Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Thermodynamic study of the enantiomeric resolution of flurbiprofen by HPLC using Chiralpak AD-RH column

H. Y. Aboul-Enein and I. Ali

The chiral resolution of (±)-flurbiprofen was achieved using water-acetonitrile (60 : 40, v/v) containing 0.1% acetic acid on a Chiralpak AD-RH column at 20 °C. The enantio-resolution was studied with different percentages of acetonitrile. Thermodynamic parameters (enthalpy, entropy and free energy) were calculated by carrying out the enantio-resolution experiments at 0 to 60 °C. The enantio-resolution was found to be exothermic in nature. Attempts have been made to explain the mechanism of chiral resolution of flurbiprofen on the Chiralpak AD-RH column.

1. Introduction

High performance liquid chromatography (HPLC) has become the most important tool in analytical science since 1980 [1, 2]. In the past two decades, HPLC has been used frequently for enantiomeric resolution of different molecules [1–10]. Various chiral stationary phases have been developed for this purpose. The important chiral selectors include Pirkle types [8, 11], derivatized linear or helical [cellulose or amylose] polysaccharides [1, 12, 13], cyclodextrin and its derivatives [14–16], protein phases [6], chiral crown ethers [17–19], macrocyclic antibiotics [20–22], ligand exchange [23] and other chiral compounds [24, 25]. Among these chiral stationary phases, polysaccharide based chiral stationary phases [CSPs] are very effective and efficient for chiral resolution of a wide variety of racemates [1, 13, 14]. The exact mechanism of chiral resolution on the polysaccharide based CSPs is not known [13, 14, 26, 27]. In the literature, thermodynamic studies have been used to describe the mechanism of the chiral selectors [2, 28]. This paper describes the determination of thermodynamic parameters of the enantiomeric resolution of flurbiprofen, a commonly used non-steroidal anti-inflammatory agent at different temperatures using Chiralpak AD-RH (polysaccharide based CSP) column. Attempts have been made to explain the enantio-resolution of this drug under the reported HPLC conditions.

2. Investigations, results and discussion

The chromatographic parameters, retention factor (k), separation factor (α) and resolution factor (Rs) for the resolved enantiomers of (±)-flurbiprofen, using water-acetonitrile [60 : 40] containing 0.1% acetic acid as mobile phases at 0, 10, 20, 30, 40, 50 and 60 °C, were calculated and are presented in Table 1. A typical chromatogram of the resolved enantiomers is shown in Fig. 1. The order of elution was confirmed by running the chromatograms of the optically pure enantiomers under identical chromato-

Table 1: Retention (k), separation (α) and resolution (R_s) factors for the enantiomeric resolution of flurbiprofen on Chiralpak AD-RH column at different temperatures

Temp. (°C)	k_1	k_2	α	Rs
0	19.75	26.75	1.35	1.10
10	18.40	23.42	1.27	1.23
20	17.50	22.50	1.29	1.80
30	16.50	21.00	1.27	1.33
40	14.80	18.40	1.24	1.14
50	13.20	16.10	1.22	1.12
60	11.90	14.10	1.19	0.88

Mobile phase: water-acetonitrile (60 : 40, v/v), containing 0.1% acetic acid, Flow rate: 0.5 ml/min, Detection: UV at 254 nm, (−)-enantiomer eluted first followed by (+)-enantiomer in all the experients

