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DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHODS FOR SIMULTANEOUS ESTIMATION AND DISSOLUTION OF OFLOXACIN AND ORNIDAZOLE IN TABLET DOSAGE FORMS

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Abstract: The aim of this work was to develop and validate simple, accurate and precise spectroscopic methods (multicomponent, dual wavelength and simultaneous equations) for the simultaneous estimation and dissolution testing of ofloxacin and ornidazole tablet dosage forms. The medium of dissolution used was 900 ml of 0.01N HCl, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by developed and validated spectroscopic methods. Ofloxacin and ornidazole showed 293.4 and 319.6nm as λ_{max} in 0.01N HCl. The methods were validated to meet requirements for a global regulatory filing. The validation included linearity, precision and accuracy. In addition, recovery studies and dissolution studies of three different tablets were compared and the results obtained show no significant difference among products.

Keywords: Dissolution • Ofloxacin • Ornidazole • Spectroscopy • Simultaneous estimation

Introduction

Chemically ofloxacin (OFL, Fig. 1) is (±) 9-fluoro-2,3,dihydro-3-methyl-10-(4-methyl-1--piperazinyl)-7-oxo-7H-pyrido[1,2,3-d,e]-1,4--benzoxazine-6-carboxylicacid, belongs to new generation of synthetic fluorinated quinolone, structurally related to nalidixic acid [1-3]. This agent is a new broad spectrum antibacterial drug active against most Gram-negative, Gram-positive bacteria, and some anaerobes [4]. Its bacterial action is based on its anti-DNA gyrase activity [5]. This broad spectrum of antibacterial activity and widespread distribution to most tissues and body fluids at relatively high concentrations after oral administration have made this drug useful for the treatment of systemic infections including urinary tract, respiratory, and gastro-intestinal infections [6-8]. Ornidazole (ORN fig. 2)1-(3-chloro−2-hydroxy)-propyl-2-methyl-5-nitroimidazole, is a nitroimidazole derivative with antiprotozoal and antibacterial properties. It is used for the treatment and prophylaxis of infections, which is induced by anaerobic and microaerophilic bacteria and protozoa [9].

Combinations of ofloxacin and ornidazole are available in the market, which are highly active against many bacterial infections of enteritis and anaerobic bacteria [23]. Both Ofloxacin and Ornidazole are almost completely absorbed from the small intestine when administered orally both having almost 100% bioavailability. Subsequent

plasma concentrations of Ofloxacin are obtained in 1-2 hours after oral administration. Peak plasma concentrations of Ornidazole are obtained within 2 hours of administration. Drug absorption from a dosage form after oral administration depends on the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilisation under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, *in vitro* dissolution may be relevant to the prediction of in vivo performance [10-12]. The dissolution test is a very important tool in drug development and quality control.

Dissolution is an official test used by pharmacopoeias for drug evaluation release of solid and semisolid dosage forms, and it is routinely used in Quality Control (QC) and Research & Development (R&D). The purpose of *in vitro* dissolution studies in QC is batch to batch consistency and detection of manufacturing deviation while in R&D the focus is to provide some predictive estimate of the drug release in respect to the *in vivo* performance of a drug product. For QC, an over-discriminatory test might be suitable to detect even small production deviations. However, for prediction of the *in vivo* performance of drug product a dissolution test should be sensitive and reliable [12]. The accomplishment of dissolution profiles is recommended as support in the development and optimization of drug formulation as well as in the establishment of *in vitro/in vivo* correlation. When dissolution test is not defined in the monograph of the dosage form, or if the monograph is not available, the selection of a dissolution medium may be based on the solubility data and dosage range of the drug product [10]. Hydrochloric acid is typical medium used to dissolution test [14], and 0.01M HCl medium was selected. Typical acceptance criteria for the amount of drug dissolved are in the range of 70 – 80 % dissolved [12].

OFL is official in BP [13], USP [14] and EP [15]. The assay procedure mentioned in these pharmacopoeias is non aqueous titration. There are many reported HPLC [16-18], UV spectrophotometry [19] and spectrofluorimetry methods for the estimation of these drugs from pharmaceutical preparations and biological fluids, and also the analytical methods are available for stability [20] studies of these drugs. ORN is official in none of the Pharmacopoeias. Also HPLC [21-22], HPTLC [23] methods are available for the estimation of this combination. At present, there are no official monographs for OFL and ORN dosage forms and no dissolution tests have been described in literature. Parameters to set up the dissolution test should be researched and defined for drugs that do not possess official monographs [12]. The present paper describes the development and validation of analytical methods for the estimation and dissolution test for OFL and ORN tablet dosage form. The best dissolution conditions were used to evaluate the dissolutions testing of three different brands of tablets.

