Influence of Mobile Phase Composition on the Preparative Separation of Profens by Chiral Liquid Chromatography

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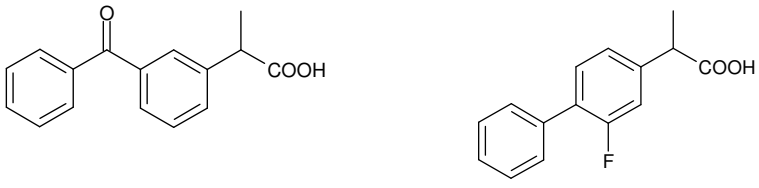
Abstract

Liquid chiral chromatography of ketoprofen and flurbiprofen enantiomers is carried out using an amylose-based stationary phase. The mobile phases used for profens chiral separations are usually a hydrocarbon-alcohol combination, with high hydrocarbon content. However, profens show poor solubilities in hydrocarbon solvents when compared to alcohols. When the final objective is high productivity preparative separations, besides retention time, selectivity and column efficiency, solubility of the racemic drug is always a mandatory aspect to take into account. This work shows that an increase of the alcoholic content in the mobile phase is possible without a decrease in selectivity and column efficiency. Considering the chiral separation of ketoprofen and flurbiprofen enantiomers, results show that the mobile phase needs only a small quantity of acidic modifier and can be composed by a high or even pure alcoholic content. Additionally, it is found that the type of alcohol to be used can differ, depending on the profen racemic mixture to be separated.

1 Introduction

The chirality of drugs is an important issue for the pharmaceutical industry, since the different enantiomers of a racemic drug may have distinct pharmacological activities, pharmacokinetic and pharmacodynamic effects. Because of its chiral selectivity, human body reacts with a racemic drug differently, and metabolise each enantiomer on separate pathways producing different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or even, in worst cases, produce unwanted effects. Flurbiprofen [2-(2-fluoro-4-biphenyl)-propionic acid] and ketoprofen [2-(3-benzoylphenyl)-propionic acid] (Figure 1), belong to a family of chemicals named 2-arylpropionic acids, or profens, an important sub-class of the frequently prescribed and used drugs called nonsteroidal anti-inflammatory drugs (NSAIDs). The main primary indications for NSAIDs therapy include rheumatoid arthritis, osteoarthritis, acute gouty arthritis, ankylosing spondylitis and dysmenorrhea (DeRuiter, 2002). The importance of profens is supported by the fact that, in the last twenty years, drugs like aspirin, phenazone derivatives or acetaminophen are being supplemented by profens (Brune and Hinz, 1998).



(a) Ketoprofen (b) Flurbiprofen

Figure 1. Chemical structures of ketoprofen (a) and flurbiprofen (b).

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Some studies refer that, while the anti-inflammatory effect and gastric toxicity is associated mainly with the S-enantiomers (Wechter et al., 2000), R-enantiomers play a major role in analgesia (Bertini and Caselli, 1999) being less toxic than the S-enantiomers or the racemic form (Geisslinger and Schaible, 1996). The pharmokinetics in terms of absorption, distribution, metabolism, protein binding and elimination may be different for the two enantiomers, leading to inter-individual variability in clinical response and drug toxicity. Therefore, there is a need for the development of a preparative-scale separation method for this class of drugs. Due to its good sensitivity, reproducibility and low chromatographic interferences, high performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) has been the most employed enantioseparation method of profens. The phenylcarbamate derivatives of polysaccharides, particularly cellulose and amylose, show high chiral recognition when used as CSPs for HPLC (Yashima, 2001). Among the many derivatives, the amylose 3,5-dimethylphenylcarbamate (e.g. Chiralpak AD, Daicel, Japan) is the most used on the separation of profen racemates. Considering the preparative separation of these class of enantiomers, the choice of the mobile phase composition is a critical issue, since directly affects the system productivity by influencing retention time, selectivity, column efficiency and solubility of the racemate (Fancotte, 2001). The objective of this work is to study how mobile phase composition, in terms of acidic and alcoholic modifiers, influences the profen enantioseparation.

2 Equipment and Materials

All analysis were performed on a Jasco HPLC system with an UV-1575 multiwavelength detector set at 260 nm, and a Rheodyne 7725(i) injection valve with a 20 µl loop. The column used was an amylose tris(3,5-dimethylphenylcarbamate) coated on a 10 µm silica-gel substrate (Chiralpak AD, 250 mm L x 4,6 mm ID) from Daicel Chemical Industries (Japan). Methanol, ethanol, isopropyl alcohol, acetonitrile and n-hexane, all HPLC grade, trifluoroacetic acid (TFA) spectrophotometric grade, 1,3,5-tri-*tert*-butylbenzene (as non-retained component), racemic flurbiprofen and racemic ketoprofen of analytical grade, were all purchased from Sigma (Madrid, Spain).

