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Separation of Profens by Chiral Liquid Chromatography – Preliminary Studies

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Fundamentos

Desenvolvimento

Aplicações

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P-61. SEPARATION OF PROFENS BY CHIRAL LIQUID CHROMATOGRAPHY – PRELIMINARY STUDIES

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In pharmaceutical industry the chirality of drugs is an important issue 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 metabolizes 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] 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. However, the use of some racemic profens has been associated with a number of undesirable side effects like gastric irritation and acute renal failure, and it is not clear to which enantiomeric form is related with. Metabolic and pharmacokinetic studies are one of the major pharmaceutical concerns, and it is imperative an individual study of each enantiomer and, eventually, the production of single enantiomer instead of racemic drugs.

In this work, the separation of profens is carried out using an amylose-based chiral stationary phase. Preliminary studies concerning this separation will be presented

taking into account the final objective of preparative production of drugs containing only one enantiomer from a racemic feed solution.

The optimization of chiral separations in these adsorbents is frequently a complex task that involves the choice of the proper mobile phase composition, organic modifier, and operating temperature. Additionally, the choice of the mobile phase composition shall take into account the solubility of the racemate, since the objective is to perform high productivity (that means high concentration) preparative enantioseparations.

SEPARATION OF PROFENS BY LIQUID CHROMATOGRAPHY

Preliminary Studies

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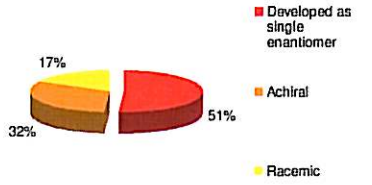
Motivation:

Why chiral separation ?

It is well known that the two enantiomers can have different therapeutical value and there is pressure of regulatory agencies to the separation of chiral drugs.



Development drugs worldwide (Stinson, C&EN, 1997).

Why chiral chromatography ?

Preparative chiral chromatography is often the fastest and most easily scalable process for obtaining initial supplies of enantiomerically pure compounds.

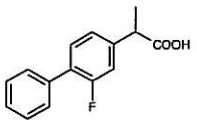
However, the technique is not limited to laboratory separations. Since the early 90's, Simulated Moving Bed (SMB) technology as been used in the pharmaceutical industry to produce large quantities of enantiomerically pure drugs.

Companies that use SMB for large-scale chiral separations

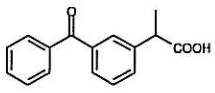
Aerojet Fine Chemicals (USA), Bayer (Germany), CarboGen Laboratories (Switzerland), Chiral Technologies (USA), Daicel (Japan), Honeywell Specialty Chemicals (Ireland), H Lundbeck A/S (Denmark), Merck (Germany), UCB Pharma (Belgium), Universal Pharma Technologies (USA)

Why profens ?

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.



FLURBIPROFEN



KETOPROFEN

Preliminary Studies:

Summary

The main objective of this work is the preparative production of drugs containing only one enantiomer from a racemic feed solution.

The optimization of the chiral separation is frequently a complex task that involves the choice of the proper mobile phase composition, organic modifier, and operating temperature. Additionally, the choice of the mobile phase composition shall take into account the solubility of the racemate, since the objective is to perform high productivity (that means high concentration) preparative enantioseparations.

HPLC Analysis

Apparatus: Jasco LC 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.

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.

Chemicals: Ethanol absolute and n-hexane, both HPLC grade, trifluoroacetic acid (TFA) spectrophotometric grade, 1,3,5-tri-*tert*-butylbenzene (as non-retained component), racemic flurbiprofen and racemic ketoprofen of analitycal grade were all purchased from Sigma (Madrid, Spain).

Formulae

Selectivity, $\alpha = \frac{t_{r_2} - t_0}{t_{r_1} - t_0}$

Capacity Factor, $k_1 = \frac{t_{r_1} - t_0}{t_0}$

Resolution, $R = \frac{t_{r_2} - t_{r_1}}{\frac{w_2 + w_1}{2}}$

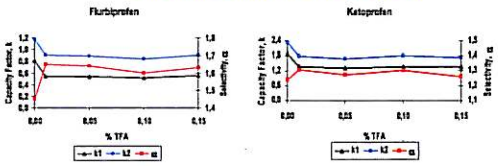
Height equivalent to theoretical plate, $HETP_i = \frac{\sigma_i^2}{t_{r_i}^2} L$

where:
t_{r_i} = retention time (0=non-retained species, 1=less retained component, 2=more retained component);
σ_i^2 = variance;
L = column length; W_i = peak width at the baseline.

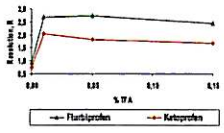
Results and Discussion

Acidic Modifier

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



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

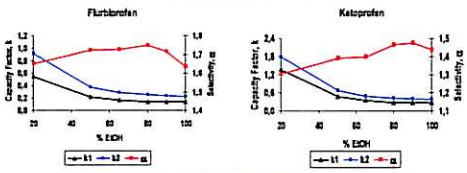


The presence of the acidic modifier (TFA) is important to increase selectivity and resolution. However, results show that a small quantity of TFA (0.01% in volume) is enough; no substantial differences in both selectivity and resolution are found for higher percentage of TFA.

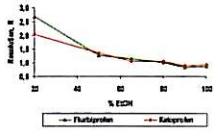
These results are similar for both profens (flurbiprofen and ketoprofen).

Alcoholic Modifier

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

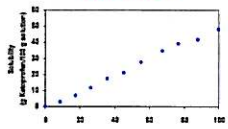


(mobile phase: %Hex / %EtOH / 0.01%TFA. T=25°C, 0.6 mL/min)



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

Solubility



The alcoholic (ethanol) content of the mobile phase drastically influences enantiomers solubility:

- Ketoprofen enantiomers are not soluble in a pure hexane solvent;
- For pure ethanol mobile phase, ketoprofen enantiomers show a solubility of 50g/100g solution.

Conclusions

The mobile phases used for profens chiral separations are usually a hydrocarbon-alcohol combination, with a higher 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 chiral stationary phases, results show that the optimum mobile phase needs only a small quantity of acidic modifier (0.01% TFA) and a higher alcoholic content (80%-100% ethanol).