Comparison of polysaccharide-based and protein-based chiral liquid chromatography columns for enantioseparation of drugs

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Abstract: Two different columns – Lux Cellulose-1 and Chiralpak CBH – were evaluated for their chiral recognition abilities for 8 drugs comprising 3 β-blockers, 1 antacid and 4 cathinones in polar-organic elution mode and reversed-phase elution mode respectively. The factors that affected the enantioseparation were tested and optimized to develop a suitable chiral separation method whose LC conditions are compatible with MS detection. In polar-organic elution mode with the Lux Cellulose-1 column, methanol and acetonitrile were tested as the main components of the mobile phase. In addition, the effects of adding isopropanol as organic modifier, acidic additives (formic acid) and basic additives (diethylamine) were evaluated. In reversed-phase elution mode with the Chiralpak CBH column, the effect of type and concentration of organic modifier (isopropanol, acetonitrile and methanol), the mobile phase pH (6.4 and 5.0) and buffer concentration (1-20 mM ammonium acetate) were evaluated. The best enantioseparation was achieved with the Chiralpak CBH column with a mobile phase composed of 5 mM ammonium acetate aqueous (pH=6.4)/methanol (95/5, v/v) at a flow rate of 0.1 mL/min and a temperature of 30°C. Under these conditions, 6 out of 8 chiral drugs were baseline separated.

Keywords:
Enantioseparation; Chiral drugs; Chiralpak CBH column; Lux Cellulose-1 column; Polar-organic mobile phase; Reversed-phas

1. Introduction

Chiral separation has recently attracted much attention, not only in the pharmaceutical field but in the environmental and biological areas as well. This is due to the increasing evidence of pharmacological, pharmacokinetic and toxicological differences between enantiomers. Over half the drugs currently on the market are chiral,[1] and for these one enantiomer is usually pharmacologically active, while the other may be less active or even can be harmful.[1-3] For example, S-propranolol is 100 times more active than R-propranolol.[3] However, many chiral drugs are still sold as racemic mixtures because of the difficulty of chiral separation, the high cost of production and the similar~~equal~~ pharmacological effects between enantiomers.[4] As a result, there is increasing demand for the enantioselective determination of chiral drugs.[5]

It is well known that enantiomeric analysis in biological systems is decisive to understand the stereoselective implications, therapeutic use and toxicology of pharmaceuticals.[6] For example, stereoselective disposition was observed for carvedilol – an antihypertensive drug – with higher excretion rates for the S-enantiomer by monitoring its enantiomer~~s~~ concentration in human plasma.[7] On the contrary, the role of stereoselectivity is normally neglected in environmental studies. However, enantiomers of chiral drugs often exhibit stereoselectivity in environmental occurrence, fate and toxicity.[1] For example, Stanley et al.[8] reported that S-fluoxetine was 9.4 times more toxic for P. promelas than R-fluoxetine. Therefore, it is also important to investigate the enantiomeric composition of chiral drugs in the environment to provide a more realistic risk assessment of chiral contaminants.[1]

LC using chiral stationary phases (CSPs) is the most commonly used technique for the enantioselective determination of chiral drugs.[2] Several chiral stationary phases have been designed using different chiral selectors (i.e. polysaccharides, proteins, macrocyclic antibiotics, cyclodextrins, Pirkle type, ion-exchangers and chiral crown ethers). Of these, polysaccharide-based CSPs are the most frequently used due to their wide chiral recognition and high loading capacity.[9,10] Various polysaccharide-based columns have been developed and are commercially available, such as the Lux series (e.g. Lux Cellulose-1) from Phenomenex (Torrance, CA, USA), and the Daicel Chiralpak and Chiralcel series (e.g. Chiralpak IA and Chiralcel OD) from Chiral Technologies (Exton, PA, USA). Polysaccharide-based columns can be used in normal-phase (NP), reversed-phase (RP), or polar-organic (PO) mode. As the NP mode is favorable for its principal mechanisms of chiral recognition (i.e. hydrogen bonding interaction), most chiral separations with polysaccharide phases are performed in NP mode using hexane and alcohol modifiers as mobile phase components.[11-13] However, these mobile phases are not compatible with MS detection. There are several studies showing that polysaccharide-based chiral columns in PO mode can achieve successful enantioseparations for a wide range of chiral compounds.[9,10,14] The PO mobile phase made up of polar-organic solvents such as acetonitrile (ACN), alcohols or a mixture of them offers several advantages including improved solubility of analytes, favorable peak shapes and LC-MS compatibility.

