Chiral separation and determination of enantiomeric purity of the pharmaceutical formulation of cefadroxil using coated and immobilized amylose-derived and cellulose-derived chiral stationary phases

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Background and objectives
In the present article, we describe the development of a simple, direct, and isocratic high-performance liquid-chromatographic method for chiral separation and the determination of the enantiomeric purity of cefadroxil. Cefadroxil has three chiral centers; the existence of eight different stereoisomers is possible. Only one of these isomers is currently under development as an antibiotic agent and, consequently, the other seven isomers are considered as unwanted chiral impurities.
Materials and methods
An analytical chiral separation was carried out to check its enantiomeric purity. The separation was carried out by exploiting the high efficiency of several coated/immobilized cellulose and amylose chiral stationary phases under normal-phase and polar-organic modes. The effects of type and concentration of the alcoholic modifiers, 2-propanol and ethanol, on the separation of stereoisomers were studied for optimum resolution.
Results and conclusion
Complete baseline separation of stereoisomers with good resolution was achieved within 40 min under normal-phase mode on Chiralpak IB column using hexane–2-propanol (60 : 40 v/v) as the mobile phase, without any organic additives, at a flow rate of 0.4 ml/min at 25°C and with the ultraviolet detection set at 268 nm. This process was found to be suitable for rapid enantiomeric purity analysis and a quality control of cefadroxil in pharmaceutical formulations without interference of the excipients. The chiral recognition mechanisms of separation were also discussed.

Keywords:
cefadroxil enantiomers, cellulose-amylose coated chiral stationary phases, cellulose-amylose immobilized chiral stationary phases, drug with multiple chiral centers, high-performance liquid chromatography

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Introduction

Chiral separation, as well as the determination of the optical purity of chiral pharmaceuticals, has attracted a great deal of attention from the healthcare and pharmaceutical industries. Most pharmaceutical researches and drug development efforts have been concentrated on the production of enantiomerically pure products because of the increasing demand for such drugs to be administered in a highly optical purified form [1,2]. The therapeutic action of a chiral drug depends on its stereospecificity, each isomeric form having its own pharmacological effect [3]. The determination of optical impurity in a drug is very important from the efficacy and safety point of view [4], and is rapidly becoming one of the key issues in the development of new drugs [5]. These impurities can be starting materials, intermediates, reaction by-products, or degradation products [6]. Besides the ethical or environmental reasons for developing single enantiomers, determination of optical impurity represents a real therapeutic benefit, and, in some cases, has been used as a strategy for extending the patient's life [7]. Regulatory authorities therefore recommend that new chiral drugs should ideally be marketed only in the form of pure enantiomers. Such differences in pharmacological activity necessitate developing adequate methodologies for quality control and analytical methods capable of determining the enantiomeric purity of drugs during chemical and pharmaceutical developments [8]. Developing optically active pure drugs poses a great challenge for researchers and scientists [9]. Drugs that are derived from natural products are usually obtained in the

optically active or pure form of a single isomer; only homochiral drugs are safe for humans. However, the drugs that are produced by chemical synthesis are usually a mixture of equal parts of two, four, or more isomers, depending on the number of asymmetric centers. Accordingly, stereoselectivity in chiral drug bioavailability, distribution, interaction with receptor sites, metabolism, and elimination results in differences of isomer activity, ranging from unwanted toxicity to no significance and finally to enhanced activity [10–12]. Currently, the enantiomeric separation of some drugs with multiple stereogenic centers is one of the most difficult tasks for pharmaceutical analysts during method development [13].

Antimicrobial agents are an important class of drugs that are most commonly used in the treatment of microbial infections. In recent years, there has been a rapid development of β-lactam antibiotics, in which the most attention has been focused on cephalosporin antibiotics. Nowadays, these antibiotics hold a large share in the global market and can be considered one of the most important and most frequently used groups of antibiotics [14]. The cephalosporin antibiotics have been important life-saving medicines in the global market for more than 20 years [15,16].

Cefadroxil, chemically a 7-{[2-amino-2-(4-hydroxyphenyl) acetyl] amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a semisynthetic β-lactam antibiotic from the group of cephalosporins. The intermediate for semisynthetic production of cephalosporins is 7-aminocephalosporanic acid, which is formed by hydrolysis of cephalosporin C produced by fermentation [17]. Cefadroxil is a first-generation drug from the cephalosporin family with good activity against gram-positive bacteria and, to a lesser extent, gram-negative bacteria. As for all β-lactam antibiotics, the bactericidal mechanism of action is the disruption of bacterial cell wall synthesis via inhibition of peptidoglycan crosslinking [18]. Cefadroxil binds to specific penicillin-binding proteins located inside the bacterial cell wall, causing the inhibition of the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefadroxil interferes with an autolysin inhibitor [19,20]. It has been shown to be effective in the treatment of mild to moderate infections caused by susceptible strains of microorganisms. In fact, it is used to treat the respiratory and urinary tracts, skin, soft tissue infections, pharyngitis, and tonsillitis [21,22]. Furthermore, it has been used in the prophylaxis of recurrent urinary tract infections in children [22]. Cefedroxil is an important member of the cephalosporin antibiotics, having a global market of ~300 tons per year [15].

