

Enantioselective HPLC-DAD method for the determination of etodolac enantiomers in tablets, human plasma and application to comparative pharmacokinetic study

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Introduction

Etodolac (ET) is a chiral NSAID with a single stereogenic carbon atom and exists in two enantiomeric forms; *R* and *S* (Fig. 1).

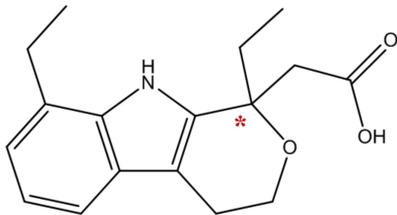


Figure 1. The chemical structure of ET, Asterisk indicates chiral center.

It is marketed as racemate and used as analgesic and for the treatment of rheumatoid arthritis and osteoarthritis [1]. ET decreases the synthesis of peripheral prostaglandins involved in mediating inflammation by inhibition of the cyclooxygenase (COX) enzyme. ET is administered orally for patients with rheumatoid arthritis at doses of 200 – 400 mg given twice daily in the form of tablets or capsules. The pharmacological properties of the two enantiomers differ profoundly in which the *S*-form is responsible for the anti-inflammatory activity [2].

The present study describes the development of a simple and sensitive HPLC-DAD method for enantioselective determination of ET enantiomers in tablets and human plasma. The proposed has been successfully applied to a clinical pharmacokinetic study following a single oral dose of 300 mg ET tablets to twelve healthy Egyptian male volunteers.

Methods

Instrument and conditions:

The HPLC-DAD system operated using kromasil Cellucoat chiral column (250mm4.6 mm,5 mm). The mobile phase consisted of hexane: isopropanol: TFA (90:10:0.1v/v/v). Detection was performed at 274 nm.

Sample preparation:

Plasma samples were submitted to the C-18 SPE cartridges (Fig. 2). Analytes were eluted using methanol after washing the sorbent from other interferences using water + 5% methanol.

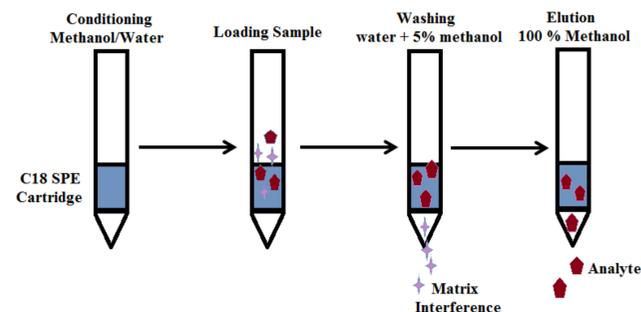


Figure 2. Solid phase extraction method using C18-Cartridge

Pharmacokinetics study:

The pharmacokinetics of etodolac tablets was studied in twelve healthy male subjects. Following an overnight fast, each subject received a single 300 mg oral dose of ET. Blood samples (4 mL) were collected at 0.0 (pre-dose), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after administration.

Results and discussion

A mobile phase composed of hexane: isopropanol (90:10 v/v) gave acceptable retention of the two enantiomers and good resolution between them in tablet and plasma samples (Fig. 3). Addition of TFA (0.1%) to the mobile phase improved the symmetry of the peaks.

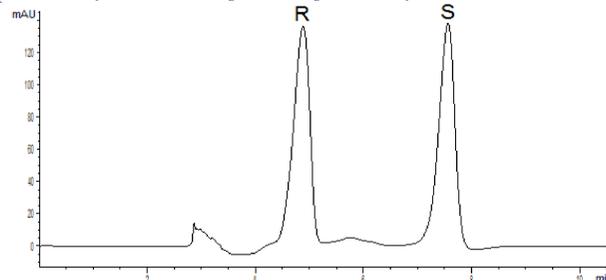


Figure 3. HPLC chromatogram of etodolac tablet showing peaks corresponding to *R*-etodolac (R) and *S*-etodolac (S).

Ketoprofen was selected as internal standard and was used to spike the plasma samples containing etodolac before SPE. The drug was eluted in between the two enantiomers of etodolac using the proposed chromatographic method (Fig. 4)

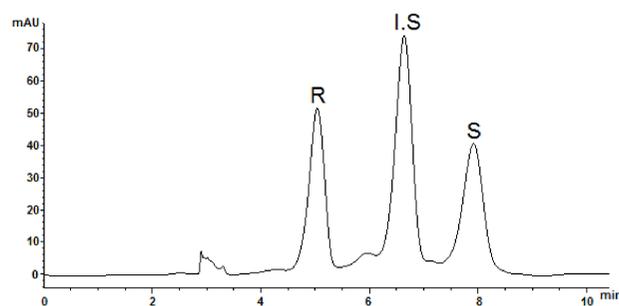


Figure 4. HPLC chromatogram of etodolac and ketoprofen I.S. in plasma after SPE.

