

VALIDATION OF REVERSE PHASE-HPLC METHOD FOR THE QUANTITATIVE ESTIMATION OF NORFLOXACIN IN PURE AND PHARMACEUTICAL FORMULATION

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ABSTRACT

An accurate, simple, reproducible HPLC method for the determination of Norfloxacin (NRF) has been developed and validated. The separation of NRF was achieved on a reversed phase Synchronis C₁₈ (10 μ m, 250 x 4.6 mm i.d) column using UV detection at 275 nm. The mobile phase was consisted of (850:150, v/v) of buffer:acetonitrile (buffer 1 cm³ orthophosphoric acid to 1000 cm³ distilled water. The linear range of detection for NRF was found to be 0.43 % μ g/ml ($r^2 = 0.9987$). Intra and inter day assay relative standard deviations were less than 1 %. The method has been applied successfully to the determination of NRF in various pharmaceutical preparations. There was no interference from drugs commonly administered with NRF. The method has been shown to be linear, reproducible, specific and rugged.

Keywords: Norfloxacin, RP-HPLC, Pharmaceutical Formulation

I. INTRODUCTION

Norfloxacin (NRF) 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-1H-quinoline-3-carboxylic acid as presented in Figure 1.

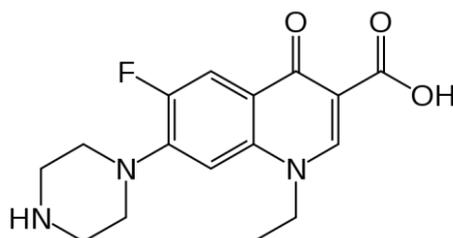


Figure 1. Chemical structure of norfloxacin

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] and UV detection [15, 16, 20, 21]. 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 [22, 23]. 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.

II EXPERIMENTAL

Materials and apparatus:

Pharmaceutical grade (> 99%) Norfloxacin was obtained from Global Calcium, Hosur, TN India. Acetonitrile (s.d Fine-Chem, Ltd., India) and water (Rankem Ltd., India) used were of HPLC grade. All HPLC measurements were made on a Shimadzu Corporation system (Analytical Instruments division, Kyoto, Japan) consisting of a LC-2010 CHC binary gradient solvent pump, SPD-10A detector and a data station with win chrome software version 3.1. The elution was performed on reversed phase Synchronis C₁₈ (10µm, 250 x 4.6 mm i.d) column. A 850:150 v/v mixture of buffer: acetonitrile was used as a mobile phase at a rate of 1.5 ml/min. Hamilton 702 µR injector with a 25 µl loop was used for the injection of the samples. Detection was done at 254 nm. The mobile phase was filtered through 0.45µ Millipore membrane filter and degassed. The separation was carried out at room temperature.

Stock solutions

NRF (12.49 mg) was accurately weighed into 50 ml calibrated flask and dissolved in the mobile phase by sonicating for 5 minutes and filled up to volume with the mobile phase. The solutions were diluted as and when required (4 ml to 20 ml with diluent).

Standard working solutions

Standard working solutions were prepared in mobile phase. A suitable aliquot from working solution was diluted with mobile phase to yield a solution with final concentration of 200 µg/ml of NRF. Studies on the stability of analytes in standard working solution showed that there was no decomposition product in the chromatogram and difference in area-ratios during the analytical procedure and even after storing for 2 days at +4°C.

Pharmaceutical preparation

Twenty tablets of the selected drug were finely powdered. An amount equivalent to 20 mg of the drug was weighed accurately and transferred into a 50 ml volumetric flask. 25 ml of mobile phase was added and the powder was completely disintegrated. The solution was filtered and the filtrate was made up to 50 ml with the mobile phase. Further transferred 3.0 ml of the solution into 25.0 ml volumetric flask, diluted up to the mark with diluents and mixed well.

III. PROCEDURE

Chromatographic conditions

LC analysis was performed by isocratic elution with flow rate of 1.5 ml/min. The mobile phase of 850:150 v/v mixture of buffer : acetonitrile was used throughout. Buffer solution was prepared by dissolving 1cm³ of orthophosphoric acid into 1000 cm³ of distilled water. After addition of buffer, the pH of mobile phase was 3.0 ± 0.05. All solvents were filtered through a 0.45µ Millipore membranes filter before use and degassed in an ultrasonic bath. The flow rate was maintained at 0.8 mL/min. Quantification was effected by measuring at 275 nm and the chromatographic run time was 5 min.