To assure patient safety and clinical efficacy, the pharmacological evaluation of stereoisomers is an integral part of new drug development. Analytical methods to determine the enantiomeric purity of new investigational drugs are often attained through a series of generic or screening methodologies [6]. The number of enantiomers in drugs with multiple asymmetric centers usually depends on the number of asymmetric centers, and usually only one is pharmaceutically active (eutomer). Therefore, the administration of racemates is undesirable as it may lead to serious side effects [9]. In addition, academicians, scientists, clinicians, and government authorities are looking for safe medication – that is, optically active pure drugs. Among the chiral analytical techniques currently used to achieve chiral separation of chiral mixtures is the high-performance liquid chromatography (HPLC) on chiral stationary phases (CSPs), which is widely employed and represents one of the most efficient, direct, and facile techniques for the determination of the optical purity and analytical separation of several enantiomeric drugs and pharmaceutical preparations [1,10,23–27]. Currently, the use of HPLC to assess the chiral purity of drugs, their synthetic intermediates, and raw materials has become a routine practice, owing to the commercial availability of a variety of CSPs for the direct separation of enantiomers [5,28].

The preference of CSPs lies in the inherent advantages of any chromatographic separation, such as the speed of the analysis, the possibility to analyze or purify the enantiomers in complex mixtures, and the reproducibility of the analysis and its flexibility [12]. CSPs have several advantages. They are easily manipulated through synthesis and separate enantiomeric mixtures without the necessity of derivatization [29]. As a consequence, a large number of CSPs are available nowadays, suitable for a variety of different solvents and conditions [25]. There is a wide variety of natural and synthetic CSPs suitable for the separation of chiral drugs, the most widely used being polysaccharide CSPs – that is, derivatized cellulose and amylose CSPs – as they are considered efficient CSPs able to separate wide range of racemic compounds [10,28,30].

The polysaccharide-based CSPs were introduced by Okamoto and his group in 1984 and are prepared by coating cellulose and amylose derivatives on pretreated

silica [30]. Enantiomeric inclusion in chiral cavities, which might be multiple, and competitive in cellulose and amylase-based CSPs seems to be responsible for the chiral discrimination [5].

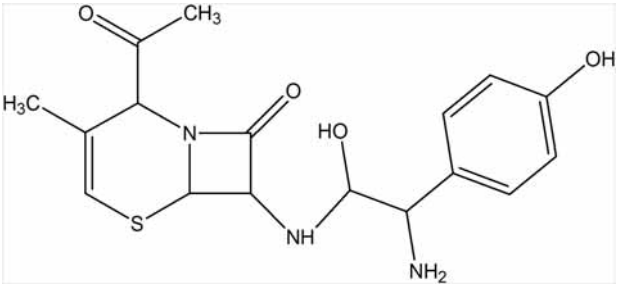
However, to the best of our knowledge, chiral separation of cefadroxil has not been reported yet except for only on one method where the capillary electrophoresis was used as a technique for the analysis of cefadroxil. Moreover, there is no literature data about the liquid chromatography (LC) chiral separation of cefadroxil in bulk drugs and pharmaceutical formulations. Consequently, the development of a new simple enantioselective chiral HPLC procedure for their determination in pharmaceutical dosage forms is necessary. The aim of this investigation was to report a simple HPLC for testing the optical purity of a commercial sample of cefadroxil antibiotic (Fig. 1) in pharmaceutical formulations. Cefadroxil possesses three stereogenic centers (Fig. 1) that allow for eight possible stereoisomers. Only one isomer of cefadroxil is active as an antibiotic which possesses the following stereochemical configuration 6R, 7R, 2R at at C-6, C-7 and C-2 respectively. Therefore, to ensure the quality of cefadroxil, the determination of optical impurities is required. This was achieved by using seven commercially available different coated and immobilized polysaccharide derivatives of cellulose and amylose CSPs, namely Chiralcel OZ-3, Chiralcel OD-H, Chiralcel OD, Chiralcel OJ, Chiralpak AD, Chiralpak IA, and Chiralpak IB. The chiral recognition mechanism and the influence of different mobile-phase solvents by using normal and polar-mode elution were also discussed.

