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# Enantioseparation of chiral pharmaceuticals in biomedical and environmental analyses by liquid chromatography: An overview

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ABSTRACT

This review aims to present the issues associated to enantioseparation of chiral pharmaceuticals in biological and environmental matrices using chiral stationary phases (CSP). Thus, it related some enantioselective methods in liquid chromatography (LC) and compares the importance given to chiral separation in biomedical and environmental fields. For that the most used CSP, the enantioselective chromatographic methods, their advantages and drawbacks were swiftly revised and compared. The recent advances and the limitations of chiral analytical methods in LC were also discussed.



## 1. Introduction – chiral pharmaceuticals

Chiral drugs are one of the most important categories of pharmaceuticals, and although chirality can be originated by a plane, an axis or centers of asymmetry, the carbon atom is by far the predominant stereogenic center in pharmaceuticals. Many chiral pharmaceuticals are administrated as racemates despite the desired pharmacological or biological activity is normally associated with only one enantiomer while the mirror image form is often inactive, less active, or presents mild to severe side effects or toxicity [1]. The recognition of an enantiomer of a chiral pharmaceutical by a receptor depends on their complementary configuration, so enantiomers can have different pharmacokinetics and pharmacodynamics properties [2]. The evaluation of the license for enantiomeric pure pharmaceuticals that were sold as racemates in the past (chiral switching), the many advantages of the use of single enantiomers and the innovation techniques to obtain and control enantiomers have been important mechanisms to increase the use of enantiomeric pure pharmaceuticals [3–5]. Regarding pharmacokinetics, pharmacodynamics and toxicological studies the advantages of single enantiomer formulations are lower therapeutic doses, higher safety margin, lower interindividual variability, less drug interactions and side effects. Nowadays the tendency is to use enantiopure forms, despite there are several pharmaceutical drugs that are both commercialized at racemic and single enantiomer, such as the antidepressants citalopram and (*S*)-citalopram (escitalopram), the non-steroid anti-inflammatory drugs (NSAID) ketoprofen and (*S*)-ketoprofen (dexketoprofen), ibuprofen and (*S*)-ibuprofen (dexibrufen), the proton pump inhibitors omeprazole and (*S*)-omeprazole (esomeprazole), the antimicrobial ofloxacin and (*S*)-ofloxacin (levofloxacin) [2,4,6,7]. The current preference to approve chiral molecules in an enantiomeric pure form is evident and in the year 2010 only single enantiomers were approved worldwide as new molecular entities (Fig. 1) [8].

In 2012 from the five top-selling pharmaceuticals in the U.S. four single enantiomer trademark drugs stand out [9]: Nexium® (esomeprazole magnesium; (*S*)-enantiomer of omeprazole), a proton pump inhibitor used as an antiulcer agent; Crestor® (rosuvastatin calcium; 3*R*, 5*S*, 6*E*-estereoisomer), an inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase, used as an antidyslipidemic agent; Advair Diskus® (fluticasone propionate; (*S*)-enantiomer + salmeterol xinafoate; racemic form), used

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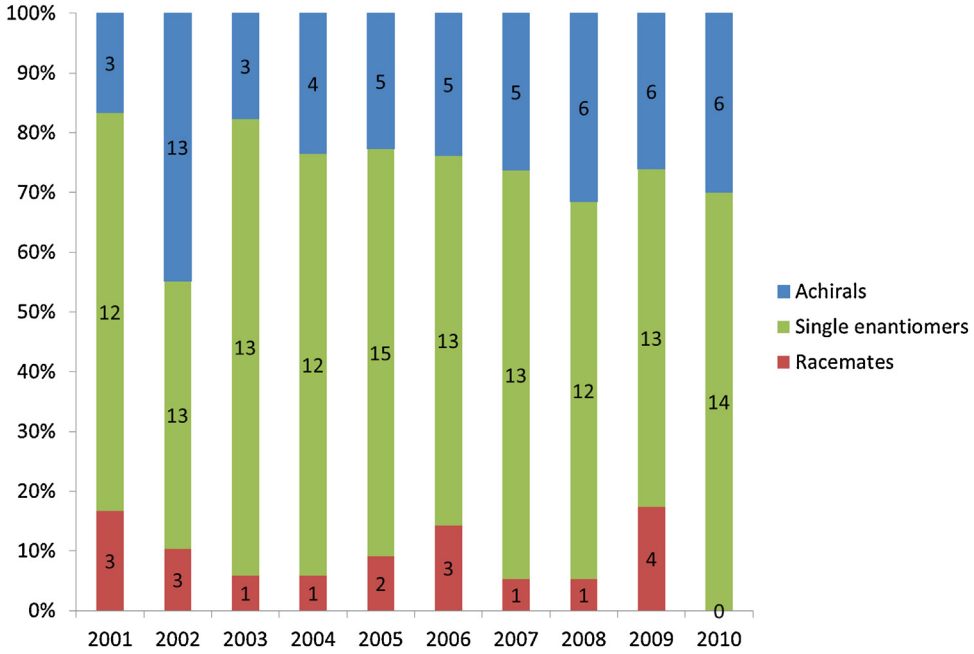