Throughout the study, the solubility of the chromatographic system was monitored by calculating the capacity factor (k), the resolution (R), the selectivity (σ) and the peak asymmetry (T).

Establishment of calibration

Working standard solutions of NRF (1.5-55 µg/ml) was prepared in mobile phase. Triplicate 20 µl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of standard was plotted against the concentration of the drug to obtain the calibration graph. The results were subjected to regression analysis to calculate calibration equation and correlation coefficients. A typical chromatogram is obtained as show in Fig. 2.

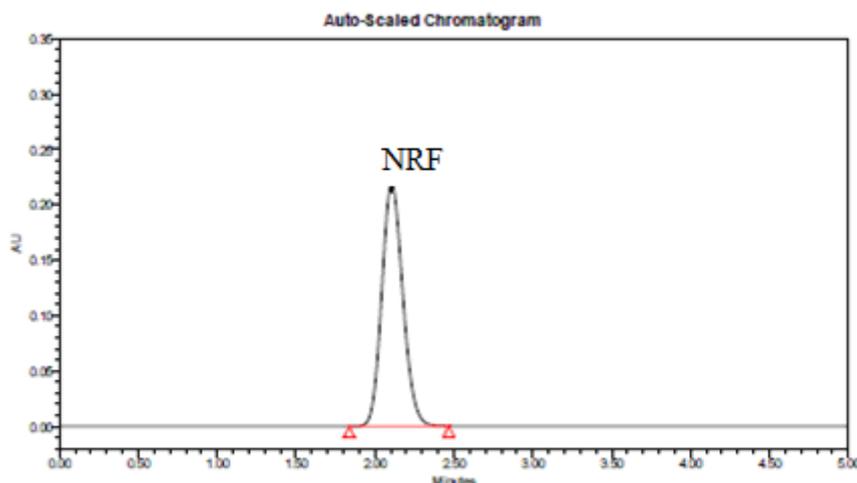


Figure 2. Chromatogram for NRF (retention time = 2.2 min)

Analysis of tablet

An aliquot of the drug obtained by following the procedure described for analysis of pharmaceutical preparations was taken and analysed. The chromatogram at 275 nm showed a complete resolution of the peak.

IV RESULTS AND DISCUSSION

Method development

The mobile phase was chosen after several trials with methanol, acetonitrile, isopropyl alcohol, triethylamine, water and buffer solutions in various proportions and at different pH values. A mobile phase consisting of buffer: acetonitrile (850:150 v/v) was selected to achieve the maximum separation and sensitivity. The effects of flow rates in the range of 0.5 and 2.0 ml/min were examined. A flow rate of 1.5 ml/min gave an optimal signal-to-noise ratio with a reasonable separation time. Using reverse phase C18 column, the retention time was observed to be of 2.2 min. The total time of analysis was less than 10 min.

The solution containing NRF exhibited maximum absorption at 275 nm and hence, this wavelength was chosen for the analysis.

Linearity

The calibration curves were linear in the studied range of 0.02-0.8 µg/ml. The calibration curve equation is $y = bx + c$ and the response was measured as peak area. The mean equation of the calibration curve ($n = 9$) obtained was $y = 33115x + 62494$. Excellent linearity was obtained for NRF between peak area and concentrations of 0.02-0.8 µg/ml with $r^2 = 0.9985$.

Limit of detection and limit of quantification

Limits of detection (LOD) were established at a signal-to-noise ratio (S/N) of 3 while limits of quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 9. The LOD and LOQ were experimentally verified by nine injections of NRF at the LOD and LOQ concentrations. The limit of detection was calculated to be 0.43 µg/ml and the limit of quantification was calculated to be 0.550 µg/ml.

Suitability of the method

The chromatographic parameters such as tailing factor, selectivity and peak symmetry were evaluated for the selected drug. The tailing factor for NRF in standard solution was found to be 1.50 while the theoretical plate was observed to be 1000. The capacity factor (k^1) was found to be 1.0 while the peak asymmetry (T) value was observed to be 1.10.

Precision

The precision (Table 1) of the method (within-day variations of replicate determinations) was checked by injecting nine times of NRF at the LOQ level. The precision of the method expressed as the relative standard deviations (R. S. D., %) at the LOQ level, were 2.41.

