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# Synthesis and chromatographic enantioresolution of anti-HIV quinolone derivatives

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ABSTRACT

The successful enantioseparation of five 6-desfluoroquinolones with three polysaccharide-based stationary phases (namely, the cellulose-based Chiralpak IB and the two amylose-based Chiralpak AD-H and Lux Amylose-2) is herein described. The investigated species differ for the nature of substituents and/or the position of the stereogenic centre on the quinolone scaffold.

The effect on the enantioseparation performance exerted by the different morphology of the cellulose-based and amylose-based polymers, was systematically evaluated for all compounds. In this frame, the impact of alternative alcoholic (ethanol, 2-ethoxyethanol, methanol, 2-propanol) and acidic (acetic, methanesulfonic and trifluoroacetic acid) modifiers as well as of a "non-standard" solvent (chloroform), was investigated in normal phase conditions along with the stereo-electronic peculiarities of the selected polymers. While 7-[4-(1,3-benzothiazol-2-yl)-2-methyl-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**1**) was enantioresolved with conventional normal-phase conditions by means of the largely employed amylose-based Chiralpak AD-H column, the recruitment of a bulky alcohol (2-ethoxyethanol) succeeded in the enantioresolution of 6-amino-1-methyl-7-[2-methyl-4-(2-pyridinyl)-1-piperazinyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (**2**) and 6-amino-1-[1-(hydroxymethyl)propyl]-4-oxo-7-(4-pyridin-2-ylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**3**) with the same column. The use of the amylose-based Lux Amylose-2 column, carrying both an electro-withdrawing (chlorine) and an electro-donating (methyl) group on the carbamate residue, allowed to get 6-amino-1-methyl-4-oxo-7-[3-(2-pyridinyl)-1-pyrrolidinyl]-1,4-dihydro-3-quinolinecarboxylic acid hydrochloride (**4**) enanantioresolved, and 6-amino-1-methyl-4-oxo-7-(3-pyridin-2-ylpiperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**5**) enantioseparated.



## 1. Introduction

Since the introduction of nalidixic acid in 1962, the quinolones have gained widespread acceptance as a class of antimicrobial agents with multiple therapeutic applications. More recently, variously functionalized quinolones have shown to possess antiviral properties [1]. In particular, the 6-desfluoroquinolones (6-DFQs), constitute a promising class of anti-HIV compounds [2]. Due to their innovative mechanism of action, they could be interesting candidate for use in association with the currently used cocktail of drugs. Indeed, the potent antiviral activity of the 6-DFQs in acutely, chronically and latently infected cells is related to the inhibition of the Tat-mediated HIV-1 transcription, one of the crucial steps in the HIV replication cycle that, to date, has not been clinically exploited.

A large series of anti-HIV 6-DFQs have been already realized and further synthetic efforts are still going on with the aim to find more potent and selective analogues.

The establishment of suitable analytical methods for chiral 6-DFQs able to distinguish between the two enantiomers is essential to determine their optical purity along the development process.

The need for suitable enantioseparation protocols is furthermore corroborated by the evidence that most of the antibacterial quinolones approved in the last years, as well as those in preclinical and clinical development, have one or multiple stereocentres [3].

Accordingly, a multitude of "chiral chromatography" (direct) approaches has been successfully employed for the enantioseparation of this class of compounds [4–16].

*Abbreviations:* 6-DFQs, 6-desfluoroquinolones; CSPs, chiral stationary phases; CLEC, chiral ligand-exchange chromatography; RP, reversed-phase; NP, normal phase; DEA, diethylamine; IPA, 2-propanol; TFA, trifluoroacetic acid; MSA, methanesulfonic acid; DMF, dimethylformamide; HPLC, high-performance liquid chromatography.

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In this scenario, only few studies dealing with the use of polysaccharide-based CSPs (both cellulose-based and amylose-based) have been published along the years [13–16]. Such a scarcity of applications does not fit with the worldwide recognized highest versatility of these materials in definitely enantiodiscriminating all classes of compounds [17,18]. Moreover, the huge variety of commercially available polysaccharidic CSPs helps to overcome all the peculiar limitations of the other CSP types. Firstly, with some exceptions, all the chromatographic regimes are allowed, which makes possible the enantioseparation of both polar and apolar compounds irrespective of the presence of specific functional groups on their scaffold. In connection to this, heterogeneous binding modes can be easily promoted through a careful selection of the eluent composition [17,18]. Moreover, the polymer morphology being susceptible to variation with the eluent composition [17,18], expands their applicability to differently sized and shaped compounds.

