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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF NIMORAZOLE AND OFLOXACIN IN PURE AND ITS PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT

A new simple, precise, rapid and accurate reverse phase high performance liquid chromatographic method had been developed for the simultaneous estimation of nimorazole (NIM) and ofloxacin (OFX) in pure and its pharmaceutical dosage form. The chromatographic separation was achieved on a phenomenex luna C18, 250 x 4.6 mm, 5µm particle size column was used with PDA detector by using mobile phase containing mixture of 0.02M Potassium dihydrogen orthophosphate (KH_2PO4) buffer : acetonitrile (95:5 % v/v pH 6.92) was used. . The flow rate was 1 ml / min and effluents were monitored at 260 nm. Chromatogram showed two main peaks corresponding to ofloxacin and nimorazole at retention times 3.96 and 11.55 min respectively. The method was linear over the concentration range of 50-250µg/ml for nimorazole and 20-100 µg/ml for ofloxacin respectively. The developed method was validated in according to ICH guidelines.

KEY WORDS

Nimorazole, Ofloxacin, RP-HPLC, Validation and ICH.

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INTRODUCTION

Ofloxacin (OFX) is a fluoroquinolone derivative with potent activity against a broad spectrum of bacteria. Chemically, it is (±)-9-fluoro-2, 3-dihydro-3-methyl-10- (4-methyl-1-piperazinyl)-7-oxo-7H-pyrido-[1,2,3-de]-1,4-benzoxazine -6-carboxylic acid[1-5] in Figure No.1. It is mainly used as an antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

Nimorazole (NIM) is a 5-nitroimidazole in Figure No.2, which is closely related to Metronidazole in structure and activity. Nimorazole is used as a hypoxic sensitizer concomitantly with radiotherapy for head and neck cancers and could from the similarities with Metronidazole theoretically lead to increased effect of anticoagulant therapy. Nimorazole chemically known as 4-[2-(5-nitro-1*H*-imidazole-1-yl)ethyl][6-8] morphine.

Literature survey stated that, few analytical methods such as, HPLC-ES MS/MS [9], LC –MS[10],HPLC [10-16], HPTLC and UV [17-24] were reported for the estimation of OFX and NIM either individually or combined with other drugs. However no method is reported in the literature for the analytical method development and validation for the simultaneous estimation of OFX and NIM.

EXPERIMENTAL

Samples

Working standards of OFX (98.21 %) and NIM (99.42%) were kindly supplied by AN therapeutics, (Pondicherry, India),and HPLC grade water, arranged from Milli-Q-Academic system, Millipore, Bangalore, India, were used throughout the experiments. The pharmaceutical formulation used in this study was Nimorazole tablets (Lupin Ltd, Mumbai, India) procured from the local market and labeled to contain 200mg OFX and 500mg NIM per tablet.

Instrumentation and Chromatographic Conditions

A shimadzu HPLC system consist of LC-10AT-vp Solvent delivery system (pump), SPDM– 10AVP photodiode array detector, Rheodyne injector with 20µL loop volume, LC Solution assisted for data collections and processing. The mobile phase consisted of 5 % of acetonitrile and 95 % of Phosphate buffer was delivered at a flow rate of 1 mL/min. Separation was achieved using a 150mm X 4.6 mm (i.d.) Phenomenex luna C18 column with an average particle size of 5µ and the column was kept at an ambient temperature. The column effluent was monitored at 260 nm. The mobile phase was filtered through 0.45µ filter before using.

Preparation of Phosphate Buffer Solution

6.8 gm of potassium di - hydrogen orthophosphate (25 mM) was dissolved in sufficient water (HPLC grade) with aid of sonicator. Then 5 ml of tri ethanol amine was added and the volume was made up to 1000ml with mobile phase. Finally, pH was adjusted to 6.9 with potassium hydroxide.

Standard stock solution

Standard stock solutions of 500 µg/ml of OFX and NIM were prepared separately in methanol. From the stock solutions, the mixed standard solutions were prepared to contain 40µg/ml of OFX and 100 µg/ml NIM.

Sample solution

Twenty tablets were accurately weighed and finely powdered. A quantity of powder weight equivalent to 20mg of OFX and 50mg of NIM were weighed and transferred to a 100 ml volumetric flask. Sufficient amount mobile phase was added and the resulting solution was sonicated for 30 minutes. Then the final volume was adjusted with mobile phase and filtered by vacuum filtration. From the filtrate 10mL was taken and transferred to a 50 ml volumetric flask, final volume was adjusted to 50mL with mobile phase so as to get working concentration of 40µg/ml of OFX and 100 µg/ml NIM. The optimized chromatogrhic condition was shown in Table No.1 and the standard chromatogram for Ofloxacin and Nimorazole were shown in Figure No.3.

