Ribeiro, A., **Pais**, L., and Rodrigues, A.

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in *XIX Encontro da Sociedade Portuguesa de Química*, Coimbra, Portugal, 15-17 Abril, 2004.
(poster)

XIX Encontro da
Sociedade **P**ortuguesa de **Q**uímica

Livro de Resumos

15-17 Abril, 2004
Coimbra, Portugal

PRELIMINARY STUDIES FOR THE PREPARATIVE SEPARATION OF PROFENS BY CHIRAL LIQUID CHROMATOGRAPHY

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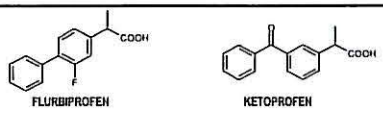
Introduction:

The increasing pressure by regulatory authorities against the marketing of racemic mixtures, resulting from considerations of clinical efficacy and consumer safety, led to current efforts in the development of sensitive and efficient techniques.

In present work, liquid chromatography of profens is carried out using an amylose-based stationary phase. The optimization of chiral separations in these adsorbents is frequently a complex task since some factors are related to the proprieties of the chiral stationary phase, while others are related to the proprieties of the racemate.

In this task the choice of the proper mobile phase is of crucial importance since selectivity, retention time and solubility of the racemate are very sensitive in changes on mobile phase composition. Additionally, in a preparative perspective, pressure drop can also be a reason to change the mobile phase composition.



FLURBIPROFEN KETOPROFEN

Flurbiprofen [2-(2-fluoro-4-biphenyl)-propionic acid] and Ketoprofen [2-(3-benzoylphenyl)-propionic acid] 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). They have potent analgesic and antipyretic proprieties and are prescribed to relieve inflammation when associated to diseases such as rheumatoid arthritis, osteoarthritis, acute gouty arthritis, ankylosing spondylitis and dysmenorrhea.

The separation of these drugs is of special interest because its use has been associated with a number of undesirable side effects and it is believed that different enantiomers have distinct pharmacological activities and consequently different clinical effects.

Instrumentation and Materials:

Apparatus:
Jasco HPLC System containing a PU-1580 pump, an UV-1575 multiwavelength detector set at 260 nm and a manual injector Rheodyne with a 20 µl loop.

Chemicals and Materials:
Ethanol absolute, methanol, isopropyl alcohol, n-hexane and acetonitrile were all of 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).
The column used was a 10 µm Chiralpack AD (250x4.6 mm) from Daicel Chemical Industries (Japan). All separations were carried out at 25°C using a water bath.

Formulae

Selectivity, $\alpha = \frac{tr_2 - tr_0}{tr_1 - tr_0}$

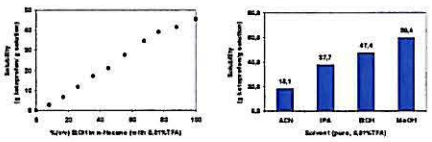
Capacity Factor, $k_1 = \frac{tr_1 - tr_0}{tr_0}$

Resolution, $R = \frac{tr_2 - tr_1}{\frac{w_2 + w_1}{2}}$

Results and Discussion:

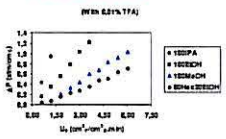
Solubility

(T=25°C)



- The alcoholic (ethanol) content of the mobile phase drastically influences enantiomers solubility;
- Ketoprofen enantiomers are not soluble in a pure hexane solvent;
- Ketoprofen enantiomers show increasing solubilities for pure acetonitrile (ACN), isopropyl alcohol (IPA), ethanol (EtOH) and methanol (MeOH).

Pressure Drop

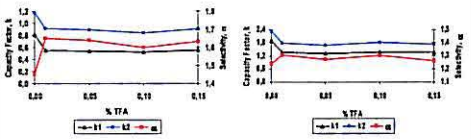


Decreasing pressure drop (pure eluents): isopropanol, ethanol, methanol, and n-hexane.

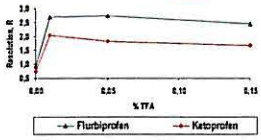
Acidic Modifier

(mobile phase: 80%Hex / 20%EtOH / %TFA, T=25°C)

Flurbiprofen Ketoprofen



(mobile phase: 80%Hex / 20%EtOH / %TFA, T=25°C, 0.6 mL/min)

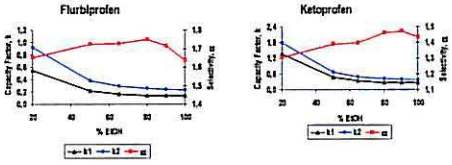


The presence of the acidic modifier (TFA) is important to increase selectivity and resolution. However, results show that a small quantity of TFA (0.01% in volume) is enough; no substantial differences in both selectivity and resolution are found for higher percentage of TFA.
These results are similar for both profens (flurbiprofen and ketoprofen).
Also, the same results are found when using methanol as solvent (results not showed).

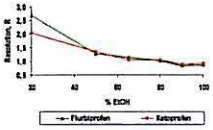
Alcoholic Modifier

(mobile phase: %Hex / %EtOH / 0.01%TFA, T=25°C)

Flurbiprofen Ketoprofen



(mobile phase: %Hex / %EtOH / 0.01%TFA, T=25°C, 0.6 mL/min)

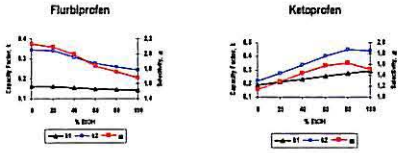


Increasing alcoholic (ethanol) content, decreases retention and resolution. However, selectivity is not significantly affected. Similar results are found for both flurbiprofen and ketoprofen.

Ethanol versus Methanol

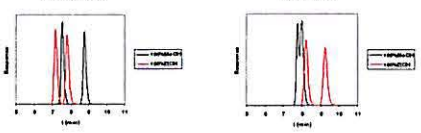
(mobile phase: %MeOH / %EtOH / 0.01%TFA, T=25°C)

Flurbiprofen Ketoprofen



(mobile phase: 100% Alcohol/ 0.01%TFA, T=25°C, Q=0,5 mL/min)

Flurbiprofen Ketoprofen



- **Flurbiprofen:** The retention of the first enantiomer is not significantly affected by the composition of the MeOH/EtOH mobile phase, while the retention of the second enantiomer increases with the increase of the MeOH content. Selectivity also increases with MeOH content. Better mobile phase for flurbiprofen separation: 100%MeOH/0.01%TFA.
- **Ketoprofen:** The retention of both enantiomers and selectivity decrease with the increase of the MeOH content. Better mobile phase for ketoprofen separation: 80%EtOH/20%MeOH/0.01%TFA.

Conclusions

The mobile phases used for profens chiral separations are usually a hydrocarbon-alcohol combination, with a high hydrocarbon content. However, profens show poor solubility 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 a high alcoholic content.
Solubility studies were carried out using different solvents and results show that solubility in methanol is 59,4 g/100 g, higher than in ethanol (47,4 g/100 g). In this way, the use of methanol instead of ethanol may be considered when the objective is to separate flurbiprofen enantiomers: besides higher solubility, the use of methanol presents higher selectivity and lower pressure drop. However, the separation of ketoprofen enantiomers must be carried out using ethanol, since the use of methanol presents very low selectivities.