International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.6, No.4, pp 1189-1194, Aug-Sept 2014

Validated and Precise Reverse Phase-HPLC Method for the Quantitative Estimation of Norfloxacin from Marketed Formulation

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Abstract: A validated reverse phase liquid chromatographic method was developed for the estimation of a popular synthetic antibacterial agent, norfloxacin from marketed pharmaceutical formulations. The method was simple, sensitive, rapid, precise, accurate and repeatable. The liquid chromatographic technique was developed by reverse phase C18 column (Xterra C18, 5µm, 150mm × 4.6mm) using a mobile phase consisting 5.3mM phosphate buffer of pH 3.5 and acetonitrile in the ratio 60:40 pumped at a flow rate of 0.5ml/min. The detection wavelength and the retention time of norfloxacin were found to be 278 nm and 2.11 min, respectively. The results of the proposed method was statistically validated for linearity, specificity, accuracy, precession, ruggedness, robustness, limit of detection (LOD) and limit of quantification (LOQ) in accordance with ICH guidelines. The method was linear for the assay of norfloxacin in the range of to 0.01 to 0.5 µg/ml. The percentage recovery of norfloxacin was estimated to be 99.99%. The results of the described RP-HPLC method showed that this method can be successfully applied for routine quality control analysis of norfloxacin in marketed formulations.
Keywords: Norfloxacin, RP-HPLC, Chromatography, Validation, Mareted formulation.

Introduction

Norfloxacin (NX) is a first generation synthetic antibacterial agent used for the treatment of common and complicated urinary tract infections[1, 2]. Other applications include prostatitis due to E.coli[3, 4] and in ophthalmic preparations for the treatment of conjunctival infections[5, 6]. It is also administered along with a nitroimidazole for the treatment of amoebiasis associated with diarrhea symptoms. It is a broad spectrum antibacterial agent and like other fluoroquinolones it inhibits bacterial DNA gyrase and Topoisomerase II and IV[7, 8]. However, norfloxacin has got limited applications due to resistance against several bacteria or its associated side effects. Chemically it is 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1*H*-quinoline-3-carboxylic acid as presented in Figure 1.

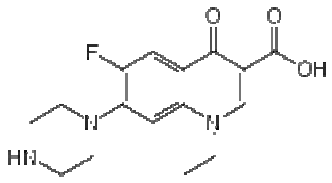

Figure 1. Chemical structure of norfloxacin

The commonly available marketed formulations of norfloxacin include tablets and ophthalmic preparations. An extensive literature review presents a large number of analytical techniques for the estimation of norfloxacin, its degradation products and its metabolites from solutions, from body fluids like blood and urine samples[9, 10], ground water samples[11-13], sediments[14] and from poultry feeds and products[15-17]. These techniques mainly involved spectrophotometric methods[18, 19], liquid-liquid micro extraction, liquid chromatography coupled with - tandem mass spectrometry[11, 12,14, 20], fluorescence detection[16, 17] and UV detection[15, 17, 21, 22]. Most of the reported chromatographic techniques mentioned the use of a C18 column and a phosphate buffer in combination with an organic modifier like acetonitrile or methanol as mobile phase. However, very few reported the estimation of NX from marketed formulations[23, 24]. Also these methods were less precise and more time consuming. In this study we reported a simple, rapid and less time consuming technique for the estimation of this drug from the marketed formulation. The simplicity of this method presents the importance of this technique in regular analysis of this drug from marketed formulations including solid dosage forms and ophthalmic preparations.

Materials and Methods

Instrumentation

The analysis was carried out using Waters Alliance auto sampler (eAlliance e2695, Waters, USA) and dual λ-absorbance detector (2489, Waters, USA) using a C_{18} (150 mm × 4.6 mm, 5 µm) X terra column. The injection volume was 10 µl. All chromatographic data were analysed using Empower 3 software.

Materials

Reference standard of norfloxacin was procured from Central Drug Laboratory, Kolkata. Norfloxacin tablets (Norfloxacin-400) were purchased from local pharmacy. All reagents were analytical grade. Potassium di-hydrogen phosphate and dipotassium hydrogen phosphate were purchased from Merck Ltd., Mumbai. All solvents were of HPLC grade. Acetonitrile and phosphoric acid were purchased from HPLC grade water was prepared from Merck Ltd., Mumbai. HPLC grade water was prepared from Aurium 611 UV water purification system of Sartorius, Germany.