RESULTS AND DISCUSSION

Method validation15: The developed method was validated as per the ICH guidelines with respect to system suitability, specificity, linearity, accuracy, precision, LOD and LOQ.

System suitability: To ensure the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions were used for the evaluation of the system suitability parameters like tailing factor, the number of theoretical plates and retention time. The system

suitability results obtained for nimorazole and ofloxacin is summarized in Table No.2.

Linearity

The linearity of the method was evaluated by analyzing different concentration of the drugs. According to ICH recommendations, at least six concentrations must be used. In the present study six concentrations were chosen and injected. The peak areas of the chromatograms were plotted against the concentration of drug to obtain the calibration curve and the corresponding calibration curve data and graph for Ofloxacin and Nimorazole shown in Table No.3 and Graph in Figure No.4 and 5 respectively.

Validation

The developed and optimized method was validated as per ICH Q2 (R1) guidelines. Specificity was performed by comparing the peaks observed in sample solution, blank and placebo (synthetic mixtures). No interference was observed. Hence observed peaks in sample solution was the actual peak of OFX and NIM shown that, the method was specific. System performance was developed by system suitability parameters such as retention time, theoretical plates, asymmetric factor and resolution were calculated and percentage RSD was found to be less than 2 % indicating the good performance of the system. The method was found to be linear over the concentration range of 20 – 100 µg/mL for ofloxacin and 50 – 250 µg/mL for Nimorazole with their correlation coefficient values (R2) of 0.991and 0.9939 respectively, indicating that good correlation existing between concentration and responses.

Accuracy

Accuracy was performed at various levels of 80, 100% and 120% of label claim. The amount of OFX and NIM recovered in all the levels were found to be close to 100%, indicative of good accuracy of the proposed method (Table No.4). Precision study was performed by injecting the sample solution 6 times at by different analysts and in different instruments the results were shown in (Table No.5). The amount of OFX and NIM present in sample solution was found to 99.3 -100.2%, 100.7 – 99.3% and100.2-99.8 %. Percentage RSD was found to less than 2%. Robustness of the method was determined by small deliberate changes were made in the method parameters such as wavelength (±2nm), flow rate (±0.1ml), mobile phase ratio (±2%) and pH (± 0.05). But these changes, not affected the method results indicated that the method was robust. Standard and sample Solutions stability were checked up to 3 days at room temperature and the responses were measured at one time on each day. Results revealed that there was no degradation of OFX and NIM.

All the validation parameters results were indicating that the developed and optimized method was specific, suitable, linear, precise, accurate and robust for the simultaneous estimation of OFX and NIM in pure and pharmaceutical dosage form.

Method application to the marketed formulation

Sample solution of the marketed formulation was prepared as per the above procedure as described in the preparation of sample solution. Six replicate injections were given in to HPLC without changing the proposed method procedure. The amount of OFX and NIM present in each tablet was calculated and found to be 199.52 mg and 500.41 mg respectively, the results were shown in Table No.6.

Table No. 1: The optimize chromatographic conditioned

Flow rate	1ml/min
Column	Phenomex luna, C18, 100 x 4.6 mm, 5µ.
Detector wave length	260 nm
Column temperature	30°C
Injection volume	5µL
Run time	15 min
Diluent	Methanol
Mobile phase	Buffer : Acetonitrile (95:5 % v/v pH 6.92)

Table No.2: System suitability tests were performed and chromatographic parameters calculated from experimental data

S.No	Parameters	Ofloxacin	Nimorazole
1	Linearity	20-100 µg ml^{-1}	50-250 µg ml^{-1}
2	Retention time	3.93	11.55
3	Resolution	--	8.212
4	Asymmetry factor	1.622	1.163
5	Tailing factor	1.34	1.86
6	No.of theoretical plates	6722	8214
7	K.Prime	3.076	3.423

Table No.3: The corresponding Linearity (Calibration curve) data

S.No	Concentration in ppm (OFX)	Peak area	Concentration in ppm (NIM)	Peak area
1	20	485262	50	184142
2	40	616781	100	263032
3	60	780137	150	315638
4	80	963493	200	420851
5	100	1098751	250	542461
	Slope Intercept Correlation co-efficient	1749 82873 0.991	Slope Intercept Correlation co-efficient	3182.8 12248 0.9939