In this paper, we discuss the enantioresolution of the 6-DFQs 7-[4-(1,3-benzothiazol-2-yl)-2-methyl-1-piperazinyl]-1-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid (**1**), 6-amino-1-methyl-7-[2-methyl-4-(2-pyridinyl)-1-piperazinyl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid (**2**), 6-amino-1-[1-(hydroxymethyl)propyl]-4-oxo-7-(4-pyridin-2-ylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**3**), 6-amino-1-methyl-4-oxo-7-[3-(2-pyridinyl)-1-pyrrolidinyl]-1,4-dihydro-3-quinolinecarboxylic acid hydrochloride (**4**) [19] and 6-amino-1-methyl-4-oxo-7-(3-pyridin-2-ylpiperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**5**) (Fig. 1), while the antiviral activity of the new compounds will be reported elsewhere.

Compounds **1** and **2** owe their chirality to the presence of a methyl group on the piperazine ring, usually present as a spacer between the quinolone core and the heteroaromatic ring. While compound **3** features a chiral substituent at the N-1 position of the quinolone nucleus, compounds **4** and **5** derive their chirality from the presence of a pyridine substituent on the different, saturated heterocyclic ring, again acting as a spacer.

Three different polysaccharide-based CSPs (that is the cellulose-based Chiralpak IB and the two amylose-based Chiralpak AD-H and Lux Amylose-2, Fig. 2) were used in this work for the enantioseparation of the above quinolone derivatives.

## 2. Materials and methods

### 2.1. Synthesis

The synthesis of the 6-DFQ target acids was accomplished starting from the appropriate quinolone or 1,8-naphthyridone scaffolds, prepared through previously reported procedures [20,21], which were reacted with selected heterocyclic side chains in dimethylformamide (DMF) at 80 °C, followed by catalytic reduction of the 6-nitro intermediates, and basic hydrolysis of ethyl 3-carboxylates. The experimental details are given in the Supporting Material.

### 2.2. Chemicals

While all the analytes were synthesized in our laboratories, analytical grade ethanol (EtOH), n-hexane, 2-propanol (IPA), 2-ethoxyethanol, chloroform, acetic acid (AcOH), trifluoroacetic acid (TFA), methanesulfonic acid (MSA) and diethylamine (DEA) were purchased from Sigma–Aldrich (Milano, Italy). All the employed mobile phases were degassed with 20 min sonication. Analytes to be injected were solubilized in the selected mobile phase.

### 2.3. Instrumentation and procedures

All the HPLC experiments were carried out on a Shimadzu (Kyoto, Japan) Class-VP equipped with a EZ Start chromatography data software, a LC-10 ATVP pump, a SCL-10AVP system controller, a FCV-10ALVP low pressure gradient formation unit, a DGU-14A online degasser and a Rheodyne 7725i injector (Rheodyne, Cotati, CA, USA) with a 20 mL stainless steel loop. The columns Chiralpak IB (250 mm × 4.6 mm I.D., containing cellulose tris(3,5-dimethylphenylcarbamate) immobilized onto a 5 μm silica gel) and the Chiralpak AD-H (250 mm × 4.6 mm I.D., containing amylose tris(3,5-dimethylphenylcarbamate) adsorbed onto a 5 μm silica gel) were purchased from Chiral Technologies (West Chester, PA, USA). The column Lux Amylose-2 (250 mm × 4.6 mm I.D., containing amylose tris(5-chloro-2-methylphenylcarbamate) adsorbed onto a 5 μm silica gel) was purchased from Phenomenex (Torrance, CA, USA). Column temperature was controlled through a Grace (Sedriano, Italy) heater/chiller (Model 7956R) thermostat. The column was used after previous conditioning with the selected mobile phase at a 1.0 mL $min^{-1}$ flow rate for at least 30 min. Unless otherwise stated, the analyses were run at 1.0 mL $min^{-1}$ flow rate and with a 35 °C column temperature.

