Thermodynamic study of the separation of racemic ibuprofen by chiral liquid chromatography

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ABSTRACT: The chiral drug (RS)-2-(4-(2-methylpropyl)phenyl) propanoic acid, known as ibuprofen, is an important non-steroidal anti-inflammatory drug, also notorious for its analgesic and antipyretic properties. This medicine is market in racemic form R-(-)-ibuprofen and S-(+)-ibuprofen, however, in some countries only the second enantiomer is applied. This paper refers to the study of the thermodynamic parameters relative for separation of the racemic mixture in analytical chiral chromatographic columns packed with cellulose tris (3,5-dimethylphenylcarbamate). In this analysis, the enthalpy (ΔH^0), entropy (ΔS^0), enthalpy difference ($\Delta\Delta H^0$), entropy difference ($\Delta\Delta S^0$), and isoenantioselective temperature (T_{iso}) was determined by van't Hoff approach.

KEYWORDS: ibuprofen, thermodynamic parameters, chiral separation, adsorption and HPLC

1.INTRODUCTION

Ibuprofen was commercially introduced as a non-steroidal anti-inflammatory drug (NSAID) in the United Kingdom in 1969, and in the United States in 1974. This drug replaced others NSAIDs that caused gastrointestinal irritation and severe intolerance in the body (Palma *et al.*, 2009). The Figure 1 shows the ibuprofen chiral structure (Chen *et al.*, 1991).

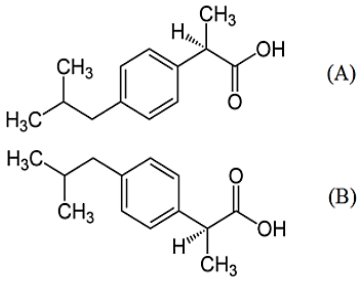

Figure 1 – Ibuprofen molecular structure: (A) R-(-)-ibuprofen; (B) S-(+)-ibuprofen.

Many countries administer the racemic structure instead of S-(+)-ibuprofen, except some places like Switzerland and Austria (Valderrama and Poppi, 2011). The S-(+)-ibuprofen is a prostaglandin and thromboxane inhibitor. This enantiomer demonstrates better clinical efficiency, less variability in therapeutic effects and toxicity, if compared with this racemic form. In addition, it is possible to reduce the amount of drug used. R-(-)-ibuprofen structure does not display pharmacological action. Moreover, it triggers a chiral inversion on human body causing toxicity by the formation of hybrid triglycerides (Yoon *et al.*, 2008).

High performance liquid chromatography (HPLC) can be used to obtain R-(-)-ibuprofen and S-(+)-ibuprofen in their pure forms, when the system has a suitable device chiral recognition. In this technique, the differential migration of compounds through the chromatographic column promotes the resolution. Separations involving chiral compound in this method must employ a chiral selector on mobile phase or in the stationary phase to promote different energies in the adsorption between the analytes and the stationary phase. These energies are originated from the formation of diastereoisomeric molecules that they are spatially oriented due the intermolecular bonds, which attractive and repulsive forces are associated. Chiral recognition proposes that one enantiomer makes three simultaneous bonding with three different active site of chiral selector in which it happens a bonding of stereochemical feature, while the other enantiomer does not interact as a stereochemical (Lämmerhofer, 2010).

The analysis of thermodynamic parameters enables better understanding of the driving forces that guide this separation phenomenon (Lämmerhofer, 2010), and it also checks whether if chemisorption or physisorption occurs (Schulte and Epping, 2005). Furthermore, the study of both enthalpy and entropy changes can help in getting nonlinear isotherm parameters as demonstrated in Asnin *et al.* (2006).

This work aims to determine the thermodynamic parameters enthalpy, entropy, enthalpy difference, entropy difference, and isoenantioselective temperature, in analytical scale to provide information about ibuprofen separation by chiral liquid chromatography. The parameters chromatographic are determined by van't Hoff approach.