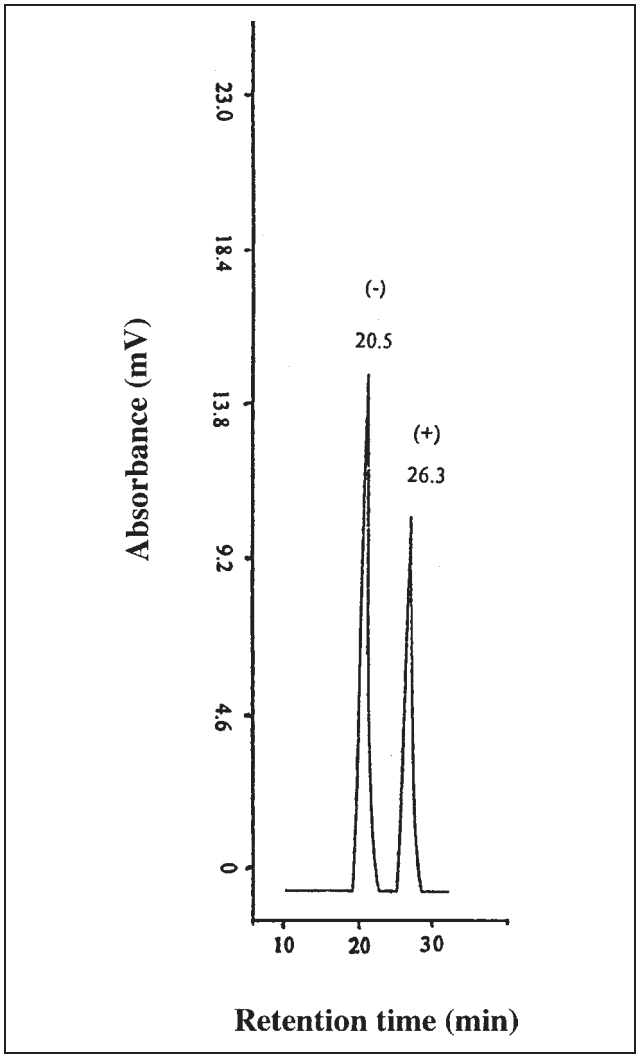

Fig. 1: The chromatograms of the resolved enantiomers of flurbiprofen on Chiralpak AD-RH column using water-acetonitrile (60 : 40,v/v) containing 0.1% acetic acid at 0.5 ml/min flow rate and 20 °C

graphic conditions. It has been observed that the R-(−)-enantiomer eluted first followed by the S-(+)-enantiomer under all the experimental conditions.

It has been observed that the peaks were broad when using water at a concentration of 70% or higher while the resolution was poor when using 50% or lower concentrations of water. The peak broadening at higher water concentrations or lower acetonitrile concentrations may be due to strong bondings between enantiomers and CSP. Therefore, water-acetonitrile (60 : 40, v/v) was selected as the best mobile phase. The effect of pH on the resolution was also studied. The enantio-separation was carried out in the pH range 2 to 6 and it was observed that the resolution was almost constant from pH 2 to 3.5 and then started to decrease. Moreover, the peaks were broad at pH 2, 2.5 and 3. For this reason pH 3.5 was selected as the best one and used throughout the study. It has also been observed that the retention factor increased with the decrease of acetonitrile ratio in the mobile phase. The values of the retention factors were also decreased at higher temperature (Table 1) indicating the exothermic nature of the interactions between the drug and CSP under the reported conditions of HPLC.

The chiral recognition mechanism at a molecular level on the polysaccharide based CSPs is still unclear although it has been reported that the chiral resolution by these CSPs is achieved through the different hydrogen, π-π and dipole induced dipole interactions between the chiral stationary phase and the enantiomers [29–31]. The structure of flurbiprofen contains electronegative atoms (oxygen and fluorine) along with two aromatic rings. Therefore, the resolution of the enantiomers of this drug is due to the different hydrogen, dipole-dipole and π-π interactions between the CSPs and the enantiomers. The enantiomers fit stereogenically into the chiral grooves of the stationary phase which is stabilized by these interactions of different magnitude and hence the resolution of enantiomers occurs. Steric effect may also plays a crucial role in the chiral resolution of this drug.