Experimental methods

Apparatus

All chromatographic separations of cefadroxil were carried out using Shimadzu Scientific Instruments' HPLC system LC-20A (Shimadzu, Kyoto, Japan) consisting of an injector with 20 µl rheodyne 1907 sample loop, a pump LC-20A, a vacuum degasser DGU-20 A_5, and a Shimadzu SPD 20. A variable-wavelength ultraviolet (UV) photodiode array detector was connected after the column. The chromatographic and integrated data were acquired, stored, and analyzed by using the LC solution software (Shimadzu, Tokyo, Japan).

Figure 1



Chemical structure of cefadroxil.

Chiral stationary phases

Separation of cefadroxil was accomplished using seven polysaccharide-based CSPs, namely Chiralcel OZ-3, Chiralpak AD, Chiralpak IA, Chiralpak IB, Chiralcel O-DH, Chiralcel OD, and Chiralcel OJ, which were obtained from Chiral Technologies Europe (Illkirch Cedex, France). The CSPs Chiralpak AD, Chiralcel OD-H, and Chiralcel OD are coated on silica and are based on tris (3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose. Chiralcel OZ-3 is coated on silica and based on cellulose tris (3-chloro-4-methylphenylcarbamate); Chiralpak IA and Chiralpak IB are immobilized on silica and are based on tris (3,5-dimethylphenylcarbamate) derivatives of amylose and cellulose, respectively; Chiralcel OJ is coated on silica and based on cellulose (4-methylbenzoate), whereas Chiralpak IB contains the same chiral selector as Chiralcel OD-H, but the polysaccharide is immobilized onto silica. The dimensions of the columns were 250×4.6 mm internal diameter except for the Chiralcel OZ-3 column, for which they were 50×4.6 mm internal diameter The particle size was 3 µm for Chiralcel OZ-3, and 5 µm for Chiralcel OD-H, Chiralpak IA, and Chiralpak IB, and 10 µm for other columns. The mobile phases were based on solvents compatible with the coated and immobilized polysaccharide CSPs.

Chemicals

All solvents used in the experiment were of HPLC or analytical grade, purchased from Riedel-de Haën (Seelze, Germany). Commercial pharmaceutical preparations in the form of Cedrox tablets (Hikma Ltd, Amman, Jordan) were provided by a local pharmacy.

HPLC operating conditions

All the experiments were carried out by using the HPLC system as described above according to the isocratic mode at a flow rate of 0.4 ml/min except in the case of Chiralcel OZ-3, for which the flow rate was 0.5 ml/min. The chromatographic analyses runs were carried out at a room temperature ~20°C. Injection volume was 20 µl and the eluted peaks were monitored at 268 nm. Various mobile-phase systems were investigated in this study. All of them were

composed of commonly used organic HPLC solvents with a multimodal operation: the mobile phase compositions for the chromatographic separations were prepared by mixing hexane and an alcohol modifier (2-propanol or ethanol) with different proportions of these solvents in the normal-phase mode. Four different solvents (methanol, ethanol, 2-propanol, acetonitrile) were used in the polar-phase mode. The mobile phase was prepared in a volume/volume relation, and before delivering into the system, it was filtered through a Millipore membrane filter (0.5 μm; Millipore, Bedford, Massachusetts, USA) and degassed daily before use.

Preparation of assay

Ten cefadroxil tablets were weighed and grinded to fine powder. Accurately weighted tablet powder equivalent to 1.0 mg active ingredient was transferred to a 10 ml volumetric flask; about 10 ml of methanol was added. The contents of the flask were subjected to ultrasonic treatment to affect complete dissolution of the powder (4–5 min). The sample was then filtered through a 0.45 μm microporous membrane. The filtrate was transferred to a 10 ml volumetric flask, which was then filled to the volume with methanol; the solution was then wrapped with aluminum foil to protect it from light, and was subjected to further HPLC analysis. Sample solutions were stable for at least 24 h.

Results and discussion

The present study was aimed at finding a simple, efficient, and rapid process for the determination of enantiomeric purity of cefadroxil in pharmaceutical formulations containing 1 g cefadroxil per tablet (Cedrox; Hikma Ltd). The determination of enantiomeric purity of cefadroxil is of great importance because of the drug's widespread use in medicine. Cefadroxil was created by adding a hydroxyl group to the para-position of cephalexin's aromatic ring [31]; it is produced by the chemical coupling of the side chain (d)-p-hydroxy phenyl glycine to the β-lactam nucleus.