Protein-bonded CSPs, which are used exclusively in RP mode, have become popular due to the character of the chiral selector that can be changed by a simple modification of the mobile-phase composition, allowing a wide range of enantiomers to be separated.[15,16] Nowadays, a broad variety of protein-based columns have been commercialized, of which the Chiralpak CBH and the Chiralpak AGP columns appear to be the most used so far.

The major aim of this study is to evaluate the effect of different parameters on the enantioseparation of 8 drugs comprising β-blockers, antacid and a group of new psychoactive drugs named cathinones. A polysaccharide-based column (Lux Cellulose-1)

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Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

Formatat: anglès (EUA)

and a protein-based column (Chiralpak CBH) were examined for their chiral recognition abilities in PO mode and RP mode respectively. The compatibility of the mobile phase with LC–MS detection was taken into account in the development of the method with a view to allowing coupling with an MS detector for determining individual drug enantiomers in environmental and biological matrices in future research.

2. Materials and Methods

2.1. Chemicals and solutions

The standard analytes – (±)-atenolol, R-(+)-atenolol, (±)-propranolol hydrochloride, R-(+)-propranolol hydrochloride, (±)-metoprolol (+)-tartrate salt and (±)-omeprazole – were purchased from Sigma–Aldrich (St. Louis, MO, USA), and (±)-2-methylmethcathinone hydrochloride (2-MMC HCl), (±)-3-methylmethcathinone hydrochloride (3-MMC HCl), (±)-4-methylmethcathinone hydrochloride (4-MMC HCl,) and (±)-4-methyl-N-ethylcathinone hydrochloride (4-MEC HCl) were purchased from LGC Standards (Luckenwalde, Germany). The structures of these compounds are shown in Figure S1.

ACN, methanol (MeOH) and isopropanol (IPA) of HPLC grade were purchased from J. T. Baker (Deventer, The Netherlands). Reagent-grade diethylamine (DEA) from Scharlau (Barcelona, Spain) and formic acid (FA) from Honeywell (Augsburg, Germany) were used as mobile-phase additives. Analytical-grade ammonium acetate (NH_4Ac) was supplied by Sigma–Aldrich and the acetic acid used to adjust the pH of the mobile phases was from J. T. Baker. Ultrapure water for the preparation of the mobile phase was obtained using a water purification system (Veolia, Sant Cugat del Vallès, Spain).

Individual stock standard solutions (1 mg/mL) were prepared in MeOH. Working solutions of each compound (20 µg/mL) were prepared by diluting the stock solution with mobile phase. All stock and working solutions were protected from light and stored in a refrigerator at 4°C.

2.2. LC instrument and chromatographic conditions

All the analyses were performed with an Agilent 1100 series LC system (Agilent Technologies, Waldbronn, Germany) equipped with a degasser, a binary pump, a temperature controller and a diode array detector. Chemstation software (Agilent Technologies) was used for data acquisition and data handling. All the analyses were performed at 30°C under isocratic conditions at a flow rate of 0.1 mL/min. UV data were collected at 220 nm or 254 nm. The injection volume was 5 µL.

The Lux Cellulose-1 column (150 x 4.6 mm, 3 µm) from Phenomenex (Torrance, CA, USA) was used in PO mode. MeOH and ACN were tested as the main components of the mobile phase. In addition, the effect of the concentration of IPA as organic modifier and the addition of FA and/or DEA as acidic/basic additives in the mobile phase were investigated. The optimum mobile phase was ACN/IPA/FA/DEA (90/10/0.1/0.1, v/v/v/v).

The Chiralpak CBH column (150 x 2 mm, 5 µm) from Chiral Technologies (Exton, PA, USA) was used in RP mode with a mobile phase consisting of a mixture of aqueous buffer and an organic modifier. The effects of the organic modifier, mobile phase pH and buffer concentration were evaluated. The satisfactory enantioseparations with appropriate retention times were achieved with the mobile phase consisting of 5 mM NH_4Ac aqueous solution (pH=6.4)/MeOH (95/5, v/v).

