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Simultaneous Estimation of Ofloxacin and Ornidazole in Combined Dosage Forms by Dual Wavelength and Ratio Spectra Derivative Methods using UV-Spectrophotometer

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Abstract: Two sensitive, validated, accurate, precise and specific methods were developed as useful alternatives for the simultaneous quantitative estimation of Ofloxacin and Ornidazole in the combined pharmaceutical formulation. The first method was based on the first derivative of ratio ultra-visible spectra. Signal intensities at 323.0nm and 260.0nm were used in this method for Ofloxacin and Ornidazole respectively. The second method was dual wavelength method in which, 266.0nm and 287.0nm were selected for estimation of Ofloxacin and 271.0nm and 319.0nm were selected for estimation of Ornidazole. In both the methods 0.1N Hydrochloric Acid is used as diluent. Calibration curve was established in the range of 1.92 - 9.6µg/ml for Ofloxacin and 4.8 - 24.0µg/ml for Ornidazole in both the methods. Both drugs show linearity in the range and furnishing near quantitative analyte recoveries. Performance characteristics of the analytical methods were evaluated by using commercial samples and both the methods shown accurate, precision and specificity. These two validated methods are easy to apply, use relatively simple equipment, require minimum analysis time and do not use polluting reagents.
Keywords: Ofloxacin, Ornidazole, UV-Spectrophotometer, Ratio Spectra Derivative, Dual wavelength.

1. INTRODUCTION

Ofloxacin (OFX) is a synthetic broad spectrum antibacterial agent. Chemically Ofloxacin a fluorinated carboxyquinolone, is aracemate, (±)- 9-fluro-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]. Ornidazole (ORN) is an anti infective / antibacterial and antiprotozaol drug. Chemically ORN is a 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)propan-2-ol [2]. Literature survey reveals that OFX and ORN can be quantified by spectrophotometrically [3-7], by HPLC [8-15], by Capillary Zone Electrophoresis [16-17], by HPTLC [18-20] in their individual and combined dosage form either with other drug or with each other. OFX and ORN in combined tablet dosage form is available in the market, has gained increasing acceptance in diarrhea, bacterial and protozoal infections. This paper presents two simple, accurate and reproducible spectrophotometric methods for simultaneous determination of OFX and ORN in tablet dosage form.

2. EXPERIMENTAL

2.1 Materials and Reagents

OFX and ORN Reference standards with 98.9% and 99.1% purity respectively, Hydrochloric Acid (Analytical Reagent Grade), Nylon 0.45µ filter paper(Millipore) and tablet formulation containing 200 mg of OFX and 500 mg of ORN were supplied by Torrent Research Center, Gandhinagar, India.

2.2 Instrumentation

A double-beam Shimadzu UV-Visible spectrophotometer, Model UV-2450 PC, equipped with 1 cm quartz cells, with a fixed slit width (1 nm), wavelength accuracy of ±0.5 nm (with automatic wavelength correction) was connected to computer loaded with UVProbe software, version 2.0 (Shimadzu). For scanning, the wavelength range was selected from 400 nm to 200 nm with medium scanning speed.

2.3 Preparation of standard and test solutions

2.3.1 Preparation of standard solution

32mg of OFX and 80mg of ORN were weighed separately and transferred to two separate 100ml volumetric flask. Each drug was dissolved in about 70ml of 0.1N Hydrochloric Acid and sonicated for 10 minutes. The volumetric flask was made up to the mark with same diluent. These solutions were further diluted with same diluent to obtain the concentration of 6.4µg/ml of OFX and 16.0µg/ml of ORN separately.

Standard solutions of both the drugs were prepared individually by dilution of the standard stock solutions with 0.1N Hydrochloric Acid to obtain the concentration range of 1.92-9.6µg/ml for OFX and 4.8-24.0µg/ml for ORN.

2.3.2 Preparation of test solution

Ten tablets were weighed and finely powdered in a mortar. A tablet powder equivalent to 160 mg of OFX and 400 mg of ORN was accurately weighed and transferred to a 250 ml volumetric flask. About 120 ml of 0.1N Hydrochloric Acid was added, and the solution was sonicated for 30 min with intermediate shaking. Volume was made up to the mark with the same diluent. The solution was filtered through 0.45 µm Nylon syringe filter. 2.0ml of the filtrate was transferred into 200ml volumetric flask and made up the volume with same diluent, to obtain concentration of 6.4µg/ml of OFX and 16µg/ml of ORN.