2. MATERIALS AND METHODS

2.1 Setting of separation and materials

Ferrari and Cremasco (2014) used high performance liquid chromatography to separate the racemic ibuprofen. In this separation, they employed a column packed with silica coated for the cellulose tris(3,5-dimethylphenylcarbamate). The mobile phase was composed for hexane and isopropyl alcohol in the ratio 99/1 and an additive, the trifluoroacetic acid (TFA). The authors observed that the R-(-)-ibuprofen is the most retained compound and that the S-(+)-ibuprofen is the less retained one.

The experiments were performed in HPLC equipment, that consists of controller (CBM-20A), UV-vis detector (SPD-20A), and pump (LC-20AT), originally purchased from Shimadzu (Japan). The column and mobile phase were temperature-controlled using Quimis circulation water bath, model Q-214m2 (Brazil).

The column Lux Cellulose-1 (250 x 4.6 mm), purchased from Phenomenex, was employed as stationary phase. Solvents hexane and isopropyl alcohol, both of HPLC grade

(TEDIA®, USA), were employed in mobile phase ratio 99/1. The additive reagent trifluoroacetic acid was used in 0.1% proportion at total volume of the mobile phase. The racemic ibuprofen and inert compound 1,3,5-tri-tert-butylbenzene (TTBB) were acquired from Sigma-Aldrich (USA). The system was operated at a flow rate of 1 ml/min and monitored at wavelength of 220 nm. Samples of ibuprofen (0.5 g/L) diluted in the mobile phase were injected at different temperatures to determine the retention factors.

2.2 Methods

To determine the thermodynamic parameters, it was adopted the chromatographic van't Hoff approach (Coym, 2010; Lämmerhofer, 2010; Nascimento *et al.*, 2012; Oliveira and Cremasco, 2013). In this method, the retention factor ($k = t'_R/t_0$, with $t'_R = t_R - t_0$) is correlated with temperature (T) to provide a linear relationship, where ΔH^0 and ΔS^0 can be obtained by slope and intercept from

$$\ell n(k) = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ell n(\phi) \tag{1}$$

with

$$\phi = \frac{1-\varepsilon}{\varepsilon} \tag{2}$$

Parameter ϕ represents the volume phase ratio between stationary and mobile phases (Jandera *et al.*, 1982; Mihlbachler *et al.*, 2002; Lämmerhofer, 2010). The total porosity (ε) was determined by first moment method (Schneider and Smith, 1968; Cremasco *et al.*, 2001).

$$t'_R = \frac{L}{v}\varepsilon \tag{3}$$

Equation 4 was used to determine the energy variation between the more and less retained compound, where a linear relationship between selectivity ($\alpha = k_j/k_i$) and temperature (T) is set (Lämmerhofer, 2010; Moreira *et al.*, 2013).

$$\ell n(\alpha) = -\frac{\Delta\Delta H^0}{RT} + \frac{\Delta\Delta S^0}{R} \tag{4}$$

Another important parameter is the isoenantioselective temperature (T_{iso}), also called isoeluotropic temperature. This temperature is that one which the chromatographic separation does not occur ($\alpha = 1$). Applying this condition in Equation 4, it is possible to obtain.

$$T_{iso} = \frac{\Delta\Delta H^0}{\Delta\Delta S^0} \tag{5}$$

3. RESULTS AND DISCUSSION

3.1Total porosity and phase ratio

The total porosity is an important parameter to determine isothermal adsorption and ratio phases. Total porosity was calculated by first moment method (Equation 3), where the graph of first moment of an unretained band profile method is shown in Figure 2. In this method, the inert compound TTBB is employed in chiral column and rate flow was changed from 0.2 up to 1.6 ml/min with step 0.2 ml/min.

