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Enantiomeric purity assay of moxifloxacin hydrochloride by capillary electrophoresis

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Abstract

A capillary electrophoresis method for determining the enantiomeric purity of moxifloxacin hydrochloride in drug substance and ophthalmic/otic drug products was developed and validated. Because moxifloxacin hydrochloride has two chiral centers, the existence of four different isomers is possible. The method was capable of separating moxifloxacin hydrochloride, which is the *S*,*S*-isomer, from its potential chiral degradation products, which are the *R*,*R*-enantiomer, the *R*,*S*-diastereomer, and the *S*,*R*-diastereomer. The separation was carried out at 20 °C in a 50 μm × 40 cm fused-silica capillary with an applied voltage of −13 kV using a 12.5 mM TEA phosphate buffer (pH 2.5) containing 5% highly-sulfated gamma-cyclodextrin (HS-γ-CD) and 6% acetonitrile. The detection wavelength was 295 nm. The method was validated with respect to its specificity, linearity, range, accuracy, and precision in the expected range of occurrence for the isomeric impurities (0.05–5%). The method was used to investigate whether racemization was a potential degradation pathway for the drug substance, both in the solid state and in solution.

Keywords: Moxifloxacin; Fluoroquinolones; Capillary electrophoresis; Validation; Enantiomeric purity

1. Introduction

Moxifloxacin hydrochloride belongs to the fluoroquinolone class of anti-infective compounds. The compound has a broad antibacterial spectrum against Gram-positive and Gram-negative organisms including anaerobic bacteria and was developed by scientists at Bayer AG for systemic treatment of respiratory tract infections [1]. Alcon Research Limited has licensed the drug from Bayer AG for development as a topical treatment of eye and ear infections.

Moxifloxacin hydrochloride (Fig. 1) possesses two chiral centers [2] and is the *S*,*S*-isomer. Its potential chiral impurities are the *R*,*R*-enantiomer, the *R*,*S*-diastereomer and the *S*,*R*-diastereomer. The FDA's Draft Guidance for Industry on the development of stereoisomeric drugs [3] states that applications for an enantiomeric drug substance or applications for drug products containing an enantiomeric drug substance should include a stereochemically specific identity test and/or a stereochemically selective assay method. However, if it can be demonstrated that stereochemical conversion does not occur during stability testing of the drug substance and drug product, then stereoselective tests may not be needed. Therefore, the aim of the present work was to develop and validate an analytical method capable of separating *S*,*S*-moxifloxacin, the parent drug, from its three potential isomeric impurities, with a quantitation limit of 0.05%, with the intent of using the method to determine if racemization was a potential degradation pathway of the drug substance, both in the solid state and in solution.

For the majority of drug tests that involve quantitation of impurities, HPLC is the preferred analytical method. Direct liquid chromatographic enantiomeric separation of several fluoroquinolones, including gemifloxacin, on a Crownpak CR(+) column has been reported [4]. A normal phase chiral LC method has been described for the separation of two isomers of (±)*cis*-8-benzyl-2,8-diazobicyclo(4.3.0)nonane, an intermediate of moxifloxacin using a Chiralcel OD-H col-

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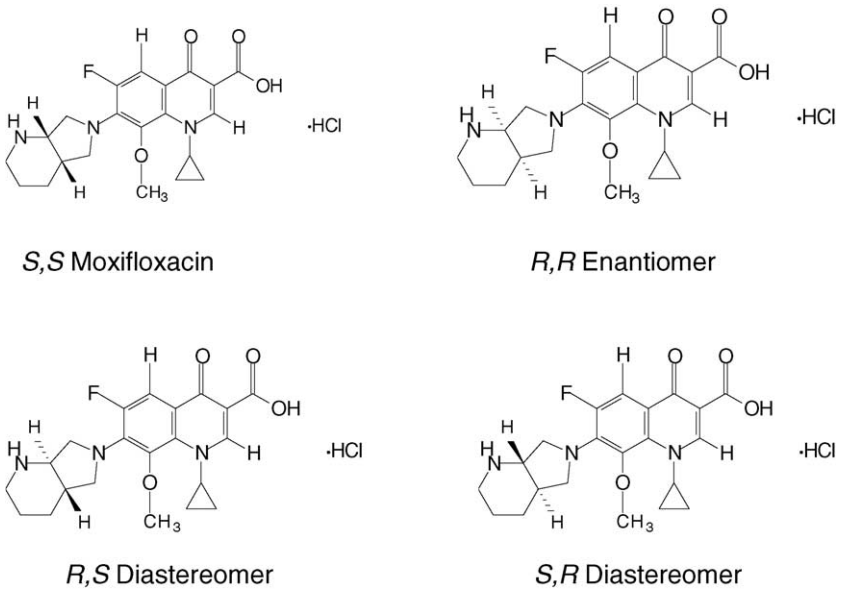