3. Results and Discussion

Two different columns (Lux Cellulose-1 and Chiralpak CBH) were evaluated using two different elution modes (PO mode and RP mode) to separate enantiomers of 8 drugs. Various LC methods for the enantioseparation of β-blockers (atenolol, propranolol and metoprolol) and omeprazole have been reported,[3,17-19] as the different pharmacological effects between their enantiomers are well known. However, the LC enantioseparations of cathinones have been less studied because of their novelty. Silva et al.[20] have reviewed the methods developed for cathinones enantioseparation, but in most cases the mobile phase systems are incompatible with MS detection. In the previous studies on chiral separation of cathinones, the Lux Cellulose-1 column was only used in NP mode,[21] and the Chiralpak CBH column was only used for chiral separation of 4-MMC.[22]

3.1. Optimization of chiral separation with the Lux Cellulose-1 column

The Lux Cellulose-1 column contains cellulose tris(3,5-dimethylphenylcarbamate) as the chiral selector, which is coated on silica gel. ~~In the Lux Cellulose-1 column, the polar carbamate group is known to be the most important adsorbing site for chiral recognition, since it can interact with the enantiomers via hydrogen bonding and dipole-dipole interaction using C=O and NH groups. In addition, as all the target analytes have phenyl ring groups, the analytes may provide an additional stabilizing effect to the analyte-CSPs complex through the insertion of the aromatic ring into the chiral cavity of the CSPs.~~[23] In the Lux Cellulose-1 column, the derivatized glucose units are regularly arranged along the helical axis with the substituents creating a helical groove. The polar carbamate groups, which are known to be the most important adsorbing site for chiral recognition, are located inside the chiral grooves, while the hydrophobic aromatic moieties are located outside the polymer chain.[23] Enantiomers are discriminated by enantioselective inclusion into chiral cavities which maybe mediated via formation of hydrogen-bonding and dipole–dipole interactions with C=O or NH of the carbamate groups as well as via steric and π–π interactions with the phenyl rings.[21,24] In our case, functional groups of OH and NH for β-blockers, S=O and NH for omeprazole and NH and C=O for cathinones, could be contributed to the interaction with the carbamate groups on CSP, resulting in chiral recognition. In addition, all the target analytes have phenyl ring groups providing an additional stabilizing effect to the analyte-CSPs complex.

In the present study the Lux Cellulose-1 column was used in PO mode, which is compatible with MS detection. The optimization of the mobile phase was conducted according to previous studies.[23,24,25] Following the literature, MeOH and ACN were tested as the main components of the mobile phase. The effect of the organic modifier (IPA), acidic additives (FA) and basic additives (DEA) were evaluated to find the optimal composition of the mobile phase. The initial flow rate was set at 0.6 mL/min. However, retention times were quite short and no analytes showed any chiral separation. Since some studies have reported successful enantioseparations of a wide variety of analytes at very low flow rates (e.g. 0.075, 0.08 and 0.1 mL/min),[22,26,27] this was reduced to 0.1 mL/min for all the analyses.

Nonethless, with the initial mobile phase for method development – 100% ACN – no analytes showed any chiral separation, and β-blockers and omeprazole did not elute as a well-defined peak during the analysis time (60 min).

3.1.1. Effect of organic modifier concentration

The alcoholic modifier is traditionally considered to be the elective modulator of the eluotropic power because it competes with the analyte for the hydrogen bonding sites of the CSPs, and thus modifies retention and enantioselectivity.[28] For this reason, the effects of the addition of 10% IPA and 20% IPA to the mobile phase were evaluated. As expected, when the IPA content increased, the retention times of the target analytes decreased, but no improvement in resolution was observed.

3.1.2. Effect of acidic and/or basic additives

Acidic and basic additives are often used to minimize peak broadening and improve enantioresolution by minimizing non-specific retention.[29,30] The influence of acidic additives on enantioseparation was investigated in the mobile phase by adding 0.1% FA to ACN with different ratios of IPA (0%, 10% and 20%). The presence of FA in the mobile phase led to a significant decrease in retention time for all the analytes. However, no enantioseparation was observed.

