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RESEARCH ARTICLE

A VALIDATED RP- HPLC METHOD FOR THE ANALYSIS OF MOXIFLOXACIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and precise RP-HPLC method was developed and validated for the determination of Moxifloxacin hydrochloride in pharmaceutical dosage forms. Chromatography was carried out on Shimadzu Item CBM-20A , control of LC-20A/10Avp/10A-Series solvent Delivery Module (Pump), SPD-M20A Photodiode Array Detector using a mixture of Buffer: Methanol (55:45%) as the mobile phase at a flow rate of 1.0 mL min^{-1}. The analyte was monitored using UV detector at 293 nm. The Retention time of the drug is 5.816 min for Moxifloxacin. The proposed method is found to be having linearity in the concentration range of 20-60µg mL^{-1} with correlation coefficient of 0.999. The developed method has been statistically validated and found simple and accurate. The mean recoveries obtained for Moxifloxacin HCL are in the range 99.3-102%. Due to its simplicity, rapidness, high precision and accuracy of the proposed method it may be used for determining Moxifloxacin HCL in bulk and dosage forms.

KEYWORDS: *Moxifloxacin HCL, RP-HPLC.*

Introduction

Moxifloxacin hydrochloride[1],is a fourth generation synthetic fluoroquinolone antibacterial agent developed by Bayer AG can be used to treat respiratory infections, including acute sinusitis, acute exacerbations of chronic bronchitis, and community-acquire pneumonia, as well as dermatological infections, as a second-line agent in tuberculosis. Its chemical designation is (+)-(1-cyclopropyl-7-((s,s)-2,8-diazabicyclo(4.3.0)non-8-yl)-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-3quinoline carboxylic acid.. The empirical formula is $C_{21}H_{24}FN_3O_4$•HCl and having a molecular weight of 437.9. The structure was shown in Fig. 1.

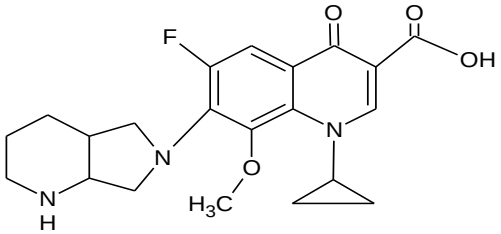

Fig. 1 Structure of Moxifloxacin HCL

Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerases, and topoisomerase IV[2], enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication. This mechanism can also affect mammalian cell replication. In

particular, some congeners of this drug family (for example those that contain the C-8 fluorine),[3]display high activity not only against bacterial topoisomerases, but also against eukaryotic topoisomerases and are toxic to cultured mammalian cells and in vivo tumor models.[4] Although quinolones are highly toxic to mammalian cells in culture, its mechanism of cytotoxic action is not known. Quinolone induced DNA damage was first reported in 1986 (Hussy et al.)[5]

Recent studies have demonstrated a correlation between mammalian cell cytotoxicity of the quinolones and the induction of micronuclei[6,7,8,9]. As such some fluoroquinolones, including moxifloxacin, may cause injury to the chromosome of eukaryotic cells [10, 11,12,13,14,15].

There continues to be considerable debate as to whether or not this DNA damage is to be considered one of the mechanisms of action concerning the severe adverse reactions experienced by some patients following fluoroquinolone therapy.

Literature survey.

As per the literature survey it is revealed that very few analytical methods were reported. Sanjay K.Motwani et al.,developed a simple, high performance thin layer chromatographic (HPTLC) method for densitometric determination of Moxifloxacin both as bulk drug and pharmaceutical formulation [9]. Shas S.A et al., [15]developed simple, sensitive and precise HPTLC method for estimation of Moxifloxacin in its tablet formulation . Sanjay K.Motwani et al., [16] developeda simple UV-Spectrophotometric method for the estimation of Moxifloxacin in bulk and pharmaceutical formulations. Fanny L.B.Guerra et al., [17] developed a microbiological assay for quantitation of Moxifloxacin in tablets. The method consisted of a cylinder-plate agar diffusion assay using Micrococcus luteus ATCC 9341 as the test microorganism and phosphate buffer (0.1M, pH 8.0) as the diluent solution. Our present plan is to develop new simple, reliable and reproducible RP-HPLC method which was developed, validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [15].

