 30 °C. A mixture of acetonitrile–10 mM sodium dihydrogen phosphate (65:35, v/v) was used as mobile phase, at a flow-rate of 1.0 ml/min.

The final apparent pH (pH_a) of the mobile phase was adjusted to 6.80 with concentrated sodium hydroxide. Prior to use, the mobile phase was filtered through a durapore membrane 0.45 μm filter and degassed with a helium sparge. UV detection was performed at 205 nm. Injection volumes of appropriate solutions (see below) were 20 μl.

The columns hold-up times (t_0) were determined from the elution of an unretained marker (toluene), using methanol as eluent, delivered at a flow-rate of 1.0 ml/min. For the CHIROBIOTIC TAG^{TM} column ($L = 250$ mm), t_0 was 2.78 min; for the Spherisorb S5 SCX column ($L = 250$ mm), t_0 was 2.84 min; for the tandem-columns arrangement of the above columns, t_0 was 5.62 min.

2.6. Sample preparation

2.6.1. Sample and reference solutions (assay and impurities determination)

Approximately 10 mg, accurately weighed, of a bulk sample of **1** were transferred into a 10 ml glass volumetric flask and then approximately 5 ml of mobile phase was added. The mixture was sonicated to dissolve and diluted to volume with mobile phase. The reference solution was prepared in the same way as the sample solution, using a reference sample of **1**. The sample solution for impurities determination was prepared, as above described, at a final concentration of about 5 mg/ml.

2.6.2. Reference impurities solution

Four stock solutions (S1, S2, S3, S4) were separately prepared for each impurity. The final concentration of stock solutions for impurities **2**, **3** and **6** was about 1 mg/ml (S1, S2, S4, respectively), while for **5**, it was 0.2 mg/ml (S3).

The stock solutions S1, S2, S3, and S4 (1.0 ml each) were transferred into a 20 ml glass volumetric flask and diluted to volume with mobile phase to obtain the final reference impurities solution (S5). The final concentration of **2**, **3** and **6** in S5 solution represented 1% by weight of the active drug substance, while, for **5**, 0.2%.

2.6.3. System suitability test (SST) solution

Approximately 20 mg, accurately weighed, of a reference sample of **1** were transferred into a 20 ml glass volumetric flask and then stock solutions S1, S2, S3, S4 (1.0 ml each) were added and diluted to volume with mobile phase.

2.7. System suitability test (SST)

The system suitability was evaluated by performing six replicated injections of the SST solution. The system was deemed suitable for use if the relative standard deviation (R.S.D.%), calculated on the capacity factors (k) and on the peak areas, was less than or equal to 2% for **1** and 5% for all the related impurities (**2**–**6**).

2.8. Stability of solutions

The stability of all the above solutions was checked over 24 h, at room temperature, by the same HPLC method here described.

3. Results and discussion

3.1. Development of the tandem-columns chromatographic method

A pharmaceutical batch of propionyl L-carnitine hydrochloride (**1**) can contain different impurities coming from the specific procedure adopted for its preparation [19]. In particular, we can suppose that the following impurities may be found in the drug substance (Fig. 1): propionyl D-carnitine hydrochloride (**2**); acetyl L-carnitine hydrochloride (**3**); *cis*-crotonoylbetaine hydrochloride (**4**); *trans*-crotonoylbetaine hydrochloride (**5**); L-carnitine hydrochloride (**6**). Compound **6** is the starting material used in the synthesis of **1** and a potential degradation product. Crotonoylbetaine hydrochloride is both a potential impurity and a degradation product of the starting material **6**; the presence of the double bond in the structure generates two isomers, the most abundant of which is (*E*) or *trans* (compound **5**), but it can also be present in trace amount the other one, (*Z*) or *cis* (compound **4**). Compound **3** is a related substance coming from the impurities included in the starting materials themselves, namely, acetyl chloride (impurity of propionyl chloride) and acetic acid (impurity of propionic acid). Compound **2**, the inactive stereoisomer of **1**, may also be present due to acylation of D-carnitine, potential chiral impurity of the starting material **6**. To sum up, in a pharmaceutical batch of **1** are present both chemically different substances, such as **3**–**6** and the enantiomer of the drug, that is, **2**. Thus, it was necessary to develop an analytical method, which had to be at the same time enantio- and chemo-selective. For the analysis of the substances closely related to **1** we chose a strong cationic exchanger column (Spherisorb S5 SCX), due to the fact that all carnitine derivatives bear a quaternary ammonium group in their structures; for the enantio-selective dosage of propionyl carnitine we successfully applied a teicoplanin aglycone (TAG) containing CSP, recently developed in our labs [20–22], which is particularly indicated for the enantiomeric resolution of very polar compounds, such as amino acids and *O*-acyl carnitine derivatives [21].

