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Spectrophotometric simultaneous estimation of ofloxacin and nimorazole in pure and pharmaceutical dosage form by vierordt's method

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Abstract: A simple, rapid, accurate, precise and economical vierordts method was developed for the simultaneous estimation of ofloxacin and nimorazole in bulk and tablet dosage form. Ofloxacin and nimorazole exhibited maximum absorbance at 284.8nm and 296nm respectively in water. The developed method was fully validated as per ICH guidelines. The concentration of ofloxacin and nimorazole were found to be linear in the range of 2 - 5 µg/ml and 5-15µg/ml respectively with satisfactory correlation coefficient (R^2) values of 0.9994 and 0.9999. The % RSD value of precision and recovery studies were found to be less than 2% indicated that the method is more accurate and precise. The limit of detection and limit of quantification for ofloxacin and nimorazole was found to be 83.57ng/mL and 253.24ng/mL and 269.63ng/mL and 817.08ng/mL respectively.
Keywords: Ofloxacin, Nimorazole, Vierordt's method, Simultaneous estimation, Method development and Validation.

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] (Fig no. 1). It is mainly used as antibacterial for the treatment of urinary tract infection and sexually transmitted diseases.

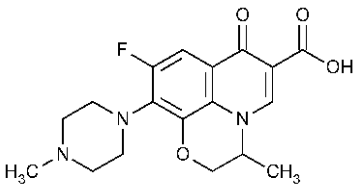

Fig. No.1. Structure of OFX

Nimorazole (NIM) is a 5-nitroimidazole, 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] morpholine [2] (Fig no. 2).

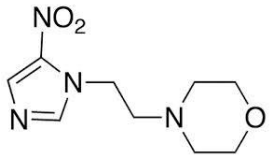

Fig. No. 2. Structure of NIM

Literature survey assured that, very few analytical methods such as, HPLC [3-7], LC –MS [8,9] HPTLC [10,11] and UV [12-18] were reported for the estimation of OFX and NIM either individually or combined with other drugs. However vierordt's method was not reported in the literature for the simultaneous estimation of OFX and NIM in pure and pharmaceutical dosage form. Hence a simple, rapid, precise, accurate and economical vierord's method was developed and validated for the simultaneous estimation of OFX and NIM in pure and tablet dosage form.

Experimental

Drugs and chemicals

Working standards of OFX (99.35 %) and NIM (99.68%) were kindly supplied by AN therapeutics, (Pondicherry,India). Triple distilled water was used throughout the experiment. The pharmaceutical formulation used in this study was NIMORAZ O tablets (Lupin Ltd, Mumbai, India) procured from the local market and labelled to contain 200mg OFX and 500mg NIM per tablet.

Instrument

Shimadzu 1650 UV-VIS double beam spectrophotometer with UV probe software was used. Absorbance measurements were recorded with a pair of 1cm matched quartz cells.

Preparation of working Standard solutions

20 mg of OFX and 50 mg NIM were weighed accurately and transferred in to a separate 100 volumetric flasks and sufficient water was added to dissolve the drug and then sonicated for 10 minutes. Finally the volume was adjusted up to the mark with water. 2ml was pippetted out from each stock solution in to a 100ml volumetric flask and the final volume was adjusted to 100mL with water, so as to get the final concentration of 4µg/mL of OFX and 10 µg/mL for NIM.

Preparation of Sample solutions

Twenty tablets were accurately weighed and powdered. A quantity of powder weight equvalent to 20mg of OFX and 50mg of NIM were weighed and transferred to a 100 ml volumetric flask. Sufficient amount water was added and the resulting solution was sonicated for 20 minutes. The final volume was then adjusted with water and filtered by vaccum filtration. From the filtrate 2mL was taken and transferred to a 100 mL volumetric flask. Final volume was adjusted to 100mL with water, so as to get the final concentration of 4µg/mL of OFX and 10 µg/mL for NIM.