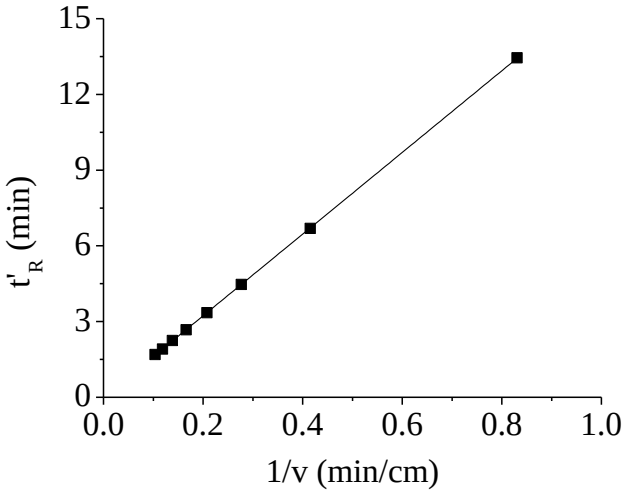

Figure 2 – Total porosity by first moment method.

The slope presented in Figure 2 provides the value of 0.647 for total porosity. The coefficient of determination was 0.999. The phase ratio, from Equation 2, is equal 0.545. Mihlbachler *et al.* (2002) found the value of 0.543 for a Chiralpak AD column, 20 μm particle-silica-based packing materials, coated with amylose tri (3,5-dimethylphenylcarbamate).

3.2Thermodynamic parameters

Thermodynamic parameters were investigated using a variation of the van't Hoff's method, where data $\ell n(k)$ *vs.* T^{-1} were linearized according to Equation 1, as shown in Figure 3. The temperatures chosen for this study were 15, 20, 25, 30, and 35 °C. This figure provides information about enthalpy difference for adsorption of the overall process (ΔH^0), while the intercept represents an entropy difference (ΔS^0). Table 1 shows the results from van't Hoff analysis.

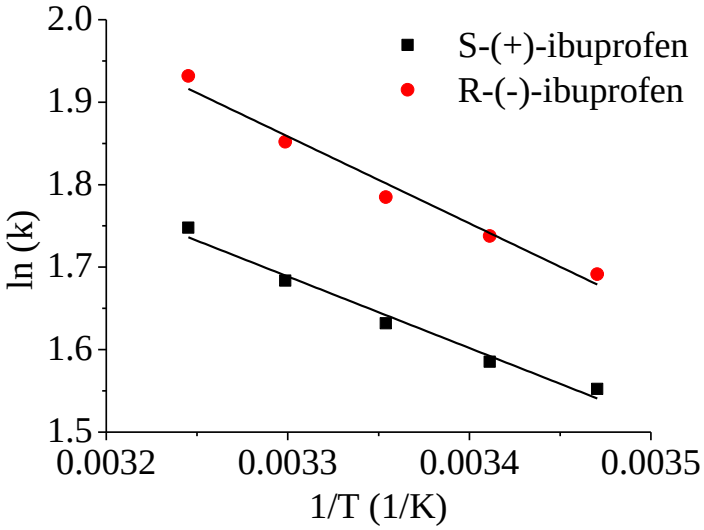

Figure 3 – Van't Hoff graphical in function of retention factor and temperature.

Table 1 – Thermodynamic parameters of the separation of racemic ibuprofen from Figure 3.

Compound	ΔH^0 (J/mol)	ΔS^0 (J/K mol)	R^2
S-(+)-ibuprofen	7212.01	42.89	0.981
R-(-)-ibuprofen	8758.95	49.40	0.979

According to Schulte and Epping (2005), the values of positive ΔH^0 suggest endothermic adsorption and values of ΔH^0 lower than 50000 J/mol characterize physical adsorption. The compound more retained, R-(-)-ibuprofen, binds more strongly in chiral stationary phase (CSP) since it has the largest value of enthalpy. Lämmerhofer (2010) affirms that in most instances van't Hoff plots reveal linear relationships (ΔH^0 invariant with T) with a strong preference for exothermic adsorption processes as it can be inferred from a negative sign of the derived ΔH^0 values, a situation that usually becomes evident in a decrease in retention with increasing temperature, what did not happened in this study. About the entropy, both enantiomers must be solvated identically in the mobile phase, and release the same number of solvent molecules when they are associated with the CSP. The S-(+)-ibuprofen has smaller values for ΔS^0 what indicates it may have fewer degrees of freedom on the CSP (it is held at more points or it is less able to move or rotate) (Péter *et al.*, 1998).