The direct chiral separation of cefadroxil has been attempted via HPLC by using different polysaccharide-type CSPs. Cefadroxil's chemical structure comprises three asymmetric centers and thus is expected to have three pairs of enantiomers (eight possible stereoisomers). The resolution of this drug is more difficult than that of the single chiral center compounds [32]; multiple stereogenic center drugs represent special challenge for chiral separation of all possible enantiomers because of the complex structure of these analytes and because chiral selectors must have the ability to differentiate the chiral centers simultaneously [33,34], especially under isocratic conditions [35]. The chiral HPLC conditions were optimized by varying chromatographic parameters to gain insight into the overall chiral recognition mechanism. Chiral separations by using a CSP column were related to the appropriate choice of the CSP column and mobile-phase composition. For simplicity, we tried to separate all the eight enantiomers of this drug using the isocratic elution technique, which involves two modes of elution (normal-phase and polar-organic).

The analytical processes were developed on various polysaccharide CSPs by using seven different polysaccharide derivatives of cellulose and amylose CSPs. The commonly used mobile phases for this kind of CSPs are pure methanol, ethanol, acetonitrile, and mixtures of alkanes with alcohols. Several types of mobile phase compositions were investigated by changing the nature and percentage of alcohol. For example, mobile phases with different ratios of hexane–2-propanol and hexane–ethanol were tried. In addition, different polar solvents such as acetonitrile, methanol, ethanol, and 2-propanol were used to achieve the best and fastest separation. As a result of exhaustive experimentation, the best solvent system was optimized. To simplify the presentation, only the chromatographic results obtained by the optimal mobile phase composition and/or the conditions that gave the best resolution (separation more than five peaks only) on different columns are presented in this article.

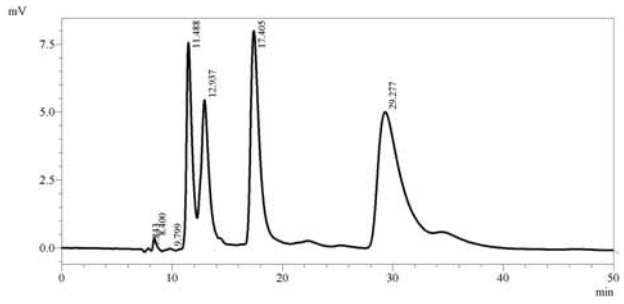
Effect of chiral polysaccharide columns

Among existing CSPs, polysaccharide-based (cellulose, amylose) stationary phases have been used extensively with success, and have proved to be very efficient in HPLC (in normal and polar mode of elution) for the resolution of either chiral drugs or diastereomers [10,36]. The structure of the CSP in LC plays an important role in the separation of stereoisomers [13]. The correct choice of the CSP in LC determines the success or failure of a chromatographic enantioselective separation. Since their introduction to chiral separation, derivatized cellulose-based and amylose-based CSPs have proved their usefulness as chiral selectors in LC [37].

To separate the stereoisomers of cefadroxil, seven different types of chiral polysaccharide-based stationary phases (CSPs) were used (both the

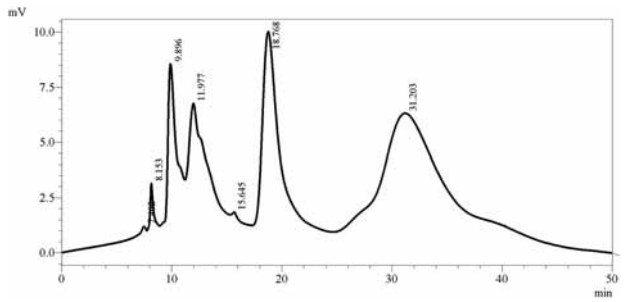
immobilized or coated type), namely Chiralcel OZ-3, Chiralcel OD-H, Chiralcel OD, Chiralcel OJ, Chiralpak AD, Chiralpak IA, and Chiralpak IB. Various experiments were conducted, with different mobile-phase compositions, on the CSPs mentioned above to select the stationary phase that would give optimum resolution and selectivity for cefadroxil stereoisomers. Some selected chromatograms are shown in Figs 2–9. Almost baseline separation with excellent resolution was obtained for cefadroxil on cellulose CSPs (Chiralcel OZ-3, Chiralcel OD-H, Chiralcel OD, Chiralcel OJ, and Chiralpak IB), whereas cefadroxil enantiomers were partially or poorly resolved on amylose CSPs. When Chiralpak® AD and Chiralpak® IA columns were examined under the conditions described above, they showed the lowest chiral discrimination ability for cefadroxil enantiomers.

