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Development of chiral methodologies by capillary electrophoresis with ultraviolet and mass spectrometry detection for duloxetine analysis in pharmaceutical formulations

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ABSTRACT

Two chiral methodologies were developed by capillary electrophoresis (CE) with UV and mass spectrometry (MS) detection to ensure the quality control of the drug duloxetine, commercialized as a pure enantiomer. Both methods were optimized to achieve a high baseline enantioresolution ($Rs > 2$) and an acceptable precision (RSD values <5% for instrumental repeatability and <10% for intermediate precision). In addition to allow the unequivocal identification of duloxetine enantiomers, the CE–MS method improved the sensitivity with respect to the use of CE–UV (LOD 200 ng/mL by CE–UV and 20 ng/mL by CE–MS) enabling to detect 0.02% of duloxetine enantiomeric impurity. This is the lowest LOD value ever reported for this drug, being this work the first one enabling to accomplish with the ICH guidelines requirements. The developed methods were validated and applied for the first time to the analysis of four pharmaceutical formulations. The content of *R*-duloxetine in all these samples was below the detection limit and the amount of *S*-duloxetine was in good agreement with the labeled content, obtaining results by the two methods that did not differ significantly (p-values >0.05).

1. Introduction

Chirality acquires special relevance in the pharmaceutical industry since drug properties are strong dependent on the ingested enantiomer as a result of the inherent enantioselectivity of the biological processes. Due to the potentially different bioactivity of the enantiomers of a chiral drug, it is mandatory for the pharmaceutical laboratories to justify the commercialization of a new drug, either as a pure enantiomer or as a racemic mixture. Therefore, a rigorous quality control must be carried out to regulate the drug enantiomeric impurities present in a pharmaceutical formulation, especially if they have adverse effects.

As many pharmaceutical compounds, duloxetine ((+)-(*S*)-*N*-methyl-γ-(1-naphthalenyloxy)-3-(2-thiopene)-propanamine) is a chiral drug. Both enantiomers of duloxetine are potent norepinephrine and serotonin reuptake inhibitors, although the *S*-enantiomer was found to be slightly more potent [1]. Since this drug is commercialized as a pure enantiomer in the treatment of major depressive disorder [2], chiral methodologies need to be developed to ensure the quality control of its optical purity. These methodologies must, on the one hand, separate the desired enantiomers of a chiral drug and, on the other hand, detect low amounts of the enantiomeric impurities (the International Conference on Harmonisation (ICH) dictates that a method must be able to detect amounts of a determined impurity lower than 0.1% [3]).

The separation and study of duloxetine enantiomers have been reported by HPLC and CE with UV detection. Regarding HPLC, Rane et al. [4] developed a chiral methodology employing a chiral stationary phase (CSP) based on amylose detecting 250 ng/mL of duloxetine impurity, which eluted in first place. Yang et al. [5] described two HPLC methods, one employing a vancomycin CSP and the other using (2-hydroxypropyl)-β-CD (HP-β-CD) as additive in the mobile phase. In both cases, the enantiomeric impurity of duloxetine was the last to elute making it harder to be detected in presence of high amounts of the major enantiomer. CE methods have also been described. Some authors reported methods employing glycogen based selectors [6,7] and erythromycin lactobionate [8,9] resulting in analysis times from 15 to 60 min. Rickard et al. reported an acceptable duloxetine enantioseparation by means of HP-β-CD in 25 min detecting up to 0.2% of enantiomeric impurity as it migrated in first place [10,11]. This is, so far, the only LOD reported for *R*-duloxetine in the bibliography; nevertheless,

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it is not in agreement with the ICH guidelines requirements [3]. This remarks the necessity to develop more sensitive methods to be able to determine duloxetine enantiomers in pharmaceutical formulations.

CE is one of the most relevant analytical techniques used in chiral separation since it offers many advantages such as high resolution power, low reagents and sample consumption, and high flexibility given the possibility to easily modify the chiral selector added to the BGE. In addition, CE can be coupled to MS to combine the advantages of CE in chiral analysis with the MS potential to identify unknown chiral compounds with unambiguous assignment and to give structural information [12,13]. The main problem of the chiral CE–MS coupling is the contamination of the ionization source as a result of the entrance of non-volatile chiral selectors, which produces a loss of the ionization efficiency and a decrease in the detection sensitivity [14,15]. Although some works demonstrate no significant decrease in the sensitivity when low concentrations of the chiral selector are employed [13,14,16], different approaches and strategies have been broadly employed to solve this problem. These strategies include the use of compatible chiral selectors in EKC or CSPs in capillary electrochromatography (CEC), or counter migration and partial filling techniques (PFT) [12,13,15,17–19].