To better describe the separation thermodynamically, it was used a second approach in which Van't Hoff was plotted $\ell n(\alpha)$ *vs.* T^{-1} from Equation 4 as indicated in Figure 4. Data results and the isoenantioselective temperature (Figure 4) were summarized in the Table 2. This table presents the values of $\Delta\Delta H^0$ and $\Delta\Delta S^0$. T_{iso} found was below temperature used in study. This justifies the increasing selectivity with increasing temperature. If the separation occurs below T_{iso}, it could have happened a reversing in the order of compounds elution. Another important factor is that the entropic energy have more influence than enthalpy on the Gibbs energy in the temperature range studied ($\left|T\Delta\Delta S^0\right| > \left|\Delta\Delta H^0\right|$ as observed by Lämmerhofer (2010). Higher temperatures favor the separation; however, the maximum temperature supported by the CSP is 313.15 K.

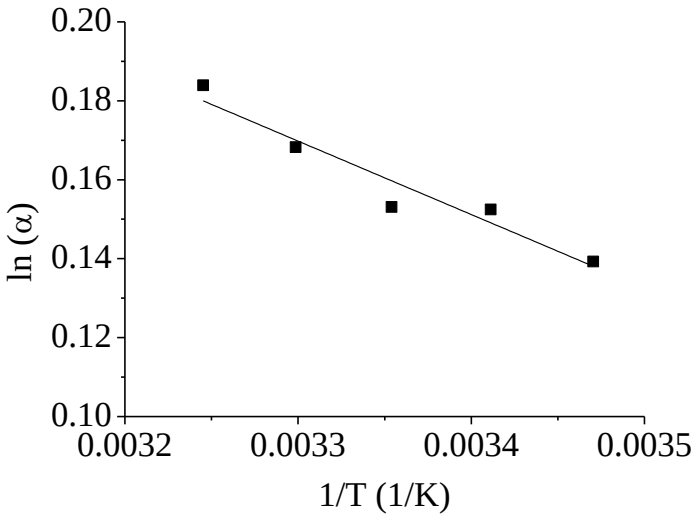

Figure 4 – Van't Hoff graphical in function of selectivity and temperature.

Table 2 – Thermodynamic parameters of the separation of racemic ibuprofen from Figure 4.

$\Delta\Delta H^0$ (J/mol)	$\Delta\Delta S^0$ (J/K mol)	R^2	T_{iso} (K)
1546.88	6.52	0.936	237.81

4. CONCLUSIONS

The results obtained for the separation of racemic ibuprofen in columns packed with cellulose tris (3,5-dimethylphenylcarbamate) demonstrated a phenomenon of endothermic physisorption. The total porosity and ratio phase was determined. As expected, the R-(-)-ibuprofen binds more energetically CSP and S-(+)-ibuprofen has a lower degree of freedom. With this work is possible conclude that the isoenantioselective temperature was below the working temperature. At higher temperature the separation is favored. Besides it, the enthalpy and mainly entropy influenced the phenomenon of adsorption in the temperature range studied.

Symbols

ΔG^0= standard Gibbs energy (J/mol)

ΔH^0 = standard enthalpy (J/mol)

ΔS^0= standard entropy (J/K mol)

ΔS^*= standard entropy change under the influence of phase ratio (J/K mol)

$\Delta\Delta G^0$ = standard Gibbs energy between compound more and less retained (J/mol)

$\Delta\Delta H^0$ = standard enthalpy energy between compound more and less retained (J/mol)

$\Delta\Delta S^0$= standard entropy energy between compound more and less retained (J/mol)

k = retention factor

L= column length (cm)

R= universal gas constant (8.3144 J/mol K)

t_0= dead time of the column (min)

t_R = retention time plus dead time (min)
t'_R = retention time (min)
T = absolute temperature (K)

T_{iso} = isoenantioselective temperature (K)

v = superficial velocity (cm/min)

Greek letters

α = selectivity

ε = total porosity

ϕ = phase ratio

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