## 3. Results and discussion

In line with the more frequent use of polysaccharide-based CSPs in the normal phase (NP) mode, this chromatographic regime was initially selected to attempt the separation of **1** enantiomers. The study was performed with the relatively new immobilized analogue of the widely employed Chiralpak OD-H [22], that is the Chiralpak IB [17]. In spite of the claimed lower enantioselectivity of the immobilized cellulose-based 3,5-dimethylphenyl carbamate-based material vs the corresponding coated version [23,24], successful enantioseparations can be however achieved with the former [17]. Accordingly, with a n-hexane/ethanol – 80/20 (v,v) containing eluent, further enriched for the presence of 0.1% diethylamine (DEA), the selected stationary phase resulted effective for the enantiomer separation (Fig. 3a).

Even though no relevant improvements in terms of column efficiency were gained with the DEA suppression of the few exposed silanols [25], the default incorporation of the basic additive was maintained thereafter for standardization purposes. The worst performance stemming from the partial or total replacement of ethanol (EtOH) with 2-propanol (IPA) led us to employ the more polar alcoholic modifier in the course of all the following runs. A noticeable amelioration of the overall chromatographic performance turned out when a reduction of the eluent flow rate (from 0.8 to 0.3 mL $min^{-1}$) was coupled with an increase of the column temperature (from 20 to 45 °C) (Fig. 3b). The result can be explained on the basis of the amplified number of theoretical plates ($N$) with a reduced eluent flow rate which combined with an improvement of the mass transfer kinetics as the column temperature was increased [26]. Only a subtle variation resulted for the enantioselectivity ($\alpha$) upon this experimental modification.

The difficulty to identify new NP conditions providing for a better chromatographic outcome stimulated us to attempt an improvement of the enantiomer separation by relying on the incorporation of a "non-standard" solvent into the NP eluent. Indeed, the generally increased robustness of the immobilized CSPs expands the solvent compatibility of the enantiodiscriminating material and, in turn, opens the way to previously unexplored selectivity profiles [17]. Interestingly, with a partial replacement (10%) of EtOH with chloroform, while keeping constant the n-hexane content, the base-line separation of the enantiomer peaks was successfully achieved ($\alpha$ = 1.17, $R_S$ = 2.41) (Fig. 3c).

An extraordinary improvement of the overall chromatographic performance turned out when the amylose-based Chiralpak AD-H column was utilized in the presence of a n-hexane/ethanol – 80/20 (v,v) plus 0.1% DEA eluent. Indeed, as a result of the different

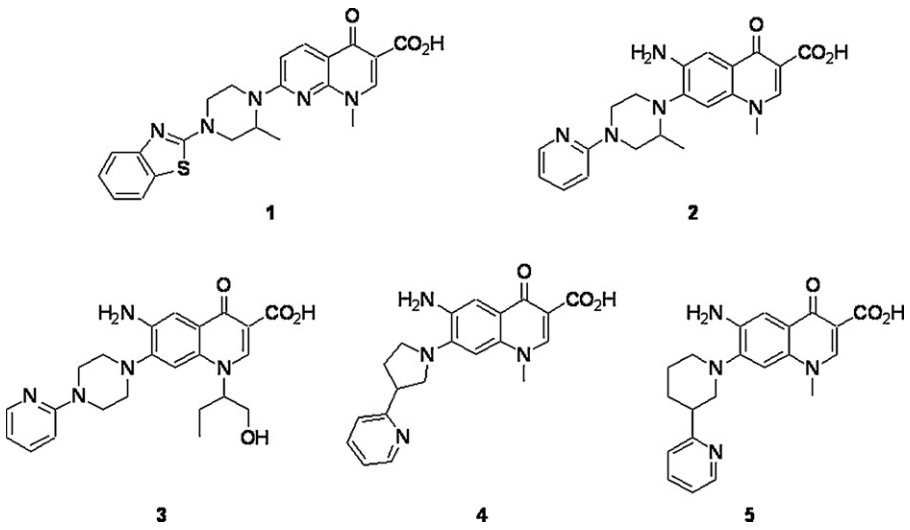


**Fig. 1.** Quinolone derivatives investigated in this study.

supramolecular structure of the corresponding enantioresolving polymer [18], this column afforded amazingly high $R_S$ (15.68) and $\alpha$ (2.03) values (Fig. 3d) with a 1.0 mL min$^{-1}$ eluent flow rate. The different access to the repertory of stereoselective binding sites can account for the remarkable progress. A 35 °C column temperature was selected with the intent to prevent a thermally induced irreversible conformational modification of the polymeric backbone as observed at higher temperatures for amylose-based CSPs [27]. This temperature was then maintained for the analyses on the other investigated compounds.