Table No.4: Accuracy results for Ofloxacin and Nimorazole

S.No	Spiking Level	Ofloxacin		Nimorazole		Recovery (%)	
		Added (mg)	Recovered (mg)	Added (mg)	Recovered (mg)	Ofloxacin	Nimorazole
1	80%	40	39.87	80	80.4	99.3	100.2
2	100%	50	50.4	100	99.5	100.7	99.3
3	120%	60	60.2	120	119.2	100.2	99.8

Table No.5: Precision of HPLC method

S.No	Components	Concentration (µg) ml^{-1})	N	System precision		Method precision	
				Peak area	RSD %	Peak area	RSD %
1	Ofloxacin	40	6	616781	0.23	616564	0.24
2	Nimorazole	100	6	263032	0.56	264161	0.53

Table No.6: The determination o f Ofloxacin and Nimorazole Tablet dosage form

Components	Label claim (mg/tablet)	N	Amount present (mg/tablet)	Percentage Label claim (% w/v)
Ofloxacin	200	6	199.52	99.7
Nimorazole	500	6	500.41	100.3

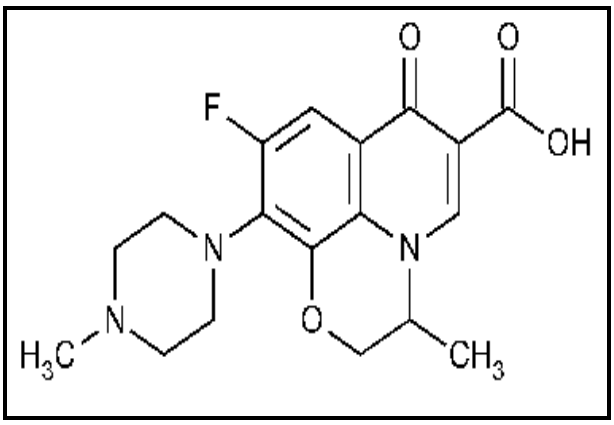

Figure No.1: Structure of OFX

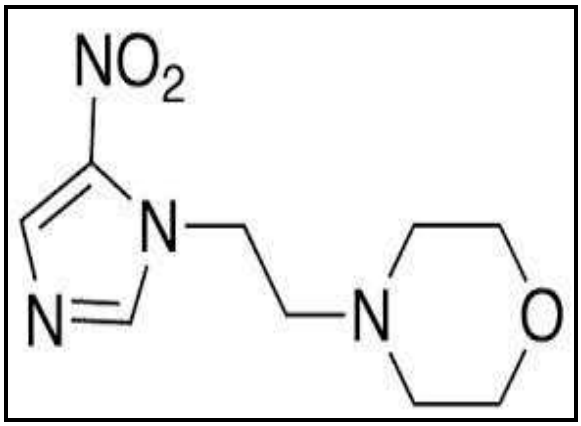

Figure No.2: Structure of Nimorazole

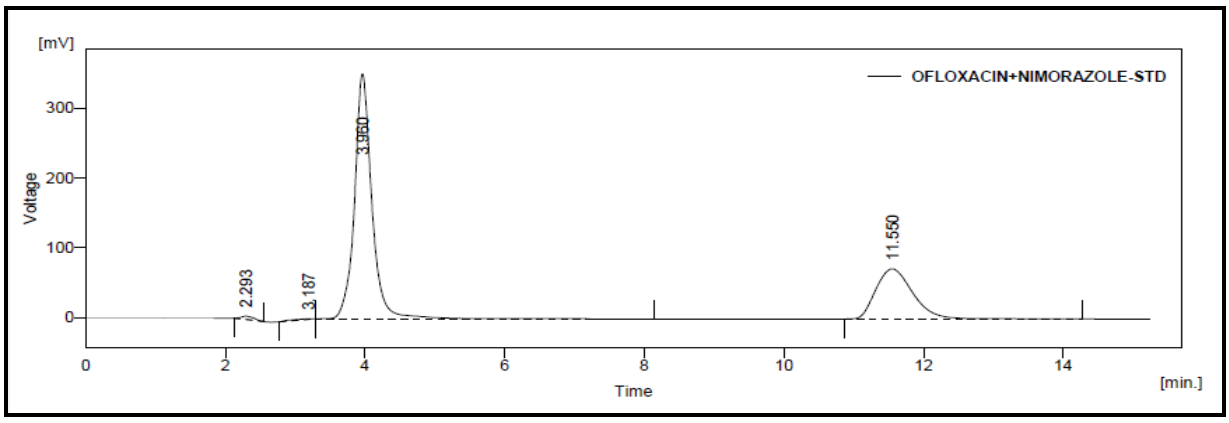

Figure No.3: Standard Chromatogram for Ofloxacin and Nimorazole

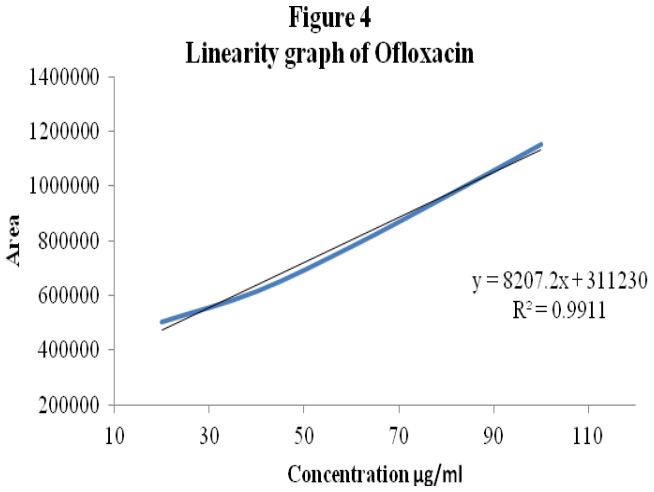

Figure No.4: Linearity graph of Ofloxacin

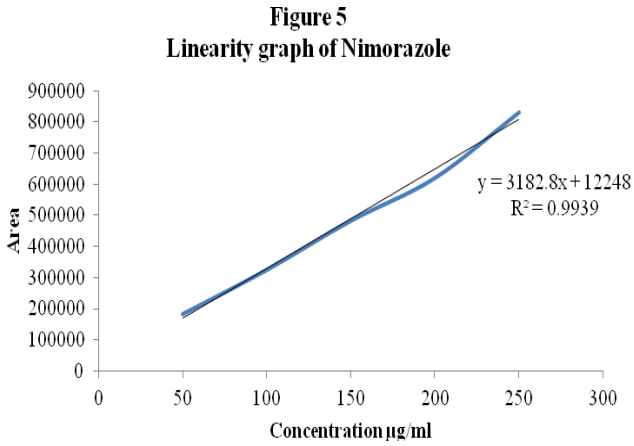

Figure No.5: Linearity graph of Nimorazole

CONCLUSION

This developed method is considered as the first method for the simultaneous estimation of OFX and NIM using RP-HPLC. The various validation characteristics were applied and determined, to assure the suitability of the method. This investigation also proved that, the chromatographic techniques provide a complete profile of separation process, making this combined technique a powerful analytical tool. Therefore, this validated RP-HPLC method can be readily adapted for the simultaneous estimation of OFX and NIM in pure and pharmaceutical dosage form as a routine quality control analysis.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Hardman J G, Limbird L E. Good Man and Gillmans, The pharmacological basis of therapeutics, 10th Edition, 2001, 1105-1108.
2. Williams D A, Lemke T L. Foye's Principles of Medicinal chemistry, *Philadelphia, Lippincott Williams and Wilkins,* 5th edition, 2005.
3. Korolkovas A. Essentials of Medicinal Chemistry, *New York: Wiley Interscience,* 2nd edition, 1998.