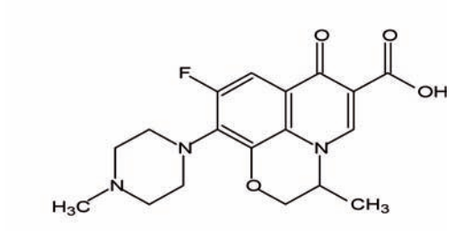

Figure 1. Chemical structure of ofloxacin

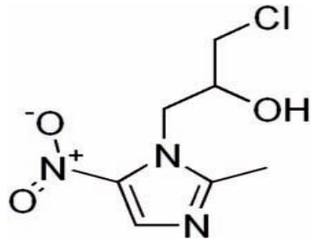

Figure 2. Chemical structure of ornidazole.

Experimental

Materials

Ofloxacin and ornidazole chemical reference substances (CRS) (assigned purity 99.7 & 99.8%) were obtained from GLPL (Vadodara, India). Tablets were purchased at the local market and were claimed to contain 200 mg OFL and 500mg ORL each. All reagents and solvents used were analytical grade. Distilled water obtained from a Lab Sil Water Distillation Unit (Bangalore, India). 0.01 M HCl solution was prepared according to USP Pharmacopoeia [14].

Instrumentation

Dissolution test was performed in a Veego dissolution test system, model VDA 6DR multi--bath (n=6), in accordance to USP Pharmacopoeia [14] general method. The mediums were vacuum degassed under house vacuum and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Vis spectrophotometer (Shimadzu, Japan) model UV – 1700 PC, with a fixed slit width (1 nm) using 1.0 cm quartz cells was used for all absorbance measurements.

Stock solutions

OFL and ORN are present in 2:5 ratio in commercial dosage forms, hence stock solution of OFL (100µg ml) and ORN (250 µg ml) were prepared in 0.01N HCl by dissolving 10mg and 25mg of drug in solvent, and volume made up to 100ml with 0.01N HCl. Appropriate dilutions were made to the stock solution with distilled water to get the working standard solutions in the same linearity ranges for all three methods.

Sample solutions

Average weight of twenty tablets was determined and these tablets were crushed to fine powder. The powder sample equivalent to 10mg of Ofloxacin and 25mg of Ornidazole was weighed and transferred to 100ml volumetric flask and dissolved in 0.01N HCl. The content was sonicated for 10min; finally the volume made up to the mark with 0.01N HCl and filtered through Whatmann's filter paper. The filtered solution was suitably diluted with distilled water to obtain mixed standards in the linearity range for each drug. The sample solutions were scanned in the selected wavelength region for respective methods, and the results were obtained are reported in the table.

Development of UV spectroscopic methods for the simultaneous estimation

Three simple, accurate spectrophotometric methods Multi component, Dual wavelength, Simultaneous equations have been developed for the simultaneous determination and dissolution studies of Ofloxacin and Ornidazole in tablet dosage forms. Ofloxacin shows absorption maximum at 293.4 nm and Ornidazole shows at 319.6nm in 0.01N HCl. Beer's law was obeyed in the concentration range of 2-12 µg ml for Ofloxacin and 5-30 µg ml for Ornidazole.

Method 1: Multi component analysis

Two sampling wavelengths 293.4nm for Ofloxacin and 319.6nm for Ornidazole were selected for the estimation in the multicomponent mode in the instrument. The absorbance spectra of mixed standards and sample solutions were measured at selected wavelength.

Method 2: Dual wavelength method

For estimation of one component, two wave lengths were selected, where the absorbances of other component were same. Therefore the difference in the absorbances in the mixed spectra at the corresponding wavelength will be directly proportional to the concentration of that component. For Ofloxacin, 303.2nm (λ_1) and 334.6(λ_2) nm, for Ornidazole 284.4nm (λ_1) and 300.8(λ_2) were selected. The difference in the absorbances at the selected wavelengths, were plotted against the respective concentration to obtain the calibration curves. The concentration in sample solutions

of each component was obtained from the calibration curves of the respective drugs.

