Journal of Chromatography A, 1497 (2017) 110–117

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Full length article

# High performance liquid chromatography with photo diode array for separation and analysis of naproxen and esomeprazole in presence of their chiral impurities: Enantiomeric purity determination in tablets

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ARTICLE INFO

*Article history:*
Received 22 December 2016
Received in revised form 24 February 2017
Accepted 19 March 2017
Available online 24 March 2017

*Keywords:*
Naproxen
Esomeprazole
Chiral impurities
Chiral HPLC-DAD

ABSTRACT

A stereoselective high performance liquid chromatographic method with diode array detection (HPLC-DAD) was introduced for S-naproxen and esomeprazole determination in tablets. The separation was achieved on a Kromasil Cellucoat chiral column using a mobile phase consisting of hexane: isopropanol: trifluoroacetic acid (TFA) (90:9.9:0.1 v/v/v). The proposed system was found to be suitable for the enantioseparation of naproxen and omeprazole biologically active isomers. After optimization of the chromatographic conditions, resolution values of 3.84 and 2.17 could be obtained for naproxen and omeprazole isomers, respectively. The method was fully validated for the determination of *S*-isomers of each drug in their dosage form. Also, the enentiomeric purity was determined in commercial tablet containing S-naproxen and esomeprazole. The enantiomeric purity was calculated for each drug and the chiral impurities (R-isomers) could be determined at 1% level. The method was validated and good results with respect to linearity, precision, accuracy, selectivity and robustness were obtained. The limits of detection (LOD) and quantification (LOQ) were 2.00, 6.50 and 0.10, 0.35 $\mu g\,mL^{-1}$ for S-naproxen and esomeprazole, respectively.



## 1. Introduction

Most of the chiral drugs are marketed as racemates. Despite that most isomers have the same chemical structure; they exhibit great differences in their biological activities such as pharmacology, pharmacokinetics, toxicology, metabolism etc. [1,2]. Moreover, the FDA demands a full documentation on the separate enantiomers activity and toxicity as well as the racemic mixture when registering chiral pharmaceuticals [2–4].

Owing to the revealed importance of using only the single active isomers and the restrict regulations concerning their analysis, different techniques were developed aiming at the enantiomeric separation and analysis of chiral drugs in pharmaceutical industry.

Polarimetry, NMR and enzyme techniques are examples of techniques that could be used to analyze enantiomers but they suffer from a number of disadvantages. The main disadvantages of such techniques are: the need of pure samples and the lack of enantiomers separation. These problems could be overcome by the use of chromatographic or electrophoretic techniques. Chiral HPLC has confirmed to be one of the best methods for the direct analysis and separation of enantiomers [5]. Unlike GC, it can deal with a wide variety of nonvolatile compounds. Electrophoretic techniques have relatively limited application in the stereoselective resolution and analysis of drugs. Chiral HPLC methods are either direct, using chiral stationary phases (CSPs) and chiral additives in the mobile phase, or indirect, involving derivatization of drugs [6,7]. The direct chiral separations using CSPs are of lower cost and higher efficacy than those using chiral additives [7–9]. So they are the most widely used in stereoselective separation and analysis.

Naproxen (NAP) is a non-steroidal anti-inflammatory drug (NSAID) of the arylacetic acid group, Fig1. Naproxen is used to relieve the pain, inflammation and stiffness. Enantiomeric purity of naproxen is important, as the R- isomer is hepatotoxic while the S- isomer is safe. Also, the S- isomer of the drug has 28 times the anti-inflammatory activity of the R- isomer [10].

Esomeprazole (ESO), the S-isomer of omeprazole (OME) (Fig1), demonstrated a pharmacological effect beyond that exhibited with the racemic parent drug. This may be attributed to the fact that, ESO is metabolized to lower extent than the racemic form, resulting in a higher plasma concentration and better pharmacokinetic profile [11]. As a result, ESO is indicated for the treatment of gastroesophageal reflux disease.

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http://dx.doi.org/10.1016/j.chroma.2017.03.059
0021-9673/

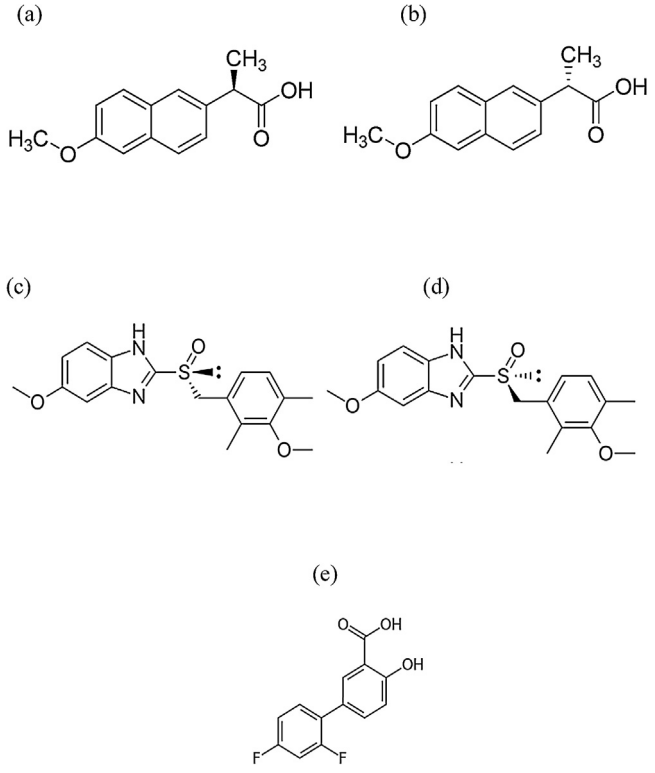


**Fig. 1.** Chemical structures of R and S isomers of (a, b) Naproxen and (c, d) Omeprazole, respectively, and (e) Diflunisal internal standard.