2.4 Methods

2.4.1 Ratio spectra derivative method

The method involves dividing the spectrum of mixture by standardized spectra of each of the analyte and thereby deriving the ratio of spectrum that is independent of concentration of analyte used as a divisor. The ratio spectra thus obtained are derivatized to first order.

Overlaid spectra of OFX and ORN are shown in Figure-1. Spectra of OFX in the range of 1.92-9.6µg/ml (Figure-2) and of ORN in the range of 4.8-24.0µg/ml (Figure-3) were collected in the range of 200nm to 400nm. All the spectra of OFX were divided by the stored 16.0µg/ml ORN spectra and ratio spectra of OFX were obtained (Figure-4). The ratio spectra thus obtained were derivatized to first order (Figure-5). Similarly all the spectra of ORN were divided by the stored 6.4µg/ml OFX spectra and ratio spectra of ORN were obtained (Figure-6). The ratio spectra thus obtained were derivatized to first order (Figure-7). 323.0nm and 266.0nm were selected for the quantification of OFX and ORN in their combined dosage form respectively. The calibration graphs of OFX and ORN were established by measuring the absorbance intensities at 323.0nm and 260.0nm respectively. Measured analytical signals were proportional to their concentration. The concentration of individual drug present in the mixture was determined against the calibration curve in quantitation mode.

Figure-1: Overlain spectra of Ofloxacin (6.4µg/ml) and Ornidazole (16.0µg/ml)