Development of Vierordt's method

Working standard solutions of both the drugs were scanned in the UV range 200-400nm. From the overlain spectra (Fig no. 3), wavelengths 284.8 nm (λ_{max} of OFX) and 296 nm (λ_{max} of NIM) were selected for analysis of both the drugs using simultaneous equation method or vierordt's method (λ_1- 284.8nm for OFX and λ_2-296 nm for NIM). Consequently, it may be possible to determine both the drugs by the technique of vierordt's method or simultaneous equation method [19-22].

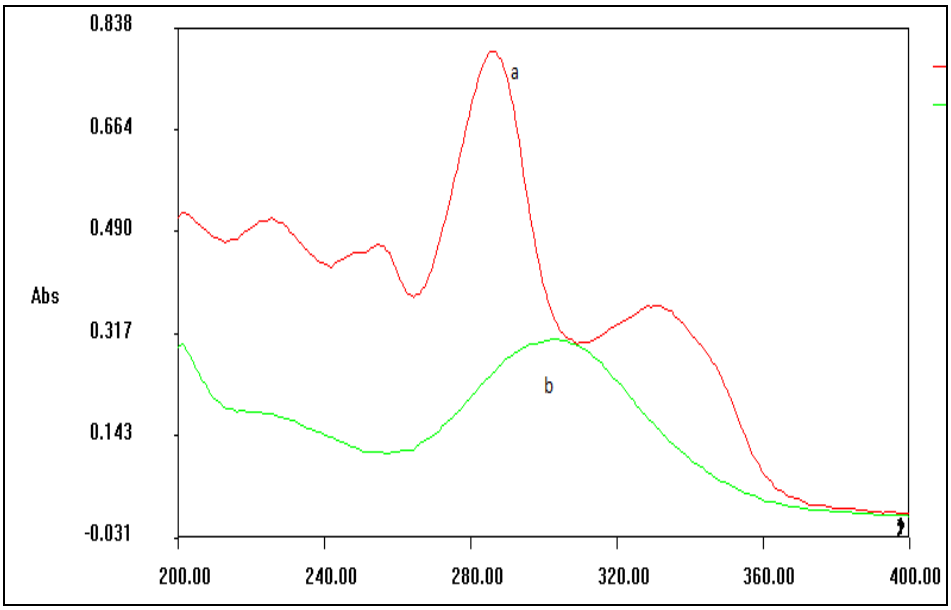

Fig. No. 3. Overlain spectra of OFX (a) and NIM (b)

Five standard solutions having concentrations of 2, 3, 4, 5 and 6 µg/mL for OFX and 5, 7.5, 10, 12.5 and 15µg/mL for NIM were prepared in water and their corresponding absorbance's were measured at 284.8nm and 296nm. The concentration of drugs X (OFX) and Y (NIM) in sample solutions were determined by the SE method or vierordt's method using the following formula:

$$C_x = A_2ay_1 - A_1ay_2 / ax_2ay_1 - ax_1ay_2$$

$$C_y = A_1ax_2 - A_2ax_1 / ax_2ay_1 - ax_1ay_2$$

Where C_x and C_y are the concentration of OFX and NIM, A_1 and A_2 are the absorbance of sample solution at 284.8nm and 296 nm respectively, ax_1 and ax_2 are absorptivity of OFX at 284.8nm and 296nm, ay_1 and ay_2 are absorptivity of NIM at 284.8nm and 296nm respectively.

Determination of absorptivity value

The absorptivity value of OFX and NIM from each solution was calculated using following formula [23] and the results were presented in table no 1 and 2:

Absorptivity = Absorbance / conc (gm/100mL)

Developed method was fully validated as per ICH guidelines [24].

Table No 1. Absorptivity value for OFX

Concentration (µg/mL)	**Absorbance λ1 -284.8 nm**	**Absorptivity λ1-284.8nm**	**Absorbance λ2-296nm**	**Absorptivity λ2-296nm**
2	0.163	815	0.104	520
3	0.239	796.666	0.151	503.333
4	0.328	820	0.214	535
5	0.411	822	0.257	514
6	0.489	815	0.311	518.333
	Absorptivity for λ1	813.733	Absorptivity for λ2	518.133

