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STUDY OF THE SEPARATION CONDITIONS OF THE RACEMIC IBUPROFEN ON HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT: Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID), also known for its antipyretic and analgesic significant properties. This drug is commercialized on its racemic structure, R-(-)-ibuprofen and S-(+)-ibuprofen, however only S-(+)-ibuprofen has the clinical activities. This paper refers to the study of the enantiomers separation conditions, and theirs eluition order on the high performance liquid chromatography, using a chiral stationary phase, cellulose tris(3,5-dimethylphenylcarbamate).

KEYWORDS: ibuprofen, chiral separation, chromatographic parameters, HPLC.

1. INTRODUCTION

The (RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid, known as ibuprofen, is a non-steroidal anti-inflammatory drug (NSAID). This chiral drug was introduced to the market in 1969 at United Kingdom to replace others NSAIDs that caused severe gastrointestinal irritation and intolerance in the body (Palma *et al.*, 2009). The ibuprofen structure is shown in the Figure 1 (Chen *et al.*, 1990).

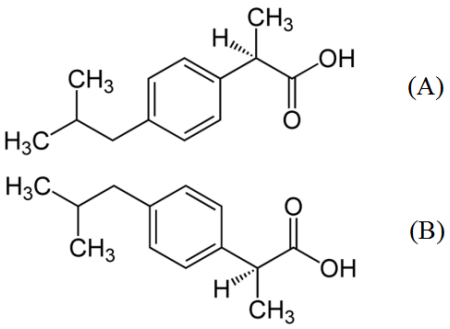

Figure 1. Ibuprofen molecular structure: (A) R-(-)-ibuprofen; (B) S-(+)-ibuprofen.

This drug is administered in the racemic structure, except in some countries, such as Switzerland and Austria, where only the S-(+)-ibuprofen is used (Valderrama and Poppi, 2011). This enantiomer is a prostaglandin and thromboxane inhibitor (Yoon *et al.*, 2008). The treatment using S-(+)-ibuprofen, when compared to the racemic mixture, demonstrates better clinical efficiency, less variability in the therapeutic effects and less toxicity, besides, it is possible to reduce the administered drug. The R-(-)-ibuprofen structure does not exhibits pharmacologic action, however, the drug goes through a chiral inversion in the human body, this way it causes toxicity by the formation of hybrids triglycerides (Yoon *et al.*, 2008).

Pure ibuprofen enantiomer can be obtained by high performance liquid chromatography (HPLC). In this technique, the separation and purification of R-(-) and S-(+)-ibuprofen can be done in analytical scale for clinical purposes, while in preparative scale the goal is the pure drug production in large scale. Chromatography is a method based on differential migration of the compounds through the chromatographic column. In the enantiomers separation that exhibits the same physic and chemical properties very close (as it happens with the racemic mixture of ibuprofen), it is necessary to work with a chiral selector to promote different energies in the adsorption phenomenon between the analytes and the stationary phase in the column (Meyer, 2004).

This work aims to establish an appropriate mobile and stationary phases for the separation of racemic ibuprofen. In this case the

chromatographic parameters were used as references.

1.1. High performance liquid chromatography

Enantiomers cannot be separated by conventional methods, such as distillation and extraction, since these techniques not have any mechanism of chiral recognition. The high performance liquid chromatography (HPLC) can provide this mechanism of recognition by chiral stationary phase (CSP) (Meyer, 2004). Usually the CSP is made of a material with chiral properties that is coated or chemically bonded onto a silica support. The capacity of chromatographic separation is influenced by particle size, porosity, surface area, flow rate, and column dimension. The CSP based on polysaccharides are the most used to resolve enantiomers (Unger *et al*., 2005).

Another important factor in chromatography is the mobile phase, where the affinity difference of analytes with the phases (mobile and CSP) contributes to compound different migration velocities (Lanças, 2009). The mobile phase choice is an essential point in liquid chromatography, which will influence directly in the separation selectivity, resolution, retention time, and racemate solubility (Francotte, 2001).