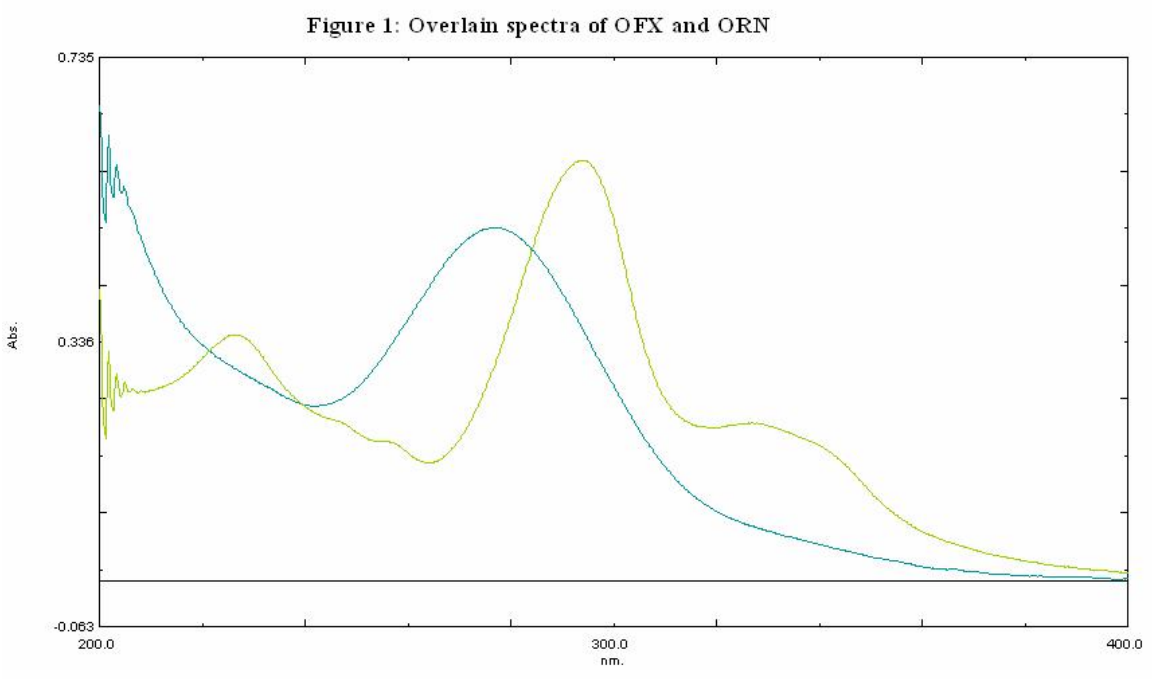


Figure-2: Spectra of OFX in the range of 1.92-9.6µg/ml

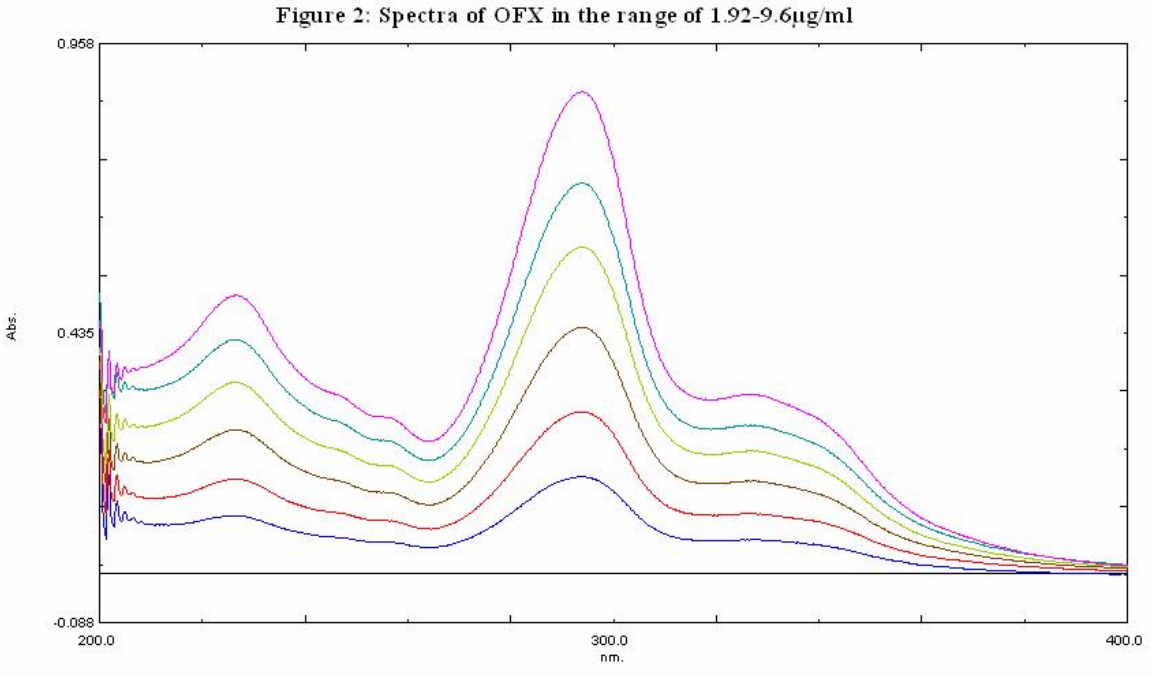


Figure-3: Spectra of ORN in the range of 4.8-24.0µg/ml

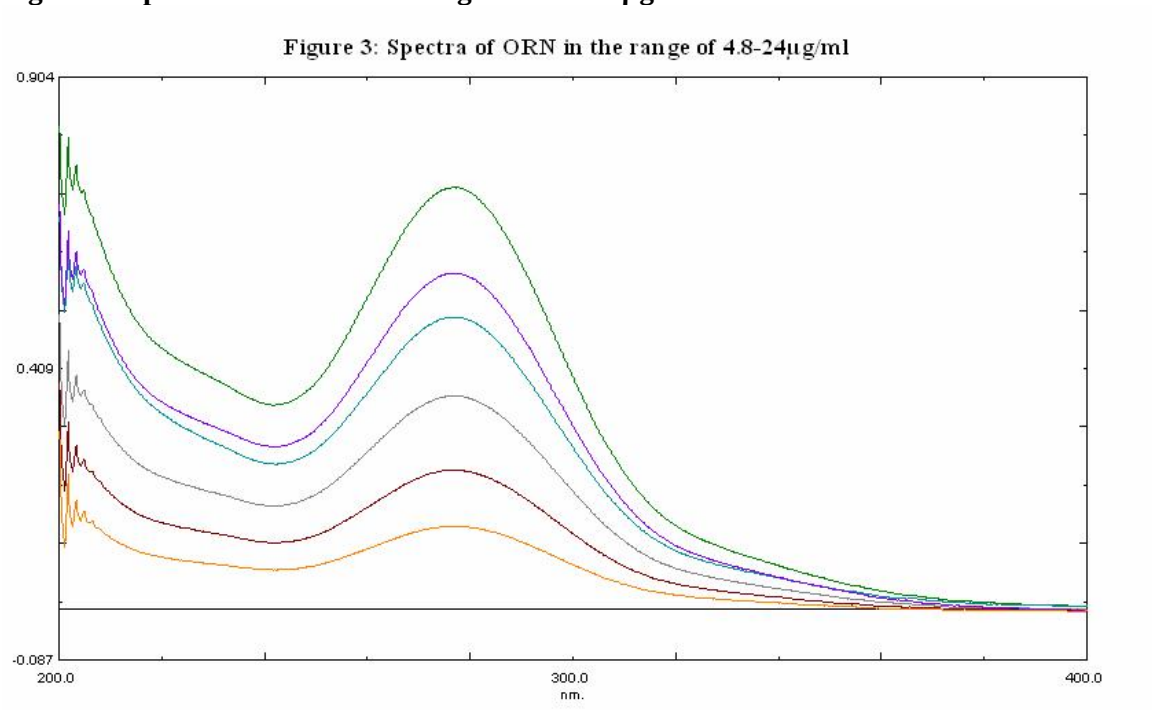


