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RESEARCH ARTICLE

The Influence of Some Parameters on Chiral Separation of Ibuprofen by High-Performance Liquid Chromatography and Capillary Electrophoresis

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Objective: The aim of the study was to compare the influence of mobile phase composition and temperature on chiral separation of racemic ibuprofen by capillary electrophoresis and high performance liquid chromatography with UV detection. Materials and methods: Racemic ibuprofen was analysed on a chiral OVM column with an HPLC system 1100 Agilent Technologies, under isocratic elution, by using potassium dihydrogen phosphate 20 mM and ethanol in mobile phase. The flow rate was set at 1 mL/min, UV detector at 220 nm and different column temperatures were tested. For electrophoresis separation an Agilent CE G1600AX Capillary Electrophoresis System system, with UV detection, was used. The electrophoresis analysis was performed at different pH values and temperatures, with phosphate buffer 25 mM and methyl-β-cyclodextrin as chiral selector. Results: The chromatograhic analysis reveals a high influence of mobile phase pH on ibuprofen enantiomers separation. An elution with a mixture of potassium dihydrogen phosphate 20 mM pH=3 and ethanol, at 25ºC, allowed enantiomers separation with good resolution in less than 8 min. Conclusions: The proposed HPLC method proved suitable for the separation of ibuprofen enantiomers with a good resolution, but the capillary electrophoresis tested parameters did not allow chiral discrimination.

Keywords: ibuprofen, enantiomers, HPLC, CE, chirality

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Introduction

Ibuprofen or (*R,S*)-2-(4-isobutylphenyl)propanoic acid is an anti-inflammatory agent with chiral structure (Figure 1). The two enantiomers have different biological behavior, the S(+)-enantiomer being the eutomer, but R(-)-ibuprofen is partially converted to form S(+) in biological environment [1,2]. There were many attempts to isolate the eutomer from racemate or to find a specific chemical synthesis of single enantiomer. The high costs of processes and their difficulties, together with the partial biological conversion of the inactive enantiomer explain why ibuprofen is used as racemase in drugs.

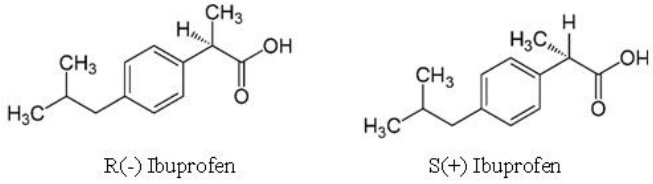

Fig.1. Ibuprofen enantiomers

There are many published methods regarding ibuprofen chiral resolution, mainly made by chromatographic methods [3-11] and few by capillary electrophoresis [12-16]. In general, the methods are designed to analyze enantiomers in biological environment for different purposes such as enantiomers conversion, differences regarding their biological behavior etc.

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Ovomucoid is a chiral chromatographic support suitable for HPLC separation of many active pharmaceutical ingredients from different pharmacological classes [17-19]. Capillary electrophoresis is today a powerful chiral separation technique with many applications in enantiomers separation of drugs.

The aim of the present work is to propose a new application of ovomucoid chiral stationary phase for HPLC separation of ibuprofen enantiomers and to test the ability of methyl-β-cyclodextrin to discriminate the same enantiomers by capillary electrophoresis.

Methods

HPLC analysis

Apparatus: The HPLC analysis was performed on HPLC system 1100 Agilent Technologies, using an Ultron ES OVM column, 150x4.6 mm, 5um (Shinwa Chemical Industries LTD., Agilent Technologies).

Materials and methods: Ibuprofen racemic working standard from Societa Italina Medicinali Scandicci, Italy, was used. For HPLC analysis, the following reagents and solvents were necessary: ethanol and methanol for chromatography, potassium dihydrogen phosphate (Merck products), and purified water obtained from a purifying system Direct Q5 (Millipore).

Methods: The stock racemic ibuprofen solution was prepared in methanol at a concentration of 0.1 % (m/V). The stock solution was further diluted in order to obtain the working solution of 10 μg/mL. The solutions were stored at 8°C to avoid any potential degradation. The mobile phase consisted of 90% potassium dihydrogen phosphate

buffer 20 mM and 10% ethanol, and delivered at a flow rate of 1 mL/min. The samples were injected in volumes of 5 µl and the analytes were detected at 220 nm.