Figure 2



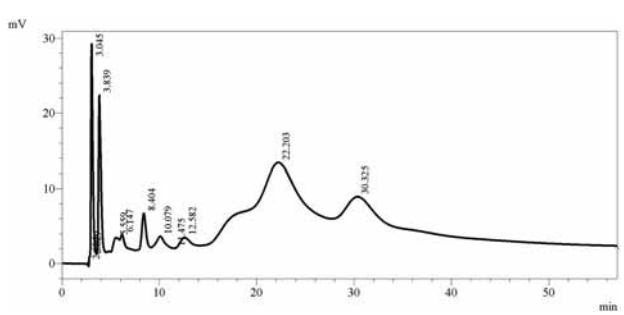
Chiral-HPLC chromatogram of cefadroxil on Chiralcel OD, mobile phase: 40% 2-propanol +60% hexane. HPLC, high-performance liquid chromatography.

Figure 3



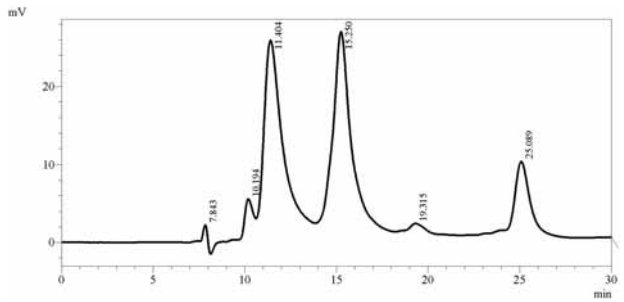
Chiral-HPLC chromatogram of cefadroxil on Chiralcel OD-H, mobile phase: 40% 2-propanol+60% hexane. HPLC, high-performance liquid chromatography.

Figure 4



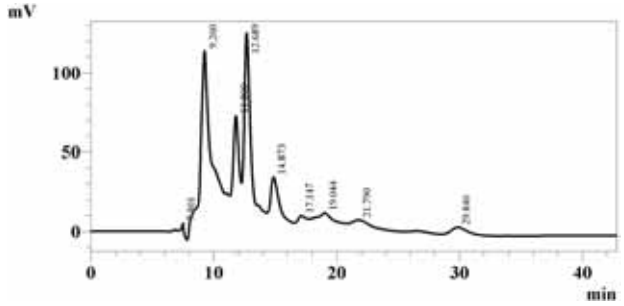
Chiral-HPLC chromatogram of cefadroxil on Chiralcel OZ-3, mobile phase: 40% 2-propanol+60% hexane. HPLC, high-performance liquid chromatography.

Figure 5



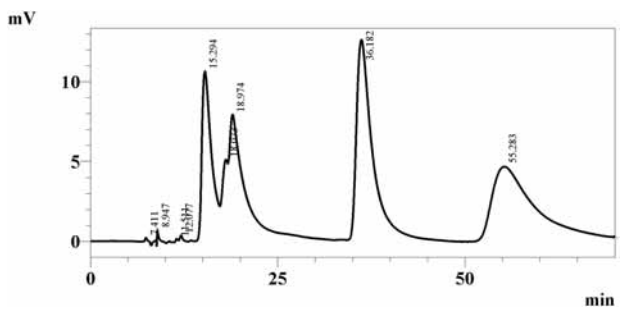
Chiral-HPLC chromatogram of cefadroxil on Chiralcel OJ, mobile phase: 40% 2-propanol+60% hexane. HPLC, high-performance liquid chromatography.

Figure 6



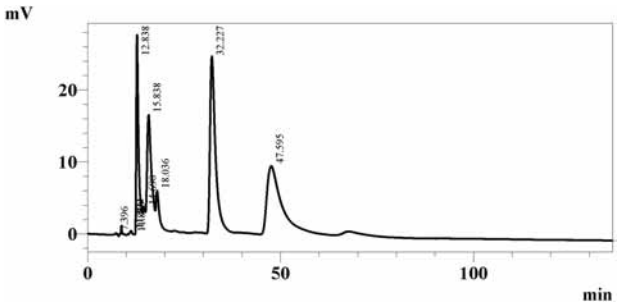
Chiral-HPLC chromatogram of cefadroxil on Chiralcel OJ, mobile phase: 40% EtOH+60% hexane. HPLC, high-performance liquid chromatography.