Figure-4 Spectra of OFX (1.92-9.6µg/ml) divided by the spectra of 16.0µg/ml ORN spectra (Ratio spectra of Ofloxacin)

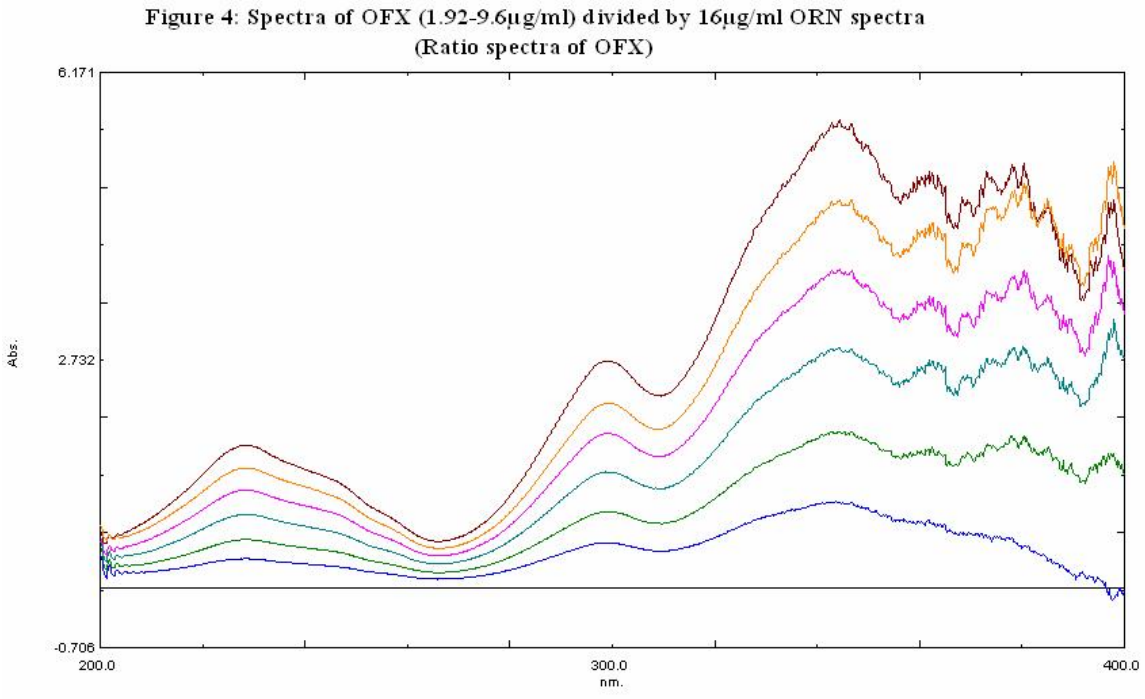


Figure-5 First order derivative spectra of ratio spectra of Ofloxacin (1.92-9.6µg/ml)