The aim of this work was the development of two CE methodologies using UV and MS detection for the enantioseparation of duloxetine, along with their validation to establish the content of duloxetine and its enantiomeric impurity in different pharmaceutical formulations.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade. Ortho-phosphoric acid 85% (v/v), dimethyl sulfoxide (DMSO), and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Methanol was obtained from Scharlau Chemie (Barcelona, Spain) and formic acid, and ammonium hydroxide from Sigma (St. Louis, MO, USA). The employed water was Milli-Q quality (Millipore, Bedford, MA, USA). β-CD; methyl-β-CD (M-β-CD) (DS 1.7–1.9); HP-β-CD (DS ∼ 4.2); 2,6-di-*O*-methyl-β-CD and 2,3,6-tri-*O*-methyl-β-CD were acquired in Fluka (Buchs, Switzerland). Acetyl-β-CD; (2-hydroxybutenyl)-β-CD (HB-β-CD); 2,3,6-tri-*O*-acetyl-β-CD; γ-CD, acetyl-γ-CD (A-γ-CD); methyl-γ-CD (M-γ-CD) (DS ∼ 12), (2-hydroxy)-butenyl-γ-CD (DS ∼ 4.5), (2-hydroxy)-butenyl-γ-CD (DS ∼ 3.2) and 2,3,6-tri-*O*-acetyl γ-CD were purchased from Cyclolab (Budapest, Hungary). M-β-CD (DS 10.5–14.7) was bought in Sigma-Aldrich (St. Louis, MO, USA).

(*R*,*S*)-duloxetine HCl, (*R*)-duloxetine HCl and (*S*)-duloxetine HCl were purchased from IS Chemical Technology (Shanghai, China). The commercial pharmaceutical formulations were acquired in pharmacies from Madrid (Spain). According to the labeled data, they contained 30 mg of duloxetine per capsule.

2.2. CE–UV conditions

Electrophoretic experiments were carried out on a HP ^{3D}CE system from Agilent Technologies (Palo Alto, CA, USA) with a diode array detector (DAD). The electrophoretic system was controlled by HP ^{3D}CE ChemStation software included the data collection and analysis. BGE employed in the CE–UV experiments consisted on 150 mM phosphate buffer (pH 3.0) containing 0.5% (w/v) of HP-β-CD. Separations were performed in an uncoated fused-silica capillary of 50 μm I.D. and a total length of 64.5 cm, acquired from Polymicro Technologies (Phoenix, AZ, USA) at 30 kV (positive polarity) and 20 °C. Injections were carried out applying 50 mbar for 20 s. Detector parameters were as follows: a response time of 1.0 s and a wavelength of 220 nm (bandwidth 35 nm) including a reference wavelength of 375 nm (bandwidth 100 nm). At the beginning of each working day the capillary was flushed with buffer solution for 10 min and at the end of the day it was flushed with Milli-Q water for 5 min. In order to ensure the repeatability between injections, the capillary was flushed with DMSO for 2 min, buffer solution for 3 min and BGE for 1 min.

2.3. CE–MS conditions

CE–MS analysis were performed in a HP^{3D}CE instrument (Agilent Technologies, Palo Alto, CA, USA) coupled through an orthogonal coaxial sheath interface (model G1607A from Agilent Technologies, Palo Alto, CA, USA) to the electrospray ionization (ESI) source of an ion trap (IT) mass spectrometer (model AmaZon SL from Bruker Daltonics, Bremen, Germany) for MS detection. For MS control and data analysis, a TrapControl Software 7.0 for AmaZon was used. BGE employed in the CE–MS experiments consisted of 150 mM ammonium formate buffer (pH 3.0). The separation was achieved in an uncoated fused-silica capillary of 104 cm and 50 μm I.D., at 30 kV (positive polarity) and 15 °C. Injections were performed applying 50 mbar for 5 s. Between analysis, the capillary was flushed by applying 1 bar with DMSO for 4 min, BGE solution for 5 min and BGE with 0.5% (w/v) of HP-β-CD during 1 min (38% of total capillary length).

Operating MS conditions consisted on a sheath liquid composition of 80:20 (v/v) methanol/water with 0.1% (v/v) of formic acid at a flow rate of 3.3 μL/min by a syringe pump (Hamilton, USA). The nebulizer and the drying gas conditions were 3 psi N_2 and 5 L/min N_2 at 200 °C. The mass spectrometer operated with the ESI source in the positive ion mode at −4.5 kV with an end plate of −500 V. The ion optical parameters were tuned in the "expert mode" and the capillary exit value was optimized to 57 V. In MS^2 experiments the Ion Charge Control (ICC) was activated with a target up to 100,000 ions using 50 ms of accumulation time and three averages. The *m*/*z* scanned range was from 100 to 400 *m*/*z* in the "Ultra-Scan" mode (32,000 (*m*/*z*)/s). The isolation width of the precursor ion (298.1 *m*/*z*) was set to 4.0 *m*/*z*. Its fragmentation was carried out by collision-induced dissociation with the helium present in the trap for 40 ms with a fragmentation amplitude of 0.5 V (with the "smart-frag" option deactivated) and a fragmentation width of 10 *m*/*z*. Extracted Ion Electrophoregrams (EIEs) were obtained extracting the product ion 153.8 *m*/*z* with an extraction window of −0.3/+0.7 *m*/*z* using a smoothed option of the software (Gauss at 4 points).

2.4. Preparation of stock and sample solutions

Stock solutions of duloxetine were prepared in DMSO and then diluted with Milli-Q water until desired concentration. Commercial pharmaceutical solutions were prepared by homogenizing the content of five capsules of the medicament and grinding it. The required amount for analysis of the grinded powder was dissolved in DMSO and centrifuged for 10 min with a rotational speed of 4000 rpm at 20 °C. The supernatant was taken and brought to a known volume. Afterwards, it was diluted to the required concentration employing Milli-Q water.

3. Results and discussion

3.1. Development of a chiral methodology for the enantiomeric separation of duloxetine by CE with UV detection

The first step in the method development was to choose a CD offering the best enantioselectivity and enantioresolution (*R*s).