3 Results and Discussion

3.1 Solubility measurements and pressure drop

Racemic ketoprofen and flurbiprofen were used for solubility measurements in different solvents. First, the influence of the composition of a mixture containing ethanol/n-hexane/0.01%TFA was studied. Later, ketoprofen solubility was measured in five different pure solvents: n-hexane, acetonitrile, isopropyl alcohol, ethanol, and methanol. All former measurements were carried out at 25 ºC. Additionally, the influence of temperature on ketoprofen solubility was studied for pure ethanol and methanol. Figure 2 shows that the alcoholic (ethanol) content in the mobile phase drastically influences profen solubility: ketoprofen enantiomers are insoluble in pure n-hexane solvent and present high solubility in pure ethanol.

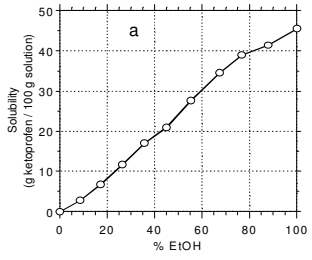

Figure 2. Effect of the alcoholic content on ketoprofen solubility (solvent: ethanol/n-hexane with 0.01%TFA; T=25ºC).

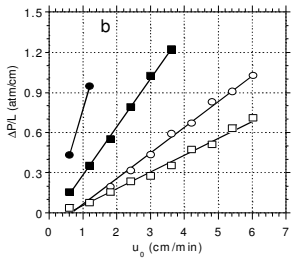

Figure 3. Pressure drop for different solvents: pure isopropanol (filled circles), pure ethanol (filled squares), pure methanol (open circles), and an 80% n-hexane/20% ethanol mixture (open squares). All solvents with 0.01% TFA.

Solubility measurements obtained for different solvents shows that, at 25 ºC, ketoprofen enantiomers have increasing solubilities for pure acetonitrile, isopropyl alcohol, ethanol, and methanol. These results confirm that racemic drugs have considerably higher solubilities in alcoholic solvents than in the traditional mobile phases used in analytical chiral separation, consisting in an alcohol-hydrocarbon combination, with a high hydrocarbon content (Miller et al., 1999). Results also shows the expected increasing solubilities with temperature.
Additional solubility measurements were carried out for flurbiprofen enantiomers. Although showing lower solubilities than ketoprofen, the flurbiprofen enantiomers present the same increase in solubility with the increase of the alcoholic content (data not shown).

In a preparative scale perspective, it is of crucial importance the pressure drop obtained in the adsorbent bed. Figure 3 shows that lower pressure drops are obtained for methanol; lower than for ethanol and even lower than for isopropyl alcohol.

3.2 Effect of acidic modifier

Concerning the separation of ketoprofen and flurbiprofen enantiomers, the effect of the acidic modifier content on the capacity and selectivity (Figures 4 and 5) and on column efficiency (Figures 6 and 7) was studied, using a mobile phase composition of 80% n-hexane/20% ethanol with TFA (0, 0.01, 0.05, 0.10, and 0.15% v/v).

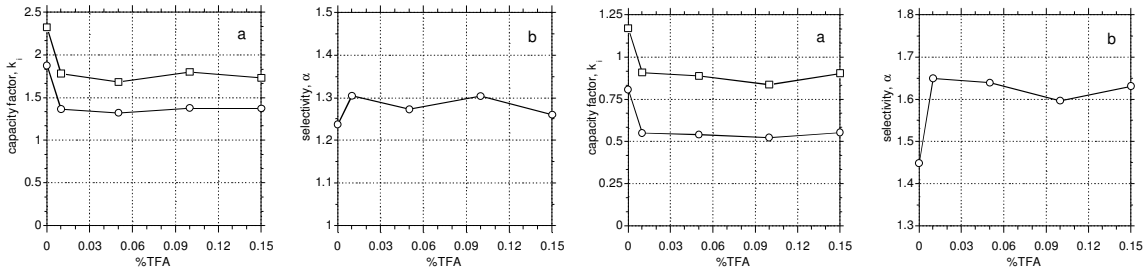

Figure 4. Effect of the acidic modifier content (%TFA) on the separation parameters for ketoprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: 80% n-hexane/20% ethanol; T=25ºC).

Figure 5. Effect of the acidic modifier content (%TFA) on the separation parameters for flurbiprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: 80% n-hexane/20% ethanol; T=25ºC).