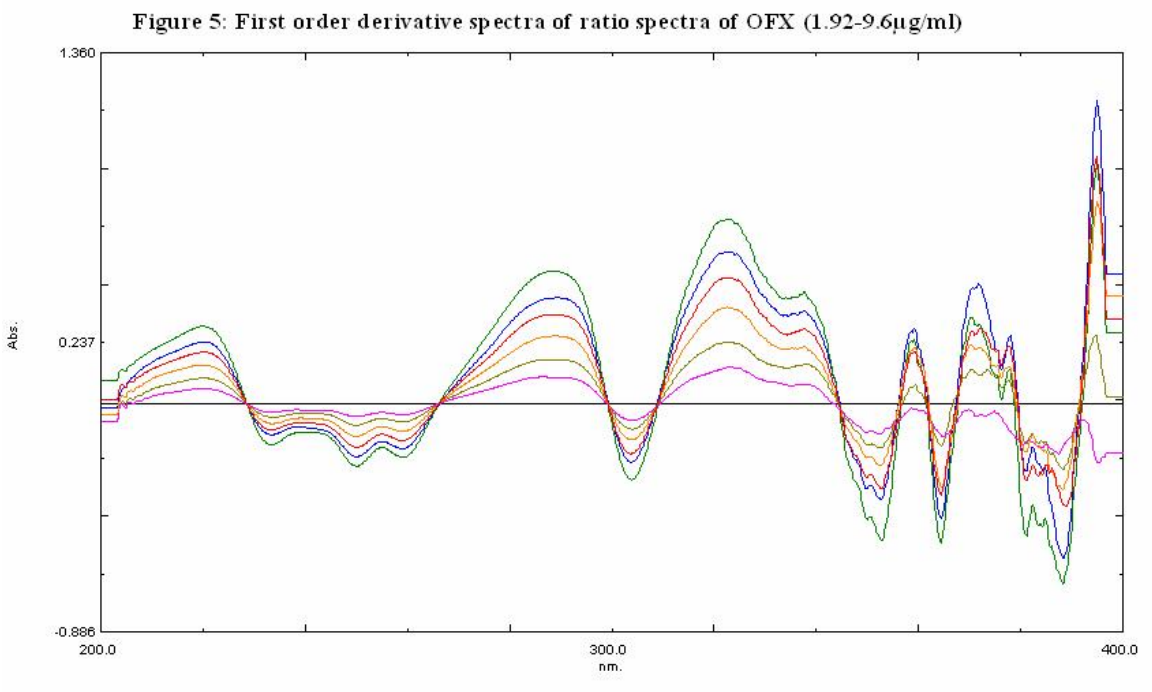


Figure-6 Spectra of ORN (4.8-24.0µg/ml) divided by spectra of 6.4 µg/ml OFX spectra (Ratio spectra of ORN)

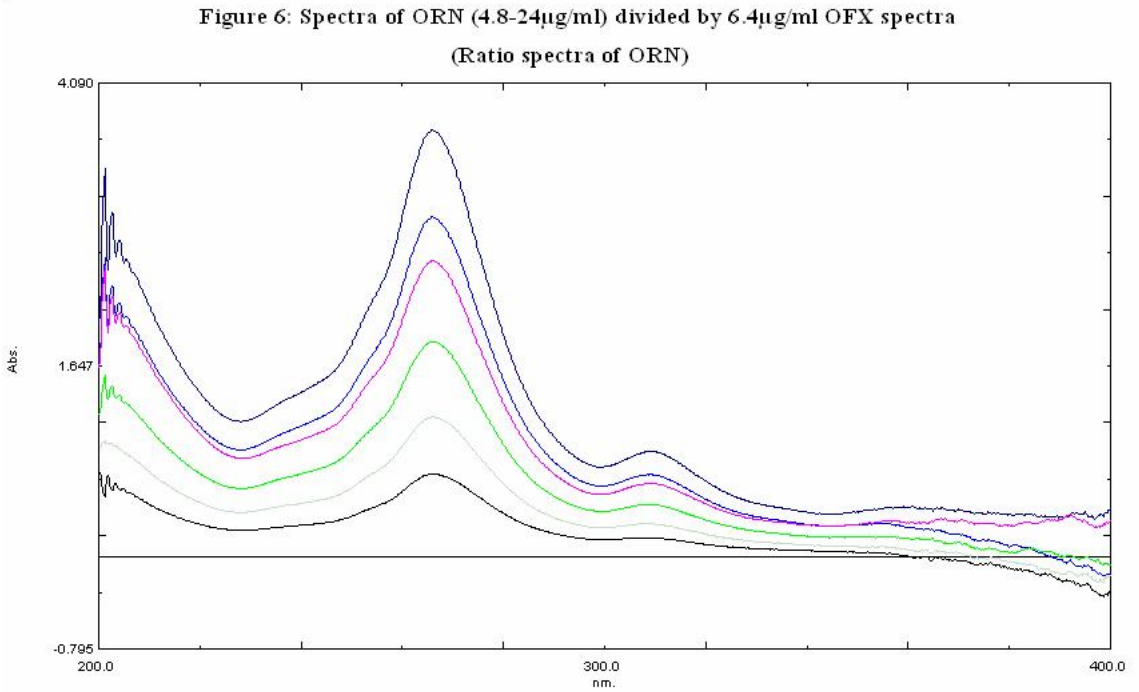


Figure-7 First order derivative spectra of ratio spectra of Ornidazole (4.8-24.0µg/ml)