EXPERIMENTAL

Instrumentation

Analysis was performed using High Performance Liquid Chromatography System (HPLC) Shimadzu Item CBM-20A, control of LC-20A/10Avp/10A-Series solvent Delivery Module (Pump), SPD-M20A Photodiode Array Detector..

Chemicals and reagents

Moxifloxacin HCL was obtained as a gift sample from Msn labs . Triethylamine and Orthophosphoric acid (AR grade) was used for preparing buffer and acetonitrile HPLC grade was purchased from Merck.

Chromatographic conditions

Mobile phase consists of buffer (P^H 2.5 with Triethylamine and Orthophosphoric acid): Methanol [55:45 (v/v)]. Buffer was prepared by dissolving 2 ml of orthophosphoric acid into 1000 ml of water and the P^H was adjusted to 2.5 with triethylamine, filtered through 0.45µm nylon membrane filter and degassed.

The mobile phase was pumped from the solvent reservoir to the column at a flow rate 1.0 mL min^{-1}. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The eluents were monitored at 293 nm.

Diluent: Mobile phase.

Methodology

400 mg of accurately weighed Moxifloxacin HCL is dissolved to 100 mL volumetric flask containing 70mL of diluents. The solution was sonicated for 20

min to dissolve the drug completely and the volume made up with mobile phase. Subsequent dilutions of this solution ranging from 20-60 µg mL^{-1} were made with the mobile phase in 10 mL volumetric flasks. These solutions were filtered through 0.45 µ membrane filter.10 µL of the filtrate was injected 6 times into the column and the corresponding chromatograms were obtained. Drug was analyzed at 293 nm. Retention time and mean peak areas were recorded for all the concentrations obtained from the chromatograms. A calibration curve of mean peak area to respective concentration was plotted; the regression of the drug concentrations over the peak area was computed using least squares method of analysis. Regression equation was used to estimate the amount of Moxifloxacin HCL in tablet formulations.

Estimation of Moxifloxacin HCL in Tablet dosage forms

Weighed and powered 20 tablets. Transferred the powder equivalent to 400 mg of Moxifloxacin into 100 ml of clean, dry, volumetric flask and, to this added 70 ml of mobile phase and sonicated for about 15 minutes, further made up the volume with mobile phase and then filtered through 0.45 micron filter. Further diluted 1 ml of the filtrate to 100 ml with mobile phase. Each of these solutions was injected twice into the system and the chromatograms were recorded. The mean peak areas of the drug of five such determinations were calculated and the drug content in the tablets was quantified using the regression equation obtained for the pure sample.

The calibration curve for Moxifloxacin HCL was drawn by plotting the mean peak area versus concentration of Moxifloxacin HCL, yielded coefficient of regression r^2=0.9990over a concentration range (20-60µg mL^{-1}) the representative linear regression equation for Moxifloxacin HCL Y= 64053x-3184 as shown in Table 1 and Fig.3.

Results and discussion

Several systematic trials were performed to optimize the Chromatographic conditions for developing a sensitive, precise and accurate RP-HPLC method for the analysis of Moxifloxacin HCL in pharmaceutical dosage forms. The present method contains mobile phase buffer (P^H 2.5 with Triethylamine and Orthophosphoric acid): Methanol [55:45 (v/v)] which was found to be the most suitable as the chromatographic peaks obtained with this system were better defined and resolved and all almost free from tailing. Under the above conditions the retention time obtained for Moxifloxacin HCL was 5.816 min. A model Chromatogram was shown in Fig. 2.