Table 1. Precision parameters

Parameters	Intra-day	% RSD	Inter-day			
			Day1	Day2	Day3	% RSD
Peak Area	9517899	0.21	9516889	9518898	9516897	0.03
Peak RT	2.20	0.10	2.12	2.11	2.11	0.47
Amount (mg/Tab)	399.82	0.21	397.45	397.28	397.58	0.05

Accuracy

A standard working solution containing NRF to give final concentration 20 μ g/ml was prepared. The prepared solution of standard was injected nine times as a test sample. From the area counts, the concentration of the NRF was calculated using the detector responses. The accuracy, defined in terms of % deviation of the calculated concentrations from the actual concentrations is listed in Table 2.

Table 2. Accuracy parameters (recovery)

Tablet Formulation	Drug	Labelled Amount of Drug (mg/tab)	Amount mg/tab found	% label claim (n=6)	Recovery Tablet Studies (n = 9)				
					Total Amt. after spiking(mg)	Amt recovered (mg) Mean \pm SD	% Recovery	% Mean Recover	% RSD
Normax Tab (Ipc)	NRF	400	399.97	99.99	360	359.53 \pm 3.97	99.77	99.99	0.254
					440	440.79 \pm 1.78	100.22		
					480	479.79 \pm 4.09	99.99		

Ruggedness

The ruggedness of the HPLC method was evaluated by carrying out the analysis of the standard working solution, the same chromatographic system and the same column on different days. Small differences in area ratios and good constancy in retention times were observed after 48 h time period. The R.S.D. value of less than 0.21% for areas was observed. The R.S.D. values for nine determinations were found to be 0.21 % (for intra-

day analysis), and 0.47 % (for inter-day analysis). The low R.S.D. values indicated the ruggedness of the method. The comparable detector responses obtained on different days indicated that the method is capable of producing results with high precision on different days.

Similarly, injecting the standard working solution into a different HPLC unit tested the ruggedness of the method. The high degree of reproducibility of the detector response and the retention times indicate that the method is fairly rugged.

Specificity of the method

Specificity of the chromatographic analysis was confirmed by the fact that the drugs such as ibuprofen, chlorpheniramine maleate, amoxicillin, cloxacillin, pseudoephedrin hydrochloride, cefadroxil, methdilazine hydrochloride dichlofenac sodium, and ciprofloxacin did not interfere in the determination, as evident from their retention times which are different from those of NRF.

Analysis of pharmaceutical preparation

The proposed method was successfully applied to the analysis of NRF in tablet (labelled to contain NRF 400 mg as active substances) and the results were shown in Table 2. The low values of relative standard deviation indicated high precision of the method.

V CONCLUSIONS

The data obtained in the proposed method demonstrated that the method has acceptable linearity, precision and accuracy over the concentration range. The method described is rapid since the developed method is relatively simple and the total chromatographic run time is about 10 min. The limit of quantification values for NRF was observed to be 0.43 µg/ml. High percent recovery values show that the method was free from interference by the excipients used in the preparations. The proposed chromatographic method can be used to analyse a large number of samples each day in analytical laboratories, since they need same reversed-phase column, mobile phase, detection system and sample preparation. Hence, the present method could be used for routine quality control as well as to analyse biological fluids.

VI ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of University Grants Commission, New Delhi, India (F. No. 42-308/2013 (SR) Dated, 28/03/2013). We thank Global Calcium Ltd., Hosur, India for supplying gift samples of Norfloxacin. Thanks are also due to the Principal, Maharani's Science College for Women, Bangalore for providing necessary infrastructural facility.

REFERENCES

- [1] Oliphant C.M., Green G.M., Quinolones: A comprehensive review, Clin. Pharmacol. 2002, 65, 455- 464. 2.
- [2] Tadesse E., Teshome M., Merid Y., Kibret B., Shimelis T., Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia, BMC Res. Notes. 2014, 7, 155.