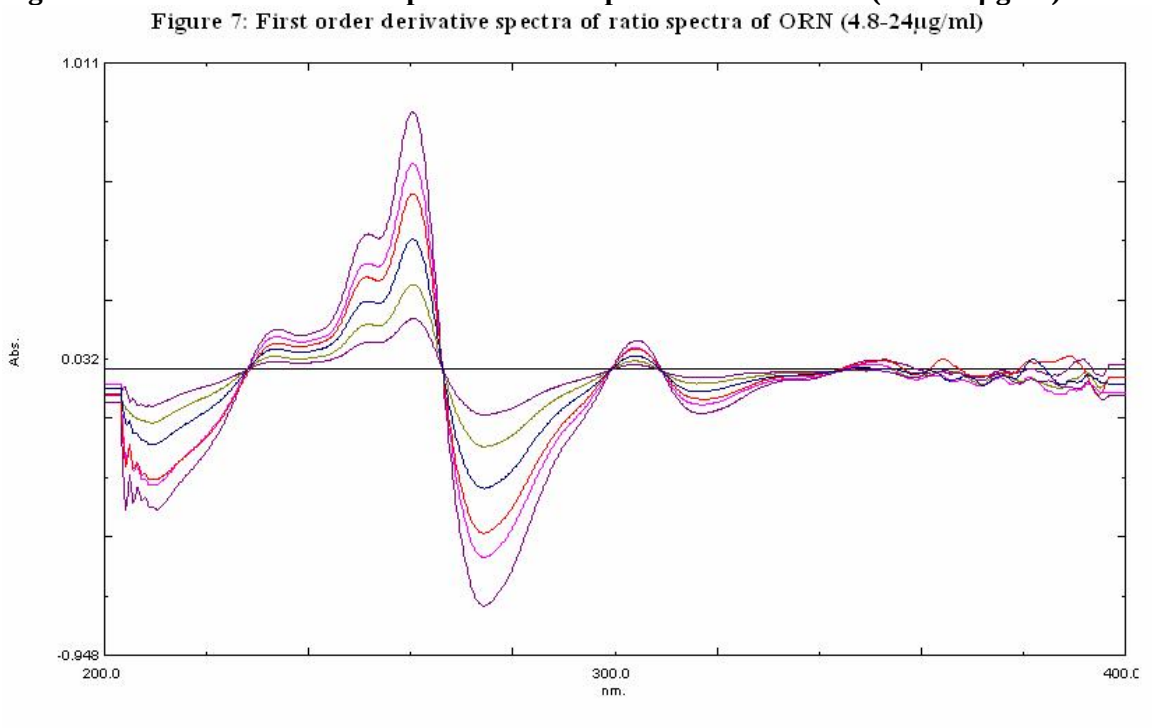
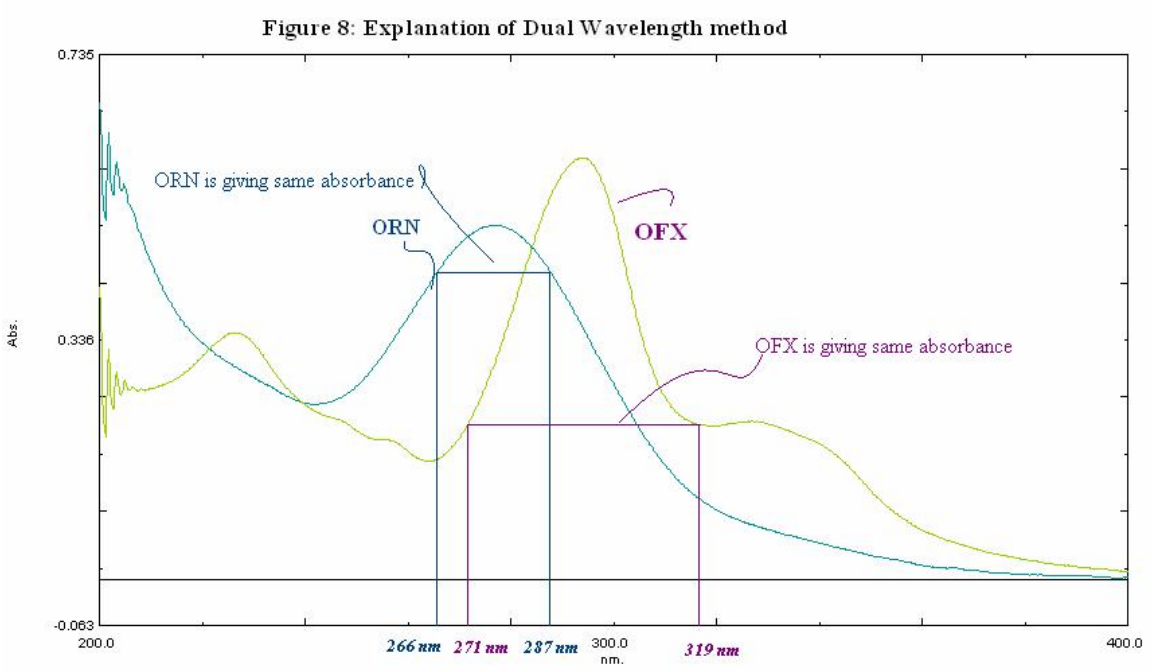