Table No 2. Absorptivity value for NIM

Concentration (µg/mL)	Absorbance λ1 -284.8 nm	Absorptivity λ1-284.8nm	Absorbance λ2-296nm	Absorptivity λ2-296nm
5	0.126	252	0.151	302
7.5	0.191	254.666	0.225	300
10	0.253	253	0.303	303
12.5	0.31	248	0.381	304.8
15	0.378	252	0.458	305.333
	Absorptivity for λ1	251.933	Absorptivity for λ2	303.026

Results and Discussion

Specificity

Specificity of the method was established by measuring the absorbance of OFX and NIM individually at 284.8nm and 296nm against the blank and synthetic excipients and their absorbances were compared with the blank and synthetic excipients. No interference was observed at 284.8nm and 296nm indicated that the method is specific.

Linearity

2, 3, 4, 5, 6 µg/mL and 5, 7.5, 10, 12.5, 15µg/mL standard solutions of OFX and NIM were prepared. Linearity of the method was established by measuring the absorbances of the above standard solutions and Calibration curves were constructed by plotting concentration versus absorbance. Calibration graphs (Fig no 4 and 5) showed linearity over the concentration range of 2 - 6µg/mL for OFX and 5- 15µg/mL for NIM with significantly high correlation coefficient values. The linear regression analysis data for the calibration graph showed good linear relationship between concentration and absorbance with their correlation coefficient (R^2) values of 0.9996 for OFX and 0.9999 for NIM.

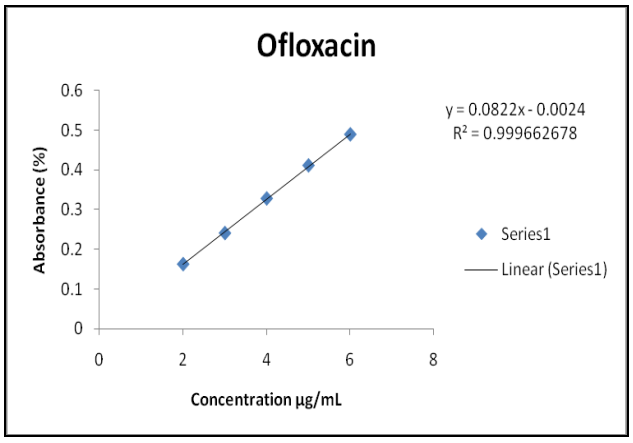

Fig. No. 3. Calibration graph for OFX

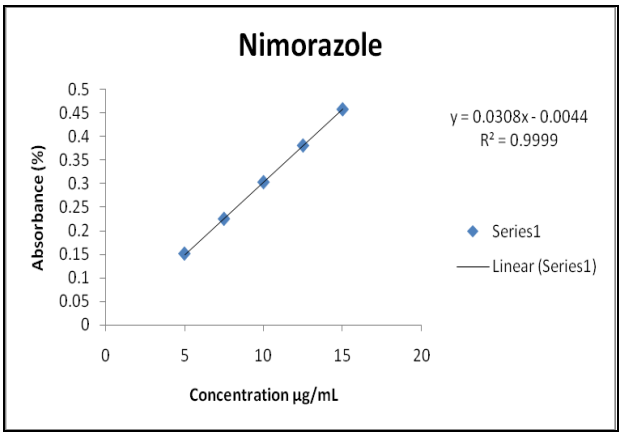

Fig. No. 4. Calibration graph for NIM

Accuracy

The recovery of the method was studied by accuracy experiments. The known amount of OFX and NIM was added 50 %, 75%, 100% 125% and 150% from the label claim to the synthetic excipients. The % recoveries in the all levels were found to be close to 100 % indicated, that the proposed method was accurate and the results are presented in table no 3.

Precision

Precision study was done by measuring the absorbance of OFX and NIM in sample solution without changing the assay procedure and the results were presented in Table no 4.