In order to evaluate the influence of basic additives on enantioseparation, instead of 0.1% FA, the same concentration of DEA was added to the mobile phase. 4-MMC was partially separated (R_s=1.1) with the mobile phase of ACN/DEA (100/0.1, v/v). In the case of propranolol, baseline separation (R_s=1.3 or 1.5) was achieved after the addition of IPA (either 10% or 20%) to the mobile phase, while a very broad single peak was obtained without IPA in the mobile phase. No enantioseparation was observed for the other 6 compounds.

The results showed that no satisfactory separations were achieved with the presence of either FA or DEA in the mobile phase. In some cases the simultaneous presence of both a basic and an acidic additive in the mobile phase improved the enantioseparation.[24,29,25,30] Therefore, the influence of a combination of 0.1% FA and 0.1% DEA on enantioseparation was also studied in the mobile phase of ACN with different ratios of IPA. The results of enantioseparation are shown in Table 1. It was observed that β-blockers and omeprazole were separated with the simultaneous presence of both 0.1% FA and 0.1% DEA in 100% ACN, while enantiomers of cathinones remained unresolved. In most cases, by increasing the IPA concentration in the mobile phase, the retention time and resolution decreased and the peaks of the enantiomers became narrower. According to the results (Table 1), ACN/IPA/FA/DEA (90/10/0.1/0.1, v/v/v/v) was found to provide the best combination between a satisfactory resolution, short retention time and good peak shapes, although the enantiomers of the cathinones were not separated.

3.1.3. Selection of the main solvent

Instead of ACN, MeOH as the main solvent was also tested with the addition of different ratios of IPA (0%, 10% and 20%) and 0.1% of both FA and DEA. No better results were obtained. A maximum of 2 analytes were separated during the optimization process for the MeOH-based mobile phase. Therefore the optimum composition of the mobile phase for the enantioseparation of the target drugs using the Lux Cellulose-1 column was ACN/IPA/FA/DEA (90/10/0.1/0.1, v/v/v/v) (Table 1). With this mobile phase, the enantiomers of 4 out of 8 analytes were separated; however, none of the studied cathinones was enantioseparated.

3.2. Optimization of chiral separation with the Chiralpak CBH column

The Chiralpak CBH column contains a protein cellobiohydrolase (CBH) as the chiral selector, which is immobilized on 5 µm particles of silica gel. As a single protein may contain a variety of chiral centers and different binding sites, the chiral recognition on the column is capable of complex mechanisms involving ion exchange, hydrogen bonding and hydrophobic interactions.[22] Particularly, CBH is effective for the enantiomer separation of basic drugs, in which the key chiral recognition mechanism has been attributed to the ion-exchange between the carboxylic acid residues on the protein and basic analytes.[31] This column is used in RP mode with a mobile phase consisting of a mixture of aqueous buffer and an organic modifier so it can easily be coupled with MS detection. ~~CBH is effective for the enantiomer separation of basic drugs.[16] The key chiral recognition mechanism of CBH has been attributed to hydrophobic interactions, hydrogen bonding and ion-exchange.[25]~~In order to achieve the best enantioseparation for the target analytes, the following parameters were optimized in this study: type and concentration of organic modifier (IPA, ACN and MeOH), mobile phase pH (6.4 and 5.0) and buffer concentration (1-20 mM NH_4Ac).

3.2.1. Effect of organic modifier concentration

Based on previous studies,[25,26,27] initially 1 mM NH_4Ac in water was chosen as the aqueous phase. In the first instance, the organic modifier concentration of the mobile phase was optimized. The IPA concentrations of 2%, 5% and 10% were evaluated, and the results are shown in Table 2. With the mobile phase composed of 1 mM NH_4Ac aqueous/IPA (98/2, v/v), the enantiomers of 6 drugs were separated within 40 min with R_s ranging from 1.4 for 4-MEC to 17.7 for atenolol. In fact metoprolol was separated, but the second enantiomer was eluted after 75 min. In the case of propranolol, no peak was eluted within the analysis time. On increasing the organic content from 2% to 5%, retention time decreased, as did enantioresolution, for all the analytes. Meanwhile, retention time increased and resolution decreased for certain analytes on increasing the organic content from 5% to 10%. A mobile phase consisting of 5% organic modifier was chosen because it provided the shortest retention times with acceptable resolutions for most of the analytes.

