Temperature and eluent composition effects on enantiomer separation of carvedilol by high-performance liquid chromatography on immobilized amylose-based chiral stationary phases

Cristina Panella a, Rosella Ferretta a, Adriano Casulli b, c, Roberto Cirilli a, *

a Centro Nazionale per il Controllo e la Valutazione dei Farmaci, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
b European Union Reference Laboratory for the Parasites, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
c World Health Organization Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis (in Animals and Humans), Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Article history:
Received 12 February 2019
Received in revised form 26 March 2019
Accepted 1 April 2019
Available online 4 April 2019

Abstract
Carvedilol is a chiral drug with potent antihypertensive and antianginal activities. Although it is clinically used as a racemic mixture, its enantiomers show different pharmacokinetic and pharmacodynamic profiles. Here, the direct chiral separation of racemic drug by high performance liquid chromatography using two immobilized-type amylose-based chiral stationary phases is presented. Some chromatographic parameters, such as retention and selectivity, were determined under multimodal eluent conditions and different temperatures. A temperature-dependent inversion of the elution order of enantiomers was observed in the operative temperature range of chiral chromatographic support. Finally, an effective direct enantioselective method was successfully applied to the separation of the enantiomers of carvedilol on a semipreparative scale.

1. Introduction
It is widely recognized that temperature is a critical parameter in enantioselective HPLC of chiral compounds. Temperature controls both thermodynamic and kinetic aspects of the enantiomer complexation process occurring onto the chiral stationary phase. While the effects on column efficiency, viscosity of mobile phase and analyte diffusivity are quite foreseeable, the effects of temperature on retention and enantioseparation factors are unpredictable and need to be evaluated on a case-by-case basis.

For a given chiral separation, the experimental determination of the retention and enantioselectivity factors at different temperatures and the subsequent application of van't Hoff analysis allow to calculate the thermodynamic parameters governing the resolution process. In particular, the following equations:

\[
\ln k = -\Delta H^\circ / RT + \Delta S^\circ / R + \ln \phi
\]  
(1)

\[
\ln \alpha = -\Delta \Delta H^\circ / RT + \Delta \Delta S^\circ / R
\]  
(2)

provide the enthalpy and the entropy of adsorption \(\Delta H^\circ\) and \(\Delta S^\circ\), respectively, and the differences in enthalpy and entropy of adsorption of the more and less retained enantiomers onto stationary phase, \(\Delta \Delta H^\circ\) and \(\Delta \Delta S^\circ\). In the Eqs. (1) and (2), \(\phi\) is the phase ratio, \(R\) is the gas constant and \(T\) the absolute temperature. The van't Hoff plots of the chromatographic data (i.e. the logarithm of the retention or enantioseparation factors versus the inverse of the absolute temperature) can be either linear or non-linear, indicating a unique retentive and selective process or mixed mechanisms governing the enantioseparation over the temperature range studied [1–3].

Thus, thermodynamic study is a useful method to investigate
chiral recognition mechanism.

The knowledge of the $\Delta H^\circ$ and $\Delta S^\circ$ quantities also permits calculation of the isoantiselective temperature ($T_{ISO}$) through the following equation:

$$T_{ISO} = \frac{\Delta H^\circ}{\Delta S^\circ}$$  \hspace{1cm} (3)

It follows from equations (2) and (3) that, in all cases of enantioselective HPLC separations in which both the terms $\Delta H^\circ$ and $\Delta S^\circ$ are characterized by equal sign, there exists a temperature ($T_{ISO}$) at which enthalpy-entropy compensation occurs and the enantiomers coelute (i.e. $\alpha = 1$). For temperatures above $T_{ISO}$ the separation of enantiomers is entropy-controlled ($\Delta H^\circ < |\Delta S^\circ|$) whereas for temperatures below $T_{ISO}$ the enantioseparation is enthalpy-controlled ($\Delta H^\circ > |\Delta S^\circ|$).

By changing the temperature from one domain to another an inversion in the enantiomeric elution order is expected.