Capillary electrophoresis separation

Apparatus: Capillary electrophoresis separation was performed using a HP AGILENT 3D CE G1600AX system with UV detection, glass capillary with optic window 8 mm, 65 cm x 75µm (PolymicroTehnology, Phoenix, AZ, USA).

Materials: ibuprofen racemic working standard, methanol (Merck for liquid chromatography), ultrapure water (18.2 MΩ·cm) disodium phosphate, monosodium phosphate, sodium borate hydrate, methylbetacyclodextrine (CycloLab).

Methods: The ibuprofen solution of 0.5 mM in methanol:water 1:1 was injected (100 µL), under 50 mbar pressure for 2 seconds, with an applied current of 20 kV. For system preconditioning, the capillary was washed with NaOH 1 M solution for 30 minutes, with water for 15 minutes, then with buffer solution (phosphate buffer 25 mM) for 5 minutes. The cyclodextrine solutions were prepared in phosphate buffer 10 mM.

Results and discussions

HPLC analysis

The ovomucoid chiral column contains a glycoprotein present in eggs with large chiral recognition ability. The recommended pH analysis domain is 3-7.5 with phosphate buffer at concentration of 20 mM. The maximum allowed proportion of organic solvent in mobile phase composition is 50% and the best resolution is provided with the flow rate of 0.8-1.2 ml/min. Acid compounds retention is maximum at isoelectric pH of analytes.

The concentration of the test solution, 10 µg/ml, is correlated with the plasmatic concentration of ibuprofen after its oral administration in low to high doses [20].

Acetonitrile, a powerful elution solvent, was not suitable for ibuprofen retention, even at high composition of aqueous phase. Because the differences between ethanol and methanol in mobile phase were not so significant and considering its lower toxicity, ethanol was used as organic modifier of the mobile phase.

The influence of pH on enantiomers separation was performed using different pH values for mobile phase: pH=3, pH=4.7, pH=6.1, with a proportion of aqueous phase of 90% and the column temperature maintained at 25°C. The best resolution of separation was obtained using a mobile phase pH=4.7, with a resolution of 2.46, but the retention times for ibuprofen enantiomers were high: tR1 = 19.80 min and tR2 = 23.49 min (Figure 2). At higher pH of 6.1, the separation was not possible (Figure 3); the low pH of 3 allowed the enantiomer separation with a very good resolution in less than 8 min (Figure 4, upper image).

As it is well known, the temperature is an important parameter of enantiomer separation, thermodinamic processes of chemical compounds retention and separation being strongly influenced by this parameter. In this case, we carried out the chromatographic separation at the optimum mobile phase pH (pH=3.00) at different column temperatures (20°C, 25°C, 30°C and 35°C). The other chromatographic conditions were the same as previously described (Figure 4).

In accordance to our results, an acid pH of 3 for aqueous mobile phase was an optimum value for a compromise between good separation and adequate retention time.

As it can be seen from Figure 4 and Table I, we can conclude that a higher column temperature did not lead to a better resolution of enantiomers separation and a column temperature similar with the room temperature is an optimum value.

Thus, we can conclude that the mobile phase consisting in phosphate buffer 20 mM, pH 3 and ethanol (90:10),