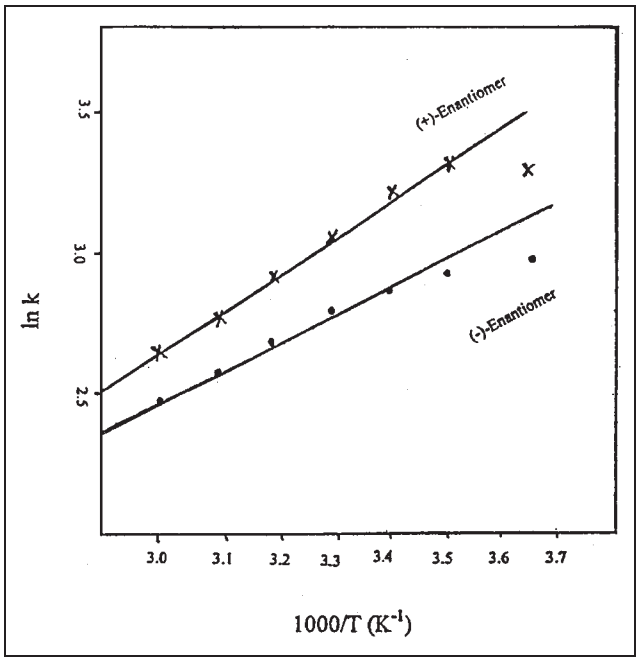

Fig. 2: Representative van't Hoff plots for the chiral resolution of flurbiprofen (between ln k and reciprocal of absolute temp.) using water-acetonitrile (60 : 40, v/v) containing 01% acetic acid as the mobile phase at 20 °C

The enthalpy (ΔH) and entropy (ΔS) for the enantiomers-CSP interactions were calculated from the retention data at different temperatures using van't Hoff relation as given below.

$$\ln k = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln \varphi \quad (1)$$

where, R and φ are gas constant and the mobile phase ratio respectively. Typical graphs of ln k vs 1/T using water-acetonitrile (60 : 40, v/v) at different temperature are shown in Fig. 2. The values of enthalpy and entropy were obtained from the slope and intercept respectively. At lower temperatures (0 and 10 °C) the graphs were non-linear, which may be due to the presence of the amylose phase in glass transition phase (at lower temperature). However, the poor resolution at higher temperature may be due to the folding or change in conformation of amylose CSP.

To understand the change in thermodynamic parameters as a function of acetonitrile ratio in the mobile phase, the enthalpies and entropies were plotted against the percentage of acetonitrile in Figs. 3 and 4 respectively. Fig. 3 and 4 indicate that the values of ΔH and ΔS of R-(−) and S-(+) enantiomers become closer at higher concentration of acetonitrile, which explains the poor enantio-resolution. This sort of behavior may be explained by water and acetonitrile chemistry. At lower concentrations of acetonitrile, the molecules of acetonitrile enter the water cavities up to a certain concentration. Above that, acetonitrile undergoes self association [32]. At higher acetonitrile concentrations the solute gets solvated with acetonitrile resulting in a lower retention and poor or no resolution.

Whenever a solute is introduced into a phase, a hole or cavity is formed into the phases which is an endothermic process [2]. The cavity formation in the mobile phase is