It is worth noting that, however, a given chiral stationary phase (CSP) is potentially able to separate the enantiomers, when the column temperature reaches the $T_{ISO}$ the enantioseparation fails. Since the commercially available chiral supports for enantioselective HPLC operate in a fairly narrow temperature range (i.e. usually from 0 to 50 °C), only a few examples of temperature-induced inversion of the enantiomer elution order have been observed [4–10]. Nevertheless, in order to obtain unambiguous data on the chiral resolving ability of a given CSP as well as for achieving the maximum degree of chiral separation, it is advisable to determine the experimental value of the isoevaluation temperature.

In this context, since both the $\Delta H^\circ$ and $\Delta S^\circ$ terms, whose values determine the magnitude of $T_{ISO}$, are critically dependent on any changes in mobile phase composition, it is conceivable that the elution order of a given pair of enantiomers, at a certain column temperature, depends on mobile phase composition.

The present paper describes multimodal eluent conditions for the HPLC enantioseparation of carvedilol (Fig. 1) on the amyle-based Chiralpak IA-3 and Chiralpak IG-3 CSPs.

Carvedilol is a chiral antihypertensive and antianginal drug with a single alcohol carbon stereocenter [11]. Although it is administered clinically as a racemic mixture, its enantiomers exhibit different pharmacological effects. The $\beta$-blocking potency of (S)-carvedilol is approximately 50–500 times greater than that of its antipode (R) [12]. Moreover, the (R)-enantiomer shows higher plasma concentrations, bioavailability and protein binding with respect to the (S)-counterpart [13,14].

Therefore, in order to evaluate the pharmacokinetic and pharmacodynamic profiles of each carvedilol enantiomer and to produce enantiopure forms for clinical evaluation, it is mandatory to develop analytical and preparative enantioselective methods.

Here, the effects of the mobile phase composition and column temperature on enantioselectivity and enantiomeric elution order of carvedilol using the immobilized-type Chiralpak IA-3 and Chiralpak IG-3 CSPs are discussed. The outcomes of the thermodynamic study, and in particular the knowledge of $T_{ISO}$, were carefully considered in the perspective to optimize the productivity of chiral separation on a semipreparative scale.

2. Experimental

2.1. Chemical and reagents

Carvedilol, HPLC-grade solvents (n-hexane, 2-propanol (IPA), ethanol, ethyl acetate (EA), dichloromethane (DCM), acetonitrile (ACN) and amines (diethylamine (DEA), triethylamine, butylamine, exylyamine, ethanolamine, ethylenediamine and ethylamine) were purchased from Sigma-Aldrich (Milan, Italy). HPLC enantioseparations were performed by using stainless-steel Chiralpak® IG-3 (250 mm × 6.6 mm, 3 µm), Chiralpak® IA (250 mm × 10 mm, 5 µm), Chiralpak® IA-3 (250 mm × 6.6 mm, 3 µm) and Chiralpak® IA (250 mm × 10 mm, 5 µm) columns (Chiral Technologies Europe, Illkirch, France).

2.2. Instruments and chromatographic conditions

The HPLC apparatus used for analytical enantioseparations consisted of a PerkinElmer (Norwalk, CT, USA) 200 LC pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 50 µL sample loop, an HPLC PerkinElmer oven and a PerkinElmer detector. The signal was acquired and processed by Clarity software (DataApex, Prague, Czech Republic).

In analytical separations, fresh standard solutions of carvedilol were prepared shortly before using by dissolving 1–3 mg of analyte in 20 mL of ethanol. The injection volume was 20 µL.

For semipreparative separation, a PerkinElmer 200 LC pump equipped with a Rheodyne injector, a 5000 µL sample loop, a PerkinElmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) was used. The feed solution for milligram enantioseparations was prepared by dissolving 49.35 mg of racemic carvedilol in 4 mL of ethyl acetate and diluting the solution with 4 mL of the mixture n-hexane-IPA-EA-DEA 50:30:20:0.1 (v/v/v/v).

