Theoretical study of the chiral separation process of atenolol and carvedilol β -blocker drugs

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Abstract

In the present work, we perform a theoretical study to understand the chiral recognition process of polysaccharide-derived stationary phases with two β -blocker drugs: atenolol and carvedilol. According to the main results, the structural and thermodynamic parameters obtained by DFT calculations pointed out the chiral discrimination, as well as the enantiomeric elution order of both β -blockers, thus explaining at the molecular level the HPLC experimental data previously obtained. Finally, the relative stability of distinct diastereoisomeric complexes was explained by the hydrogen bonds and π - π stacking interactions formed between β -blockers enantiomers and their respective chiral selectors.

1. Introduction

Enantioselective separation analyses have been frequently conducted by conventional separation techniques such as Capillary Electrophoresis (CE), Gas Chromatography (CG), and High-Performance Liquid Chromatography (HPLC), combined with the use of appropriate chiral selectors such as chiral crown ethers, proteins, cyclodextrins, antibiotics, and polysaccharides [1, 2].

In a standard chiral separation procedure by HPLC, the analyte is injected in the chromatographic system as a racemic mixture. So, both enantiomers traverse a medium (stationary phase) filled by a liquid fluid (mobile phase), establishing different equilibria in both phases. The differential balance of the enantiomers between these two phases generates different velocities of retention, thus allowing their separation [3].

Enantioselective separation is based on the formation of diastereomeric complexes (DCs) present in the thermodynamic equilibrium [4]. DCs are complexes generated by selective intermolecular interactions, such as hydrogen bonds, established between the analyte enantiomers and chiral stationary phases. So, in the chromatographic system each enantiomer will interact differently with its chiral selector (CS) leading to the formation of DCs which will present distinct spatial configurations and complexation energies, i.e., one of the two formed DCs will be more stabilized than the other in the thermodynamic equilibrium [5]. Therefore, the enantiomeric elution order (EEO) evidenced in the chromatograms will strongly depend on the chemical affinity of the CS for the analyte enantiomers.

In this context, aiming to provide an auxiliary analysis to the chiral separation by HPLC as well as assisting to explain at the molecular level the EEO, computational studies have been reported in an attempt to comprehend the molecular properties involved in the formation of DCs, providing relevant information on the electronic structures and thermodynamics properties related to the complexation process [6–16]. Recently, our research group has been successfully performed theoretical studies via Density Functional Theory (DFT) calculations regarding the enantioselective separation of three distinct chiral analytes [17–19]. In these works, we were able to elucidate and explain at the molecular level the experimental EEO.

Over the years, a large number and type of CSs have been tested to assess their chiral resolution capabilities [20]. Considering specifically chiral drugs and their metabolites, one can note in the literature the success of those CSs based on polysaccharide derivatives (amylose or cellulose), which present a helical structure containing carbamate or benzoate groups located inside and hydrophobic aromatic groups located externally (Fig. 1). Thus, chiral discrimination will depend on the spatial accommodation of the enantiomers in this polysaccharide structure, as well as on the interaction with the carbamate or benzoate groups [21].

It is agreed that for the CSs based on the amylose tris(phenylcarbamates) (ADMPC) or cellulose tris(phenylcarbamates) (CDMPC), chiral analytes will interact with the stationary phase via hydrogen bonds by the NH and C = O key groups [22, 23]. Besides, π - π stacking interactions can also be established between the CSs and aromatic racemates. It is worth mentioning that these intermolecular interactions are strongly influenced by the location and chemical nature of the analyte functional groups [24].

Focusing on the chiral separations of two specific β -blockers, atenolol (ATL) and carvedilol (CVL) (Fig. 2), which is a class of drugs widely used for the treatment of cardiovascular diseases [25], it is possible to find various papers reporting different chromatographic conditions concerning the stationary and mobile phases used [26–37]. In this context, we dedicated this work to analyze theoretically the chiral separation process of ATL and CVL by substituted polysaccharides. Structural and thermodynamic properties of the respective DCs formed were obtained via DFT calculations in an attempt to explain the experimental EEO previously estimated [26, 33]. The main idea of our study was to understand through quantitative evidence at the molecular level the enantioselective separation process of such β -blockers.