Table 1. Enantioseparation results obtained on Lux Cellulose-1 column with presence of both 0.1% FA and 0.1% DEA in ACN based mobile phase.

	ACN/FA/DEA (100/0.1/0.1,v/v/v)				ACN/IPA/FA/DEA (90/10/0.1/0.1,v/v/v/v)				ACN/IPA/FA/DEA (80/20/0.1/0.1,v/v/v/v)			
Compound	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s
Atenolol	41.05	52.01	1.33	3.6	21.67	26.02	1.32	2.9	14.31	16.50	1.35	2.2
Propranolol	26.65	33.77	1.38	6.0	16.83	22.50	1.66	7.5	12.72	16.92	1.91	8.3
Metoprolol	16.92	28.13	2.30	12.8	12.80	18.70	2.30	10.2	10.46	14.06	2.90	8.9
Omeprazole	15.01	15.55	1.08	1.3	12.69	13.01	1.08	1.0	11.44	-	-	-
2-MMC	13.42	-	-	-	11.92	-	-	-	10.56	-	-	-
3-MMC	13.86	-	-	-	11.98	-	-	-	10.98	-	-	-
4-MMC	14.35	-	-	-	12.03	-	-	-	10.13	-	-	-
4-MEC	12.54	-	-	-	10.85	-	-	-	9.66	-	-	-

Table 2. Enantioseparation results obtained on Chiralpak CBH column with mobile phase consisting of 1 mM NH_4Ac buffer and different ratio of IPA (2%, 5%, 10%).

	1 mM NH_4Ac aqueous/IPA (98/2, v/v)				1 mM NH_4Ac aqueous/IPA (95/5, v/v)				1 mM NH_4Ac aqueous/IPA (90/10, v/v)			
Compound	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s	t_1 (min)	t_2 (min)	α	R_s
Atenolol	21.97	39.79	2.00	17.7	19.58	35.27	2.01	17.0	21.82	40.44	2.05	18.7
Propranolol	-	-	-	-	-.	-	-	-	-	-	-	-
Metoprolol	39.86	> 75	-	-	31.34	> 75	-	-	31.31	> 75	-	-
Omeprazole	14.77	18.47	1.33	4.3	10.84	13.15	1.31	3.3	7.78	8.74	1.26	2.1
2-MMC	19.38	25.88	1.43	4.8	17.45	22.68	1.39	4.5	20.40	25.67	1.32	4.0
3-MMC	21.08	24.73	1.22	3.8	18.40	21.11	1.19	3.3	22.34	25.10	1.15	2.9
4-MMC	20.81	23.52	1.16	2.9	18.18	20.34	1.15	2.6	22.35	25.07	1.15	2.9
4-MEC	20.44	21.51	1.06	1.4	17.01	17.80	1.06	1.1	21.60	22.64	1.06	1.3

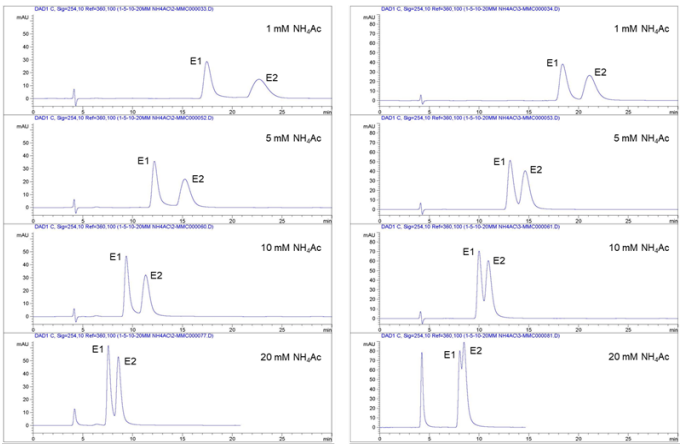

Figure 1. Effect of buffer concentration on enantioseparation using Chiralpak CBH column. (a): 2-MMC; (b): 3-MMC.