The polarimetric analysis of the enantiomers of carvedilol collected on a semipreparative scale was carried out measuring the specific rotations at five wavelengths by a PerkinElmer polarimeter model 241 equipped with Na/Hg lamps. The volume of the cell was 1 mL and the optical path was 10 cm. The system was set at a temperature of 20 °C.

2.3. Enantiomer elution order

In order to establish the enantiomeric elution order of carvedilol on the Chiralpak IG-3 and Chiralpak IA-3 CSPs, enantiomers of known stereochemistry, which were separated by HPLC on the Phenomenex Lux-Cellulose 4 (250 mm × 0.46 mm, 5 µm) column using the mixture n-heptane-2-propanol 40:60 (v/v) as a mobile phase [15], were analyzed in the experimental conditions described in Table 1. As previously reported, under normal-phase mode, the first eluting enantiomer on Phenomenex Lux-Cellulose 4 CSP has (R)-configuration and the second eluting enantiomer (S)-configuration [15].

3. Results and discussion

3.1. Analytical HPLC enantioseparation in normal-phase, polar organic and reversed-phase conditions

Previous reports indicate that the CSPs based on cellulose and amylose derivatives exhibit a good enantiomer resolving ability towards carvedilol [15–20]. In particular, good results were obtained with the chiral packing materials formed by amylose tris-(3,5 dimethylphenyl-carbamate) (ADMPC) either coated [18,19] or
immobilized [20] on μm silica particles. Bodoki et al. [20] have reported on the resolution of carvedilol using the immobilized-type ADMPC-based Chiralpak IA CSP under normal-phase mode. The enantioselective HPLC analysis was optimized with a mobile phase consisting of n-hexane-IPA-ethylenediamine with an alcohol gradient ranging from 20% to 50%. In these conditions, resolution factor value was 10.9 and the second eluting enantiomer was eluted within 54 min.

These results and the complete solvent compatibility of IA CSP encouraged us to extend the study of chiral recognition to non-standard mobile phases containing DCM and EA. The Chiralpak IA-3 CSP produced a baseline enantioseparation compared to DEA.

The chromatographic results obtained are listed in Table 1. The Chiralpak IA-3 CSP under normal-phase mode. The chromatographic results obtained at 25 °C. The presence of 0.1% DEA in the mobile phases was helpful to improve peak shape and efficiency. A preliminary study carried out adding a 0.1% content of different basic additives such as triethylamine, buthylamine, exylamine, ethanolamine and ethylenediamine to n-hexane-IPA 50:50 (v/v) didn’t provide better enantioresolution compared to DEA.

The chromatographic results obtained at 25 °C using a 250 mm × 4.6 mm i.d. Chiralpak IG-3 column in combination with the standard mixtures n-hexane-IPA-DEA 50:50:0.1 (v/v/v) and n-hexane-ethanol-DEA 50:50:0.1 (v/v/v) and non-standard mixtures are shown in Table 1.

The Chiralpak IA-3 CSP produced a baseline enantioresolution under all elution modes exploited.

The best chiral resolving ability was observed with n-hexane-IPA-DEA 50:50:0.1 (v/v/v) mixture (entry 2, α = 2.65, Rs = 6.80) while the lowest enantioselectivity was recorded using the n-hexane-ethanol-DEA 50:50:0.1 (v/v/v) mixture (entry 1, α = 1.17). The partial replacing of IPA with EA or DCM still led to rather high enantioselectivity values (entries 3 and 4, α about 1.8).

The next step of our work was to examine the performance of Chiralpak IA-3 CSP in polar organic mode. For this purpose, three mobile phase compositions were selected: methanol-DEA 100:0.1 (v/v), ethanol-DEA 100:0.1 (v/v) and acetonitrile-DEA 100:0.1 (v/v). The chromatographic results obtained are listed in Table 1. The former mobile phase could produce the highest enantioselectivity and resolution values (entry 6, α = 2.32 and Rs = 6.28). In contrast, the use of acetonitrile produced poor efficiency and not well resolved enantiomeric peaks (entry 7, Rs = 0.57).