Owing to the structural analogy between **1** and **2**, the best chromatographic conditions identified for the enantioseparation of the former were initially selected to attempt the separation and resolution of the latter. Although sufficiently separated ($\alpha$ = 1.17), the base-line separation still missed with the adopted eluent system (Fig. 4a). The higher polarity of **2** in respect to **1** accounts for its longer retention into the column. With the task to get the resolution in an usable analysis time, differently composed mobile phases were successively appraised.

In this frame, a reduction of the EtOH content (from 20% to 13%) in the eluent was firstly investigated. The change in the polymer structure, clearly diagnosed with solid-state NMR studies [28,29] upon such a variation of eluent composition, did not reveal gainful for our purpose. Indeed, the only marginal improvement in $R_S$ (from 2.20 to 2.43) accompanied with an almost doubled retention time of both peaks and an increase of the peak tailing as well.

Even though the retention was markedly affected by the EtOH content, the scanty variation of the $\alpha$ value (from 1.17 to 1.18)

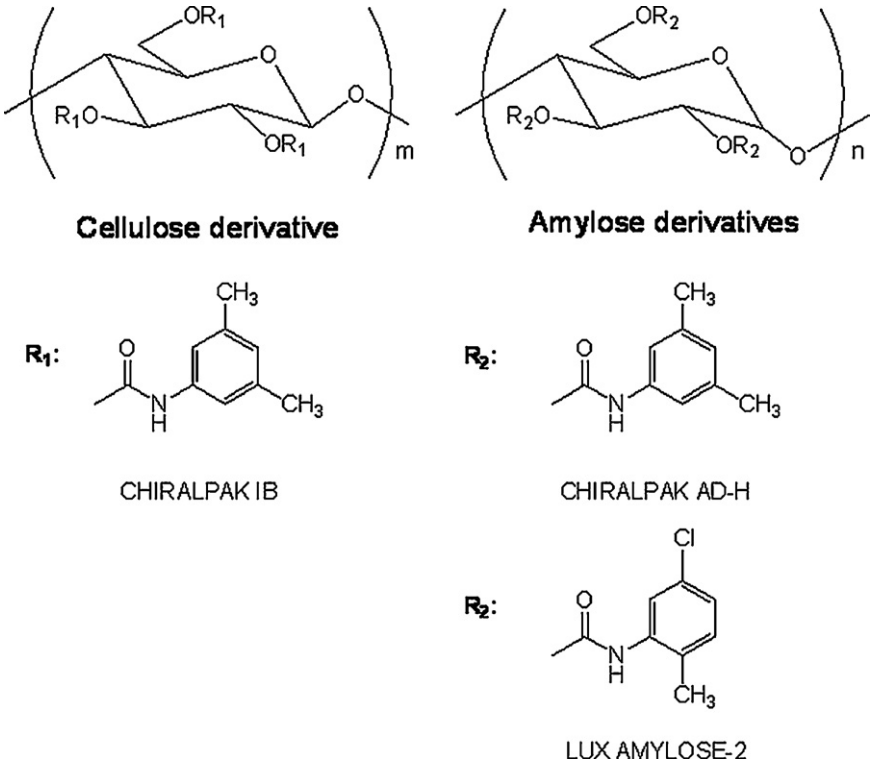


**Fig. 2.** Structures of polysaccharide type CSPs employed in the study.

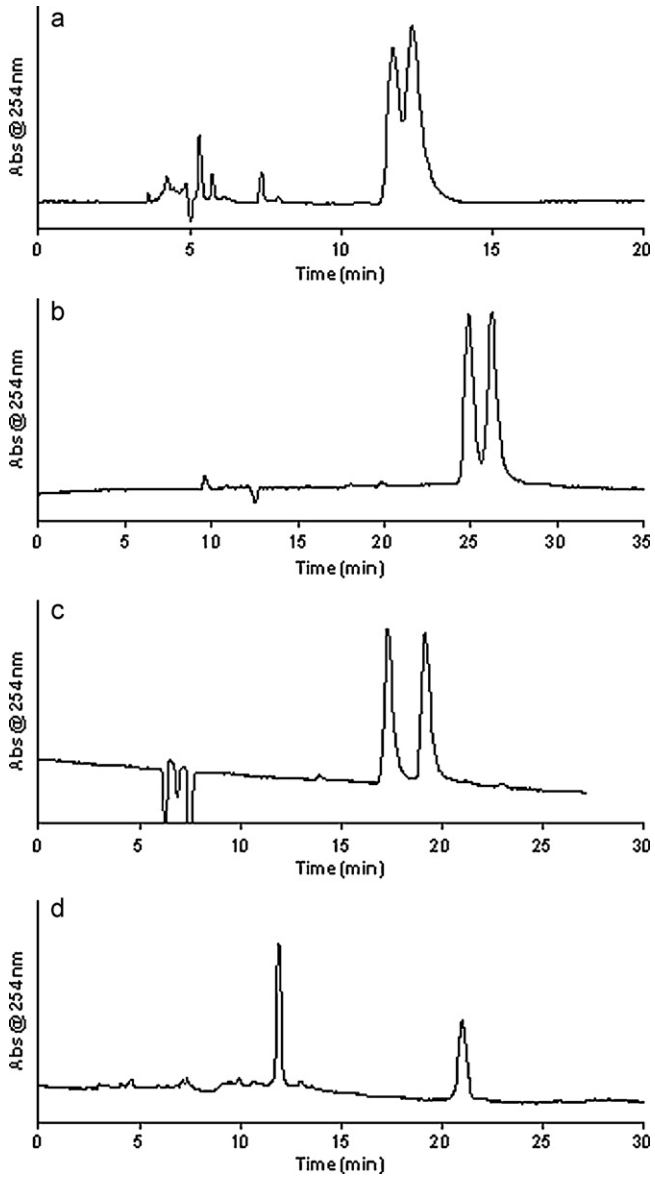


**Fig. 3.** Chromatographic traces of **1** obtained with: (a) column: Chiralpak IB, eluent: n-hexane/EtOH/DEA – 80/20/0.1 (v/v/v), flow rate: 0.8 mL min$^{-1}$, column temperature: 20 °C; (b) column: Chiralpak IB, eluent: n-hexane/EtOH/DEA – 80/20/0.1 (v/v/v), flow rate: 0.3 mL min$^{-1}$, column temperature: 45 °C; (c) column: Chiralpak IB, eluent: n-hexane/EtOH/$CHCl_3$/DEA – 80/10/10/0.1 (v/v/v/v), flow rate: 0.5 mL min$^{-1}$, column temperature: 30 °C; (d) column: Chiralpak AD-H, eluent: n-hexane/EtOH/DEA – 80/20/0.1 (v/v/v), flow rate: 1.0 mL min$^{-1}$, column temperature: 35 °C.

clearly indicated a mechanism of enantioselectivity principally based on other interactions than hydrogen bonding [30]. This evidence was also observed during the separation of **1** enantiomers. Interestingly, for **2**, the reduction of the EtOH content into the eluent, caused an increase of the tailing factor from 1.80 to 2.15 for the second eluted enantiomer. Thereby, as a matter of fact, the presence of a lower alcoholic content in the eluent emphasized heterogeneous mass transfer kinetic processes [31]. Basing on the observation that the higher the bulkiness of the alcohol, the smaller the quantity required to fix a stable polymer conformation [28], an eluent with 2% of 2-ethoxyethanol was tested. A satisfactory result both in terms of $R_S$ (2.91) and $\alpha$ (1.22) was finally achieved within an usable run time (Fig. 4b).

The Chiralpak AD-H column, in combination with n-hexane/EtOH – 80/20 (v,v) plus 0.1% DEA as the eluent, proved

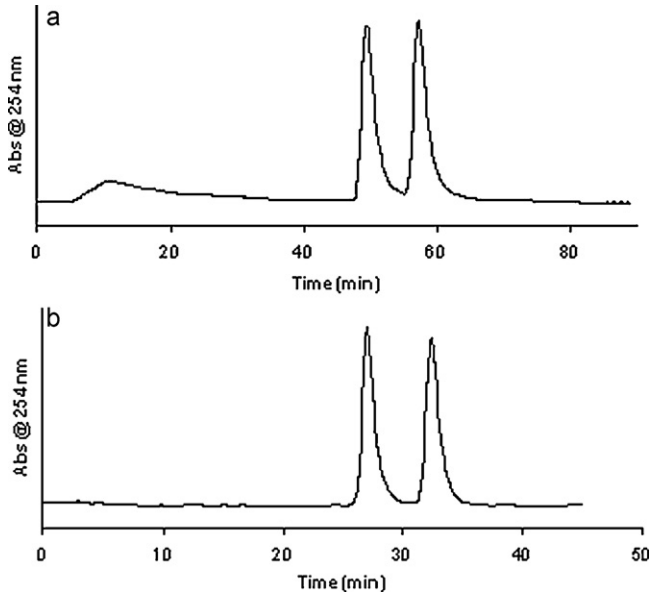


**Fig. 4.** Chromatographic traces of **2** obtained with: (a) see caption of Fig. 3d; (b) column: Chiralpak AD-H, eluent: n-hexane/EtOH/2-ethoxyethanol/DEA – 78/20/2/0.1 (v/v/v/v), flow rate: 1.0 mL min$^{-1}$, column temperature: 35 °C.