Chromatographic conditions

Chromatographic separation was performed using an equilibriated C_{18}, X terra column (particle size 5 µm, 150 mm × 4.6 mm ID).Acetonitrile : 5.3 mM phosphate buffer in the ratio of 60:40 (v/v) was used as mobile phase and was filtered through 0.45 µm membrane filter (Millipore) and sonicated for 15 min. The pH of the buffer solution was adjusted to 3.5 ± 0.1 with orthophosphoric acid. A rheodyne injector with a 10 µl loop was used to deliver standard and sample solutions at a flow rate of 0.5 ml /min. The eluents were monitored at 278 nm.

Preparation of stock and standard solution

The standard stock solution of norfloxacin was prepared by transferring 12.5 mg of the drug in 25 ml volumetric flask. 10 ml of HPLC grade water and 1 drop of conc. HCl were added and sonicated for 15 minutes to dissolve the drug completely. The final volume was made by mobile phase. Finally the working standard solution containing 0.02 mg/ml was prepared from the above stock solution by diluting it with mobile phase. The contents of standard solution were filtered through 0.45 µm syringe filter before injection.

Preparation of sample solution

Twenty tablets containing norfloxacin were weighed individually, mean weight was determined and crushed into fine powder. Powder equivalent to 12.5 mg of norfloxacin was weighed accurately and transferred to a 25 ml volumetric flask. About 10 ml of HPLC grade water and 1 drop of conc. HCl were added to the volumetric flask and sonicated for 15 minutes. The final volume was made up to the mark by mobile phase. The resulting solution was filtered through Whatman filter paper no. 1. From the filtrate 1 ml was pipette and transferred into a 25 ml volumetric flask and the solution was made up to the volume by mobile phase to get final concentrations of 0.02 mg/ml of norfloxacin (theoretical value). The contents of sample solution were filtered through 0.45 µm syringe filter.

Results and Discussion

System suitability studies

10 µl of the freshly prepared solution of reference standard was injected under optimized chromatographic conditions to evaluate the system suitability parameters like retention time, tailing factor, theoretical plate etc. The results were presented in Table 1.

Table 1. System suitability parameter

Parameters	Norfloxacin
Wavelength of max absorbance (nm)	278
Retention time (min)	2.11
Tailing factor	1.21
Theoretical factor	11345
LOD (µg/ml)	0.08
LOQ (µg/ml)	0.23

Analysis of tablet formulation

10 µl standard and sample solutions were injectded on HPLC system after setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline. Chromatograms of standard solution (six replicates) and sample solutions (two replicates) were recorded. A representative chromatogram was given in Figure 2. The retention time was 2.11. The concentration of norfloxacin in tablet was calculated by comparing area of the sample solution with that of standard solution. The percentage assay of norfloxacin was given in Table 2.

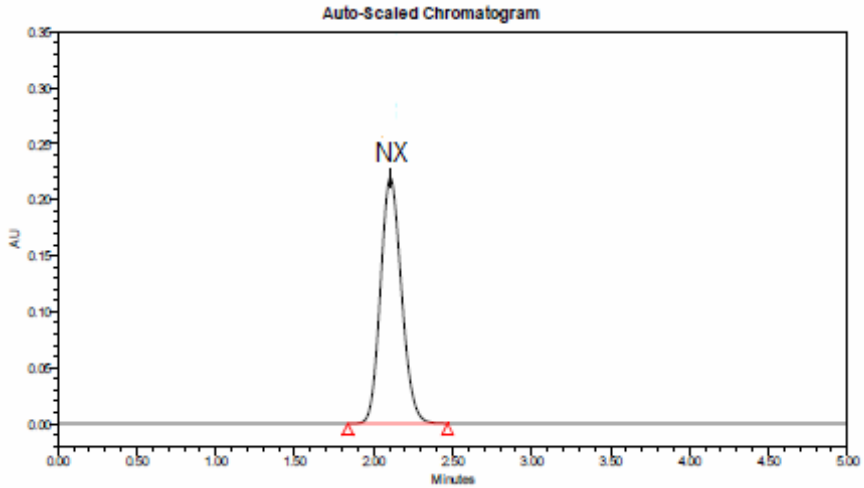

Figure 2. Representative chromatogram for NX (retention time = 2.106)

Table 2. Assay parameters

Tablet Formulation	Drug	Amount of Drug (mg/tab)		% of Label Claim	% RSD
		Labelled	Estimated		
Normax Tab (Ipca)	Norfloxacin	400	399.98	99.99	0.45

Method validation

The proposed analytical method was validated as per recommendation of USP and ICH guidelines[25, 26] for the parameters like linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ by the following procedures.