In order to explore whether the chiral resolving ability of the polysaccharide-based CSP in aqueous conditions was preserved, the enantioselectivity was evaluated after addition of small volumes of water to 100 vol of methanol-DEA 100:0.1 (v/v) and ethanol-DEA 100:0.1 (v/v).

The increased content of water in the mobile phase yielded a progressive increase in retention (data not shown) and a small reduction in resolution with respect to polar organic condition. By using methanol-water-DEA 100:10:0.1 (v/v/v) as a reversed-phase mobile phase the enantioseparation and resolution factors were 1.83 and 5.42, respectively.

It is well documented that the replacing of a methyl group of the phenyl moiety of ADMPC by a chlorine atom gives rise to an effective and complementary chiral selector (i.e. amyllose tris(3-chloro-5-methylphenylcarbamate)) (ACMPC) [21–27]. The differences in chiral resolving ability between the amyllose-based IA-3 and IG-3 CSPs depend basically on their different 3D structures and electronic density to aromatic and carbamate moieties which are two portions critically involved in the formation of intramolecular forces with selectand.

As the parent amyllose-based IA-3 CSP, the immobilized-type Chiralpak IG-3 CSP can operate enantiorecognition under multimodal standard and non-standard conditions [22].

Afterwards, we selected the same mobile phases used in combination with the IA-3 CSP and evaluated the enantioselectivity of the chiral alcohol carvedilol on the chlorine-containing amyllose-based IG-3 CSP.

Table 1 summarizes the retention, enantioseparation and resolution factors obtained at 25 °C with the 250 mm × 4.6 mm i.d. Chiralpak IG-3 column.

As a general trend, the recognition ability of the chlorinated IG-3 CSP towards carvedilol appeared to be less effective than the parent IA-3 CSP. However, even if the enantioselectivity was apparently lost using the n-hexane-ethanol-DEA 50:50:0.1 (v/v/v), a good enantioresolution was recorded in normal phase mode with the mixture n-hexane-IPA-DEA 50:50:0.1 (v/v/v) and its non-standard modification n-hexane-IPA-EA-DEA 50:30:20:0.1 (entries 11 and 12, Rs = 3.50 and Rs = 4.35, respectively). A baseline enantioresolution was also observed under reversed phase mode (entry 17, Rs = 3.28).
It is worth emphasizing that for drug analysis by direct injection of biological matrices like serum, plasma and urine, the aqueous elution condition is particularly advantageous with respect to normal-phase and polar organic modes.

3.2. Effect of temperature on retention and enantioselectivity

In order to evaluate the impact of temperature on retention and enantioseparation factors of carvedilol on the IA-3 and IG-3 CSPs the column temperature was changed from 5 to 45 °C in 10 °C
increments. The following mixtures were selected as mobile phases: n-hexane-IPA-DEA 50:50:0.1 (v/v/v), n-hexane-ethanol-DEA 50:50:0.1 (v/v/v), n-hexane-IPA-EA-DEA 50:30:20:0.1 (v/v/v/v), ethanol-DEA 100:0.1 (v/v) and methanol-DEA 100:0.1 (v/v).

The van’t Hoff plots for retention and enantioseparation factors are depicted in Figs. 2 and 3. As can be noted, under the system eluent n-hexane-IPA-DEA 50:50:0.1 (v/v/v) the van’t Hoff plots followed a classical linear trend and either the retention or enantioselectivity decreased as the column temperature increased. Since the column temperature was below the computed $T_{iso}$ values,