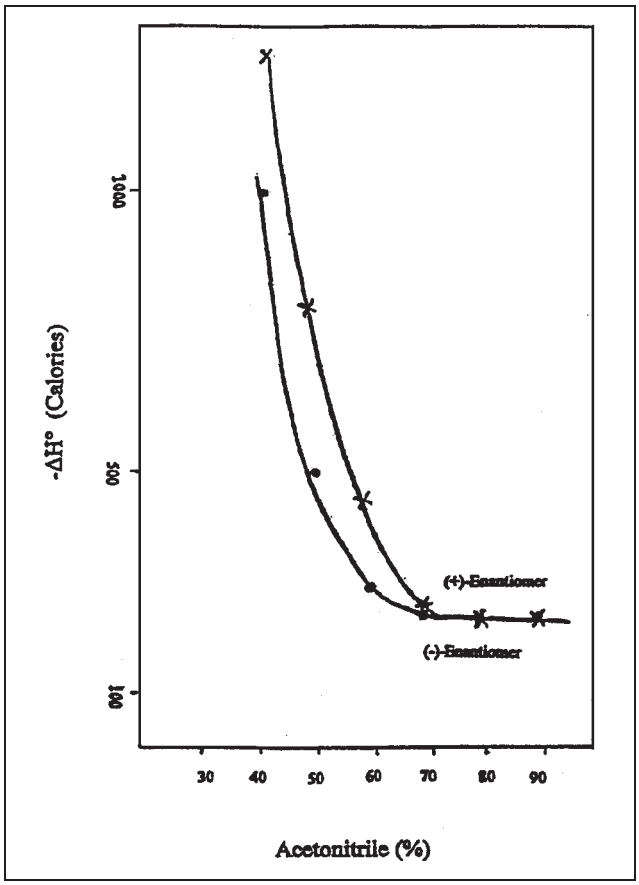

Fig. 3: The relationship between enthalpies (ΔH°) of transfer and the percentage of acetonitrile for the chiral resolution of flurbiprofen

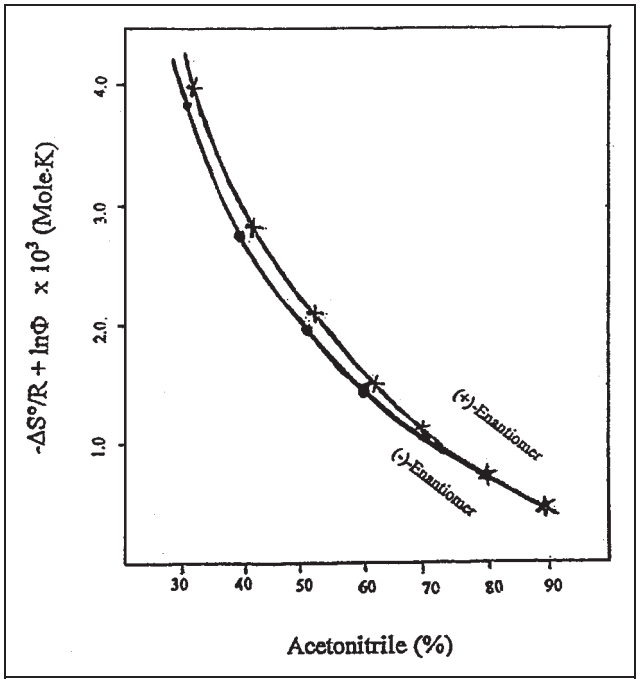

Fig. 4: The relationship between entropic term ($\Delta S^\circ/R + \ln \varphi$) and the percentage of acetonitrile for the chiral resolution of flurbiprofen

much larger than in the stationary phase because several interactions such as hydrogen bonding, π-π, dipole induced dipole etc. are stronger in the mobile phase than the stationary phase in the reversed phase mode. Therefore, the analyte prefers to remain in the mobile phase rather than to bind to the stationary phase. It may be concluded that the cavity formation in the mobile phase is favored by higher concentration of acetonitrile which reduces the chances of the interaction of enantiomers with the stationary phase. The poor interactions of enantiomers with the stationary phase resulted in lower retention and poor resolution. The higher capacity of cavity formation at higher acetonitrile concentrations may be due to cluster formation.

The free energy of the enantiomer-CSP interaction was calculated using the following equation.

$$\Delta G^\circ = -RT \ln k \qquad (2)$$

where, R, T and k are the gas constant, absolute temperature and retention factor. The values of enthalpy, entropy and free energy of the enantiomer-CSP interaction at different ratios of acetonitrile are given in Table 2. The change in enthalpy is greater than in entropy indicating that enthalpy is responsible for the enantio-separation. Table 2 shows that the differences of enthalpy and free energy of the enantiomers are lower at lower concentrations of acetonitrile while the differences of entropy are higher at 60, 70 80 and 90% acetonitrile respectively which are thus responsible for the poor enantio-resolution at these acetonitrile concentrations.