Fig.2. Representative Chromatogram for Moxifloxacin Hcl

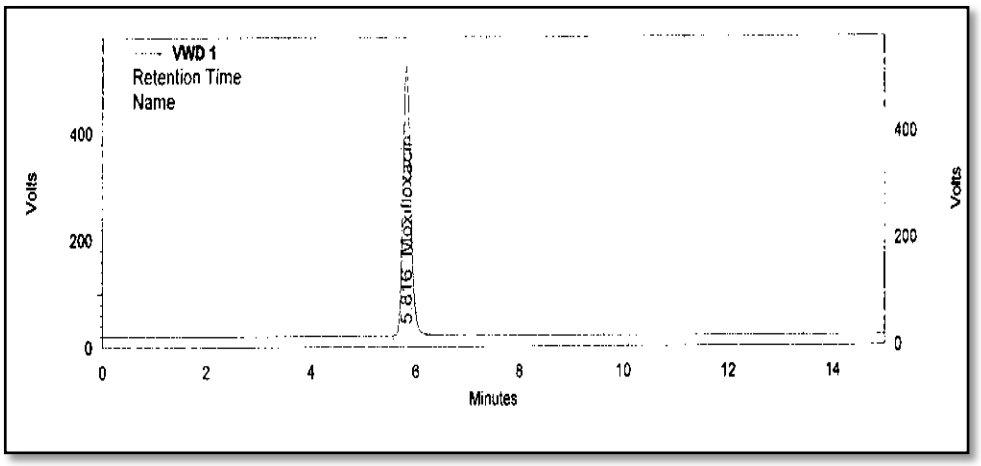

Table 1. Calibration of the proposed HPLC method

Solution No.	Conc. (µg)	Mean Area
1	20	1274173
2	24	1530117
3	32	2051283
4	40	2551840
5	48	3094460
6	56	3589786
7	60	3821015

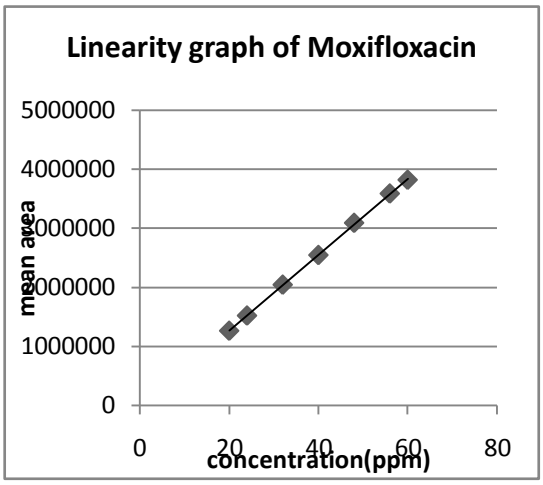

Fig.3Linearity plot for Moxifloxacin HCL

To study the accuracy of the proposed analytical method, recovery experiments were conducted using standard addition method. To discover whether excipients interfered with the analysis, known amount of pure drug at a different concentration levels were added to the Moxifloxacin HCL formulation and the mixtures were analyzed by the proposed method. The accuracy of the method was demonstrated at three different concentration levels in triplicate. The analysis carried out at 50%, 100%, 25% and 150%of specification limit. The analyzed samples were getting high recovery values from the developed method. The % recovery results of the method are given in Table 2.

Table 2 Recovery data of standard solutions added to the samples analyzed by using the proposed HPLC method

Amount of drug added (µg mL^{-1})	Amount found (µg mL^{-1}) (N=3)	%Recovery (N=3)
10	9.83	98.3
20	19.9	99.5
25	24.65	98.6
30	30.6	102

The developed HPLC method in the present study has also been used to quantify Moxifloxacin in the tablets dosage forms. Moxifloxacin HCL was quantified using the proposed analytical method and the results are given in Table. 3.

Table 3. Assay of Moxifloxacin HCL in tablets dosage forms by proposed HPLC method

Labeled amount (mg)	Observed amount (mg)	% Purity
400	398.96	99.74

From the obtained result it can be concluded that this method is quite precise and accurate. The absence of additional peaks in the Chromatogram indicated that there is no interference of the common excipients used in the Moxifloxacin HCl. The proposed HPLC method is sensitive and reproducible for the analysis ofMoxifloxacin HCL in pharmaceutical dosage forms. The method was duly validated by using required statistical parameters.

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