---

**Fig. 3.** Plots of $\ln k$ and $\ln a$ vs $1/T \times 10^3$ for carvedilol on the Chiralpak IA-3 CSP. Chromatographic conditions: Column, Chiralpak IA-3 (250 mm × 4.6 mm i.d.); mobile phase, as indicated in figure; column temperature, 5, 15, 25, 35 and 45 °C; flow rate, 1.0 mL/min; detection, UV at 280 nm. MeOH: methanol; EtOH: ethanol; IPA: 2-propanol; EA: ethyl acetate; DEA: diethylamine.
both recognition processes were enthalpy-driven. The substitution of IPA with ethanol as the organic modifier in the mobile phase led to a substantial decrease of T$_{ISO}$ from 97 to 29 °C in the case of the IG-3 CSP whereas the diminution was less pronounced for the IA-3 CSP (i.e. from 79 to 52 °C). As a consequence, only for the IG-3 CSP the computed temperature of isoelution value falls within the temperature range studied. This means that the lacking of recognition ability of the IG-3 CSP recorded at 25 °C (Table 1, entry 10) is only due to the proximity of the temperature at which the competitive enthalpic and entropic terms are balanced. Actually, due to enantiomeric peak width, it would be more realistic to refer to a cryptoenantioselectivity range rather to a single value of temperature. Such a coalescence temperature range centered on T$_{ISO}$ is unpredictable a priori and changes with the characteristics of CSP/eluent system. Going back to carvedilol resolution on the IG-3 CSP with n-hexane-ethanol-DEA 50:50:0.1 (v/v/v), within enthalpic domain, with increasing temperature the chiral separation deteriorated until, at the critical temperature of 25 °C, enantiomers coeluted. Above isoelution temperature, the enantioseparation was again clear and it became gradually larger as the temperature increased. Beyond the cryptoenantioselectivity range the enantioseparation was entropy-driven and the elution order of enantiomers switched (i.e. the (R)-enantiomer was eluted before than (S)-enantiomer, (R)<(S)). Unlike the ln k vs. 1/T × 10$^3$ plots, the van’t Hoff graph displaying the effects of the temperature on the enantioseparation showed a characteristic V shape. Consequently, the $\Delta H^\circ$ and $\Delta S^\circ$ quantities were obtained by the slopes and intercepts calculated by linear regression of the ln k vs. 1/T × 10$^3$ plots [28] according to following equations:

$$\ln k_{R \ or \ S} = -\Delta H_{R \ or \ S}^{\circ} / RT + \Delta S_{R \ or \ S}^{\circ} / R + \ln \phi$$ (4)

$$\Delta \Delta H_{R,S} = -R(\text{slope}_R - \text{slope}_S)$$ (5)

$$\Delta \Delta S_{R,S} = R(\text{intercept}_R - \text{intercept}_S)$$ (6)

Under the same elution mode, the van’t Hoff plots recorded for the IA-3 CSP were monomodal and the elution order of the enantiomers was always (S)<(R).

Replacing part of IPA content in mobile phase with ethyl acetate,
which is a solvent with a different ability to establish hydrogen bonding with the carbamate sites of CSPs and to induce conformational changes in the helical structure of the polysaccharide, the values of $T_{50}$ returned above 80 °C and the van’t Hoff plots revealed again the characteristic linear trend commonly established for monomodal enthalpy-driven enantioseparations. It is worthwhile noting in view of the transferring of the analytical enantioselective conditions to semipreparative level that the values of resolution achieved with the non-standard eluent at 5 °C ($R_s = 6.41$ on IA-3 CSP and $R_s = 4.90$ on IG-3 CSP) were higher than those observed at room temperature with the same elution mode (Table 1, entries 3 and 12).

Figs. 2 and 3 show the van’t Hoff plots obtained in alcoholic polar organic conditions. With the mobile phase ethanol-DEA 100:0.1 (v/v) the chiral resolution was entropy-controlled for both CSPs. A closer look on the computed $T_{50}$ values reveals that the coalescence temperatures are largely below from the practical temperature range of the amylose-based CSPs. Therefore, in the explored temperature range no change in elution order was observed ($R_s<S$).