The results clearly show that the introduction of the acidic modifier decrease retention of both enantiomers and increase selectivity. However, it was found that a small concentration of TFA (0.01%) is enough to ensure separation and no better performances are obtained with higher TFA contents.

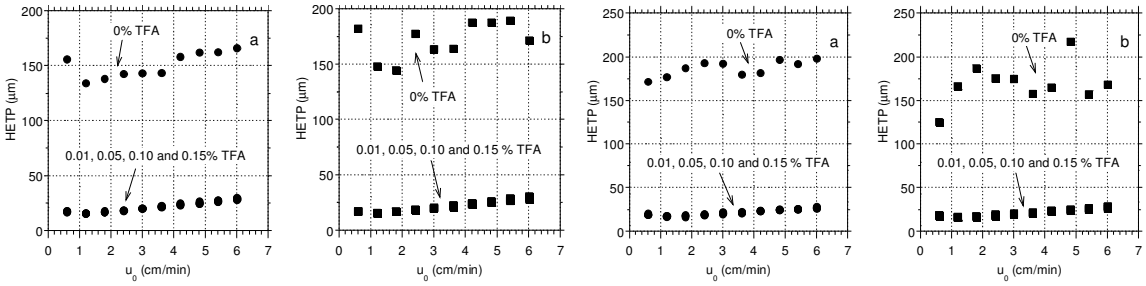

Figure 6. Effect of the acidic modifier content (%TFA) on column efficiency for ketoprofen enantiomers: (a) less retained; (b) more retained enantiomer (mobile phase: 80% n-hexane/20% ethanol with 0, 0.01, 0.05, 0.10, and 0.15% TFA; T=25ºC).

Figure 7. Effect of the acidic modifier content (%TFA) on column efficiency for flurbiprofen enantiomers: (a) less retained; (b) more retained enantiomer (mobile phase: 80% n-hexane/20% ethanol with 0, 0.01, 0.05, 0.10, and 0.15% TFA; T=25ºC).

This fact is clearly shown in Figures 6 and 7: for both ketoprofen and flurbiprofen enantiomers, the column efficiency is low ($HETP = 150 - 200$ μm) if no TFA is added. Introducing a 0.01% TFA content, the $HETP$ for both profens and both enantiomers varies between 15 and 30 μm. No better results are obtained for higher TFA contents. Additional experiments were carried out for a mobile phase containing pure methanol and TFA modifier, and a similar conclusion was found: a 0.01% TFA content is enough to ensure separation of profen enantiomers on a Chiralpak AD stationary phase.

3.3 Effect of alcoholic modifier

3.3.1 Hydrocarbon – alcohol mixtures

Experiments were carried out using different n-hexane/ethanol ratios: 80/20, 50/50, 35/65, 20/80, 10/90 (%v/v), and pure ethanol; all mixtures containing 0.01% TFA. The results obtained are presented in Figures 8 to 11.

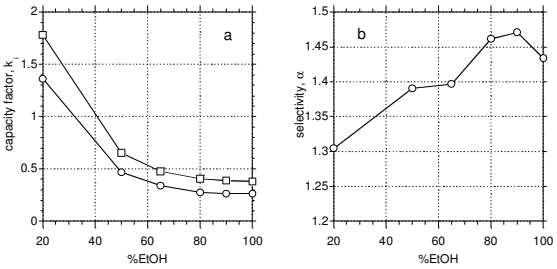

Figure 8. Effect of the alcoholic modifier content (%EtOH) on the separation parameters for ketoprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: n-hexane/ethanol mixtures, with 0.01% TFA; T=25ºC).

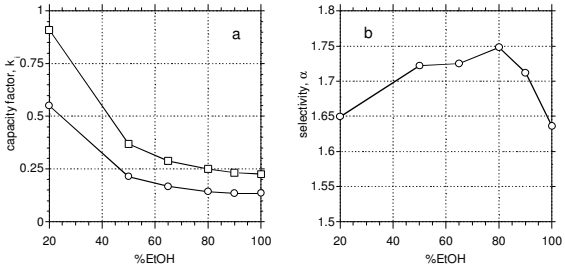

Figure 9. Effect of the alcoholic modifier content (%EtOH) on the separation parameters for flurbiprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: n-hexane/ethanol mixtures, with 0.01% TFA; T=25ºC).