Method 3: Simultaneous equations method

The absorbances of the both the drugs at both wavelengths (respective absorption maximums 293.4 nm and 319.6nm) were measured, and the absorptivity and molar absorptivity values were determined for OFL and ORN.

Dissolution test conditions

Dissolution testing was carried out according to conventional dissolution procedures recommended for immediate release products, using paddle (USP Apparatus 2) at 50 rpm. Sampling aliquots of 5.0 ml were withdrawn at 0, 15, 30, 45 and 60 minutes, and replaced with an equal volume of the fresh medium to maintain a constant total volume. After the end of each test time, samples aliquots were filtered, diluted in distilled water, when necessary, and quantified. The assay of the three tested products was performed using previously developed and validated spectrophotometric methods. The contents results were used to calculate the percentage release on each time of dissolution profile. The cumulative percentage of drug dissolved was plotted against time.

Method validation and recovery studies

The developed UV spectrophotometric methods in 0.01M HCl were validated for linearity, precision, accuracy, and the dissolution study in medium 0.01M HCl was validated for precision according to USP Pharmacopoeia [14] and ICH guidelines.

Recovery studies were carried out at 80%, 100% and 120% levels on a pre analysed tablet solution. The percent recovery of Ofloxacin and Ornidazole in the sample mixture were determined and reported in the table.

Results and discussion

As described in the experimental section, pure drugs and their mixture standards were scanned in UV-Visible Spectrophotometer in 200-400nm wavelength region, the overlay spectra of mixture (2ug/ml of OFL+ 5ug/ml of ORN, to 12ug/ml of OFL+ 30ug/ml of ORN) is shown as Figure 3.

The overlay spectra of pure drug substances OFL (2-12 ug/ml) and ORL (5-30 ug/ml), shown as Figure 4.

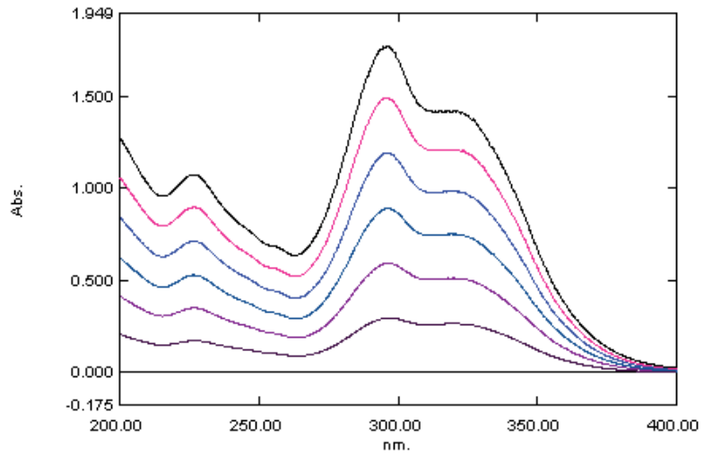

Figure 3. Overlay spectrum of mixture containing OFL and ORN (2+5 to12+30 ug/ml)

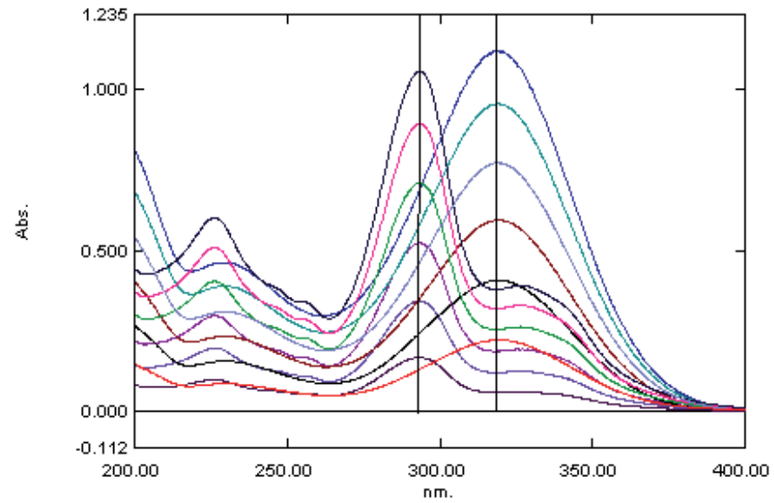

Figure 4. Overlay spectrum of ofloxacin (2-12 ug/ml) and ornidazole (5-30 ug/ml)

Method validation

The developed methods were validated for linearity, accuracy and precision.