Table No 3. Recovery results for OFX and NIM

Concentration (%)	Added AMT amount (mg)		Amt recovered (mg)		Amt recovered (%)	
OFX / NIM	**OFX**	**NIM**	**OFX**	**NIM**	**OFX**	**NIM**
50	10	25	9.98	25.12	99.80	100.48
75	15	37.5	14391	37.98	99.39	101.27
100	20	50	20.08	50.63	100.42	101.26
125	25	62.5	25.17	62.74	100.66	100.38
150	30	75	29.91	76.47	99.68	101.95

Table No 4. Precision results for OFX and NIM

Parameters	Sampling time	OFX			NIM		
		Amount present (mg)	Amount present (%)	RSD (%)	Amount present (mg)	Amount present (%)	RSD %
Repeatability	0 hrs	200.07	100.35	1.08	505.49	101.09	1.00
	8^{th} hrs	200.23	100.11	1.33	504.38	100.87	1.28
	16^{th} hrs	198.71	99.35	1.36	502.09	100.41	0.65
Intermediate precision	I^{st} Day	30.47	101.57	0.35	10.18	101.80	0.46
	2^{nd} day	30.21	100.70	0.58	10.10	101.03	0.63
	3^{rd} day	29.99	99.97	0.52	10.03	100.37	0.84
	Analyst -1	198.30	99.15	0.89	507.94	101.58	0.65
	Analyst -2	202.80	101.40	0.49	506.28	101.25	1.61
	Instrument -1	200.09	100.04	0.66	508.47	101.69	1.08
	Instrument -2	200.06	100.03	0.67	0.49	98.73	1.05
Reproducibility	Lab-1	200.07	100.37	1.22	507.35	101.47	1.10
	Lab-2	201.50	100.76	1.11	505.44	101.08	0.75

Repeatability

This study was performed with a minimum of three replicate measurements of absorbance of sample solution at 0 hrs, 8 hrs and 16 hrs in a same day.

Intermediate Precision

Intermediate precision was performed by measuring the absorbance of sample solution in three different days, by different analysts and in different instruments.

Reproducibility

Reproducibility of the method was checked in two laboratories and the results were compared.

The low % RSD (< 2 %) for OFX and NIM indicated that the method is precise.

Robustness

Robustness of the method was determined by changing the wavelength ± 1nm from 284.8nm and 296nm and the results were presented in table no 5.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the response (50% concentration solution) and the slope of calibration graph. LOD and LOQ for OFX and NIM were found to be 83.57ng/mL and 253.24ng/mL and 269.63ng/mL and 817.08ng/mL respectively.

Stability

Sample and standard solutions were stored at room temperature for three days. Stability of the sample solution was determined by measuring the absorbance of sample and standard solution every day. The amount of OFX and NIM present in standard and sample solutions were calculated and the results confirmed that the drugs in the standard and sample solutions were stable for the 3days.

Developed method was applied to the marketed dosage form

Assay sample solution was prepared according to the above described procedure (Preparation of sample solution) and the absorbance of the sample solution was measured at 284.8nm and 296 nm. Amount of OFX and NIM present in the each tablet was found to be 198.22mg and 504.22mg and the results were presented in the table no 6.

Table No 5. Results observed by changing the wavelength ± 1nm

	OFX			**NIM**			
Wavelength (nm)	**Amount present (mg)**	**Amount present (%)**	**RSD %**	**Wavelength (nm)**	**Amount present (mg)**	**Amount Present (%)**	**RSD %**
283.8	199.53	99.76	0.76	295	501.99	100.39	0.57
285.8	200.42	100.21	0.49	297	513.67	102.73	0.83

Table No 6. Assay results of tablet dosage form

OFX		**NIM**	
Amount present (mg)	**Amount present (% Label claim)**	**Amount present (mg)**	**Amount present (% Label claim)**
197.35	98.67	505.71	101.14
200.83	100.41	496.46	99.29
199.10	99.55	506.02	101.20
198.88	99.44	504.75	100.95
198.44	99.22	502.20	100.44
194.74	97.37	510.17	102.03
AVG	99.11	AVG	100.84
SD	1.11	SD	1.01
% RSD	1.12	% RSD	1.00

Conclusion

The developed vierordt's method is simple, rapid, accurate, precise and economical method for the routine analysis of OFX and NIM in pure and pharmaceutical dosage form without any interference from the excipients. This new simple and economical method can be used routinely for the estimation these drugs.

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