2. Theoretical Methodology

DFT calculations employing the B97D [38] functional, with the Pople's split valence 6-311G(d,p) basis set containing polarization functions on all atoms (B97D/6-311G(d,p)) were performed to fully optimize the following structures: (i) ATL and CVL enantiomers named (-)-(R)-ATL, (+)-(S)-ATL, (-)-(R)-CVL, and (+)-(S)-CVL; (ii) the reduced molecular models for the CDMPC and ADMPC chiral selectors; (iii) the respective DCs formed between them named (-)-(R)-ATL-CDMPC, (+)-(S)-ATL-CDMPC, (-)-(R)-CVL-ADMPC, and (+)-(S)-CVL-ADMPC. During the geometry optimization processes no symmetry constraints were imposed. It is important to clarify that the representative reduced molecular models for the stationary phases have been successfully used in our recent works, as can be found in refs. [17–19].

Our choice for the B97D is due to our background with this type of dispersion-corrected density functional. Interactions of dispersive nature are always present in organic macromolecular systems, such as CDTs based in polysaccharides. In this regard, dispersion corrections can lead to an improvement in describing and obtaining more accurate molecular properties.

Once the optimized structures were obtained for the ATL and CVL enantiomers, the respective Molecular Electrostatic Potential Maps (MPEs) [39] were generated (Fig. 3), which revealed regions of different electronic densities. One of them is that graphically evidenced by hot coloration which possesses atoms

capable of acting as hydrogen bond donors as O-1, O-2, O-3, N-1 and N-2 for ATL and O-1, O-2, O-3, O-4, N-1 and N-2 for CVL (see Fig. 3). The other region is that graphically represented by cold coloration which contains hydrogen atoms covalently bonded to O-2, N-1, and N-2 atoms from ATL and O-1, N-1, and N-2 from CVL, capable of acting as hydrogen bond receptors (see Fig. 3).

B97D/6-311G(d,p) harmonic frequency calculations were performed for the equilibrium structures, characterizing them as true minima on the potential energy surface (all frequencies were real). Besides, the B97D/6-311G(d,p) harmonic frequency were also used to calculate the enthalpy (ΔH) and Gibbs free energy (ΔG) thermodynamic properties. ΔH is predominantly associated with the number of intermolecular interactions established between the interacting molecules in a complex and it can be fairly estimated as a natural measure of the overall change in the number and/or strength of interactions on going from the isolated to the complexed form [40]. On the other hand, ΔG is related to the spontaneity or non-spontaneity of a complex formation and theoretical point of view, this important thermodynamic property is correlated to the entropy change on complex formation calculated from the rotational, translational, electronic, and vibrational partition functions assessed using well-known statistical thermodynamics equations [41].

The solvent effect was considered implicitly by the Solvation Model based on Density (SMD) [42]. It was used the *n*-hexane ($\epsilon = 1.88$) and methanol ($\epsilon = 32.61$), since experimentally the mobile phases contained these solvents in greater proportion as can be seen in refs. [26, 33].

In DFT calculations for chemical systems with a huge occurrence of non-covalent interactions, the basis set superposition error (BSSE) emerges to play a major unwanted role particularly when the association magnitudes are considered, overestimating the interactions forces. In this sense, the BSSE was evaluated using the Boys and Bernardi approach [43]. Besides, the Wiberg bond orders (Wiberg BO) were computed using NBO methodology [44] to show the strength of the intermolecular interactions [45].

All calculations were performed in the Gaussian 09 quantum mechanical package [46]. The molecular models were generated and viewed on the GaussView 5.0 graphical interface.

3. Results And Discussions

3.1. Theoretical analysis of the ATL chiral separation

Firstly, taking into account the MPE generated for the ATL (Fig. 3-B) it was possible to obtain the respective CDTs formed by its enantiomers and CDMPC which was the CS used in the ref. [26]. In this case, the complexation mode proposed involved ATL portion containing the O-2, O-3, and N-2 atoms (spatially closer to the chiral center) as can be seen in Fig. 3-B. Thus, two distinct CDTs could be obtained whose optimized structures is shown in Fig. 4. Table 1 shows the ΔH and ΔG values calculated in *n*-hexane medium for the respective CDTs.