1.2. Chromatographic parameters

The main chromatographic parameters, selectivity, resolution and number of theoretical plates are influenced by several variables, such as fluid dynamics, mass transfer and thermodynamics. The interaction of each adsorbed compound in the stationary phase is proportional to the time each enantiomer remains retained in the chromatographic column (Meyer, 2004).

In the chromatographic approach, the selectivity (α) can be describe as the ratio between the retention factor (k_i) of the more and the less retained compound. For the chromatographic separation system the minimum value required of selectivity is 1.2, which can be calculated it by Equation 1 (Meyer, 2004; Schulte and Epping, 2005):

$$\alpha = \frac{k_j}{k_i} = \frac{t_{Rj} - t_M}{t_{Ri} - t_M} \qquad (1)$$

where t_M is the hold-up time, t_{Ri} and t_{Rj} are the times of less and more retained compound.

Resolution (R_s) evaluates the separation quality. R_s is obtained from ratio between the distance that separates the maximum points of the chromatographic peaks and the width at half height ($w_{h/2i}$ and $w_{h/2j}$) of the compounds to be separated, according to the Equation 2 (Collins, 2006).

$$R_s = 1{,}177 \frac{(t_{Rj} - t_{Ri})}{w_{h/2i} + w_{h/2j}} \qquad (2)$$

Resolution values equal 1.0 indicates that the peaks are separated with 2% of overlap. At a resolution of 1.25 the separation is good enough for qualitative ends, and at a resolution of 1.50 or more there is a complete separation (Schulte and Epping, 2005; Collins, 2006).

The separation efficiency in chromatographic columns can also be estimated by the number of theoretical plates (N_i). A theoretical plate is related to an equilibrium stage of the solute between the stationary and mobile phases. In this way, the higher the number of theoretical plates higher will be the column efficiency, and consequently, better the separation. The value of N_i is determined by Equation 3 (Collins, 2006).

$$N_i = 5{,}545 \left(\frac{t'_{R_i}}{w_{h/2i}} \right)^2 \qquad (3)$$

with: $t'_{R_i} = t_{R_i} - t_M$.

For the column efficiency evaluation values of theoretical plates above 2000 are acceptable in the drugs resolution (Dantus and Wells, 2005).

2. MATERIALS AND METHODS

The experiments were performed in HPLC equipment that consists of a controller (model CBM-20A), a UV-vis detector (SPD-20A), and two pumps (LC-10AD, LC-20AT), purchased from Shimadzu (Japan). To the identification of the more and less retained compound it was used a circular dichroism, from Jasco (Brazil), model CD-

2095plus. The column and mobile phase were temperature-controlled using a Quimis circulation water bath, model Q-214m2 (Brazil).

In the present work, two columns were used, both polysaccharides, packed with chiral stationary phase cellulose tris(3,5-dimethylphenylcarbamate). Columns packed with this stationary phase are called columns OD. The first column used OD, made of stainless steel, 150 mm of length and 4.6 mm of inner diameter. The packed silica particles in the stationary phase were 20 µm in average diameter, coated with CSP. The second column was the OD Lux 5u Cellulose-1 purchased from Phenomenex, made of stainless steel, average particle diameter of 5 µm and 250 mm of length and 4.6 mm of inner diameter.

The solvents employed as mobile phase were n-hexane, isopropyl alcohol, ethanol, and n-butanol, all of them of HPLC grade, purchased from TEDIA® (USA). The ion-pairing reagent, trifluoroacetic acid (TFA), 99%, purchased from Sigma-Aldrich (USA), and it was used in 0.1% proportion above the total volume of the mobile phase. As inert compound, in order to obtain the hold-up volume of the columns, the 1,3,5-tri-tert-butylbenzene (TTBB), 97%. The racemic mixture of ibuprofen (98% purity) was acquired from Sigma-Aldrich (USA). The ibuprofen injections responses were determined by the resulting chromatogram from the UV-vis detector, monitored at 254 nm. The values of retention time and peak base width were used to determine the chromatographic parameters: selectivity (α), calculated by Equation 1; resolution (R_s) by Equation 2, and number of theoretical plates (N_i) by Equation 3. In the runs were injected a volume of 20 µL of racemic ibuprofen at a dilution of 0.5 g L, diluted in the tested mobile phase, at a flow rate of 1 mL min.