Figure-8 Overlaid spectra of OFX and ORN (Explanation of Dual Wavelength)



2.4.2 Dual wavelength method

The mechanism of this method is that the difference between absorbance at two particular wavelengths where one component shows no difference in absorbance and the other shows the significant difference and vies versa. This difference between absorbance is directly proportional to the concentration of the components of interest, while other component will not show any difference in absorbance even though concentration is changed.

Spectra of OFX in the range of 1.92-9.6µg/ml and of ORN in the range of 4.8-24.0µg/ml were collected in the range of 200nm to 400nm. At wavelengths of 266.0nm and 287.0nm, ORN was shown same absorbance and OFX was shown significant difference in absorbance, hence were selected for estimation of OFX. At wavelengths of 271.0nm and 319.0nm, OFX was shown same absorbance and ORN was shown significant difference in absorbance, hence were selected for estimation of ORN (Figure-8). To establish two separate curves for both the drugs, difference in absorbance at 266.0nm and 287.0nm were plotted against the concentration of OFX and difference in absorbance at 271.0nm and 319.0nm were plotted against the concentration of ORN.

2.5 Method Validation

The accuracy studies were carried out at different concentrations by spiking a known concentration of standard drug to the placebo at four levels (30%, 80%, 100%, and 120%) on three different preparations and analyzed by the developed methods. Precision was studied by analyzing six replicates of sample preparations. Intermediate precision was determined in a similar manner on the next day using a different instrument.

Table-1: Data showing linearity of the developed methods

Methods	**Ratio spectra derivative**		**Dual Wavelength Method**	
Parameters	**OFX**	**ORN**	**OFX**	**ORN**
Linearity range	1.92-9.6 µg/ml	4.8-24.0 µg/ml	1.92-9.6 µg/ml	4.8-24.0 µg/ml
Slope	0.0745	0.0353	0.0563	0.0238
Intercept	0.0070	0.0073	0.0040	0.0005
Correlation Co-efficient	0.9999	0.9998	0.9999	0.9996

Table-2: Data showing precision of the developed methods

Methods	**Ratio Spectra Derivative Method**		**Dual Wavelength Method**	
Parameters	**OFX**	**ORN**	**OFX**	**ORN**
Intraday Precision (% Assay)*	100.5	100.0	100.7	99.8
Intraday Precision (% RSD) **	0.12	0.25	0.94	0.81
Interday Precision (% Assay) *	101.5	99.5	101	99.1
Interday Precision (% RSD) **	0.71	0.82	0.17	0.58