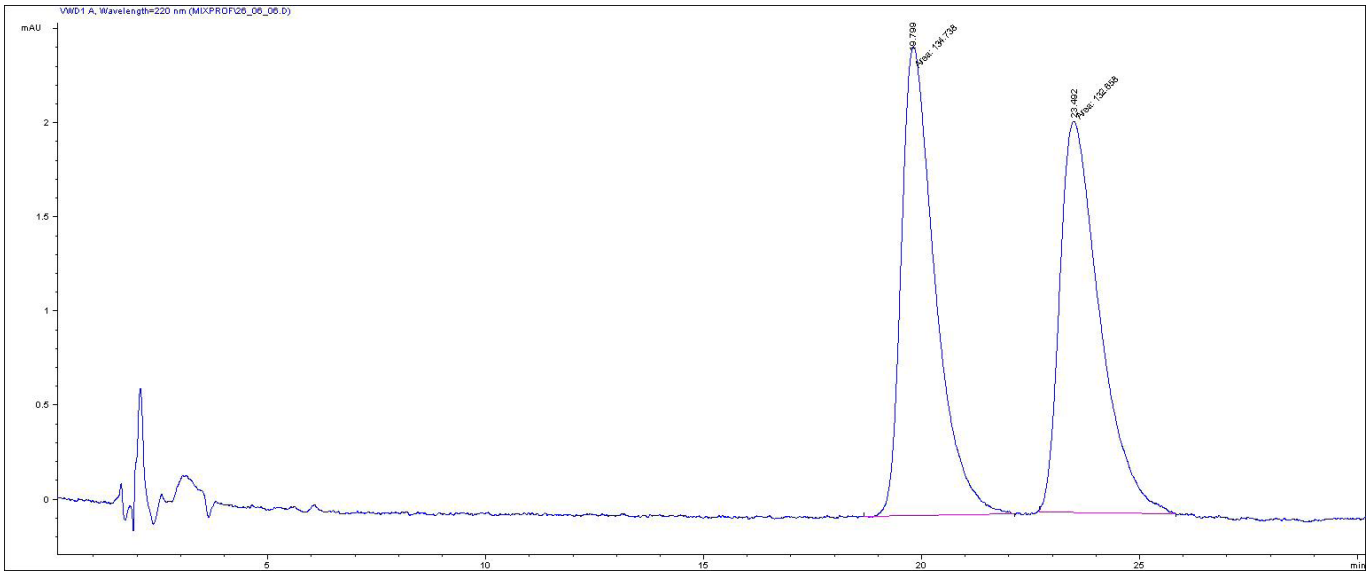

Fig. 2. Ibuprofen enantiomers separation with mobile phase A - KH2PO4 20 mM (pH=4.7), B - ethanol, 90%A:10%B, flow rate 1 ml/min, detection at 220 nm, column

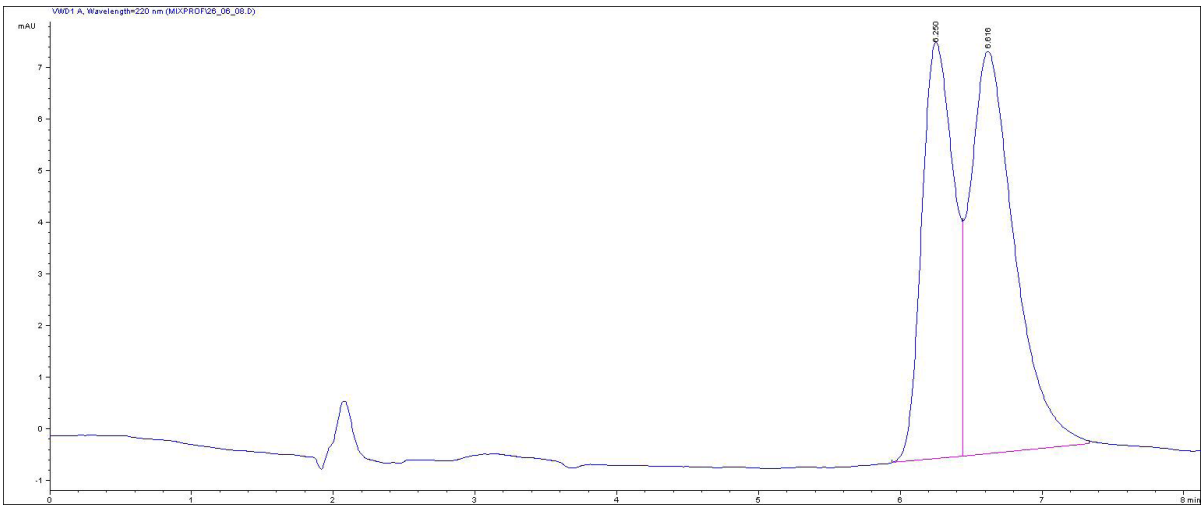

Fig. 3. Ibuprofen enantiomers separation with mobile phase A - KH2PO4 20 mM (pH=6.1), B - ethanol, 90%A:10%B, flow rate 1 ml/min, detection at 220 nm, column