3. RESULTS AND DISCUSSION

3.1 Column OD 20 µm (150 x 4.6 mm)

This subsection provides studies in the chromatographic column (150 mm x 4.6 mm) packed with particles coated with a 20 µm cellulose tris(3,5-dimethylphenylcarbamate). The first step was the separation using 100% n-hexane to further ibuprofen to interact with the CSP. The TFA was employed to obtain good resolution for chromatographic peaks. However, in this initial test, the compounds remained retained for a long time and there was not significant separation. After the initial test for the mobile phase, it was chose a binary mixture. The study was based on mixture of n-hexane and an alcohol, besides the TFA additive. The alcohols used were ethanol, isopropanol and n-butanol. The injections results of recemic ibuprofen 0.5 g L^{-1} at 25 °C are shown in Table 1 which still represents the evaluation of chromatographic parameters when the type of alcohol is changed.

Table 1. Chromatographic parameters on column OD 20µm and 150 mm length.

Mobile phase	α	R_s	N_i	N_j
n-hexane (99%) ethanol (1%)	1.16	-	-	-
n-hexane (99%) isopropanol (1%)	1.22	-	-	-
n-hexane (99%) n-butanol (1%)	1.24	0.79	442	433

It can be seen from Table 1 that only the mobile phase employing ethanol did not reach the required selectivity value (1.2) for chromatographic separation. The resolutions and the number of theoretical plates for mobile phases composited of ethanol or isopropanol were not calculated because there was not significant separation. The resolution of the system used n-butanol in the mobile phase was calculated, however, the value found did not reach the required minimum of 1.50. Moreover, it was noted that the values for the number of theoretical plates for both compounds, more retained (N_j) and for the less retained (N_i), are lower than reference value of 2000, reflecting the lower efficiency of the column for the study concerned.

In order to obtain the separation in question, it was carried out a study of temperature influence. The mobile phase chosen was composed of 99% n-hexane and 1% n-butanol, and the additive TFA due to this phase showed the best evidence of separation from the others two phases tested. The study consisted of performing chromatographic separations in the temperatures of 15, 20, 25, 30, and 35 °C. Table 2 shows the results.

Table 2. Effect of temperature on separation selectivity and resolution of racemic ibuprofen.

Temperature	α	R_s	N_i	N_j
15 °C	1.21	-	-	-
20 °C	1.23	-	-	-
25 °C	1.24	0.79	442	433
30 °C	1.24	0.91	436	471
35°C	1.26	1.05	509	504

It can be seen in Table 2, the values for selectivity in all tests are presented greater than 1.2. Values of resolutions and numbers of theoretical plates for separation at 15 °C and 20 °C were not calculated. This it was due to the inability to determine the values of the widths of the peaks at half height, in view of poor quality of the separation. Resolution values at 25 °C, 30 °C and 35 °C are less than 1.50. Moreover, it was found that increasing the temperature in the experimental tests, provides an increasing in the values of such parameters.

3.2 Column OD 5 µm (250 x 4.6 mm)

There was no satisfactory resolution using the OD (150 x 4,6 mm) column, packed with 20 µm particles. In order to increase the separation efficiency, therefore it was used another column with longer in effective length and packed with smaller particles when compared with previous column. The new column was a Lux 5µ Cellulose-1, tby Phenomenex, packed with 5 µm particles. This OD column was coated with cellulose tris(3,5-dimethylphenylcarbamate). The main objective when using this column was to offer a larger surface area of contact between enantiomers and CSP, this way it is possible increase the adsorption of the drug in the chiral selector.