Accuracy of the method was found on the basis of bias measurement and the precision of the method was determined by measuring the repeatability (intra-day precision) and the intermediate precision (inter-day precision), both expressed as RSD (%).The results were shown in tables 1&2.

Table 1. Validation parameters includes linearity, limits of detection and quantifications

Regression and analytical parameters						
Parameter	Multi component method		Dual wavelength method		Simultaneous equation method	
	Mixture		OFL	ORN	OFL	ORN
λ max / nm	296nm		293.4nm	319.6nm	293.4nm	319.6nm
Linearity, µg/ml	2 - 12µg	5 - 30µg	2 - 12µg	5 - 30µg	2 - 12µg	5 - 30µg
LOD, µg/ml	0.065	0.16	0.082	0.15	0.082	0.15
LOQ, µg/ml	0.22	0.54	0.27	0.48	0.27	0.48
Regression equation, y*						
Intercept (a)	0.592	0.1481	0.0293	0.0111	0.1464	0.0462
Slope (b)	0.0018	0.0018	-0.003	0.0283	-0.0041	0.048
Correlation coefficient (r)	0.9999	0.9999	0.9996	0.9968	0.9999	0.9992

* y = a+bx, where y is the absorbance and x the concentration in µg ml^{-1}

Table 2. Validation results includes accuracy and precision

Taken (ug/ml)	Intra-day [a]			Inter-day [b]		
	Found [c]	Precision [d]	Accuracy [e]	Found [c]	Precision [d]	Accuracy [e]
MULTI COMPONENT METHOD						
OFL 6	6.03±0.07	1.15	0.48	6.07±0.16	1.57	1.25
ORN 15	15.08±0.17	1.15	0.55	15.20±0.39	0.62	1.32
DUAL WAVE LENGTH METHOD						
OFL 6	6.02±0.03	0.5	0.31	6.04±0.02	0.32	0.63
ORN 15	15.14±0.2	1.12	0.36	15.21±0.25	0.35	0.95
SIMULTANEOUS EQUATION METHOD						
OFL 6	6.15±0.36	1.14	0.45	6.20±0.12	1.62	0.75
ORN 15	15.31±0.21	1.26	0.94	15.24±0.36	1.25	0.54

[a] n = 6; [b] n = 6; [c] mean ± standard error; [d] relative standard deviation, %; [e] bias %: (found − taken/taken)×100

Estimation by spectroscopic methods

For the multicomponent and dual wave-length methods, calibration curves were prepared at respective wavelengths selected, and were used for the measurement of samples.

Determination by simultaneous equations method

The absorptivity values and molar absorptivity values for OFL and ORN are determined (shown in Table 3) and molar absorptivity values for OFL at 293.4 and 319.6 nm were 31589.37 and 11331.30 cm^{-1} mol^{-1} lit^{-1}, while respective values for ORN at 293.4 and 319.6 nm were 5351.96 and 8706.18 cm^{-1} mol^{-1} lit^{-1}. Molecular weight of OFL and ORN is 361.4 and 219.625 respectively.

$$A1 = 5351.96C1 + 31589.37C2 \quad (1)$$

and

$$A2 = 8706.18C1 + 11331.30C2 \quad (2)$$

where A1 and A2 are the values of absorbance of sample at 293.4 and 319.6 nm respectively, and C1 and C2 are concentrations of OFL and ORN in moles lit^{-1} respectively.

Table 3. Absorptivity values of OFL and ORN for simultaneous equations method

Conc (µg/ml)	Absorptivity for OFL		Conc (µg/ml)	Absorptivity for ORN	
	293.4nm	319.6nm		293.4nm	319.6nm
2	874.00	314.00	5	244.00	399.00
4	873.50	314.50	10	244.25	397.00
6	873.33	313.33	15	245.33	396.67
8	874.00	312.00	20	242.50	396.00
10	876.00	312.90	25	243.20	395.80
12	873.67	314.50	30	242.83	394.00
Mean	874.08	313.54	Mean	243.69	396.41
S.D	0.98	0.99	S.D	1.05	1.64
R.S.D	0.11	0.32	R.S.D	0.43	0.41
M.A *	31589.37	11331.30	M.A *	5351.96	8706.18

*M.A. is molar absorptivity in cm^{-1} $moles^{-1}$ lit^{-1} determined from mean value of absorptivity

Three different tablet dosage forms were analysed by the developed methods and the results were shown in table 4. Recovery studies carried out on the pre analyzed tablets and the results were shown in table 5.