Linearity

In order to check the linearity for the developed method, solution of six different concentrations ranging from 0.01 µg/ml to 0.50 µg/ml were injected. A linear relationship between areas *versus* concentrations (Figure 3) was observed in above linearity range with regression co-efficient 0.98. The results were represented in Table 3.

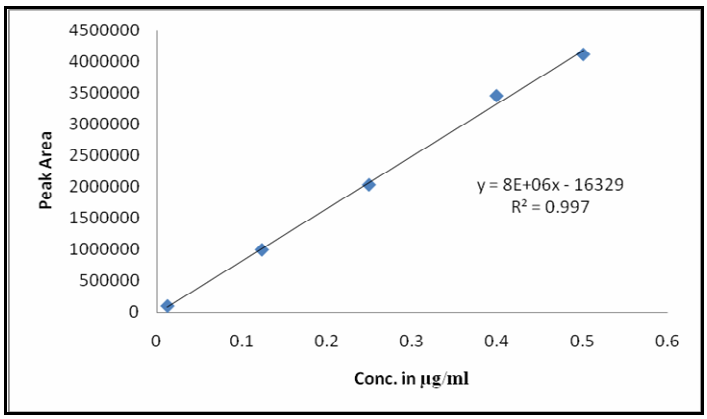

Figure 3. Linear relationship between peak areas *versus* concentrations.

Table 3. Linearity parameters

Parameters	Norfloxacin
Linearity range (µg/ml)	0.01 to 0.50
Regression coefficient	0.98
Slope	1789
Intercept	1345

Accuracy

To study the reliability, suitability and accuracy of the proposed method recovery experiments were carried out. A known amount of standard solution of drug (90, 110 and 120%) was added to pre-analyzed sample solution. These solutions were subjected for analysis. The lower values of relative standard deviation (RSD) indicate the method was accurate. The mean recovery of norfloxacin was 99.99% and RSD value was 0.45 (Table 4).

Table 4. Accuracy parameters (recovery)

Tablet Formulation	Drug	LabeledAmt of Drug (mg/tab)	Amount mg/tab found	% label claim (n =6)	Recovery Studies (n = 3)				
					Total Amt. after spiking(mg)	Amt recovered (mg) Mean ± SD	% Recovery	% Mean Recover	% RSD
Normax Tab (Ipca)	NX	400	399.98	99.99	360 440 480	359.23±3.99 440.89±1.77 479.99±4.09	99.79 100.20 99.99	99.99	0.45

Precision

The preision of an analytical method is the degree of agreement among the individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of the method was examined by intra-day and inter-day variation studies. In the intra-day studies three repeated injections of standard and sample solutions were made and the percentage RSD with respect to peak area, peak retention time and amount were calculated. Inter-day variation studies were evaluated by analysing the same sample in the same way on three consecutive days. The results were tabulated in Table 5. From the data obtained, the developed RP-HPLC method was found to be precise.

Table 5. Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	2017821	0.21	2011312	2010309	2012001	0.04
Peak RT	2.12	0.10	2.11	2.12	2.10	0.47
Amount (mg/Tab)	399.71	0.20	398.42	398.22	398.56	0.04

Robustness and ruggedness

The robustness of the proposed method was examined by evaluating the system suitability parameter data after varying individually, the pump flow rate (± 0.05), wavelength (±1) and pH of the mobile phase (±0.1). Solutions were injected 3 times for each change. Mean and SD were calculated for each peak, %RSD was calculated for each component during each change.

The ruggedness of the method was determined by carrying out the experiment on different instruments like Jasco HPLC, Merck Hitachi HPLC systems with different operators using different columns of similar type. No significant changes in the chromatograms were observed which demonstrated that the developed RP-HPLC method was robust and rugged.

LOD and LOQ

Limit of detection (LOD) and limit of quantification (LOQ) for norfloxacin were estimated by injecting a series of dilute solutions with known concentrations. The parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation. The LOD and LOQ of the drug were found to be 0.08 µg/ml and 0.23 µg/ml respectively (Table 1).

Conclusion

From the above study we can conclude that a convenient rapid, accurate, precise and economical RP-HPLC method has been developed for estimation of norfloxacin in bulk and tablet dosage form. The assay provides a linear response across the wide range of concentrations and it utilizes a mobile phase which can be easily prepared. The proposed method can be used for the routine analysis of norfloxacin in bulk preparation of the drug and in pharmaceutical dosage forms without interference of excipients.

Acknowledgments

The authors gratefully acknowledge Dey's Medical, Kolkata for providing necessary facilities for carrying out this study.

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