4. Banker G S, Anderson N R. Tablets. In: Lachman L, Libermann H A, Kanig J L, editors. The Theory and Practice of Industrial Pharmacy, *New Delhi, Varghese Publishing House,* 3rd edition, 1987, 293-299.
5. Lachman L, Lieberman A and Kinig J L. The Theory and Practice of Industrial Pharmacy, *Bombay, Varghese Publishing House,* 4th edition, 1991, 67-68.
6. Battue S K, Repay M A, Maunder S and Rio M Y. *Drug Dev. Ind. Pharm*, 2007, 33.
7. Sweetman S C. In Martindale, the Complete Drug Reference, *Pharmaceutical Press, London*, 32nd edition, 1999, 233.
8. Bjarnason N, Christiansen M, Specht L. The nimorazole regimen in patients with head and neck cancer can increase the effect of vitamin K antagonists, *Journal of Acta Oncologica*, 4, 2008, 150-156.
9. Byrro R M, De Oliveira Fulgencio G, Da Silva Cunha A J R, Cesar I C, Chellini PRnPianetti G A. Determination of ofloxacin in tear by HPLC-ESI-MS/MS method: comparison of ophthalmic drug release between a new mucoadhesive chitosan films and a conventional eye drop formulation in rabbit model, *Journal of Pharmaceutical and Biomedical Analysis*, 70, 2012, 544-548.
10. Premanand D C, Senthilkumar K L, Senthilkumar B, Saravanakumar M, Thirumurthy R. A Validated RP-HPLC Method for Simultaneous Estimation of Nitazoxanide and Ofloxacin in Pharmaceutical Formulation, *Der Chemica Sinica*, 1(2), 2010, 1-5.
11. Rege P V, Ramesh M. Simultaneous Quantification of ofloxacin and ornidazole from Combined pharmaceutical drug formulation by HPLC, *International journal of pharma and bio sciences*, 2(4), 2011, 51-58.
12. Godse Vijaya P, Bafana Y S, Deshapande S Y, Vyas M R, Bhosale A V. Validated Stability indicating HPLC method for simultaneous estimation of ofloxacin and satranidazole pharmaceutical dosage form, *International Journal of Applied Biology and Pharmaceutical Technology*, 1(3), 2010, 1220-1229.
13. Arun M, Kashid, Navin S. Dawra, Anup A, Dhange, Afroj I, Mulani, Deepali A. Ghorpade, Shashikant C. Dhawale. RP-HPLC Method development and validation for nimorazole, *American Journal of pharmtech research*, 2(6), 2012, 818-823.
14. Meredith S A, Smith P J, Norman J, Wiesner L. An LC-MS/MS method for the determination of ofloxacin in 20 µl human Plasma, *Pharmaceutical Biomedical Analysis*, 25, 2012, 177-81.