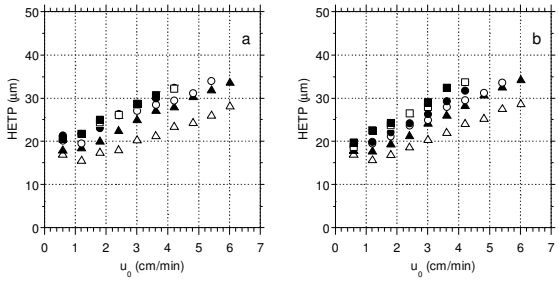

Figure 10. Effect of the alcoholic modifier content (%EtOH) on column efficiency for ketoprofen enantiomers: (a) less retained; (b) more retained enantiomer (mobile phase: n-hexane/ethanol mixtures, with 0.01% TFA ; T=25ºC). Ethanol content: 20% (open triangles); 50% (filled triangles); 65% (open circles); 80% (filled circles); 90% (open squares); 100% (filled squares).

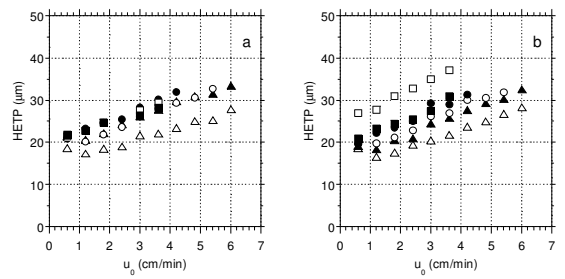

Figure 11. Effect of the alcoholic modifier content (%EtOH) on column efficiency for flurbiprofen enantiomers: (a) less retained; (b) more retained enantiomer (mobile phase: n-hexane/ethanol mixtures, with 0.01% TFA; T=25ºC). Ethanol content: 20% (open triangles); 50% (filled triangles); 65% (open circles); 80% (filled circles); 90% (open squares); 100% (filled squares).

Analysing Figures 8 and 9, we conclude that retention (capacity factors) diminish with the increment of the alcoholic (ethanol) content. However, selectivity remains under relatively constant values. The same occurs in terms of column efficiency (Figures 10 and 11). These results reveal that the use of pure alcoholic solvents is possible for chiral separations, and beneficial at a preparative scale.

3.3.2 Alcohol – Alcohol mixtures

The effect of the content of ethanol/methanol mobile phases on separation was studied for both ketoprofen and flurbiprofen systems. The study was carried out using different ethanol/methanol composition ratios: 0/100, 20/80, 40/60, 60/40, 80/100 and 100/0 (%v/v); all with 0.01%TFA. Figures 12 and 13 show distinct results respectively for ketoprofen and flurbiprofen enantiomers.

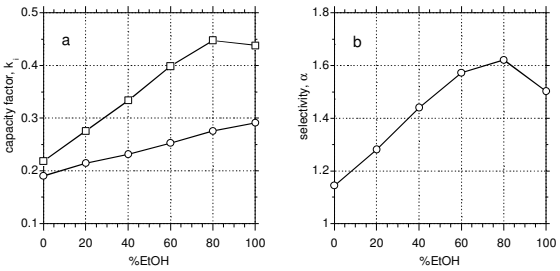

Figure 12. Effect of the content of an ethanol/methanol mobile phase on the separation parameters for ketoprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: ethanol/methanol mixtures, 0.01% TFA; T=25ºC).

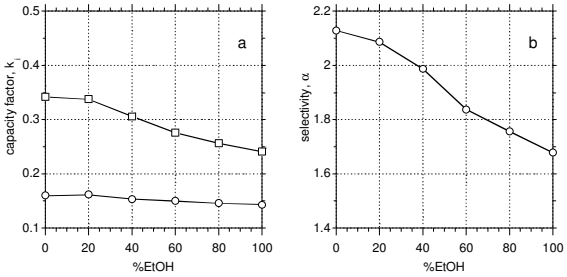

Figure 13. Effect of the content of an ethanol/methanol mobile phase on the separation parameters for flurbiprofen enantiomers: (a) capacity factors (circles for the less and squares for the more retained enantiomer); (b) selectivity (mobile phase: ethanol/methanol mixtures, 0.01% TFA; T=25ºC).

For ketoprofen enantiomers, both retention and selectivity generally increase with the increase of the ethanol content. Low selectivities and resolutions are obtained for a high methanol content. For flurbiprofen enantiomers, the retention of the first enantiomer is only slightly affected by the composition of the ethanol/methanol mobile phase, while the retention of the second enantiomer increases with the increase of the methanol content. Selectivity also increases with the increment of the methanol content. We conclude that the chiral separation of ketoprofen enantiomers can be obtained using a pure ethanol mobile phase, while separation of flurbiprofen enantiomers can be better accomplished with a pure methanol solvent. Both solutions are obtained with low retentions, which is an advantage in a preparative scale perspective.