3.2.2. Effect of buffer concentration with 5% IPA

The effect of the buffer concentration on separation was studied by varying the concentration of NH_4Ac with 5% IPA in the mobile phase. Figure 1 presents the chromatograms of the trend of the enantioseparation of 2-MMC and 3-MMC, which show that a higher buffer concentration resulted in shorter retention times, lower enantioresolutions, and narrower peaks. As a compromise, a concentration of 5 mM was selected as the optimal buffer concentration.

3.2.3. Effect of mobile phase pH

The buffer pH can affect not only the dissociation/protonation of analytes but also the ionization of functional groups of the chiral selector or even of the residual silanol groups of the silica gel support, thus providing a variety of possible stereoselective interactions.[3932] The effect of pH was evaluated using a mobile phase consisting of 5 mM NH_4Ac buffer and 5% IPA. A solution of 5 mM NH_4Ac in water had a pH of 6.4 and this same solution was adjusted to pH 5 with acetic acid. When the pH was reduced from 6.4 to 5.0, the retention times and enantiomeric resolution for all analytes significantly decreased except for propranolol and omeprazole. For example, R_s for atenolol decreased from 14.7 to 1.8 and t_2 reduced from 22.65 min to 5.27 min when the mobile phase pH decreased from 6.4 to 5.0. In the case of propranolol, the use of a mobile phase at pH 5 led to baseline separation (R_s=26.9) within 60 min, while at pH 6.4 no peak was eluted in that time. In spite of this, since the R_s decreased for the rest of the analytes on decreasing the pH of the mobile phase from 6.4 to 5.0, a pH of 6.4 was chosen.

3.2.4. Selection of organic modifier type

The effect of organic modifier type was also evaluated. MeOH and ACN were used instead of IPA, and the results are shown in Table S1. With the replacement of IPA by ACN or MeOH, the R_s for all the compounds decreased (but they were still larger than 1.9), except for 2-MMC and 3-MMC. In the case of 2-MMC and 3-MMC, the replacement of IPA by ACN or MeOH gave rise to a significant increase in resolution: R_s increased from 3.6 to 6.3 and 5.6 for 2-MMC respectively, and from 1.9 to 4.5 and 4.0 for 3-MMC respectively (Figure S2). No significant differences in R_s were found between MeOH and ACN, but MeOH provided shorter retention times for most of the analytes. Even though the enantiomers of 4-MEC were partially separated with IPA, the resolution was quite poor (R_s=0.8). Taking these results into account, 5% MeOH was selected as a compromise. Castrignanò et al. [22] also reported that better chiral recognition was achieved for 56 drugs using MeOH rather than ACN or IPA with the Chiralpak CBH column.

The effect of the buffer concentration was also confirmed when MeOH was used as the organic modifier in a similar trend as when IPA was used: by increasing the buffer concentration in the mobile phase, retention time and resolution decreased in most cases (Table S2). Finally, an aqueous solution of 5 mM NH_4Ac (pH=6.4)

with 5% MeOH was chosen as a compromise. With the optimized mobile phase, 6 out of 8 target drugs were baseline separated within 45 min, and the chromatograms are shown in Figure 2. The enantioseparation of propranolol was not resolved because very strong interactions between propranolol and the CBH column resulted in very long retention times (>120min).

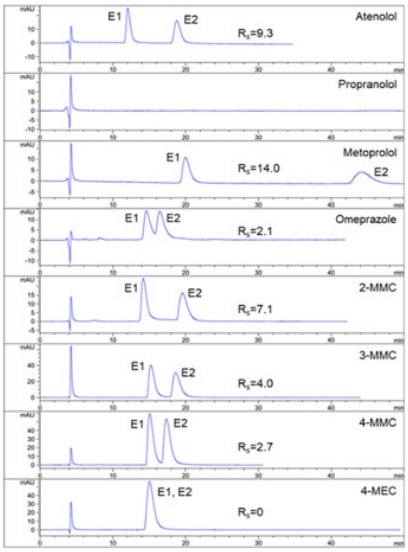

Figure 2. Chromatograms of the eight chiral drugs on Chiralpak CBH column with the mobile phase of 5mM NH_4Ac buffer (pH 6.4)/MeOH (95/5, v/v).