Table 2: Enthalpy, entropy and free energy of enantio-resolution of flurbiprofen on Chiralpak AD-RH coulmn using different ratios of water and acetonitrile at 20 °C

Acetonitrile	Water	Thermodynamic properties (J/mol)		
	(ml)	$-\Delta\Delta H^\circ$	$-\Delta\Delta TS^\circ$	$-\Delta\Delta G^\circ$
30	70	3352	255	571
40	60	1257	319	482
50	50	1047	383	405
60	40	419	415	287
70	30	96	447	201
80	20	57	479	179
90	10	48	957	99

Other experimental conditions as in Table 1.

In conclusion, this study indicates that water-acetonitrile (60 : 40, v/v) containing 0.1% acetic acid is the best mobile phase for the resolution of flurbiprofen enantiomers on Chiralpak AD-RH column at 20 °C. The chiral resolution occurs due to the distribution of the enantiomers between the cavities of the mobile and the stationary phases. The enantiomers are retained in the chiral cavities of stationary phase by hydrogen, π-π and dipole induced dipole interactions. The phenomenon of cavity formation in the mobile phase is essential to influence the resolution of (±)-flurbiprofen enantiomers. Since cavity formation is constant in the stationary phase while it varies in the mobile phase, depending upon the concentration of acetonitrile accordingly the cavity formation in the mobile phase is favored by higher percentage of acetonitrile. The differences of the values of enthalpy and free energy become closer at higher concentrations of acetonitrile which are responsible for poor or no enantio-resolution. It has also been observed that the enantio-resolution is exothermic in nature.

3. Experimental

3.1. Chemicals and reagents

The racemic mixture and optically pure enantiomers of flurbiprofen were kindly donated by BASF, plc, Nottingham, UK. The stock solution of flurbiprofen (0.01 mg/ml) was prepared in ethanol. Ethanol of HPLC grade was obtained from Merck, Darmstadt, Germany. Acetonitrile and acetic acid of HPLC grade were purchased from Fisher Scientific, USA and Sigma Chem. Co., St. Louis, MO, USA respectively.

3.2. Chromatographic conditions

Solutions (20 µl) were injected into the HPLC system consisting of a Waters solvent delivery pump (model 510), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484) and Waters integrator (model 740). The experiments were carried out at 0, 10, 20, 30, 40, 50 and 60 °C. Temperature from 20 to 60 °C was controlled by a column heater module (Waters, Milford, USA) while a home made ice bath was used to control temperatures from 0 to 20 °C. The order of elution of the enantiomers was confirmed by running the chromatograms of the individual enantiomers of flubiprofen i.e. S-(+) and R-(−)flurbiprofen under identical chromatographic conditions. The column used was Chiralpak AD-RH (15 cm × 0.46 cm) [Amylose tris 3,5-dimethylphenylcarbamate] and was obtained from Daicel Chemical Industries, Tokyo, Japan. The mobile phases used were different ratios of water and acetonitrile (70 : 30, 60 : 40, 50 : 50, 40 : 60, 30 : 70, 20 : 80 and 10 : 90, v/v). The pH was adjusted with acetic acid. The mobile phases were filtered and degassed before use. The flow rate of the mobile phase was 0.5 ml/min. The chart speed was kept constant at 0.1 cm/min. Detection was carried out at 254 nm. The chromatographic parameters such as retention factor (k), separation factor (α) and resolution factor (Rs) were calculated using standard equations.

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Professor Hassan Y. Aboul-Enein
Pharmaceutical Analysis Laboratory
Biological and Medical
Research Department (MBC-03-65)
King Faisal Specialist
Hospital and Research Center
P.O. Box 3354
Riyadh-11211
Saudi Arabia
enein@kfshrc.edu.sa