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Separation of Profens by Chiral Liquid Chromatography – Preliminary Studies


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Fundamentos
Desenvolvimento
Aplicações

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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 therapeutic value and there is pressure of regulatory agencies to the separation of chiral drugs.

![Chiral separation diagram]

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 90s, Simulated Moving Bed (SMB) technology has been used in the pharmaceutical industry to produce large quantities of enantiomerically pure drugs.

Companies that use SMB for large-scale chiral separations:
- Aerogel Fine Chemicals (USA), Bayer (Germany),
- CarboGen Laboratories (Switzerland), Chiral Technologies (USA), Daicel (Japan), Honeywell Specialty Chemicals (Ireland), H. Lundbeck A/S (Denmark), Merck (Germany), UCS Pharma (Belgium), Universal Pharma Technologies (USA)

Why profens?
Flurbiprofen [2-(2,6-dioxo-4-phenyl-1,3-dihydroisindol-2-yl) propionic acid] and Ketoprofen [1-(3-benzoyl-2-phenyl-propionic acid) belong to a family of chemicals named 2-arylopropanic acids, or profens, an important subclass of the frequently prescribed and used drugs called nonsteroidal anti-inflammatory drugs (NSAIDS). They have potent analgesic and antipyretic properties and are prescribed to relieve inflammation when associated to diseases such as rheumatoid arthritis, osteoarthritis, acute gouty arthritis, ankylosing spondylitis and dysmenorrhea.

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 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 racemic, 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-1675 multi-wavelength detector set at 260 nm and a manual injector Rheodyne with a 20 µL loop. The column used was a 10 µm Chiracel 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) spectroscopic grade, 1,3,5-trifluoro-2,4-butylenepiperazine (as racemic component), racemic flurbiprofen and racemic ketoprofen of analytical grade were all purchased from Sigma (Madrid, Spain).

Formulae

- Selectivity, S = \( \frac{t_R}{t_S} \)
- Capacity Factor, K = \( \frac{t_R - t_m}{t_m} \)
- Resolution, R = \( 2 \frac{K}{1 + K} \)

- Height equivalent to theoretical plate, \( \text{HETP} = \frac{D}{2R_{eq}} \)

- Flurbiprofen: \( \alpha = 2.5 \), \( K = 1.2 \), \( R = 1.5 \)
- Ketoprofen: \( \alpha = 2.5 \), \( K = 1.2 \), \( R = 1.5 \)

Solubility

The alcoholic (ethanol) content of the mobile phase drastically influences enantiomers solubility:
- Ketoprofen enantiomers are not soluble in a pure hexanoic solvent.
- For pure ethanol mobile phase, ketoprofen enantiomers show a solubility of 56/100g solution.

Results and Discussion

Acidic Modifier (mobile phase: ethanol/1% TFA, 25°C)

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

Alcoholic Modifier (mobile phase: n-hexane/1% TFA, 25°C)

The presence of the acidic modifier (TFA) is important to increase selectivity and resolution. However, results show that a small quantity of TFA (0.5%) is enough: no substantial differences in both selectivity and resolution are found for higher percentage of TFA. These results are similar for both products (flurbiprofen and ketoprofen).

Conclusions

The mobile phases used for pure enantiomer 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 (0.6% TFA) and a higher alcoholic content (80%-100% ethanol).