The chromatographic profile of a solution of **1** and related impurities (**2**–**6**) on the Spherisorb S5 SCX column is reported in Fig. 2 (top trace), and the chromatographic parameters obtained are collected in Table 1. It has been clearly showed that the column is highly chemo-selective (α ranged between 1.14 and 1.31), but is obviously unable to discriminate the two enantiomers of propionyl carnitine (absence of enantioselectivity, $\alpha_{1/2} = 1.00$). The chromatogram obtained for the analysis of the same solution of **1** and related impurities (**2**–**6**) on the CHIROBIOTIC TAG^{TM} column (Fig. 2,

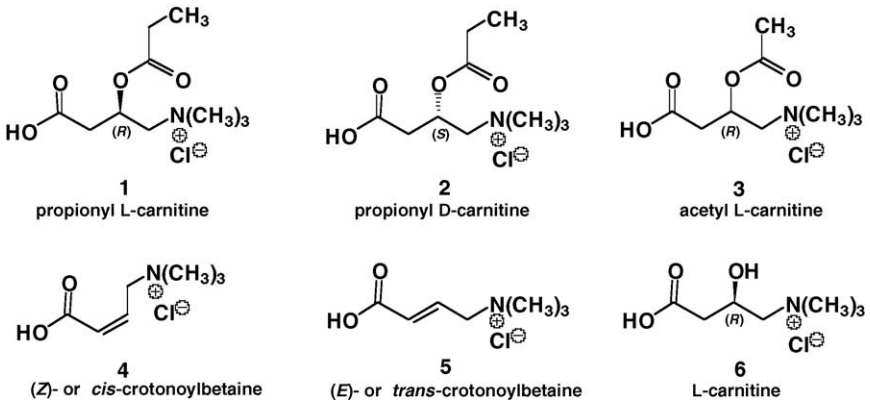

Fig. 1. Chemical structures of propionyl L-carnitine hydrochloride (**1**) and related impurities as their hydrochloride salts (**2**–**6**).