Figure 14 shows the correspondent column efficiency (*HETP*) for ketoprofen and flurbiprofen enantiomers, using the selected pure alcohol mobile phase.

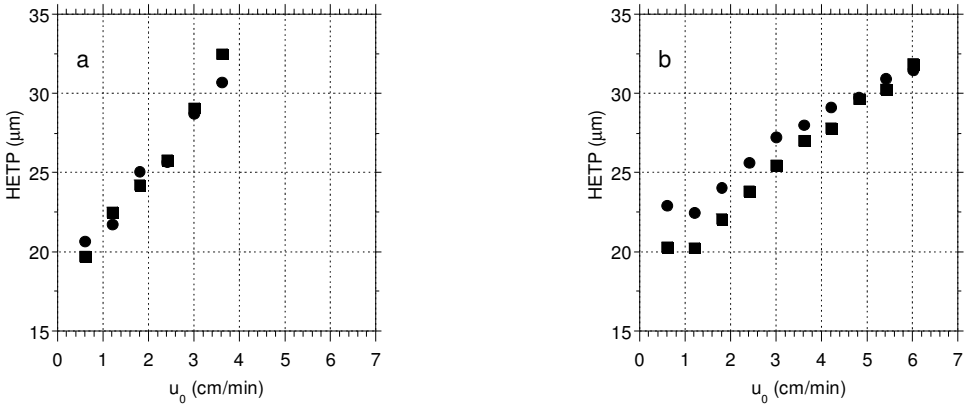

Figure 14. van Deemter curves (column efficiency versus superficial velocity) for: (a) ketoprofen chiral separation in pure ethanol; (b) flurbiprofen chiral separation in pure methanol. Circles for the less and squares for the more retained enantiomers. Both mobile phases with 0.01% TFA; T=25ºC.

4 Conclusions

The mobile phases used for profens chiral separations are usually a hydrocarbon-alcohol combination, with a high hydrocarbon content. However, profens show poor solubilities in hydrocarbon solvents. When the final objective is the high productivity preparative separations, solubility of the racemic drug is of crucial importance. The results presented show that an increase of the alcoholic content in the mobile phase is possible without a decrease on selectivity. Considering the preparative production of pure profen enantiomers using an amylose-based chiral stationary phase, results show that the optimum mobile phase needs only a small quantity of acidic modifier (0.01% TFA) and can be obtained under pure alcohol content. The use of pure alcohol solvents increases solubility of the racemate and decreases retention time, both advantages in a preparative scale point of view. Besides, the use of pure solvents also simplifies its reutilization in a production separation process. Considering the chiral separation of profen racemic mixtures, this work shows that the choice of the better mobile phase is not a straightforward task. Pure methanol (with a low quantity of TFA acidic modifier) should be used to separate flurbiprofen enantiomers: besides higher solubility, the use of methanol presents higher selectivity and lower pressure drop. However, considering the separation of ketoprofen enantiomers, pure methanol should be replaced by pure ethanol, since the former mobile phase presents low selectivities for this system.

Acknowledgements

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References

Bertini, R., Caselli, G. (1999). Analgesic Effect of Ketoprofen Is Mainly Associated to its R-Enantiomer: Role of Cytokine Modulation. *Analgesia* 4, 181-186.

Brune, K., Hinz, B. (1998). 2-Arylpropionsauren stellenwert in der schmerztherapie. *Der Schermz* 6, 383-388.

DeRuiter, J. (2002). *Principles of drug action 2*, Fall 2002 (available at http://www.duc. auburn.edu/~deruija/nsaids_2002.pdf)

Francotte, E. (2001). Enantioselective chromatography as a powerful alternative for the preparation of drug enantiomers. *J. Chromatogr. A* 906, 379-397.

Geisslinger, G., Schaible, H. (1996). New insights into the site and more of antinoceptive action of flurbiprofen enatiomers. *J. Clin. Pharmacol.* 36, 513-520.

Miller, L.,Orihuela, C., Fronek, R., Murphy, J. (1999). Preparative chromatographic resolution of enantiomers using polar organic solvents with polysaccharide chiral stationary phases. *J. Chromatogr. A* 865, 211-226.

Wechter, W., Leipold, D., Murray, E., Quiggle, D., McCracken, J., Barrios, R., Greenberg, N. (2000). E-7869 (R-Flurbiprofen) Inhibits Progression of Prostate Cancer in the TRAMP Mouse. *Cancer Res.* 60, 2203-2208.

Yashima, E. (2001). Polysaccharide-based chiral stationary phases for high-performance liquid chromatography enantioseparation. *J. Chromatogr. A* 906, 105-125.