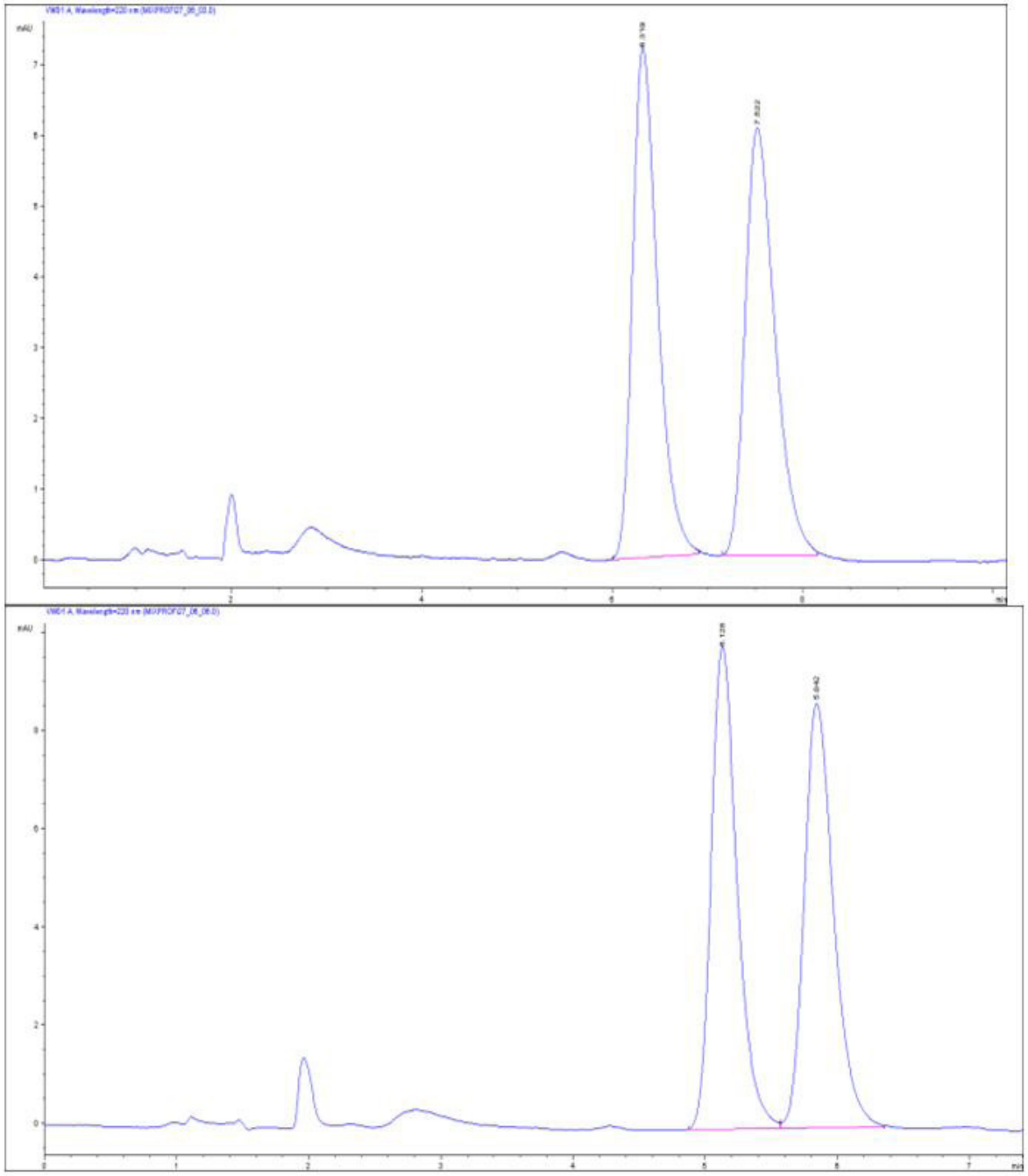

Fig. 4. Ibuprofen enantiomers separation with mobile phase A - KH2PO4 20 mM (pH=3.0), B - ethanol, 90%A:10%B, flow rate 1

Table I. The influence of temperature on chromatographic parameters of ibuprofen enantiomers

Parameters	Temperature			
	20°C	25°C	30°C	35°C
tR1, min	6.81	6.32	5.69	5.13
tR2, min	8.25	7.52	6.64	5.84
Rs	2.45	2.33	2.20	1.93

delivered at a flow rate of 1 ml/min, with a column temperature around room temperature and detection at 220 nm, are the separation conditions required for optimal resolution and detection of ibuprofen enantiomers. The separation process revealed first the R enantiomer and secondly the S enantiomer.

Capillary electrophoresis

The influence of buffer pH, temperature, cyclodextrine concentration and the nature of buffer was tested. Evaluation of different pH values for the buffer solution at room temperature has revealed a peak characteristic for ibuprofen racemate at pH=6.8. In this case, a lower pH of the buffer solution did not improve the separation. Furthermore, different temperatures of separation, 20°C, 25°C, 30°C, were tested. Unfortunately, no tendency of separation was noticed (Figure 5). Other tests were performed using different cyclodextrine concentrations (15 mM, 10 mM and 5 mM), and we observed a minimum of retention at 10 mM without separation of enantiomers. By replacing the phosphate buffer with borate buffer, the separation was not achieved.