The major factor limiting the use of NSAID such as NAP is the accompanied gastrointestinal problems, ranging from ulcers to serious complications such as perforation, obstruction and bleeding [12]. As a result, it is recommended to use selective cyclooxygenase-2 (cox-2) drug along with proton pump inhibitors in order to decrease these side effects [12]. Recently, the FDA approved a fixed-dose combination of enteric-coated NAP (500 mg) and immediate release ESO (20 mg) (Vimovo; AstraZeneca). It is indicated in the treatment of rheumatoid arthritis, ankylosing spondylitis and osteoarthritis in patients at risk for developing NSAID-associated gastric ulcers.

Several HPLC methods were reported for the analysis of NAP enantiomers using different CSPs, such as: molecularly imprinted polymer[13], human serum albumin [14] and cyclodextrin [15]. The imprinted polymers usually suffer from some disadvantages as peak tailing and tedious preparation procedures of the imprinted polymer. Also, the protein in the stationary phase shows poor stability and low column capacity. On the other hand, the cyclodextrin based stationary phase also has the disadvantages of poor peak shape and limited column capacity. The polysaccharide derivatives based columns are the most popular CSPs in enantiomers separation. They are widely used to separate and analyze various kinds of chiral compounds due to their good stability and high separation ability [10].

For the HPLC enantiomeric analysis of OME in pharmaceuticals using CSPs, polysaccharide derivatives based columns were used such as amylose derivatives based columns [16] and cellulose derivatives based columns [17]. Also, chiral columns with immobilized proteins were investigated for the separation of omeprazole enantiomers and compared with amylose based chiral columns [18].

Few reports were found in literature analyzing only the S-isomers of each drug in plasma [19] or in their pharmaceutical preparations [20,21]. To the best of our knowledge, no report was found for the simultaneous separation and analysis of the four isomers in pharmaceutical formulations with the assessment of enantiomeric purity and detection of their R-isomers as chiral impurities.

The aim of the present work is to introduce a stereoselective method for the simultaneous separation of the four isomers of NAP and OME with the quantitation of the active isomer of each of them using an internal standard (IS): diflunisal (DIF). This was applied successfully in the analysis of Vimovo® tablets. Enantiomeric purity was applied to tablets using the proposed method. The high detector sensitivity with baseline stability was essential for the detection of trace amounts of biologically inactive isomers at a level of (1%) of the main peak. Method optimization was performed in order to achieve sufficient resolution and validation of the method was done according to the International Conference on Harmonisation (ICH) guidelines [22].

## 2. Experimental

### 2.1. Materials

Working standard of pharmaceutical grade OME (the racemic form) was obtained as generous gifts from Egyptian International Pharmaceutical Industries Co (EIPICO), Egypt and certified to contain 99.85%. ESO and NAP (S- isomer of each) were obtained as a gift from EvaPharma Pharmaceutical Company, Cairo, Egypt and Nile Co. for Pharmaceuticals and Chemical Products, Cairo, Egypt, respectively (certified to contain 99.80% and 99.85%, respectively). The racemic form of NAP (with 99.7% purity) was purchased from Sigma-Aldrich, Cairo, Egypt. The internal standard, DIF (99%, purity), was obtained as a gift from Rameda Pharmaceutical Company, Second Industrial Zone, Sixth of October, Egypt. All reagents used were of HPLC grade. Hexane, trifluoroacetic acid and the alcohols used (isopropanol, ethanol and methanol) were purchased from Sigma-Aldrich (Cairo, Egypt). The commercial product namely Vimovo® delayed-release tablets containing 20 mg ESO and 500 mg S-NAP per tablet, produced by AstraZeneca, UK were purchased from the commercial market.

### 2.2. Instrumentation and chromatographic conditions

The HPLC–DAD system (Agilent Technologies, Palo Alto, CA, USA) consisted of quaternary pump G1311A which comprises a solvent cabinet, vacuum degasser G1322A, a four-channel gradient pump and photo diode array. The chromatographic system is equipped with thermostated column compartment G1316A and manual injector that uses a Rheodyne 7725i 7-port sample injection valve fitted with a 20 μL sample loop. All of them are Agilent 1200 series. The chromatographic separations were performed on Kromasil Cellucoat RP chiral column (tris-[3,4–b] carbamoylcellulose, 250mm × 4.6 mm, 5 μm). The column was thermostated at 25° C during analysis. Agilent Chemstation software for LC was used for data acquisition and analysis.

For the chromatographic analysis of the two enantiomers of naproxen and omeprazole, the mobile phase consisted of hexane: isopropanol: TFA (90:9.9:0.1 v/v/v). The flow rate of the mobile phase under isocratic elution was 1 mL/min. The injection volume was 20 μL and the runtime was 30 min. Detection of analytes and internal standard was performed at 305 nm. The samples solutions were filtered using 0.45 μm disposable filters before injection.