The van’t Hoff plots of carvedilol markedly differed in presence of methanol-DEA 100:0.1 (v/v) mobile phase. At 5 °C the enantiomers of carvedilol were not resolved on the IG-3 CSP. This is because the column temperature was again within the cryptoe-nantioselectivity range ($T_{50} = -1$ °C). Upon heating the chromatographic system at 15 °C, a peak splitting was clearly visible. As typically observed for entropy-driven enantioseparations, further temperature increases led to a better chiral discrimination. Differently, in the case of the IA-3 CSP, the enantioselectivity was substantially lowered after heating the column. It is also worth mentioning that with methanol-DEA 100:0.1, the enantiomer elution order on the IA-3 CSP was again ($R_s<S$), although the recognition process was enthalpically driven (Fig. 4). Typical chromatograms obtained using the IG-3 CSP under different thermodynamic domains are depicted in Fig. 5. Fig. 5 also shows the effects of mobile phase composition on isoenantioselective temperature.

### 3.3. Semipreparative HPLC enantioseparation

The results of the enantioselective HPLC obtained on an analytical scale were fully considered in the perspective of scaling-up the enantioseparation to a semipreparative level and collecting the enantiomers of carvedilol in multi-milligram quantities.

Solubility of carvedilol in mobile phase was the first factor to be considered. Since carvedilol is practically insoluble in n-hexane and sparingly soluble in ethanol and 2-propanol, conventional normal-phase n-hexane–alcohol mixtures, which have demonstrated to produce good level of enantioseparation on polysaccharide-based CSPs (see Table 1 and references [18,20]), were rejected. Our choice for semipreparative resolutions was to adopt the non-standard mobile phase containing EA which is capable of dissolving and dilute the racemic sample at the appropriate volume. Thus, the n-hexane-IPA-EA-DEA 50:30:20:0.1 (v/v/v/v) mixture was selected as the mobile phase to achieve the best performance in terms of sample loading, resolution and analysis times. The feed solution was prepared by dissolving 49.35 mg of racemic carvedilol in 8 mL of the ethyl acetate-mobile phase 1:1 (v/v) diluent.

The column temperature was the second parameter evaluated for mg-scale applications. As described above, by using the EA-based non-standard mobile phase the $T_{50}$ for the IG-3 and IA-3 CSPs is 82 °C and 85 °C respectively, and the enantioseparation is enthalpy-driven. Further examination of the temperature-chiral recognition relationship indicates that although a negative impact of low temperatures on column efficiency occurred, at 5 °C the values of resolution of carvedilol were higher than those observed at room temperature. Thus, with the goal of developing an effective protocol to resolve the carvedilol on a milligram scale, the column temperature was set at the unusual value of 5 °C.

The loading effect on both the 1-cm i.d. IG and IA columns was investigated by gradually increasing the injection volume of the feed solution (concentration = 6.17 mg/mL). As shown in Fig. 6, the maximum limit of sample injection onto the 1-cm i.d. Chiralpak IG column (i.e. 8.02 mg of carvedilol dissolved in 1.3 mL of diluent) was substantially higher with respect to that obtained with the Chiralpak IA column (i.e. 6.17 mg of carvedilol dissolved in 1.0 mL of diluent), whereas the time required for the resolution of racemic sample was the same (i.e. 12 min). Both enantiomeric separations gave yields of about 98% and enantiomeric excess of both enantiomers >99%.

Therefore, considering that the chromatographic run was completed within 12 min, by using the Chiralpak IG column on a lab-scale, a total of about 18 mg for each enantiomer per hour could be produced.

Polarimetric analysis indicated the first eluting (S)-enantomer on the Chiralpak IG column was levorotatory in methanol solution and the pertinent approximate optical rotation dispersion (ORD) curve calculated at five wavelengths was monotonic (Fig. 7). As
expected, the ORD curve of the second eluting (R)-(+)-enantiomer was perfectly specular to that of the (S)-(−) antipode.

4. Conclusions

The immobilized ACMPC-based and ADMPC-based CSPs have been tested in HPLC enantioseparation of carvedilol.