Table 1
Enthalpy (ΔH), Gibbs free energy (ΔG), and Wiberg bond orders (Wiberg BO) calculated for the formation process of CDTs in *n*-hexane medium via SMD calculations. BSSE corrected values in kcal mol⁻¹.

CDTs	ΔH	ΔG	Wiberg BO
(-)-(R)-ATL-CDMPC	-14,3	1,1	-6.5.10 ⁻⁷
(+)-(S)-ATL-CDMPC	-18,2	-2,2	0.016

Analyzing the results presented in Table 1, it is possible to observe that the CDT formed by the (+)-(S)-ATL enantiomer is more stable than the complex formed by the (R)-ATL. The difference between them is of approximately 4.0 kcal mol⁻¹ for ΔH and 3.0 kcal mol⁻¹ for ΔG . This fact can be explained at the molecular level by the intermolecular interactions established in the complex formation.

As can be seen in Fig. 4, the (-)-(R)-ATL-CDMPC presents only one hydrogen bond established between the C = O group of the SQ (hydrogen bond receptor) and the -NH group (hydrogen bond donor) of ATL. The (+)-(S)-ATL-CDMPC complex, on the other hand, reveals not only this same hydrogen bond between the C = O and the -NH, but also a π - π stacking interaction between the aromatic rings perpendicular to each other (T-shaped) (see Fig. 4). T-shaped π - π stacking interactions are generated from the polarization of π clouds displaced both in distance and angle. It is worth emphasizing that although π - π stacking interactions between aromatic rings perpendicular to each other is not a well explored topic in the literature, this mode of interaction concerning aromatic rings is even more common and more energetically favorable than those well-known parallel π - π stacking [47]. Therefore, the shorter hydrogen bond (2.16 Å) as well as the additional π - π stacking interaction provide relevant quantitative evidence at the molecular level that contributes to explain the higher stabilization of (+)-(S)-ATL-CDMPC in comparison to the (-)-(R)-ATL-CDMPC.

Corroborating this result and analyzing the computed bond orders for the CDTs in Table 1, it was possible to verify that the hydrogen bond established in (-)-(R)-ATL-CDMPC complex present a Wiberg BO not only negative, but also much bigger than (+)-(S)-ATL-CDMPC, showing that this complex is undoubtedly less expected to form in comparison to its antipode. This result agrees with the thermodynamic data obtained. As can be seen in Table 1, ΔG for (+)-(S)-ATL-CDMPC is negative, while ΔG for (-)-(R)-ATL-CDMPC is positive, thus indicating a not favorable formation of this CDT.

From this theoretical structural, electronic and thermodynamic analysis via DFT calculations, we could explain the enantiomeric elution order of ATL obtained experimentally by Iha *et al.* [26], which shows the (S)-ATL being eluted after the (R)-ATL enantiomer. Our results suggest that the higher retention of (S)-ATL in the chromatographic system, is due to the formation of a more stable CDT than its (R)-ATL antipode.

3.2. Theoretical analysis of the CVL chiral separation

As in the previous section, taking into account the MPE generated for the CVL (Fig. 3-A) it was possible to obtain the respective CDTs formed by its enantiomers and ADMPC which was the CS used in the ref. [33]. The complexation mode proposed involved the CVL portion containing the O-2, O-3, and N-2 atoms (spatially closer to the chiral center) as can be seen in Fig. 3-A. Thus, two distinct CDTs could be obtained whose optimized structures is shown in Fig. 5. Table 2 shows the ΔH and ΔG values calculated in methanol medium for the respective CDTs.

Table 2
Enthalpy (ΔH), Gibbs free energy (ΔG), and Wiberg bond orders (Wiberg BO) calculated for the formation process of CDTs in methanol medium via SMD calculations. BSSE corrected values in kcal mol⁻¹.

CDTs	ΔH	ΔG	Wiberg BO
(-)-(R)-CVL-ADMPC	-18,8	0,7	-4.2.10 ⁻⁶
(+)-(S)-CVL-ADMPC	-23,5	-3,5	0.025

Analyzing the results presented in Table 2, it is possible to verify that the CDT formed by the (+)-(S)-CVL enantiomer is more stable than the complex formed by its antipode. The difference between them is of approximately 5.0 kcal mol⁻¹ for ΔH and 4.0 kcal mol⁻¹ for ΔG . This fact can be explained at the molecular level by the intermolecular interactions established in the complex formation.