* Average of six determinations; ** Estimated on six determinations

Table-3: Data showing recovery from the Ratio Spectra Derivative method

		OFX						ORN					
		Amount						Amount					
% Level	Preparation	Added (mcg/ml)	Found (mcg/ml)	Recovery	Max	Min	%RSD	Added (mcg/ml)	Found (mcg/ml)	Recovery	Max	Min	%RSD
30	Recovery-1	1.884	1.9212	98.06				4.812	4.8111	100.02			
30	Recovery-2	1.928	1.9278	100.01	100.01	98.06	1.02	4.832	4.88214	98.97	100.02	98.97	0.56
30	Recovery-3	1.912	1.9214	99.51				4.804	4.8124	99.83			
80	Recovery-1	5.14	5.1384	100.03				12.804	12.8023	100.01			
80	Recovery-2	5.112	5.1482	99.30	100.03	99.3	0.38	12.844	12.8214	100.18	100.47	100.01	0.23
80	Recovery-3	5.084	5.0912	99.86				12.856	12.7956	100.47			
100	Recovery-1	6.416	6.3885	100.43				16.092	16.1281	99.78			
100	Recovery-2	6.448	6.3851	100.99	100.99	100.25	0.38	16.056	16.0324	100.15	100.68	99.78	0.46
100	Recovery-3	6.416	6.4001	100.25				16.236	16.1257	100.68			
120	Recovery-1	7.728	7.7231	100.06				19.224	19.2115	100.07			
120	Recovery-2	7.604	7.5984	100.07	100.07	99.63	0.25	19.248	19.1586	100.47	100.47	100.07	0.22
120	Recovery-3	7.684	7.7124	99.63				19.204	19.1852	100.10			

Table-4: Data showing recovery from the Dual Wavelength Method

		OFX						ORN					
% Level	Preparation	Amount Added (mcg/ml)	Amount Found (mcg/ml)	Recovery	Max	Min	%RSD	Amount Added (mcg/ml)	Amount Found (mcg/ml)	Recovery	Max	Min	%RSD
30	Recovery-1	1.924	1.9245	99.97				4.924	4.8852	100.79			
30	Recovery-2	1.948	1.9267	101.11	101.11	99.97	0.95	4.804	4.8153	99.77	101.57	99.77	0.9
30	Recovery-3	1.94	1.9258	100.74				4.86	4.7851	101.57			
80	Recovery-1	5.152	5.1458	100.12				12.804	12.8124	99.93			
80	Recovery-2	5.124	5.1241	100.00	100.12	99.52	0.51	12.768	12.6985	100.55	100.55	99.93	0.31
80	Recovery-3	5.1	5.1247	99.52				12.816	12.7992	100.13			
100	Recovery-1	6.528	6.5284	99.99				16.496	16.4851	100.07			
100	Recovery-2	6.44	6.4021	100.59	100.59	99.999	0.49	16.1	16.2174	99.28	100.07	98.4	0.84
100	Recovery-3	6.408	6.3968	100.18				16.06	16.3214	98.40			
120	Recovery-1	7.684	7.6021	101.08				19.284	19.2351	100.25			
120	Recovery-2	7.612	7.5824	100.39	101.77	101.08	1.08	19.204	19.5247	98.36	100.25	98.36	1.04
120	Recovery-3	7.656	7.5231	101.77				19.18	19.4521	98.60			

Table-5: Results of analysis of commercially available tablet dosage forms containing OFX and ORN

Methods	Ratio Spectra Derivative Method		Dual Wavelength Method	
Parameters	**OFX**	**ORN**	**OFX**	**ORN**
% Assay*	100.7	99.77	99.3	99.88
% RSD	0.81	0.19	0.35	0.68

* Average of six replicates; RSD: Relative Standard Deviation

3. RESULTS AND DISCUSSION

Because of good solubility and stability, 0.1N Hydrochloric Acid is used as the diluent. As per ICH validation guideline [21], specificity, linearity, accuracy and precision were performed for these two methods. There is no any interference observed from blank and placebo in these methods.

These methods show good linearity in the range of 1.92-9.6µg/ml for OFX and 4.8-24.0µg/ml for ORN. The concentration of individual drug present in the mixture was determined against the calibration curve in quantitation mode. In Ratio spectra derivative method, the correlation co-efficient was 0.9999 for OFX and 0.9998 for ORN. In Dual wavelength method, the correlation co-efficient was 0.9999 for OFX and 0.9996 for ORN. The results are given in Table 1.

For evaluation of precision, repeatability of results were evaluated by six replicate determinations. For intermediate precision, the same study was carried out on different day using different instrument. The results are given in Table 2.

Accuracy of the methods was assured by the use of standard addition technique. Certain amount of drugs was added to the placebo. The resulting mixtures were assayed, and the results obtained for both drugs from mixtures were compared with those expected. Accuracy study was done on four levels and three determinations were used in each level. The results are given in Table 3 and Tablet 4.

The proposed validated methods were successfully applied to estimate OFX and ORN in pharmaceutical dosage forms. The results are given in Table 5.

4. ACKNOWLEDGEMENT

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