Figure 7



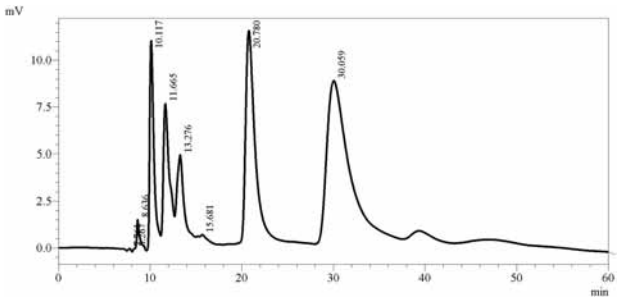
Chiral-HPLC chromatogram of cefadroxil on Chiralpak IB, mobile phase: 20% 2-propanol+80% hexane. HPLC, high-performance liquid chromatography.

Figure 8



Chiral-HPLC chromatogram of cefadroxil on Chiralpak IB, mobile phase: 30% 2-propanol+70% hexane. HPLC, high-performance liquid chromatography.

Figure 9



Chiral-HPLC chromatogram of cefadroxil on Chiralpak IB, mobile phase: 40% 2-propanol+60% hexane. HPLC, high-performance liquid chromatography.

Under normal-phase mode, analysis was carried out using hexane–2-propanol (40 : 60 v/v) as the mobile phase with amylose-based CSPs. On Chiralpak AD, no separation was obtained. Cefadroxil was unresolved since only a single peak was observed, and on Chiralpak IA a slight separation was observed. Cefadroxil was resolved partially and three peaks were obtained (data not shown). Chiralpak AD is amylose 3,5-dimethylphenylcarbamate derivative, which is physically coated on the silica gel. Chiralpak IA is the immobilized version of Chiralpak AD [36,38].

Separation was achieved on Chiralpak IB, Chiralcel OJ, and Chiralcel OD columns since an improvement in the peak shape and resolution of stereoisomers of cefadroxil was observed within 55 min. Chiralpak IB column, a 3,5-dimethylphenylcarbamate derivative of cellulose (the immobilized version of Chiralcel OD) [36], yielded better separation and has shown excellent enantioselectivity power for the cefadroxil stereoisomers. However, less resolution with broad peaks was observed on Chiralcel OZ-3 and Chiralcel OD-H, as shown in Figs 3 and 4, respectively.

These differences in chiral recognition mechanism between the amylose and cellulose derivatives are due to the different configurations of the glucose residues (and linkages) and higher-order structures of CSPs of cellulose CSP and amylose CSP columns [39]. The nature and structure of chiral polymer bound to the support (cellulose-based CSP/amylose-based CSP) influence the formation of transient diastereomeric complexes (analyte-CSP), which consequently must change their chiral-resolving ability. The differences in the results between the cellulose and the amylose derivatives can be explained by the conformation of the helix of the polysaccharide derivatives: left-handed three-fold (3/2) helix for cellulose and left-handed four-fold (4/1) helix for amylose [36]. Cellulose has well-defined grooves providing a chiral surface to the enantiomers [40]. It seems clear in this case that the amylose derivative, which possesses a more helical configuration, did not effectively contribute to the separation of cefadroxil enantiomers compared with the cellulose derivative, which has a more rigid and linear configuration resulting in its ability to resolve all the enantiomers.

Effect of mobile phase

As to the mobile phase of the HPLC analysis, a number of solvents were used for chiral separation of cefadroxil. During the development of the normal-phase elution system, the influence of alcohols, namely 2-propanol and ethanol, and their concentration in hexane were studied to give baseline-resolved cefadroxil stereoisomers.

The effect of the concentration of 2-propanol was studied. The amount of 2-propanol in the mobile phase varied and different compositions were prepared and used as mobile phases to improve the separation performance and to assess its effect on the resolution of enantiomers. Taking a systematic approach decreased the concentration of organic alcoholic modifier concentration in 10% increments, from hexane–2-propanol (80 : 20 to 60 : 40 v/v). Figures 7–9 show the influence of the percentage of 2-propanol on resolution. The resolution was improved with an increase in 2-propanol concentration. The highest enantioresolution was observed at 60 : 40 v/v proportions. At this condition, selectivity and resolution increased significantly. Baseline separation of the stereoisomers was achieved in 40 min in these conditions (Fig. 9). These results were consistent with the decreasing ability of the solvent to displace the solute from CSPs, due to a decrease in solvent polarity. The solvent strength of a mobile phase influences separation efficiency and even selectivity.