15. Chaitanya Krishna A, Vignesh R, Chelladurai R, Rajaram S Patil. A highly specific, sensitive and high throughput method for the determination of ofloxacin in human plasma by liquid chromatography coupled with tandem mass, *Asian Journal of Pharmaceutical Clinical Research*, 5(3), 2012, 174-179.
16. Khandagle K S, Gandhi S V, Deshpande P B, Kale A N, Deshmukh High P R. High Performance Thin Layer Chromatographic determination of Cefixime and Ofloxacin in combined tablet dosage form, *Journal of Chemical and Pharmaceutical Research*, 2(5), 2010, 92-96.
17. Janhavi Rao, Kamini Sethy, Savita Yadav. Validated HPTLC method for simultaneous quantitation of cefixime and ofloxacin in bulk drug and in pharmaceutical formulation, *International Journal of Comprehensive Pharmacy*, 2(4), 2011, 1-4.

18. Arun Kumar Dash T, Siva Kishore, Loya Harika, Umadevi Kothapalli. A Validated UV-Spectrophotometric Method for the Estimation of Ofloxacin in Bulk and Pharmaceutical Dosage Form, *International Journal of Pharmaceutical and Biological Archives*, 2(4), 2011, 1157-1161.
19. Senthilraja M. Simultaneous UV Spectrophotometric Method for the Estimation of Nitazoxanide and Ofloxacin in Combined Dosage, *Research J. Pharm. and Tech*, 1(4), 2008, 469-471.
20. Lalit Kishore, Ashok Kumar, Anroop Nair, Navpreet Kaur. Kinetic Spectrophotometric, Method for the Estimation of Ofloxacin in Pharmaceutical Formulations, *J. Mex. Chem. Soc*, 56(4), 2012, 355-361.
21. Avanija Dube, Sujit Pillai, Sumit Sahu, Naina Keskar. Spectrophotometric estimation of cefixime and ofloxacin from tablet dosage form, *International journal of pharmacy and life sciences*, 2(3), 2011, 629-632.
22. Madhuri D. Game, Sakarkar D M. Simultaneous Spectrophotometric Estimation of Nitazoxanide and Ofloxacin in Tablets, *Indian Journal of Pharmaceutical Sciences*, 73(1), 2011, 70-74.
23. Gandhi V M, Nair S B, Menezes C, Narayan R. Development of UV-spectrophotometric method for the quantitative estimation of ofloxacin and ornidazole in combined liquid oral dosage form by simultaneous equation method, *International journal of research in pharmacy and chemistry*, 3(1), 2013, 6-11.
24. Patel Sanket A, Patel Satish A. Dual Wavelength Spectrophotometric Method for Simultaneous Estimation of Ofloxacin and Cefpodoxime Proxetil in Tablet Dosage Form, *Asian Journal of Pharmacy and Life Science*, 1(3), 2011, 261-268.

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