Saudi Pharmaceutical Journal (2015) 23, 85–94

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Development and validation of stability-indicating high performance liquid chromatography method to analyze gatifloxacin in bulk drug and pharmaceutical preparations

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Received 2 April 2014; accepted 18 June 2014
Available online 28 June 2014

KEYWORDS

Gatifloxacin;
HPLC;
Method validation;
Accelerated stability;
Solid lipid nanoparticles

Abstract Quantitative determination of gatifloxacin in tablets, solid lipid nanoparticles (SLNs) and eye-drops using a very simple and rapid chromatographic technique was validated and developed. Formulations were analyzed using a reverse phase SUPELCO® 516 C-18-DB, 50306-U, HPLC column (250 mm × 4.6 mm, 5 μm) and a mobile phase consisting of disodium hydrogen phosphate buffer:acetonitrile (75:25, v/v) and with orthophosphoric acid pH was adjusted to 3.3 The flow rate was 1.0 mL/min and analyte concentrations were measured using a UV-detector at 293 nm. The analyses were performed at room temperature (25 ± 2 °C). Gatifloxacin was separated in all the formulations within 2.767 min. There were linear calibration curves over a concentration range of 4.0–40 μg.mL^{-1} and correlation coefficients of 0.9998 with an average recovery above 99.91%. Detection of analyte from different dosage forms at the same R_t indicates the specificity and stability of the developed method.

1. Introduction

Gatifloxacin (GTX) is a synthetic broad spectrum fluoroquinolone antibiotic obtained from nalidixic acid, used to treat various a wide range of infectious diseases (Fig. 1). Fluoroquinolones are quinolones having fluorine at the 6th position of the naphthyridine ring. Published structure–activity data show that the presence of fluorine atom (F) at C6 position

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.jsps.2014.06.005

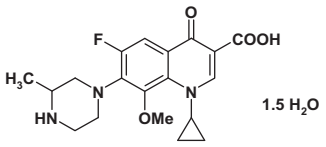

Figure 1 Chemical structure of gatifloxacin (GTX).

broadens their activity spectrum against both Gram-negative and Gram-positive pathogens (Arteseros et al., 2002). The methyl substituent on the piperazine ring contributes to its Gram-positive activity, prolongs the half-life allowing for once daily dosing, provides metabolic stability as evident by primary renal elimination of unchanged drug, and may limit potentially adverse interaction with the drug metabolizing enzyme. GTX has a cyclopropyl group at the N1 position like ciprofloxacin and sparfloxacin which boosts Gram negative activity and provides some Gram positive activity. Like ciprofloxacin and sparfloxacin, GTX has a cyclopropyl group at the N1 position that enhances Gram-negative activity and provides some Gram-positive activity. GTX lacks the 2,4-difluorophenyl group at the N1 position that was postulated to induce hepatic and hematologic toxicities associated with trovafloxacin and temafloxacin, respectively. Most notably, GTX is characterized by the presence of a methoxy (—OCH_3) group at the C8 position. This substituent also possessed by moxifloxacin, confers enhanced activity against DNA-*gyrase* and *Topoisomerase*-IV (Fukuda and Hiramatsu, 1999) and may be important in limiting the potential for the development of bacterial resistance. In addition, lack of a halide at the C8 position reduces the potential for phototoxicity.

