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atadorno@iq.unesp.br
Universidade Estadual Paulista Júlio de Mesquita Filho
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Mashru, R. C.; Saikumar, S.V.

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

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Universidade Estadual Paulista Júlio de Mesquita Filho
Araraquara, Brasil

Conclusão

As águas do Rio São Francisco Falso não apresentaram condições propícias à produção de biomassa, uma vez que prevaleceu em suas águas o estado oligotrófico. As espécies de nitrogênio e o fósforo total tiveram suas concentrações elevadas durante campanhas marcadas pela influência de chuvas, indicando presença de fontes difusas. O NO_2 em todas as amostras apresentou valores de concentração acima do recomendado pela CCME para proteção da via aquática. Por sua vez, a NH_3 não representa um risco potencial à vida aquática em função das baixas concentrações. Em comparação com o Rio Ocoí, o Rio São Francisco Falso demonstra estar em melhor condição de preservação, o que pode ser em parte explicado pela ausência de atividades urbano-industriais.

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Abstract: an environmental assessment about phosphorus and nitrogen species was carried out in waters of São Francisco Falso River, tributary of Itaipu Reservoir. Results from four field campaigns showed that trophic state vary of oligotrophic to mesotrophic, being the latter observed under rain influence. Among nitrogen species, NO_2 presented concentration above guide-value recommended by Canadian environmental agency, what means a risk for aquatic life. In comparison to Ocoí River, another tributary of Itaipu Reservoir, São Francisco's waters showed better quality.

Keyword: eutrophication, hydrographic basin, trophic state index.

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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.

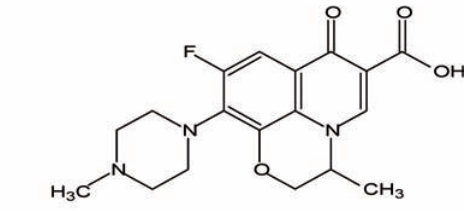

Figure 1. Chemical structure of ofloxacin

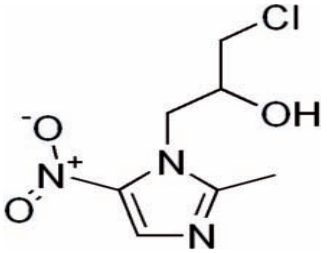

Figure 2. Chemical structure of ornidazole.

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.

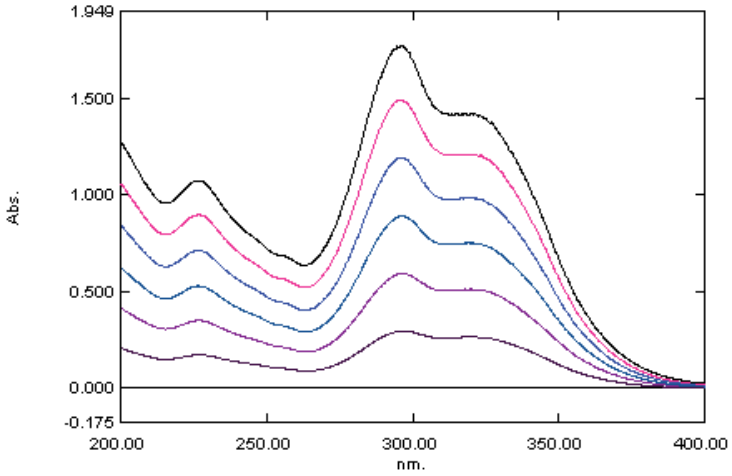

Figure 3. Overlay spectrum of mixture containing OFL and ORN (2+5 to12+30 ug/ml)

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 wavelength 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 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

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.

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