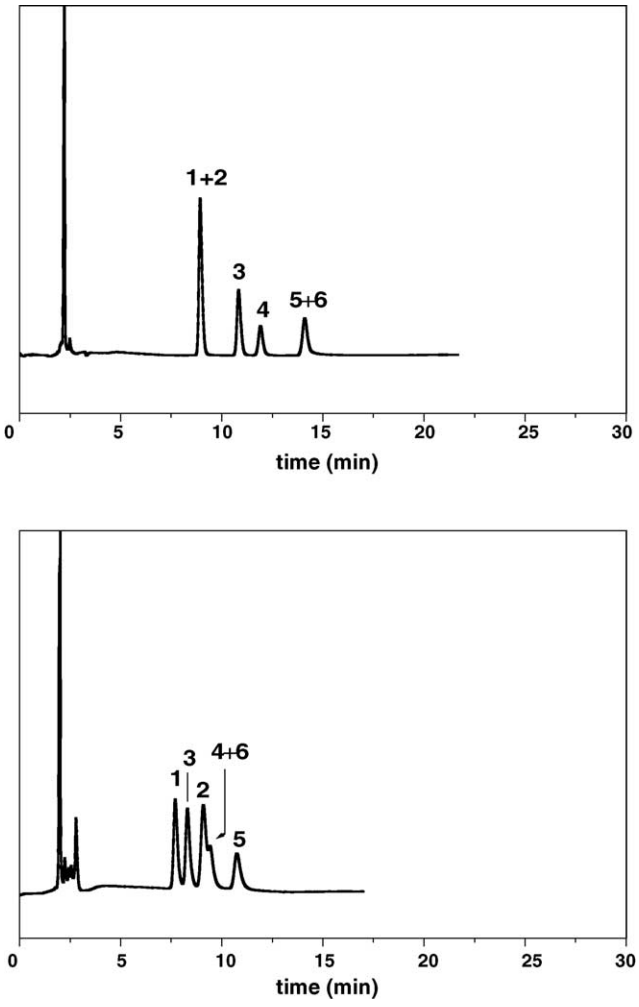

Fig. 2. Chromatographic profiles of a solution of **1** (1 mg/ml), **2** (1 mg/ml), **3** (1 mg/ml), **4** (0.005 mg/ml), **5** (0.005 mg/ml) and **6** (0.15 mg/ml) in mobile phase. Top trace, column Spherisorb S5 SCX (250 mm × 4.6 mm i.d.); bottom trace, column CHIROBIOTIC TAG™ (250 mm × 4.6 mm i.d.). For chromatographic conditions, see Section 2.

bottom trace) indicates that the couple of enantiomers of propionyl carnitine are well separated ($\alpha_{1/2} = 1.28$), but the level of chemo-selectivity exhibited by the column is unsatisfactory (α ranged between 1.14 and 1.20). The chromatographic parameters obtained are collected in Table 1. Considering the above results, the two columns were connected in series via a zero dead volume column coupler, and only in this case it was possible to baseline resolve all the six peaks contained in the mixture under investigation (Fig. 3, solid line). The corresponding chromatographic parameters are collected in Table 1. The serial order of the two columns did not affect chromatographic parameters (k, α, R_s).

3.2. Optimization of selectivity

The overall selectivity factor (α) of a general columns series can be easily calculated from the following mathematical formula,[1] at constant column temperatures [23]:

$$\alpha = \frac{k_{AB}(p)}{k_{AB}(n)}$$

where k_{AB} are the retention factors of compounds n and p on the tandem-columns arrangement of columns A and B, which can be derived by the following equations:

$$k_{AB}(p) = k_A(p) + X_B[k_B(p) - k_A(p)]$$

$$k_{AB}(n) = k_A(n) + X_B[k_B(n) - k_A(n)]$$

where X_B is a factor, calculated as follows:

$$X_B = \frac{V_{0(B)}}{V_{0(A)} + V_{0(B)}}$$

where $V_{0(A)}$ is the hold-up volume of column A and $V_{0(B)}$ is the hold-up volume of column B.

From the above equations, it follows that the selectivity of the column series AB can be tuned by the contribution of

[1] For calculations and simulations, we used a previously described lab-made computer-assisted procedure ("Simulation for Multi-Columns Chromatography", SMCC) [1], based on these simple mathematical formulas. The program can be available upon request.

Table 1
Chromatographic data obtained for the analysis of **1** and related impurities (**2**–**6**)

#	Retention factor (k)			Selectivity factor (α)			Resolution factor (R_s)		
	SCX	TAG	Tandem	SCX	TAG	Tandem	SCX	TAG	Tandem
1	2.14	1.77	1.77						
2	2.14	2.27	2.01	$1.00_{(1,2)}$	1.28	1.14	$-_{(1,2)}$	4.86	4.86
3	2.81	1.99	2.13	$1.31_{(2,3)}$	1.14	1.06	$6.27_{(2,3)}$	2.47	2.32
4	3.20	2.39	2.47	$1.14_{(3,4)}$	1.20	1.16	$3.22_{(3,4)}$	4.16	6.51
5	3.96	2.87	3.03	$1.24_{(4,5)}$	1.20	1.23	$6.31_{(4,5)}$	6.08	10.71
6	3.96	2.39	2.66	$1.00_{(5,6)}$	1.20	1.14	$-_{(5,6)}$	6.08	8.61

Hold-up times: column Spherisorb S5 SCX (250 mm × 4.6 mm i.d.), t_0 = 2.84 min; column CHIROBIOTIC TAG™ (250 mm × 4.6 mm i.d.), t_0 = 2.78 min; columns in the tandem arrangement: CHIROBIOTIC TAG™ (250 mm × 4.6 mm i.d.) + Spherisorb S5 SCX (250 mm × 4.6 mm i.d.), t_0 = 5.62 min.