In pharmaceutical preparations quantitative determination of fluoroquinolones has been reported by several analytical methods, like capillary electrophoresis (Flurer, 1997), UV spectrophotometry (Gouda et al., 2008; Amin et al., 2007), titrimetry (Belal et al., 1999), on-line solid phase extraction and fluorometric detection by HPLC (Tasso and Costa, 2007), stability indicating high-performance thin-layer chromatographic method for determination of GTX as bulk drug (Motwani et al., 2006) and HPLC (Mirza et al., 2008). Liquid chromatography with column switching technique for GTX determination in serum was developed by Nguyen et al., 2004, but column switching is a difficult and multistep technique so it is not feasible in all analytical laboratories. Mostly HPLC techniques were either very exhaustive or applicable in the identification of fluoroquinolones in biotic fluids, nourishing animal products, feeds (supplements) and to a lesser extent, in pharmaceutical formulations. Majority of the described techniques involve troublesome mobile phase (buffers) and difficult detection methods (fluorescence or mass detectors) (Samanidou et al., 2003 and Joshi, 2002). The main objective of this procedure is to develop and validate an economical, rapid and sensitive method for the quantitative determination of the drug in commercial eye drops and tablets as well as prepared solid lipid nanoparticles where all formulations containing the same fluoroquinolone molecule gatifloxacin could be determined on a single chromatographic system at the wavelength, 293 nm. Although other HPLC analysis methods for GTX have been reported, this proposed method carries two main advantages: (i) versatility proven by the validity of our method to determine GTX level in different pharmaceutical forms including bulk drug, tablets, eye drops, and solid lipid nanoparticles (SLNs) using the very same defined conditions with the same mobile phase, and (ii) the method is considerably fast where GTX retention time was as short as 2.67 min.

2. Materials and methods

2.1. Materials

Gatifloxacin sesquihydrate was kindly gifted by Wockhardt Pharmaceuticals, Aurangabad, India, and used as reference standard without further purification. Acetonitrile (Spectrochem Pvt. Ltd. Mumbai, India), orthophosphoric acid (CDH Pvt. Ltd. New Delhi, India), methanol (MerkSpecialities, Pvt. Ltd. Mumbai, India), and analytical grade disodium hydrogen phosphate (CDH Pvt. Ltd. New Delhi, India) were purchased. Milli-Q, Millipak® 40, Millipore System was used to purify water. Purification of all solvents and solutions was done by using the membrane filter (Millipore®Millex-HV filter units, Durapore-PVDF, polyethylene, 0.45 μm pore size) and degassed before use by ultrasonication (Ultrasonicator, PCI, Mumbai, India). All other solvents used were of HPLC and reagents were of analytical grade. Different pharmaceutical dosage forms of different manufacturers, presented in Table 1 were used as samples for the analysis by the developed method.

2.2. Instrumentation and chromatographic conditions

Quaternary pump high pressure liquid chromatograph model Shimadzu® LC-9A, equipped with UV–visible detector model SPP-6A, controlling system SCL-6B, connected to a microcomputer with "Chemstation" Shimadzu® Class LC-10 Version 1:62 is used for integration and processing of chromatograms. A reversed phase SUPELCO® 516 C-18-DB, 50306-U, HPLC Column of dimension 250 mm × 4.6 mm, 5 μm was used as the analytical column. All analysis was done at ambient temperature (25 ± 2 °C). The mobile phase is a mixture of 0.02 M disodium hydrogen phosphate buffer:acetonitrile (75:25, v/v) and using orthophosphoric acid pH was adjusted to 3.3. The flow rate was 1.0 $mL.min^{-1}$ and volume of injection was 20 μL. Before using all solutions the mobile phase was sonicated for 30 min and UV detection was performed at 293 nm for GTX.

2.3. Calibration curves

Accurately weighed amount of standard of gatifloxacin sesquihydrate bulk, equivalent to 10 mg of free base was transferred to a 100 mL volumetric flask. The volume was completed with the mobile phase. The prepared solutions were sonicated during 30 min and filtered through a membrane filter (0.45 μm). Final concentration was 100 $\mu g.mL^{-1}$. Aliquots of each solution were accordingly diluted with the mobile phase in order to obtain solutions with a final concentration of 12 $\mu g.mL^{-1}$. Every day solutions are prepared freshly. Ten different concentrations of GTX bulk (4, 8, 12, 16, 20, 24, 28, 32, 36 and 40 $\mu g.mL^{-1}$) were obtained of each standard solution and diluted with mobile phase. 20 μL of each solution was injected in the chromatographic system ($n = 3$) and mean values of peak areas were plotted against concentrations.