The definition of the mobile phase started with the choose of two from three mobile phases present in Table 1. The mobile phases were the mixtures of n-hexane and isopropanol, n-hexane and n-butanol, both in the proportions 99:1, with the TFA addition. The most suitable temperature was 35 °C (see Table 2). Table 3 shows the results.

All parameters values present on this table are above the levels required for effective chromatographic separation (α > 1.20, R_s > 1.50 and N_i > 2000).

Table 3. Chromatographic parameters on column OD 5µm and 250 mm length.

Mobile phase	α	R_s	N_i	N_j
n-hexane (99%) isopropanol (1%)	1.22	3.37	6196	6759
n-hexane (99%) n-butanol (1%)	1.25	3.82	6259	6842

When compared Tables 1 and 3, it is possible to observed that selectivity value remains practically the same for both columns. The resolution and number of theoretical plates values are higher than that ones from OD 20 µm/150 mm length column. Thus, it was noted that with column length increasing and particle size decreasing there is column efficiency increasing. The mobile phase using isopropanol is the most appropriate, because this alcohol is more volatile than n-butanol, contributing to the recovery after enantiomeric separation.

3.3 Elution Order

With the separation conditions set out in section 3.3, it was determined the enantiomers elution order by detector circular dichroism. Figure 2 shows separation chromatograms.

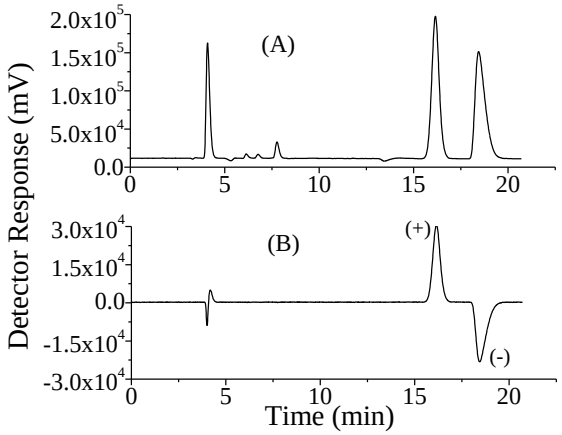

Figure 2. Chromatogram of racemic ibuprofen separation:
(A) UV-vis; (B) circular dichroism.

The chromatogram from detector UV-vis (Figure 2A) allows the identification of the peaks of the enantiomers, which should have the same retention times when using circular dichroism detector, whose chromatogram is shown in Figure 2B, from which is possible to identify S and R enantiomers. In Figure 2B, S-(+)-ibuprofen is the first enantiomer to elute, so is the least retained compound (compound i), whereas the compound more retained by the CSP is R-(-)-ibuprofen (component j).

4. CONCLUSION

The study was performed in an enantiomeric separation of racemic ibuprofen by HPLC, using a chiral stationary phase cellulose tris(3,5-dimethylphenylcarbamate). The OD column 20 µm (150 x 4.6 mm) packed with this CSP proved capable of enantioselectively interacting with racemate. However, only the chiral recognition was not enough for complete separation. For this purpose, alternatives were found to increase the efficiency of separation, in which the influence of the temperature was studied and the characteristics of the adsorption column. The solution found was to use the column Lux 5u Cellulose-1 (greater length and less particle diameter). The most suitable mobile phase was composed of 99% n-hexane, 1% isopropanol and additive TFA (0.1% solution). From these phases, it was found that S-(+)-ibuprofen is the least retained and the compound R-(-)-ibuprofen is the most retained by the CSP.

With those results, it is possible to do thermodynamic study in analytical chromatographic columns to obtain adsorption isotherms. Furthermore, the assessment of the effects of mass transfer in semi-preparative columns in order to obtain both the S-(+) ibuprofen as R-(-)-ibuprofen of high purity, and in a larger scale compared to those ones from analytical column.

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