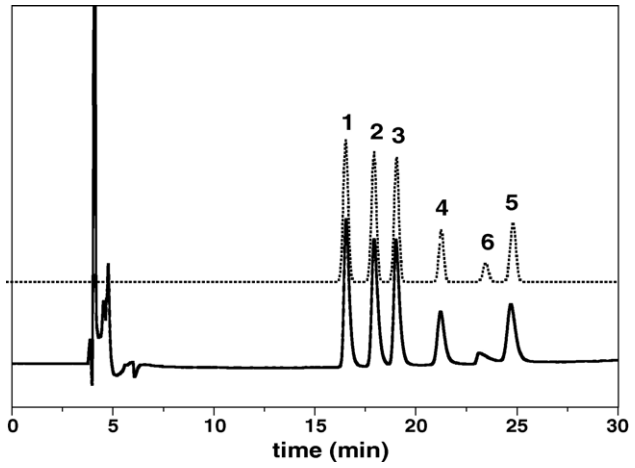

Fig. 3. Chromatographic profiles of a solution of **1** (1 mg/ml), **2** (1 mg/ml), **3** (1 mg/ml), **4** (0.005 mg/ml), **5** (0.005 mg/ml) and **6** (0.15 mg/ml) in mobile phase on the tandem-columns chiral–achiral arrangement of the CHIROBIOTIC TAG™ column (250 mm × 4.6 mm i.d.) with the Spherisorb S5 SCX column (250 mm × 4.6 mm i.d.). Solid line, experimental (for chromatographic conditions, see Section 2); dotted and vertically shifted line, simulated by the computer-assisted procedure SMCC [1].

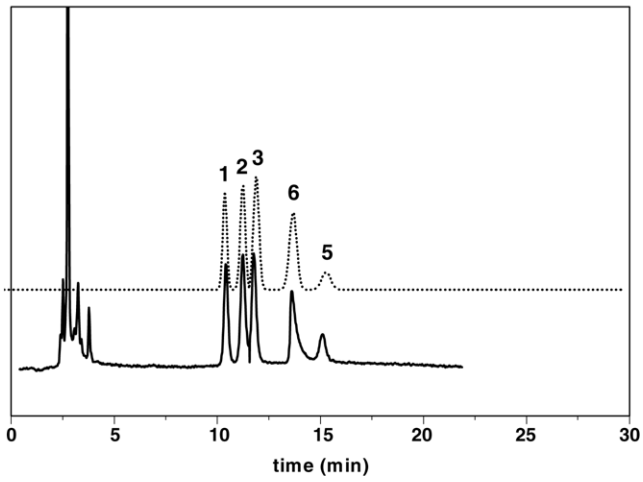

Fig. 4. Chromatographic profiles of a solution of **1** (1 mg/ml), **2** (1 mg/ml), **3** (1 mg/ml), **4** (0.005 mg/ml) and **6** (0.15 mg/ml) in mobile phase on the tandem-columns chiral–achiral arrangement of the CHIROBIOTIC TAG™ column (150 mm × 4.6 mm i.d.) with the Spherisorb S5 SCX column (150 mm × 4.6 mm i.d.). Solid line, experimental (for chromatographic conditions, see Section 2); dotted and vertically shifted line, simulated by the computer-assisted procedure SMCC [1].

the individual columns (A and/or B), expressed by the factor X_B, thus by changing the relative hold-up volumes, which are strictly correlated to columns length (L_A and L_B, respectively). As shown in Fig. 3 (dotted line), we obtained a good correlation between experimental and simulated profiles for the coupling of the two above 0.25 m columns.