**Fig. 1.** Annual distribution of worldwide-approved new molecular entities according to chirality in the period 2001–2010 (*including diastereomeric mixtures) Adapted from [8].

as antiasthma, anti-inflammatory or bronchodilator combination; and Cymbalta® (duloxetine hydrochloride; (*S*)-enantiomer), an antidepressant acting as selective serotonin and norepinephrine reuptake inhibitor. Other single-enantiomer drugs, such as Singulair® (montelukast sodium; (*R*)-enantiomer), Plavix® (clopidogrel hydrochloride; (*S*)-enantiomer) and Januvia® (sitagliptin phosphate; (*R*)-enantiomer), are included in the first twenty 2012 top-selling pharmaceuticals [9].

The fate of chiral pharmaceuticals in biological systems can be studied by monitoring their enantiomeric ratio during various biological processes. This information is important and has been used to design and develop safe chiral drugs. Despite the well-established role of enantioselectivity on pharmacokinetic, pharmacodynamic and toxicology in biological processes and in biomedical analyses [1,10], the stereochemistry is often neglected in environmental research [11,12]. Concerning biodegradation, ecotoxicity and environmental fate, the recognition of enantioselectivity is essential to provide a more realistic risk assessment of chiral compounds [2,13,14]. Thus, this review relates the importance of enantioseparation of chiral pharmaceuticals in biological and environmental matrices. It intends to give an overview about the analyses of chiral pharmaceuticals in both matrices. The most used chiral stationary phases and enantioselective chromatographic methods, their advantages and limitations will be discussed.

## 2. Chiral stationary phases

The development of the enantioselective chromatography has greatly contributed to increase the available enantiopure pharmaceutical formulations. The control of the enantiomeric purity and the monitoring of biological samples by chiral analyses are essential for developing and designing new drug. Liquid chromatography (LC) with chiral stationary phases (CSP) has achieved a high reputation in enantioselective separation in both analytical and preparative mode [15–17].

There are many different types of commercial chiral columns that were recently reviewed in an extensive way by Lämmerhofer (2010) and others [17–21]. The most important chiral selectors pointed to: Pirkle type, polysaccharide derivatives, cyclodextrin, protein, macrocyclic glycopeptides antibiotics-based and others based on synthetic polymers [17,22,23]. The success of an efficient enantioseparation, however, is primarily determined by the chiral discriminative power of the CSP employed. Nowadays, polysaccharide-based, macrocyclic antibiotics-based and Pirkle-type CSPs are pointed out as the most useful and broadly applied [17,20,21,24,25]. Many authors start the trial-error challenge with polysaccharides or macrocyclic antibiotics-based and Pirkle-type Whelk-O1 because they are versatile and suitable for all elution modes [22,26–34]. The old trend of separation in normal mode has changed along the late decade to reversed, polar organic and polar ionic mode [29,30,32–34], with the advantage of being compatible with LC coupled with mass spectrometry (LC–MS).

The range of application of polysaccharide-based CSPs is broader than macrocyclic antibiotics-based CSPs concerning the number of compounds enantioseparated by both CSPs [20,25,27]. However, macrocyclic antibiotic-based CSPs are complementary to polysaccharide-based CSPs in its ability to resolve important classes of pharmaceutical compounds [20,25]. Pirkle's-type CSPs are useful for more specific applications and can offer the chiral selector on both enantiomeric form (*R* or *S*), which is important for enantiomeric ratio determination in absence of reference samples [22,35].

### 2.1. Polysaccharide-based CSPs

Okamoto et al. were the first to coat polysaccharide derivatives successfully onto silica gel [36]. Since the 1980s, a large number of polysaccharide-based CSPs have been prepared to efficiently enantioseparate thousands of different chiral compounds [37,38]. This type of CSPs is recognized as the most successful for both analytical [39–42] and preparative separations [42–46]. Among them, the CSPs based on tris-3,5-dimethylphenylcarbamate of cellulose and amylose are the most important [47]. The more significant recent advancement was the introduction of immobilized polysaccharide CSPs [15,48]. These new CSPs are more stable, can be used