The outcomes of the enantioselective HPLC analysis can be resumed as follows: i) both polysaccharide-derived selectors could baseline separate the enantiomers of carvedilol under normal-phase, polar organic and reversed phase conditions; ii) a non-standard EA-based mobile phase was employed in semi-preparative applications; iii) temperature exerted a significant influence on the chromatographic performance of the IA-3 and IG-3 CSPs; in particular, the temperature of isolution was a critical parameter to be considered for optimizing enantioseparation either analytical or semi-preparative scale; iv) the enantiomer elution order was strongly affected by the type of solvents used as mobile phase and the column temperature.

In our opinion, the findings of this study allow having an efficient approach to the HPLC isolation of enantiopure forms of carvedilol and support preclinical evaluations on the pharmacokinetic and pharmacodynamic profiles of both enantiomers. Furthermore, the described susceptibility of enantiomer separation of carvedilol to column temperature highlights the importance to control this parameter in view to effectively establishing the chiral recognition mechanism of polysaccharide-based chiral stationary phases.

Acknowledgments

The authors are grateful to Mr. L. Zanitti Centro nazionale per il controllo e la valutazione dei farmaci, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy for his technical assistance.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] T. O'Brien, L. Crocker, R. Thompson, et al., Mechanistic aspects of chiral discrimination on modified cellulose, Anal. Chem. 69 (1997) 1999–2007.
[2] F. Wang, R.M. Wenslow Jr., T.M. Dowling, et al., Characterization of a thermally induced irreversible conformational transition of amylose tris(3,5-dimethylphenylcarbamate) chiral stationary phase in enantioseparation of dihydroperimidine acid by quasi-equilibrated liquid chromatography and solid-state NMR, Anal. Chem. 75 (2003) 5877–5885.
[3] R. Ferretti, A. Mai, B. Gallinella, et al., Application of 3 μm particle-based amylose-derived chiral stationary phases for the enantioseparation of potential histone deacetylase inhibitors, J. Chromatogr. A 1218 (2011) 8394–8398.
[4] K. Fulde, A.W. Frahm, Temperature-induced inversion of elution order in the enantioseparation of ketoprofen on a cellulobiohydrolysate 1-based stationary phase, J. Chromatogr. A 858 (1999) 33–43.
[5] Y. Bixia, Z. Fengping, Y. Guanguan, et al., Temperature-induced inversion of elution order in the chromatographic enantioseparation of 1,1-bis-2-naphthol on an immobilized polysaccharide-based chiral stationary phase, J. Chromatogr. A 1216 (2009) 5429–5435.
[6] K. Balmér, P.O. Lagerström, B.A. Persson, et al., Reversed retention order and other stereoselective effects in the separation of amino alcohols on Chiracel OD, J. Chromatogr. 592 (1992) 331–337.
[7] W.H. Pirkle, P.G. Murray, An instance of temperature-dependent elution order of enantiomers from a chiral brush-type HPLC column, J. High Resolut. Chromatogr. 16 (1993) 285–288.
[8] L. Chankvetadze, N. Ghibradze, M. Karchkhadze, et al., Enantiomer elution order reversal of fluorenlymethoxycarbonyl-isoleucine in high-performance liquid chromatography by changing the mobile phase temperature and composition, J. Chromatogr. A 1218 (2011) 6554–6560.
[9] A. Azani, L. Iliz, Z. Patáj, et al., High-performance liquid chromatographic enantioseparation of 1-(phenylethylamino)- or 1-(naphthylethylamino) methyl-2-naphthol analogs and a temperature-induced inversion of the elution sequence on polysaccharide-based chiral stationary phases, J. Chromatogr. A 1218 (2011) 4869–4876.
[10] F. Gasparinni, F. Marinii, D. Misiit, et al., Temperature dependent elution order of enantiomers on a two-armed receptor HPLC chiral stationary phase, Enantiomer 4 (1999) 325–332.