highly effective for the separation ($\alpha$ = 1.27) and resolution ($R_S$ = 3.77) of **3** enantiomers (Fig. 5a). Despite a reduced $R_S$ value (2.87), a profitable gain in retention time and peak symmetry was still reached with the addiction of 2% 2-ethoxyethanol at expenses of n-hexane. In this case, the tailing factor of the second eluted enantiomer passed from 1.82 to 1.40 (Fig. 5b). In contrast with the previous case, the higher complexity of the eluent did not turned out into a variation of the enantioselectivity (1.27 vs 1.24), meaning a reduced access to the non-stereoselective but retentive contacts.

Following the same methodological procedure as before, a mobile phase consisting of n-hexane/EtOH/DEA with a 80/20/0.1 (v,v,v) composition was firstly used in combination with the Chiralpak AD-H column to attempt the NP enantioseparation of **4**.

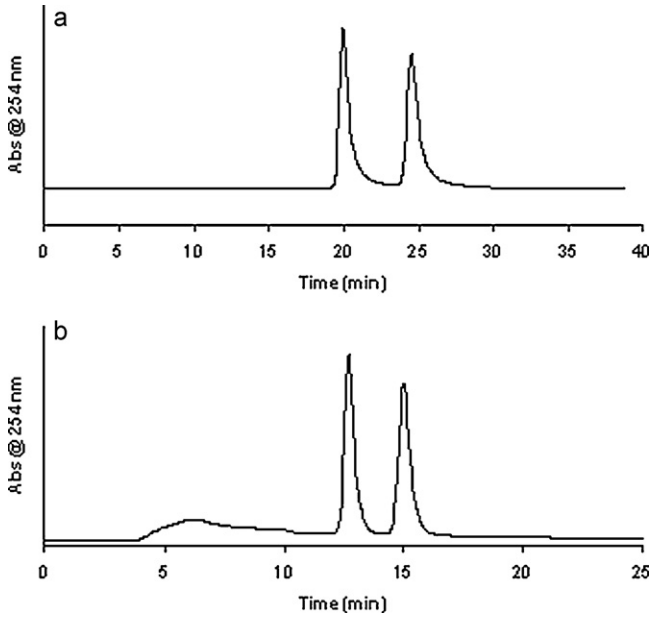


**Fig. 5.** Chromatographic traces of **3** obtained with: (a) see caption of Fig. 3d; (b) see caption of Fig. 4b.

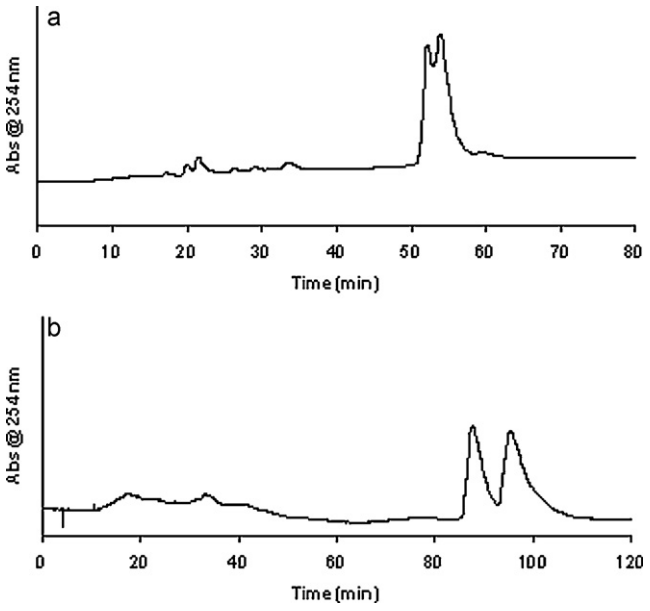


**Fig. 6.** Chromatographic traces of **4** obtained with: (a) see caption of Fig. 4b; (b) column: Lux Amylose-2, eluent: n-hexane/EtOH/2-ethoxyethanol/DEA – 78/20/2/0.1 (v/v/v/v), flow rate: 1.0 mL min$^{-1}$, column temperature: 35 °C.