Fig. 1. Chemical structures of *S,S*-moxifloxacin and its potential isomeric impurities.

umn [5]. However, we found these columns unable to separate the moxifloxacin isomers. Twenty-three other HPLC columns were also screened (including four polysaccharide, eight cyclodextrin, three macrocyclic glycopeptide and eight Pirkle-type phases), but none were found to be suitable. We, therefore, investigated the method of capillary electrophoresis (CE) as a practical alternative, because the technique is considered to be complementary to HPLC. Several validated CE methods have been reported for the separation of enantiomeric drugs [6–9] and CE is beginning to gain acceptance within the regulated environment of the pharmaceutical industry. A general test chapter on CE has recently been added to both the US Pharmacopeia [10] and European Pharmacopeia [11]. An enantiomeric separation of the fluoroquinolone drug, ofloxacin, by cyclodextrin-mediated CE has been reported [12]. However, to the best of our knowledge, the work presented here is the first literature report which describes the development and validation of a stereoselective analytical method for moxifloxacin and/or for any fluoroquinolone drug possessing two chiral centers. An unpublished CE method for the separation of the moxifloxacin *S,S*- and *R,R*-enantiomers using heptakis(2,6-di-*O*-methyl)-β-cyclodextrin has been developed and validated by Bayer AG. However, the use of highly-sulfated gamma-cyclodextrin (HS-γ-CD) reported here was able to provide separation of all four moxifloxacin isomers.

2. Experimental

2.1. Materials and reagents

Fused-silica capillaries were purchased from Polymicro Technologies Inc. (Phoenix, AZ). Acetonitrile (HPLC grade), boric acid, citric acid, hydrochloric acid (concentrated), hydrogen peroxide (30%), sodium borate decahydrate, sodium citrate dihydrate, sodium phosphate monobasic monohydrate, sodium phosphate dibasic anhydrous, and sodium hydroxide solution (50%) were purchased from EM Science (Gibbstown, NJ). Sodium hydroxide (0.1N) was purchased from Ricca Chemical Co. (Arlington, TX). Triethylammonium phosphate buffer (50 mM, pH 2.2) and HS-γ-CD (20% (w/v) aqueous solution) were purchased from Beckman Coulter (Fullerton, CA). Moxifloxacin hydrochloride (*S,S*-isomer) and its isomeric impurities (*R,R*-enantiomer, *R,S*-diastereomer, and *S,R*-diastereomer) were kindly provided by Bayer AG (Wuppertal, Germany). The diastereomers were supplied as a racemic mixture. All buffer, standard and sample solutions were prepared using purified water (USP).

2.2. Instrumentation and method conditions

Method development, validation, and degradation pathways studies were carried out on a Beckman P/ACE MDQ capillary electrophoresis instrument equipped with a capillary cartridge cooling system and a photodiode array detector. Data was acquired and processed using Beckman ^{32}Karat software, version 4.01. The capillary was 40 cm long (30 cm effective length) with a 50 μm internal diameter. The applied voltage was 13 kV (0.17 min ramp time) using reverse polarity. The capillary was thermostated at 20 °C. Samples were pressure injected for 15 s at 1 psi followed by a pressure injection of a water plug for 10 s at 0.3 psi. The photodiode array detector was used in single wavelength acquisition mode at 295 nm with a data sampling rate of 4 Hz, a bandwidth of 10 nm, and normal filtering. The capillary was preconditioned each day by flushing with the follow-