The effect of ethanol and 2-propanol on the chiral separation of optical isomers was investigated. The type and concentration of organic modifier was found to influence the resolution of cefadroxil enantiomers; when the mobile-phase modifier was changed from 2-propanol to ethanol, the resolution decreased. For example, the selectivity and resolution of cefadroxil enantiomers with 2-propanol and ethanol on Chiralcel OJ columns are shown in Figs 5 and 6, respectively, where the content of ethanol in the mobile phase was similar to that of 2-propanol. Cefadroxil was partially resolved with ethanol (used as a modifier) on the Chiralcel OJ column, resulting in a severe worsening of the peak shapes, whereas sharp peaks with higher resolution of cefadroxil on polysaccharide columns can be achieved by the use of 2-propanol. 2-Propanol had very strong effects on the chiral separation; the nature of the phase itself offers the potential for a change in selectivity. However, the use of 2-propanol in mobile phase provided better selectivity and resolution for cefadroxil enantiomers when compared with ethanol. The effect of the structure of the alcohol modifier used in the mobile phase in normal-phase mode has profound influence on chiral selectivity of polysaccharide-based CSPs [28]. It could be concluded that the hexane/2-propanol system had a greater ability of elution than did hexane/ethanol and it was found to be more advantageous for the chiral separation of cefadroxil. This is because 2-propanol is a low-polar modifier, whose adsorption on the CSP gets weakened by the interaction between 2-propanol and the stationary phase, leading to the formation of more stable diastereomeric complexes of the enantiomers, which may cause longer retention with better selectivity. 2-Propanol could be inserted into the cavity of the CSP more easily than ethanol, thus decreasing the ability of ethanol to displace the solute from binding sites as the chiral cavity might change its geometry and/or size according to the kind of alcohol modifier used [41]. The observed changes in retention and stereoselectivity were likely due to the steric differences between the two solvent molecules, which may result in quite different chiral surfaces on the stationary phases [41].

Incomplete separation, worst resolution, and poor peak shapes were achieved under polar-organic phase mode on all CSPs. Methanol, acetonitrile, ethanol, and 2-propanol were also tried but a partial separation with poor resolution or no resolution was obtained, which could be due to poor affinity of the enantiomers to the CSP or due to the difficulty of the inclusion of analyte into the chiral cavity.

The mobile phase selection is one of the critical parameters as it encourages the solute and the stationary phase interactions [13]. Mobile-phase strength, composition, and organic modifier have been shown to play important roles in the chromatographic separation of stereoisomers [13]. Mobile phase without organic additives may offer the advantages of alternative chiral recognition mechanisms and higher solubility. In addition, they are easily removed. A comparison of the system suitability results, obtained using normal-phase and polar-organic mode, clearly indicate that 2-propanol with hexane in normal phase is the solvent of choice. Hexane with 2-propanol imparts greater resolution to the CSP than does ethanol. The alcohol type plays a significant role in improving peak symmetry, chromatographic efficiency, and resolution of the stereoisomers. The effect of 2-propanol concentration on resolution and selectivity were examined in this study. For the enantiomeric separation of stereoisomers of cefadroxil on different cellulose-based CSPs, hexane–2-propanol (60 : 40 v/v) was chosen as the mobile phase, as better results were achieved as compared with other solvents. Hexane–2-propanol (60 : 40 v/v) at a flow rate of 0.4 ml/min, with the column maintained at ambient temperature, was the only combination that resolved the stereoisomers satisfactorily and efficiently on all columns used.

Mechanism of the chiral separation

A number of studies have described the power of resolution for cellulose chiral phases and its analog of amylase, and also their complementary resolving abilities [36]. The enantioselectivity of derivatized amylose and cellulose CSPs is generally attributed to the degree of steric fit of the enantiomers in the 'chiral cavity' of the CSP [30,36]. The chiral selector has chiral grooves providing a stereoselective environment to the enantiomers; the enantiomers fit in these chiral grooves to different extents as per the lock and key arrangement. The stabilities of these enantiomers on chiral grooves are facilitated by different interactive forces [42]. In the CSPs such derivatized cellulose or amylose phases, the binding of the solutes to the CSPs process involves different types of interactions between the solutes and the polar carbamate groups on the CSPs [30], together with the rigid structure (cellulose-based CSP) or helical structure (amylose-based CSP) of the chiral polymer bound to the support [5]. These polysaccharides contain a large number of chirally active sites and thus have a relative high probability of interaction with the solute, leading to the separation of the stereoisomers. Peak tailing is a result of frequent interactions between the solute and stationary phase constituents [13].

In general, the mechanism of chiral discrimination of polysaccharide phases has not yet been satisfactorily elucidated [30,39], because of the difficulties in the spectroscopic studies of these chiral selectors [42]. It has been assumed that the separation of racemates on amylose-based and cellulose-based CSPs was due to the formation of transient short-lived diastereomeric solute–CSPs complexes through the inclusion of enantiomers into the chiral cavities in the higher-order structures of the CSPs [28,30,41].