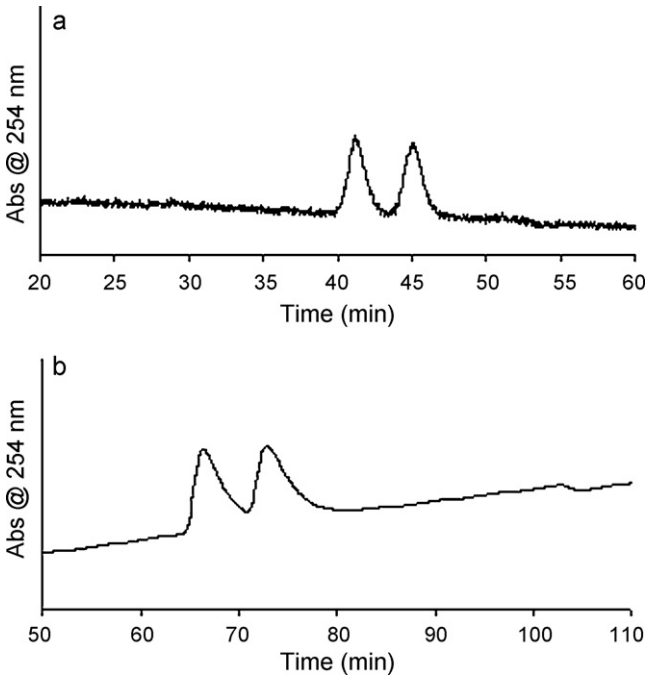


**Fig. 7.** Chromatographic traces of (a) **4** obtained with: column: Lux Amylose-2, eluent: n-hexane/EtOH/IPA/DEA – 78/20/2/0.1 (v/v/v/v), flow rate: 1.0 mL min$^{-1}$, column temperature: 35 °C; (b) **5**, see caption of Fig. 6b.

While peak co-elution was observed with this system, a hint of separation ($\alpha$ = 1.03) turned out by adding 2% of 2-ethoxyethanol (Fig. 6a). Unsuccessful results achieved with other mixtures of the three eluent components, stimulated us to evaluate the impact of the hydrochloride nature of this compound on the chromatographic behaviour. With the intent to exploit advantageous local pH effects and/or profitable alterations of non-specific binding sites [32], the effect of three different acids as minor additives to the eluent (never exceeding the 0.1%) was separately investigated. The incorporation of acidic additives into the eluent was also pursued in order to suppress the ionization of the analyte carboxylic group and to avoid the "salt breaking" phenomenon that could take place as the HCl salt moves along the column [33]. The tentative suppression of the on-column equilibrium between salt and free base form was performed by means of acidic additives endowed with different strength: acetic acid (AcOH, p$K_a$ 4.75), trifluoroacetic acid (TFA, p$K_a$ 0.77) and methanesulfonic acid (MSA, p$K_a$ −1.89). In this frame, numerous mobile phases obtained by changing the modifier identity and acid species were screened. With only one of the tested acidic eluents, a partial separation of **4** enantiomers was obtained. Specifically, the mobile phase consisted of n-hexane/IPA/MeOH (75/22.5/2.5, v/v/v) containing 0.05% of AcOH. Evidently, the higher p$K_a$ of AcOH allowed a beneficial salt break in the vicinity of the polymer binding sites [33] which promoted stereoselective contacts. No selectivity turned also out when the 0.1% of DEA was added to the AcOH containing mobile phase.