Conclusions

The proposed HPLC chiral method is suitable for the separation of ibuprofen enantiomers. Further tests are necessary to conclude that the proposed method is suitable for chiral separation of ibuprofen in biological samples (i.e. plasma samples). None of the attempts regarding chiral capillary electrophoresis separation was successful.

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Conflict of interest

None to declare.

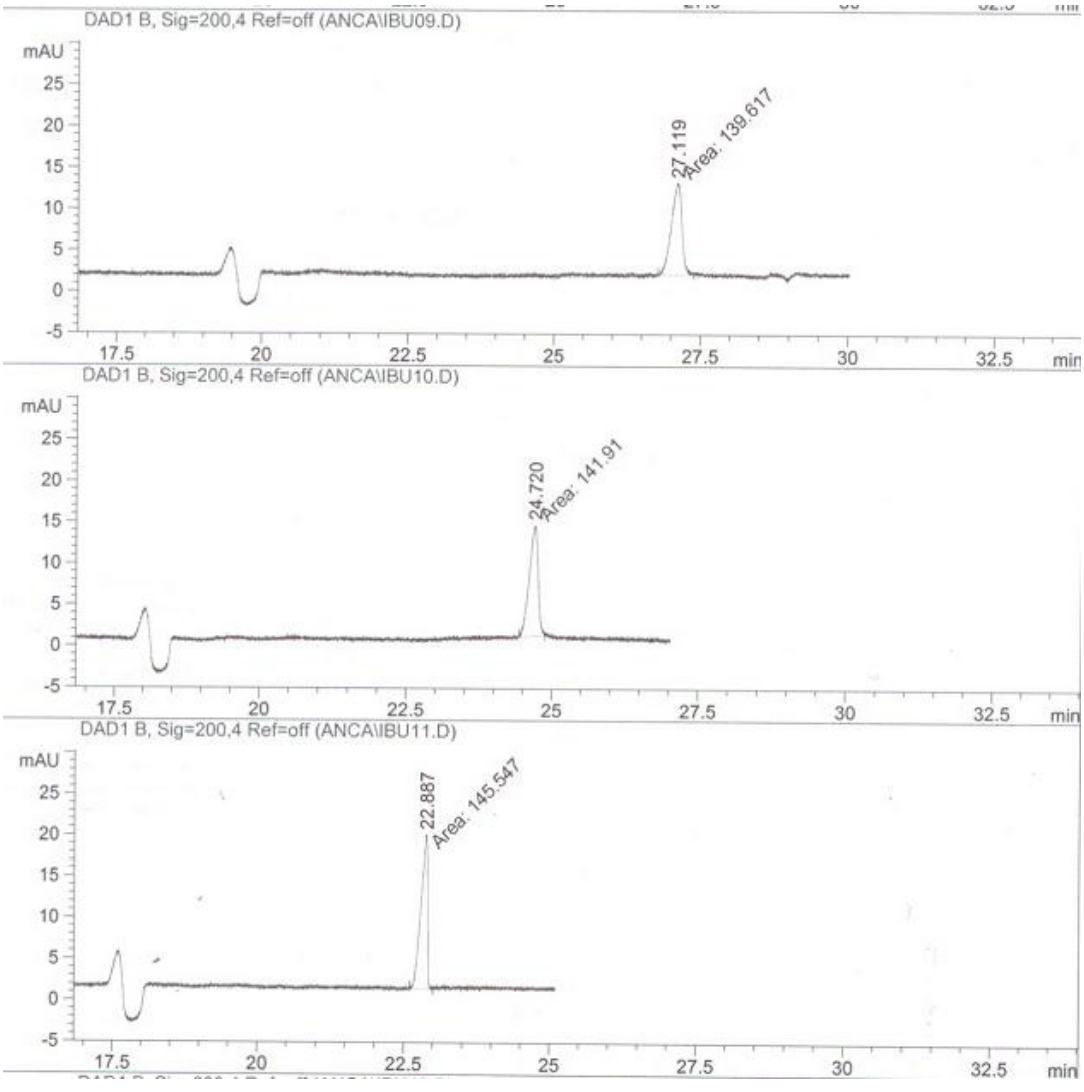

Fig 5. Ibuprofen electropherogram with phosphate buffer pH=6.8, methylbetacyclodextrine 10 mM, at 20C (up), 25C (middle), 30C (down)

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