Additionally, also with a coupling CHIROBIOTIC TAG™-Spherisorb S5 SCX with L = 0.15 m + 0.15 m, we could predict the overall separation of the mixture (Fig. 4, dotted line). In this case, we considered, for simplicity, only impurity **5**, which is the most abundant isomer. We experimentally realized the prediction by using the above columns with L = 0.15 m connected in series (Fig. 4, solid line), obtaining comparable results as in the case of the longer columns, with shorter times analysis (15 min).

3.3. Validation the tandem-columns chromatographic method

3.3.1. Selectivity

Selectivity was demonstrated either by the peak purity determination and by evaluating the chromatographic parameters (Table 1), with the aim to show that peak **1** was baseline resolved from other peaks. Peak purity determination, performed by using the "spectral-contrast technique" [24], confirmed the absence of interfering substances during the determination of the active drug substance. A typical chromatogram of **1** and related impurities (**2**–**6**) is shown in Fig. 3 (solid line).

3.3.2. Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated according to the signal-to-noise approach. A signal-to-noise ratio between 4:1 and 3:1 for LOD and 9:1 and 8:1 for LOQ were considered acceptable. Results are collected in Table 2.

3.3.3. Linearity

The linearity of peak area response versus concentration was checked in a range from 0.83 to 1.24 mg/ml for propionyl L-carnitine (**1**); from 5.12 to 62.00 μg/ml for propionyl D-carnitine (**2**); from 5.10 to 61.50 μg/ml for acetyl L-carnitine (**3**); and from 0.31 to 12.06 μg/ml for *trans*-crotonoylbetaine

Table 2
Limits of detection (LOD) and quantitation (LOQ)

Limits	Compounds			
	2	**3**	**5**	**6**
LOD				
Injected amount (ng)	12.8	13.8	0.2	30.4
Relative amount (%)	0.026	0.028	0.001	0.061
LOQ				
Injected amount (ng)	103.5	103.9	2.0	426.4
Relative amount (%)	0.207	0.208	0.004	0.852

(**5**). The squared correlation coefficient values for calibration curve ($n = 3$) were >0.998 for each peak. The corresponding regression functions were: for **1**, $y = 3.88 \times 10^5 x - 6129$ ($r^2 = 0.998$); for **2**, $y = 1.81 \times 10^4 x - 51$ ($r^2 = 0.998$); for **3**, $y = 3.76 \times 10^5 x - 514$ ($r^2 = 0.998$); for **5**, $y = 2.79 \times 10^4 x - 4285$ ($r^2 = 0.998$); for **6**, $y = 3.14 \times 10^5 x - 628$ ($r^2 = 0.999$).

3.3.4. Accuracy

Accuracy was checked in a range from about 0.8–1.2 mg/ml for **1**, from 40 to 60 μg/ml for **2**, **3**, **6** (0.8–1.2% of **1**) and from about 8 to 12 μg/ml for **5** (0.16–0.24% of **1**). The accuracy was calculated as percent recovery of the analyte with respect to the theoretical value. The mean values for each compound, summarized in Table 3, showed good level of accuracy (>95%).

3.3.5. Precision

Precision was evaluated by checking both injection and analysis repeatability. Injection repeatability was assessed on reference solution of **1** and on reference impurities solution S5 (Section 2), by performing six replicated injections of the same sample, under the same analytical conditions and by the same analyst. The relative standard deviations (R.S.D.%), calculated on capacity factors (k) and peak areas for each substance, were all less than 1%. R.S.D.% calculated on the same samples used for accuracy was considered as analysis repeatability (Table 3); all the values never exceed 2%.

Table 3
Accuracy and precision of the method: statistical data

#	Theoretical concentration (mg/ml)	Mean measured concentration (mg/ml)	Accuracy (%)	S.D.[a]	R.S.D. %
1	0.83	0.82	99.5	0.01	1.25
	1.03	1.03	100.3	0.00	0.17
	1.24	1.24	100.1	0.00	0.14
2	0.041	0.039	95.1	0.00	0.69
	0.052	0.050	96.3	0.00	0.72
	0.057	0.060	97.0	0.00	1.60
3	0.041	0.041	99.3	0.00	1.43
	0.051	0.052	101.1	0.00	1.13
	0.062	0.062	100.6	0.00	1.75
5[b]	8.00	7.69	96.1	0.11	0.43
	10.00	9.70	97.0	0.06	0.60
	12.00	12.06	100.1	0.11	0.92
6	0.042	0.042	99.3	0.00	0.87
	0.053	0.052	98.3	0.00	0.34
	0.063	0.062	98.5	0.00	0.16

[a] Standard deviation.
[b] Data for **5** are expressed as μg/ml.