The chiral recognition ability of a polysaccharide-based CSP being markedly affected by the electronic nature and position of the substituents on the aromatic ring [34–36] of the carbamate portion led us to evaluate the enantioseparative potential of an interesting amylose tris(5-chloro-2-methylphenylcarbamate) [34,35,37] based column, marketed as Lux Amylose-2. A comparison of this material and that of Chiralpak AD-H was firstly done with the n-hexane/EtOH/2-ethoxyethanol/DEA (78/20/2/0.1, v/v/v/v) eluent. Together with an increase in retention of both peaks, the stationary phase carrying both an electro-donating and electro-withdrawing group also furnished a relevant improvement both in terms of separation and resolution factor ($\alpha$ = 1.09, $R_S$ = 1.12) (Fig. 6b). The experimental outcome can be explained on the basis of the different number of free N–H and C=O groups in the two amylose-based polymers [38]. Indeed, the type and number of substituents on the phenyl ring dictate the aliquot of N-H and C=O groups involved in intramolecular H-bonding interactions, thus influencing the morphology of the helix [38]. The introduction of halogen substituents increases the fraction of free carbamate groups with a consequent enhancement of the analyte retention which accompanies with a positive influence on the peak efficiency [38]. The replacement of 2-ethoxyetyhanol with another alcoholic additive allowed to identify IPA as capable to elute base-line separated peaks ($\alpha$ = 1.10, $R_S$ = 1.67) (Fig. 7a). Despite the higher polarity of 2-ethoxyethanol ($c$ log $P$ 0.028) in respect to IPA ($c$ log $P$ 0.42), a higher retention was produced by the former.

The absence of a direct correlation between the polarity of the minor H-bond competitor and the produced retention was also confirmed by the analyses with the above alcohols. Evidently, other factors influence the chromatographic behaviour of **4** enantiomers.

Owing to the relevant structural similitude between **4** and **5** (Fig. 1), the n-hexane/EtOH/2-ethoxyethanol/DEA (78/20/2/0.1, v/v/v/v) eluent also provided the enantioseparation of the latter ($\alpha$ = 1.10, $R_S$ = 1.37) (Fig. 7b). Interestingly, in sharp contrast with the analysis on **4**, the use of IPA in place of 2-ethoxyethanol for the enantioseparation of **5**, resulted into a "regular" enhancement of the enantiomeric retention, along with an overall worsening of the chromatographic performance. Evidently, other factors than the polarity of the eluent can contribute to rule the enantiorecognition process.

## 4. Conclusions

In spite of the worldwide recognized highest versatility of the polysaccharide-based stationary phases in enantiodiscriminating almost all classes of compounds, only few studies deal with their application to quinolone derivatives. In our case, the use of three CSPs (the cellulose-based Chiralpak IB and the two amylose-based Chiralpak AD-H and Lux Amylose-2) allowed the successful enantioseparation of five 6-DFQs (**1**–**5**). Owing to their structural

diversity, dedicated experimental conditions were required for the analysis of the investigated compounds. Interestingly, the chromatographic performance turned out being markedly affected by the polymer morphology as well as the selected elution regime.

The widely employed amylose-based Chiralpak AD-H column combined with conventional NP conditions [n-hexane/EtOH/DEA – 80/20/0.1 (v/v/v)] provided the extraordinary enantioresolution of **1** ($\alpha$ = 2.03, $R_S$ = 15.68). However, the different winding of the cellulose-derived polymer in the Chiralpak IB column also furnished the peak resolution ($\alpha$ = 1.17, $R_S$ = 2.41), when running in the presence of a minor percentage of chloroform as the "non-standard" eluent component [n-hexane/EtOH/$CHCl_3$/DEA – 80/10/10/0.1 (v/v/v/v)].

A relevant gain in terms of analysis time along with of the overall chromatographic performance, was achieved for **2** and **3** upon the recruitment of the bulky 2-ethoxyethanol. While for the former, the eluent n-hexane/EtOH/2-ethoxyethanol/DEA – 78/20/2/0.1 (v/v/v/v) produced $\alpha$ and $R_S$ values equal to 1.22 and 2.91, respectively, the same factors were respectively computed as 1.24 and 2.87 for the latter.

The use of the amylose-based Lux Amylose-2 column, carrying both an electro-withdrawing (chlorine) and an electro-donating (methyl) group on the carbamate residue, allowed to get **4** enantioresolved and **5** enantioseparated. Indeed, by flowing a n-hexane/EtOH/IPA/DEA – 78/20/2/0.1 (v/v/v/v) based eluent, a satisfactory performance was achieved for the former ($\alpha$ = 1.10, $R_S$ = 1.67), while a n-hexane/EtOH/2-ethoxyethanol/DEA (78/20/2/0.1, v/v/v/v) eluent allowed the best enantioseparation of the latter ($\alpha$ = 1.10, $R_S$ = 1.37).

The exploration of different polysaccharidic CSPs as well as of peculiar selectivity profiles can open the way to future semi-preparative enantioisolations of quinolone derivatives, as fruitful alternative to enantioselective synthetic protocols.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.06.047.

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