- [3] Seo Y., Lee G., Antimicrobial resistance pattern in *Enterococcus faecalis* strains isolated from expressed prostatic secretions of patients with chronic bacterial prostatitis. *Korean J. Urol.* 2013, 54, 477-481.
- [4] Andriole V.T., Use of quinolones in treatment of prostatitis and lower urinary tract infections, *Eur. J. Clin. Microbiol. Infect. Dis.* 1991, 10, 342-350.
- [5] Chisari G., Sanfilippo M., Reibaldi M., Treatment of bacterial conjunctivitis with topical ciprofloxacin and norfloxacin: a comparative study, *Infez. Med.* 2003, 11, 25-30.
- [6] Miller I.M., Vogel R., Cook T.J., Wittreich J., Topically administered norfloxacin compared with topically administered gentamicin for the treatment of external ocular bacterial infections. The World wide Norfloxacin Ophthalmic Study Group, *Am. J. Ophthalmol.* 1992, 113, 638-644.
- [7] Abraham D.J., *Burger's medicinal chemistry and drug discovery*, 6th ed. John Wiley and Sons. 2003, 5, 583-587.
- [8] Williams D.A., Lamke T.L., *Foye's principles of medicinal chemistry*. Lippincott Williams and Wilkins. USA. 5th ed., pp.829-830.
- [9] Nada A.H., Sharaf M.A., El Gholmy Z.A., Khalafallah N.M., Comparative bioavailability of norfloxacin tablets based on blood and urine data, *Med. Princ. Pract.* 2007, 16, 426-431.
- [10] Mascher H.J., Kikuta C., Determination of norfloxacin in human plasma and urine by highperformance liquid chromatography and fluorescence detection, *J. Chromatogr. A.* 1998, 812, 381-385.
- [11] Zhou J.L., Kang Y., Matrix effect in high-performance liquid chromatography-tandem mass spectrometry analysis of antibiotics in environmental water samples, *J. Sep. Sci.* 2013, 36, 564-571.
- [12] Vázquez M.M., Vázquez P.P., Galera M.M., García M.D., Determination of eight fluoroquinolones in groundwater samples with ultrasound-assisted ionic liquid dispersive liquid-liquid microextraction prior to high-performance liquid chromatography and fluorescence detection, *Anal. Chim. Acta.* 2012, 748, 20-27.
- [13] Ramos-Payán M., Villar-Navarro M., Fernández-Torres R., Callejón-Mochón M., Bello-López M.A., Electromembrane extraction (EME)—an easy, novel and rapid extraction procedure for the HPLC determination of fluoroquinolones in waste water samples, *Anal. Bioanal. Chem.* 2013, 405, 2575-2584.
- [14] Mutavdžić Pavlović D., Pinušić T., Periša M., Babić S., Optimization of matrix solid-phase dispersion for liquid chromatography tandem mass spectrometry analysis of 12 pharmaceuticals in sediments, *J. Chromatogr. A.* 2012, 1258, 1-15.
- [15] Moema D., Nindi M.M., Dube S., Development of a dispersive liquid-liquid microextraction method for the determination of fluoroquinolones in chicken liver by high performance liquid chromatography, *Anal. Chim. Acta.* 2012, 730, 80-86.
- [16] Galarini R., Fioroni L., Angelucci F., Tovo G.R., Cristofani E., Simultaneous determination of eleven quinolones in animal feed by liquid chromatography with fluorescence and ultraviolet absorbance detection, *J. Chromatogr. A.* 2009, 1216, 8158-8164.
- [17] Yi Y.N., Li G.R., Wang Y.S., Zhou Y.Z., Zhu H.M., Simultaneous determination of norfloxacin and lomefloxacin in milk by first derivative synchronous fluorescence spectrometry using Al (III) as an enhancer, *Anal. Chim. Acta.* 2011, 707, 128-134.

- [18] Liu C., Nanaboina V., Korshin G.V., Jiang W., Spectroscopic study of degradation products of ciprofloxacin, norfloxacin and lomefloxacin formed in ozonated wastewater, *Water Res.* 2012, 46, 5235-5246.
- [19] Maia A.S., Ribeiro A.R., Amorim C.L., Barreiro J.C., Cass Q.B., Castro P.M., Tiritan M.E., Degradation of fluoroquinolone antibiotics and identification of metabolites/transformation products by liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A.* 2014, 1333, 87-98.
- [20] Córdoba-Borrego M., Córdoba-Díaz M., Córdoba-Díaz D., Validation of a high-performance liquid chromatographic method for the determination of norfloxacin and its application to stability studies (photo-stability study of norfloxacin), *J. Pharm. Bio. Anal.* 1999, 18, 919-926.
- [21] Nageswara Rao R., Nagaraju V., Separation and determination of synthetic impurities of norfloxacin by reversed-phase high performance liquid chromatography, *J. Pharm. Biomed. Anal.* 2004, 34, 1049- 1056.
- [22] Oliveira P.R., Bernardi L.S., Mendes C., Cardoso S.G., Sangoi M.A., Silva M.R., Liquid chromatographic determination of norfloxacin in extended-release tablets, *J. Chromatogr. Sci.* 2009, 47, 739- 744.
- [23] Oliveira P.R., Mendes C., Klein L., Sangoi Mda S., Bernardi L.S., Silva M.A., Formulation development and stability studies of norfloxacin extended-release matrix tablets, *Biomed. Res. Int.* 2013, 2013, 716736.