3.4. Application of the tandem-columns chromatographic method

The tandem-columns chiral–achiral approach just described was successfully applied in the analysis of a bulk sample of propionyl L-carnitine hydrochloride (Fig. 5). The procedure enabled a precise and accurate quantitation by peak area integration. In particular, we found the following contents: 98.5% for **1** (drug substance); 0.1_5% for **3**; 0.1% for **5**; and 0.2% for **6**. The enantiomeric excess (e.e.%) measured for the drug substance was 98.9%.

3.5. Application of the mixed-bed column

A binary mixture of the same stationary phases used in the tandem-columns approach was prepared in a 1:1 ratio (Section 2) and packed into a single mixed-bed column, in order to investigate the potential of such system for the mixture under investigation. The constituent stationary phases were exactly the same present in the single columns coupled in series. They showed similar chemical stability and solvent compatibility (reversed-phase, polar-organic and ion-exchange modes). For optimization of the bed composition, we based on the simple mathematical formulas previously described, about the combination of the two columns: the better results were achieved when the contribution of the single columns (chiral and achiral) to the overall separation was 1:1. When the ratio

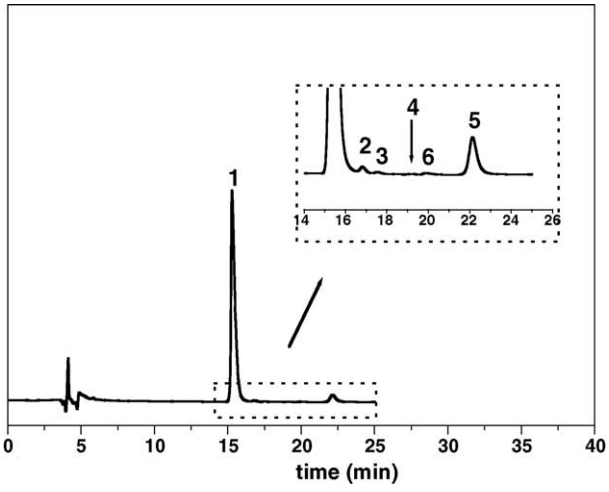

Fig. 5. Chromatographic profile of a bulk sample of **1** (sample solution) in mobile phase on the tandem-columns chiral–achiral arrangement of the CHIROBIOTIC TAG™ column (250 mm × 4.6 mm i.d.) with the Spherisorb S5 SCX column (250 mm × 4.6 mm i.d.). For chromatographic conditions, see Section 2.

was 2:1 for the chiral or for the achiral column, no complete resolution was accomplished. The single mixed-bed column (300 mm × 4.6 mm i.d.) gave similar chromatographic results as the tandem-columns approach (Fig. 4, solid line), and thus, offered an easy alternative solution to the separation of the considered mixture. Whereas tandem-columns have the advantage that they can be individually examined and replaced at will, for routine applications the convenience of a single column packed with ad hoc mixed sorbents might prove attractive, for its simplicity and practicability.

4. Conclusions

We described a special combination of CHIROBIOTIC TAG^{TM} and Spherisorb S5 SCX columns to develop an easy and convenient procedure for the enantio- and chemo-selective dosage of propionyl L-carnitine and relative impurities, which allowed for the simultaneous separation and quantitation within 30 minutes. Then we showed another type of column selectivity by the preparation of a mixed-bed (chiral and achiral) chromatographic column, thus demonstrating that the mixing of stationary phases having similar retentive properties but different selectivities can be an excellent expedient for tuning column selectivity without loss of separating capacity. Finally, we have realized, for the first time, the successful and useful coupling of a strong cationic exchange mechanism with the chiral recognition mechanism of CHIROBIOTIC TAG^{TM} column [21,25].

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