As can be seen in Fig. 5, the (-)-(R)-CVL-ADMPC exhibits two hydrogen bonds, being one of them established between the C = O group of the CS and the -NH group of the CVL; the other one occurs between the -NH group of the CVL and -NH group of the selector. Furthermore, a parallel π - π stacking type interaction between the CVL and SQ aromatic rings can also be detected. The (+)-(S)-CVL-ADMPC complex, on the other hand, reveals an additional hydrogen bond when compared to its antipode, which is established between the -NH group of the CVL with the -OH group of the SQ. This additional interaction causes a higher stabilization for the (+)-(S)-CVL-ADMPC.

Same bond orders analysis was carried out for the ATL CDTs. Corroborating this result and analyzing the Wiberg BO in Table 2, it was possible to verify that the intermolecular interactions established in (-)-(R)-CVL-CDMPC complex present a Wiberg BO not only negative, but also much bigger than (+)-(S)-CVL-ADMPC, showing that this complex is undoubtedly less expected to form in comparison to its antipode. This result again agrees with the thermodynamic data obtained. As can be seen in Table 2, ΔG for (+)-(S)-CVL-ADMPC is negative, while ΔG for (-)-(R)-CVL-ADMPC is positive, thus indicating a not favorable formation of this CDT.

Given the structural, electronic, and thermodynamic parameters obtained, we could explain the enantiomeric elution order of CVL obtained experimentally by Jiang *et al.* [33], which shows the (S)-CVL being eluted after the (R)-CVL enantiomer. Our results suggest that the higher retention of (S)-CVL in the chromatographic system, is due to the formation of a more stable CDT than its (R)-CVL antipode.

4. Conclusions

In HPLC enantioselective separation analyses, analysts are not able to elucidate on the molecular level what is indeed occurring in the formation of diastereomeric complexes and, consequently, with the chiral discrimination mechanism. For example, what differentiates in structural and energetic terms one complex from another? What are the intermolecular interactions involved? In this sense, computational studies have been extremely useful as complementary analyses.

Therefore, with this theoretical work, we could obtain via DFT calculations structural, electronic, and thermodynamic parameters related to the chiral separation of ATL and CVL β -blocker drugs. The results pointed out the chiral discrimination as well as the EEO for both drugs, explaining at the molecular level experimental data. Hydrogen bonds and π - π stacking interactions which was supported by bond order analysis played a key role in the CDTs stabilization.

Finally, this work highlights the importance of computational chemistry as a complementary tool for enantioselective separations analysis, which is able to support quantitatively the understanding of chiral recognition process.

Declarations

Author contribution: We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. In addition, a declaration of the role of each author is mentioned as follows: Prof. Clebio S. Nascimento Jr. participated in the Supervision, Validation, Analysis of the Results, Writing - Original draft preparation, Writing- Reviewing, and Editing; Miss Camila F. Silva in the Investigation, Methodology, Software, and Data curation; Miss Pollyanna P. Maia in the Investigation, Analysis of the Results, and Discussion; Miss Camila A. Nascimento in the Analysis of the Results and Discussion. All authors read and approved the final manuscript.

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Figures

Figure 1

In (A) is shown a longitudinal section of a helical polysaccharide structure and in (B) a longitudinal section of the chiral selector based on an amylose chain containing a carbamate substituent group.

Figure 2

ATL and CLV enantiomers structures. In (A) (-)-(R)-ATL; (B) (+)-(S)-ATL; (C) (-)-(R)-CVL and (D) (+)-(S)-CVL.

Figure 3

Optimized Structures (on the left) and Molecular Electrostatic Potential Maps (on the right) for: (A) CVL and (B) ATL.

Figure 4

Optimized Structures (on the left) and Molecular Electrostatic Potential Maps (on the right) for: (A) CVL and (B) ATL.

Figure 5

CDTs optimized structures at the B97D/6-31G(d,p) level. In (A) (-)-(R)-CVL-ADMPC and (B) (+)-(S)-CVL-ADMPC. The main established intermolecular interactions are shown with dotted lines.

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