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ORIGINAL ARTICLE

Enantioselective analysis of fluoxetine in pharmaceutical formulations by capillary zone electrophoresis

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Abstract Fluoxetine is an antidepressant, a selective serotonin reuptake inhibitor (SSRI) used primarily in the treatment of major depression, panic disorder and obsessive compulsive disorder. Chiral separation of racemic fluoxetine is necessary due to its enantioselective metabolism. In order to develop a suitable method for chiral separation of fluoxetine, cyclodextrin (CD) modified capillary electrophoresis (CE) was employed. A large number of native and derivatized, neutral and ionized CD derivatives were screened to find the optimal chiral selector. As a result of this process, heptakis (2,3,6-tri-O-methyl)-β-CD (TRIMEB) was selected for enantiomeric discrimination. A factorial analysis study was performed by orthogonal experimental design in which several factors are varied at the same time to optimize the separation method. The optimized method (50 mM phosphate buffer, pH = 5.0, 10 mM TRIMEB, 15 °C, + 20 kV, 50 mbar/1 s, detection at 230 nm) was successful for baseline separation of fluoxetine enantiomers within 5 min. Our method was validated according to ICH guidelines and proved to be sensitive, linear, accurate and precise for the chiral separation of fluoxetine.

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1. Introduction

Fluoxetine ((±)-N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine) (Fig. 1) is a widely marketed selective serotonin (5-hydroxytryptamine) reuptake inhibitor (SSRI) used in the treatment major depressive disorder, obsessive-compulsive disorder, panic disorder, bulimia nervosa and premenstrual dysphoric disorder (Cheer and Goa, 2001).

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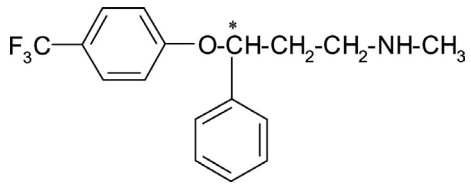

Figure 1 Fluoxetine chemical structure (* denotes the chiral center).

Fluoxetine has a chiral center in its structure resulting into the existence of two enantiomers, R-fluoxetine and S-fluoxetine.

The two enantiomers of fluoxetine are similarly effective in blocking serotonin reuptake. However these enantiomers are metabolized differently. Fluoxetine is extensively metabolized by cytochrome P450 enzyme system through demethylation into the active chiral metabolite norfluoxetine, allowing a more prolonged biological action of the drug. *R*-fluoxetine and *S*-fluoxetine have different metabolic rates, as the clearance of *R*-fluoxetine is about four times greater than the one of *S*-fluoxetine; these differences translate into differences in half-life, the half-life of *S*-fluoxetine being one quarter than that of *R*-fluoxetine. In the case of norfluoxetine only the *S*-enantiomer has similar potency as the parent drug (Brosen, 1998; Sproule et al., 1997).

The use of *R*-enantiomer was expected to result in less variable plasma levels of fluoxetine and its active metabolites compared to those observed with racemic fluoxetine, but the clinical development of *R*-fluoxetine for the treatment of depression was stopped because of a small but statistically significant prolongation of the QT interval with high doses (McConathy and Owens, 2003).

Taking into consideration the aspects presented above the elaboration of new methods for the enantioseparation of fluoxetine represents a necessity and also a challenge.

Capillary electrophoresis (CE) has become an interesting alternative, but also complementary to the more frequently used high performance liquid chromatographic (HPLC) methods, with advantages related to the low solvent and analyte consumption, short analysis time, rapid method development and high selectivity. Enantioseparations by CE are achieved by direct addition of chiral selectors, into the background electrolyte (BGE); as the enantioseparation takes place due to the different non-covalent molecular interaction of the enantiomers with a chiral selector, whose electrophoretic mobility is different to that of the enantiomers (Amini, 2011; Chankvetadze, 2007).

The most frequently additives used are cyclodextrins (CDs) because they are commercially available, UV-transparent and relatively low cost. CDs can form complexes with molecules based on their inclusion into the hydrophobic cavity; secondary interactions may include hydrogen bonding or dipole–dipole interactions with the hydroxyl groups on the CDs, or with other polar substituents of the CDs (Rezanka et al., 2014).

In chiral CE the host-guest complexation between the CD and the enantiomers is responsible for the enantioresolution and the electrophoresis and electroosmosis permit differential migrations of the host-guest complexes (Dubsky et al., 2010).

The analytical methods used for the determination of fluoxetine are mainly HPLC methods with UV (Gatti et al., 2003), fluorescence (Guo et al., 2003) or mass spectrometry (Sutherland et al., 2001) detection; enantioseparation being done using indirect methods such as derivatization (Guo et al., 2003) and direct methods using CDs (Yu et al., 2002) or chiral stationary phases (Yu et al., 2006).

Few reports on the application of CE methods for separation of fluoxetine enantiomers were found in the literature. These methods present usually the enantioseparation in acidic condition using negatively charged CDs or combined neutral and negatively charged CDs. A systematic approach to enantiomeric separations in CE and HPLC with chiral mobile phase additives or a chiral stationary phase was described in a study of fluoxetine and norfluoxetine with CDs as chiral selectors (Piperaki et al., 1995). High detection sensitivity CE was used for the stereoselective analysis of fluoxetine and norfluoxetine in plasma and serum samples using a CD-modified phosphate buffer at pH 2.5 and a dual CD system containing dimethylated-β and phosphated-γ-CDs (Desiderio et al., 1999). A complex screening including 11 neutral and charged CDs was carried out for the enantioseparation of fluoxetine and four of its structural analogs; several negatively charged CDs showed enantioresolution abilities at pH 2.5 due to the high electrophoretic mobility of these CDs in the opposite direction to fluoxetine as well as due to the enhanced binding with positively charged fluoxetine (Inoue and Chang, 2003). In order to prevent the absorption of the basic compound fluoxetine on the negatively charged capillary wall in low pH buffer and to improve enantioresolution, guanidine as cationic additive can be added to a phosphate buffer containing sulfobuthylether-β-CD at pH 2.5 (Javid et al., 2013). An electrokinetic chromatography–counter current procedure for the separation of fluoxetine enantiomers using a phosphate buffer at pH 8.0 and highly sulfated β-CD was developed and applied to the determination of the enantiomers in pharmaceutical formulations (Asensi-Bernardi et al., 2013). Sulfated maltodextrin as a novel anionic chiral selector was used as an alternative to CDs for the separation of several basic drugs including fluoxetine (Tabani et al., 2015).

The aim of the study was the development of a new, simple and rapid alternative method for the chiral separation of fluoxetine enantiomers using a CZE method and CD as chiral selectors; and also the optimization of analytical conditions and validation of the newly developed method according to ICH guidelines. For the optimization process we used an experimental design approach, a methodology of experimental research in which the variables under study are simultaneously changed inside an experiment; these strategy being aimed to guide the researcher in selecting regions of interest inside a large experimental region, with a minimum number of experiments (Orlandini et al., 2014).

2. Materials and methods

2.1. Chemicals and reagents

R,*S*-fluoxetine and *S*-fluoxetine of pharmaceutical grade were acquired from Solmag (Mulazzano, Italy). Phosphoric acid (85%), disodium hydrogenophosphate, and sodium dihydrogenophosphate were purchased from Merck (Darmstadt,