The pharmacokinetics study conducted on twelve healthy volunteers after oral dose administration showed higher levels of the inactive *R*-enantiomer over the *S*-enantiomers throughout the whole period of study (24 h). (Figs. 5).

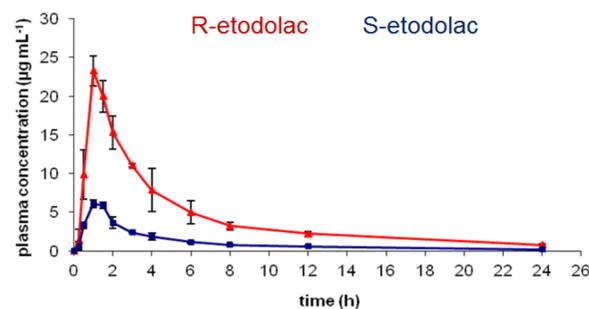


Figure 5. Mean Plasma concentration-time profile of etodolac enantiomers.

Our suggestion to explain the higher plasma concentration of the *R*-enantiomer compared to the *S*-enantiomer is based on considering the interaction of etodolac with the enzyme and the structural conformation of the etodolac. Etodolac possesses an alicyclic ring directly attached to the anionic site. The alicyclic group could exist in chair conformers where the carboxylic acid group could be on the same plane of the lipophilic planar area of the drug or out of plane (Fig. 6).

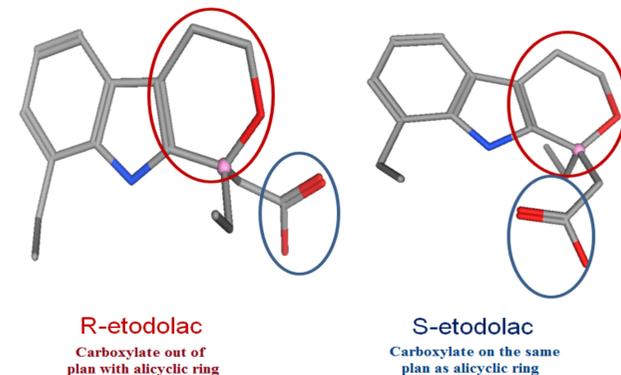


Figure 6. Configuration of *R*- and *S*-enantiomers of etodolac.

We believe that such conformation would affect the interaction of the drug with COX-2 enzyme, so the higher concentration of the *R*-enantiomer in plasma is due to the stronger bonding of the *S*-enantiomer with the enzyme (Fig. 7).

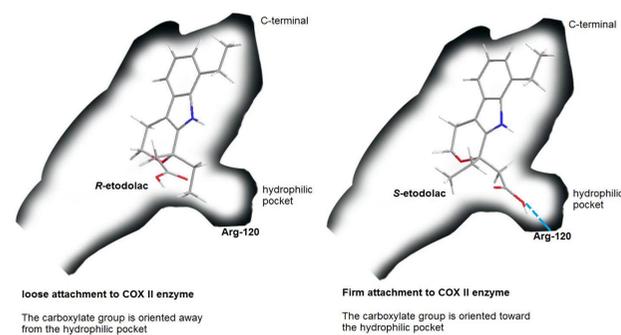


Figure 7. The interaction of etodolac enantiomers with COX II enzyme

Conclusion

The enantiomers of a chiral drug may differ significantly in their pharmacokinetics, potency and selectivity for receptors, transporters and/or enzymes, and toxicity. The use of single enantiomer drugs can potentially lead to simpler and more selective pharmacologic profiles, improved therapeutic indices, simpler pharmacokinetics

The active *S*-enantiomer was assumed to be the form that binds to the targeted COX enzyme. It is concluded that the use of the enantiopure *S*-ET would provide a superior medication compared to the racemate ET.

References

[1] C.R. Craig, R.E. Stitzel, eds. Modern pharmacology with clinical applications, sixth edition, Lippincott Williams and Wilkins, Philadelphia, USA, 2004.

[2] J.P. Boni, J. Korth-Bradley, K. McGoldrick, A. Appel, S. Cooper, Pharmacokinetic and pharmacodynamic action of etodolac in patients after oral surgery, Clin. Pharmacol. 39 (1999) 729–737.