Table 4. Results for OFL and ORN in three different tablet dosage forms

Dosage form	Labelled amount (mg)	Found [a] (% of nominal amount ± SD)		
		METHOD 1	METHOD 2	METHOD 3
ORNI O Tablet	OFL 200	98.85±1.45	99.27±2.17	98.28±0.68
	ORN 500	102.71±1.10	101.21±1.50	98.21±1.44
OSNO O Tablet	OFL 200	99.76±0.86	99.16±1.69	100.74±1.62
	ORN 500	101.68±1.45	101.32±1.64	98.35±1.73
OFLOX OZ Tablet	OFL 200	101.22±1.86	101.87±1.54	102.75±1.71
	ORN 500	102.32±1.54	101.61±1.55	97.97±1.81

Method 1 - Multicomponent method
Method 2 -Dual wave length method
Method 3- Simultaneous Equation method
[a] Mean value of six determinations

Table 5. Recovery results for OFL and ORN by using three different tablet dosage forms

MULTICOMPONENT METHOD								
Dosage form	OFL in dosage form (µg/ml)	ORN in dosage form (µg/ml)	pure OFL added (µg/ml)	pure ORN added (µg/ml)	total OFL found (µg/ml)	total ORN found (µg/ml)	pure OFL recovered % ± S.D*	pure ORN recovered % ± S.D*
ORNI O tab	5	12.5	4	10	9.07	22.67	100.78±1.17	101.56±1.60
	5	12.5	5	12.5	10.14	24.52	101.37±1.78	97.85±1.57
	5	12.5	6	15	11.24	27.08	102.20±0.87	98.66±1.54
OSNO O tab	5	12.5	4	10	8.97	22.86	99.68±0.65	102.55±1.35
	5	12.5	5	12.5	10.04	24.79	100.24±1.08	99.45±0.79
	5	12.5	6	15	11.1	27.39	101.57±0.67	99.56±1.31
OFLOX OZ Tab	5	12.5	4	10	9.12	23.01	101.53±1.12	103.35±1.40
	5	12.5	5	12.5	9.95	24.71	99.15±0.81	98.56±1.23
	5	12.5	6	15	11.31	28.12	102.81±0.96	103.65±1.35
DUAL WAVELENGTH METHOD								
Dosage form	OFL in dosage form (µg/ml)	ORN in dosage form (µg/ml)	pure OFL added (µg/ml)	pure ORN added (µg/ml)	total OFL found (µg/ml)	total ORN found (µg/ml)	pure OFL recovered % ± S.D*	pure ORN recovered % ± S.D*
ORNI O tab	5	12.5	4	10	8.94	22.45	99.68±1.32	99.56±0.54
	5	12.5	5	12.5	10.15	24.97	102.35±2.21	99.97±0.41
	5	12.5	6	15	11.15	27.58	102.31±1.31	100.56±0.67
OSNO O tab	5	12.5	4	10	9.21	22.68	101.54±0.59	101.38±0.98
	5	12.5	5	12.5	10.26	25.39	102.49±0.85	102.65±2.14
	5	12.5	6	15	11.05	27.35	100.56±0.47	101.23±0.65
OFLOX OZ Tab	5	12.5	4	10	8.89	22.61	98.75±0.97	101.96±1.25
	5	12.5	5	12.5	10.18	24.97	102.59±1.36	99.85±1.56
	5	12.5	6	15	11.26	27.47	102.36±1.50	99.79±0.69
SIMULTANEOUS EQUATION METHOD								
Dosage form	OFL in dosage form (µg/ml)	ORN in dosage form (µg/ml)	pure OFL added (µg/ml)	pure ORN added (µg/ml)	total OFL found (µg/ml)	total ORN found (µg/ml)	pure OFL recovered % ± S.D*	pure ORN recovered % ± S.D*
ORNI O tablet	5	12.5	4	10	8.85	22.45	98.35±1.45	99.65±1.41
	5	12.5	5	12.5	9.96	25.68	99.57±0.58	98.76±1.36
	5	12.5	6	15	10.75	27.69	97.65±1.25	98.65±1.52
OSNO O tab	5	12.5	4	10	9.08	22.65	100.85±0.69	101.56±1.51
	5	12.5	5	12.5	10.24	24.21	96.58±1.39	97.54±1.39
	5	12.5	6	15	11.21	27.31	102.35±1.24	102.65±0.37
OFLOX OZ Tab	5	12.5	4	10	9.24	22.36	102.63±0.59	99.25±0.77
	5	12.5	5	12.5	10.58	25.34	103.26±1.39	97.38±0.64
	5	12.5	6	15	11.39	27.85	103.25±0.15	102.36±2.15