3.3. Comparison of the Lux Cellulose-1 and Chiralpak CBH column performances

According to the results obtained, atenolol, metoprolol and omeprazole were enantioseparated in both columns. However, the Chiralpak CBH column provided better chiral separation for these target analytes than the Lux Cellulose-1 column. For example, R_s for atenolol enantiomers was 9.3 in the case of the Chiralpak CBH column and only 2.9 in the case of the Lux Cellulose-1. In addition, 2-MMC, 3-MMC and 4-MMC showed no enantioseparations in the Lux Cellulose-1 column, whereas they were enantioseparated with the Chiralpak CBH. Experiments carried out with the Lux Cellulose-1 column only showed higher enantioselectivity for propranolol. In summary, the Chiralpak CBH column in RP mode provided better enantioseparation for the target analytes than the Lux Cellulose-1 column in PO mode. The more successful chiral separation on the Lux Cellulose-1 column might be achieved in NP mode but this possibility was not examined in the present study because NP mode is not compatible with MS detection. Moreover, in the previously reported method[22] on the chiral separation of 4-MMC using CBH column, a similar resolution (R_s= 1.4) was achieved, however the suitability for other cathinones was not tested.

The Lux Cellulose-1 and Chiralpak CBH columns were used in PO and RP mode respectively. Using PO mode offers several advantages, including enhanced solubility of polar analytes, favorable peak shape and more compatibility with the solvent from previous extractions. However, the mobile phases have large eluent strengths leading to shorter retention times (i.e. limited interaction with the CSPs), and therefore the success rate of enantioseparation is lower in this mode.[9] RP conditions are especially useful for determining the enantiomeric ratios of drugs in biological matrices[3,33] because they are aqueous and can be directly injected. Moreover, this involves the use of less costly solvents.

The elution order of atenolol and propranolol enantiomers was studied on two columns by spiking racemic standard solution with R-(+)-enantiomer. In the case of atenolol, the same elution order was obtained on both columns with the R-(+) enantiomer eluted before the S-(−)-enantiomer, which indicated that the R-(+) enantiomer binds to the two CSPs with lower affinity than its S-(−)-enantiomer. In the case of propranolol, the R-(+)-enantiomer also eluted before the S-(−)-enantiomer on the Lux Cellulose-1 column. As propranolol was not separated on the Chiralpak CBH column with the optimal mobile phase, its elution order was not determined on this column.

4. Conclusions

In this paper, the recognition ability of two chiral columns (Lux Cellulose-1 and Chiralpak CBH, which are polysaccharide and protein-based respectively) towards the enantiomers of eight chiral drugs was studied in PO elution mode and RP elution mode respectively.

With the Lux Cellulose-1 column, by increasing the concentration of organic modifier, the retention time and enantioresolution decreased. The addition of 0.1% FA or 0.1% DEA alone did not bring about as much improvement as a combination of both. As a result, the optimized mobile phase was ACN/IPA/FA/DEA (90/10/0.1/0.1, v/v/v/v). With this mobile phase, β-blockers were baseline separated and omeprazole was partially separated, while cathinones were not separated.

With the Chiralpak CBH column, by increasing the concentration of organic modifier and buffer solution, the retention time and enantioresolution decreased. The decrease of mobile phase pH led to a significant reduction in retention time and enantioresolution. The optimized mobile phase was 5 mM NH_4Ac aqueous solution (pH=6.4)/MeOH (95/5, v/v). With this mobile phase, the enantiomers of 6 out of 8 studied drugs were baseline separated.

In summary, the Chiralpak CBH column in RP mode was more effective for the target compounds than the Lux Cellulose-1 in PO mode. In addition, since the solvents and additives used are MS compatible, both chiral LC methods developed in this study can be directly coupled with an MS detector for the enantiomeric determination of chiral drugs at low concentration.

Acknowledgements

The authors would like to thank the Ministerio de Economía, Industria y Competitividad, the Agencia Estatal de Investigación and the European Regional Development Fund (ERDF) (Project CTQ2017-88548-P) for the financial support received.

Supporting information

Supplementary data associated with this article can be found in the online version of this article at the publisher's website.

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Graphical Abstract