However, binding of the solute to the CSPs is achieved through the interactions between the solutes and the polar carbamate groups on CSPs [24,41]. Solutes can bind to the carbamate groups on the CSPs forming transient diastereomers through different hydrogen bonding using the C=O and N-H groups, through dipole–dipole interaction using the C=O moiety, and through π–π interactions [8,24,36]. The electronegative atoms such as nitrogen, sulfur, and oxygen, along with the aromatic rings of cefadroxil, might participate in hydrogen bonding and dipole–dipole-induced interactions with CO and NH groups of carbomate moieties of cellulose-derived stationary phases. In addition, some other achiral weak forces like Van der Waal's forces and ionic bonds may also contribute to the process of chiral resolution [42].

In the present study, cefadroxil had available S, C–OH, C–NH_2, C=O, and HN–C–OH functional groups, and these could well be contributing to the interactions with the carbamate groups on the CSPs, resulting in separation. The aromatic ring on the solute could provide additional stabilizing effect to the solute–CSP complex [24] through the insertion of the aromatic ring into the chiral cavity. In the present case, this type of stabilization effect may be possible due to the presence of the aromatic functionality on the solutes [5,39].

This fact also points to the importance of a free α-amino group in the chemical species that reacts with CSPs to generate stable diastereomeric complexes with stereoisomers.

Furthermore, there could be interactions of different magnitudes between the substituted phenyl moieties of carbomate and the aromatic rings of cefadroxil enantiomers. Both the solute molecules and the organic modifier compete for the active sites of the CSP. This phenomenon could be attributed to the difference in the steric bulkiness around the hydroxyl moiety of the mobile phase modifier. The less bulky alcohols could be inserted into the cavity of the CSP more easily than bulkier alcohols. The insertion of the mobile-phase modifier into the chiral cavities of the CSP could induce changes in the dominant chiral recognition mechanism, leading to the formation of more stable diastereomeric complexes with the stereoisomers. Thus, in the presence of 2-propanol, cefadroxil stereoisomers might interact strongly with the stationary phase leading to higher resolution [39]. The overall results of this study indicate that the mechanism of chiral recognition for cefadroxil on cellulose derivatives of polysaccharides is complex and should be elucidated in future work.

While carrying out the procedures described above for the analysis of cefadroxil in the pharmaceutical dosage forms, the influence of the commonly used pharmaceutical excipients in the dosage forms of cefadroxil was verified before determining the enantiomeric purity of antibiotics in their tablet dosage forms. The interference of excipients such as croscarmellose sodium, povidone, cellulose microcrystalline, and magnesium stearate on separation in pharmaceutical formulations has been evaluated by examining the purity of the sample on C18 column under the same conditions described above and analysis by using a spectrophotometer. Results indicate that the excipients do not interfere with the separation of stereoisomers in the formulations. An analysis of cefadroxil was conducted to see any interaction between the drug and all other excipients [43]. The proposed procedures could be applied to the determination of cefadroxil in their tablet formulations. The stereoisomers were very well separated in the developed conditions. These results also suggest that there was no interferences observed of the used excipients in determining the enantiomeric purity of cefadroxil in formulation.

This study was not intended to show quantitative data as rigorous validation was not carried out and we could not determine the absolute configuration of the individual cefadroxil enantiomers. Thus, the chromatographic peaks were identified according to their optical rotation sign. Further studies are required to determine important parameters such as efficiency, precision, accuracy, linearity, limit of detection, and limit of quantification to demonstrate the applicability of this method for the quantitative determination of cefadroxil stereoisomers in bulk and pharmaceutical dosage forms.

Conclusion

The present study describes, for the first time, a successful chiral separation and enantiomeric purity

determination of cefadroxil by using HPLC with UV detection using polysaccharide derivatives as chiral selectors. A comparison was performed on both polysaccharide-type CSPs, namely cellulose and amylose derivatives, where cellulose derivatives demonstrated efficient capabilities in resolving cefadroxil, which possesses three stereogenic centers. The type and concentration of organic modifier had a great influence on enantioseparation and the best results were obtained under the normal-phase mode. In addition, the major advantage was that the procedure did not require a sample preparation step. The efficient chromatographic resolution of cefadroxil stereoisomers was achieved in the commercial pharmaceutical dosage forms. Tablet excipients did not interfere with the analysis. The method described above can be used for quality control analysis of cefadroxil in pharmaceutical formulations.

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Conflicts of interest
There are no conflicts of interest.

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