* N=3

Dissolution testing

Dissolution was carried in 0.01N HCl for three different tablets, samples were collected at 0, 15, 30, 45 and 60 min intervals and the amount of drug dissolved was calculated by the developed three methods. The cumulative percentage of drug dissolved was plotted against time, shown in figures 5 & 6.

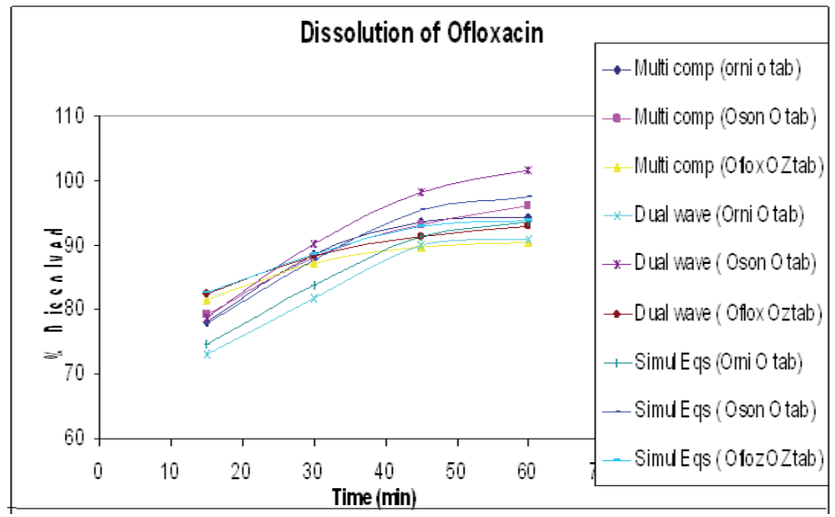

Figure 5. Dissolution profile comparison for OFL in three tablets by three methods

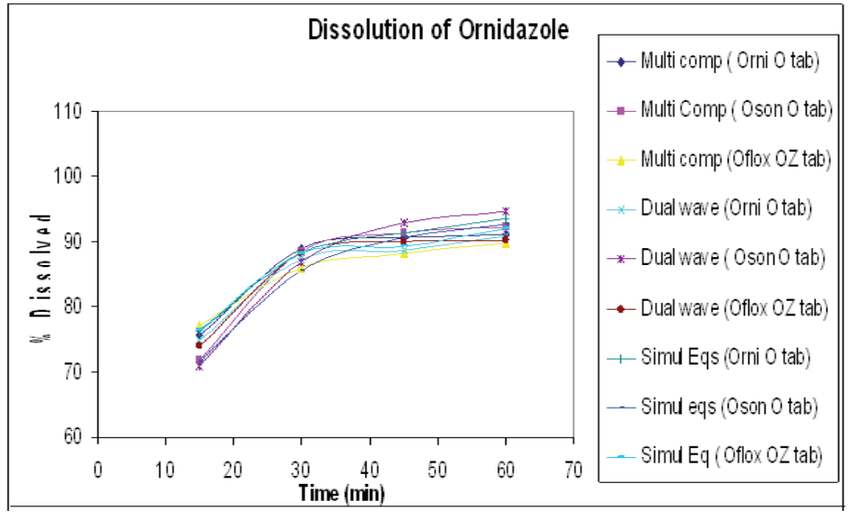

Figure 6. Dissolution profile comparison for ORN in three tablets by three methods

Conclusions

The analytical methods developed and validated for the simultaneous estimation and for dissolution testing of ofloxacin and ornidazole tablet dosage forms were considered satisfactory. The conditions that allowed the dissolution determination were 900 ml of 0.01M HCl, at 37.0 ± 0.5 ºC, paddle apparatus, 50 rpm stirring speed. The % drug delivery was higher than 80% in 30 minutes for all evaluated products. The analysis of variance of the recovery of dosage forms and dissolution values showed that the methods developed were similar ($p<0.05$). The methods were validated and showed to be linear, precise and accurate.

Acknowledgments

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