[11] V.V. Ranade, J.C. Somberg, Chiral cardiovascular drugs an overview, Am. J. Therapeut. 12 (2005) 439–459.
[12] R.R. Ruffolo, M. Gellai, J.P. Hieble, et al., The pharmacology of carvedilol, Eur. J. Clin. Pharmacol. 38 (1990) S22–S38.
[13] G. Neugebauer, A. Watan, B. Kaufmann, et al., Stereoselective disposition of carvedilol in man after intravenous and oral administration of the racemic compound, Eur. J. Clin. Pharmacol. 38 (1990) S108–S111.
[14] D. Tenero, S. Boihe, D. Boyle, et al., Steady-state pharmacokinetics of carvedilol and its enantiomers in patients with congestive heart failure, J. Clin. Pharmacol. 40 (2000) 844–853.
[15] E. Sweeth, C. Vijitha, C. Veeresham, HPLC method development and validation of (S)-carvedilol from API and formulations, Am. J. Anal. Chem. 6 (2015) 437–445.
[16] S. Magiera, W. Adolf, I. Baranowska, Continuous enantioseparation and determination of carvedilol in 5’-hydroxyphenyl carvedilol enantiomers from human urine by high performance liquid chromatography coupled with fluorescent detection, Cent. Eur. J. Chem. 11 (2013) 2076–2087.
[17] A. Medvedovic, F. Albo, C. Georgita, et al., Achiral–chiral LC/LC–FLD coupling for determination of carvedilol in plasma samples for bioequivalence purposes, J. Chromatogr. B 850 (2007) 327–335.
[18] M. Saito, J. Kawana, T. Ohno, et al., Enantioselective and highly sensitive determination of carvedilol in human plasma and whole blood after administration of the racemate using normal-phase high-performance liquid chromatography, J. Chromatogr. B 873 (2006) 71–77.
[19] J. Jiang, L. Tian, Y. Huang, et al., Enantioselective and sensitive determination of carvedilol in human plasma using chiral stationary-phase column and reverse-phase liquid chromatography with tandem mass spectrometry, J. Chromatogr. B 960 (2014) 92–97.
[20] R.C. Moldovan, G.S. Dascal, V. Mirel, et al., Chiral separation of 16 beta-blockers on immobilized polysaccharide chiral stationary phases, Farmacia 63 (2015) 909–912.
[21] L. Da Costa, E. Scheeres, A. Colucia, et al., Structure-based drug design of potent pyrazole derivatives against rhinovirus replication, J. Med. Chem. 61 (2018) 8402–8416.
[22] A. Ghanem, C. Wang, Enantioselective separation of racemates using CHIR-ALPAC K as an amyllose-based chiral stationary phase under normal standard, non-standard and reversed phase high performance liquid chromatography, J. Chromatogr. A 1532 (2018) 89–97.
[23] R. Cristilli, P. Guglielmi, E.R. Formica, et al., The sodium salt of the enantiomers of nicobendazole: preparation, solubility, and chiroptical properties, J. Pharm. Biomed. Anal. 139 (2017) 1–7.
[24] L. Da Costa, E. Scheeres, A. Colucia, et al., Heterocyclic pharmacochemistry of new rhinovirus antiviral agents: a combined computational and experimental study, Eur. J. Med. Chem. 140 (2017) 528–541.
[25] R. Ferretti, L. Zanitti, A. Casulli, et al., Unusual retention behavior of omeprazole and its chiral impurities B and E on the amylose tris(3-chloro-5-methylphenylcarbamate) chiral stationary phase in polar organic mode, J. Pharm. Anal. 8 (2018) 234–239.
[26] R. Cristilli, S. Carradori, A. Casulli, et al., A chromatographic study on the retention behavior of the amylose tris(3-chloro-5-methylphenylcarbamate) chiral stationary phase under aqueous conditions, J. Sep. Sci. 41 (2018) 4014–4021.
[27] X. Yuan, X. Li, P. Guo, et al., Simultaneous enantiomeric analysis of chiral non-steroidal anti-inflammatory drugs in water, river sediment, and sludge using chiral liquid chromatography–tandem mass spectrometry, Anal. Meth. 10 (2018) 4404–4413.
[28] J.N. Akahaya, D.R. Taylor, The effect of temperature on the resolution of racemates on a chiral bonded packing incorporating N-formylphenylalanine, Chromatographia 25 (1988) 639–642.