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Simultaneous estimation of ofloxacin and tinidazole in tablet dosage form by RP-HPLC

M Rama Kotaiah*[1], Shaik Harun Rasheed[1], Y Narasimha Rao[1], Y Venkateswarlu[1], Konda Ravi Kumar[2]

[1] Department of Pharmaceutical Analysis,. Don Bosco College of Pharmacy, Pulladigunta, Guntur-522017, Andhra Pradesh, India.
[2]Department of Pharmaceutical Chemistry, Siddartha Institute of Pharmaceutical Sciences, Narasaraopet, Andhra Pradesh, India.

ABSTRACT

A simple reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Ofloxacin and Tinidazole in combination. The separation was carried out using a mobile phase consisting of 2mM phosphate buffer and Acetonitrile with pH 3.0 adjusted with phosphoric acid in the ratio of 75: 25%v/v. The column used was Phenomenex C_{18}, (250 mm x 4.6 mm i.d, 5μm) with flow rate of 1 ml / min using PDA detection at 303 nm. The described method was linear over a concentration range of 5-50 μg/ml and 15-150 μg/ml for the assay of Ofloxacin and Tinidazole respectively. Ambroxol (50 μg/ml) was used as internal standard. The retention times of Ofloxacin, Tinidazole and Ambroxol were found to be 2.3, 4.1 and 5.1min respectively. Results of analysis were validated statistically and by recovery studies. The limit of quantification (LOQ) for Ofloxacin and Tinidazole were found to be 10 and 30 μg/ml respectively. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Ofloxacin and Tinidazole bulk drug and in its pharmaceutical dosage form.

Keywords: Ofloxacin, Tinidazole, Ambroxol.

Corresponding author
Email: mrk.pharma@gmail.com

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INTRODUCTION

Ofloxacin is a broad spectrum, fluorinated quinolone antibacterial drug, chemically it is a 9-fluro-2, 3-dihydro - 3-methyl – 10 - (4-methyl – 1 - piperazinyl) - 7-oxo - 7H – pyrido [1, 2, 3–de]-1,4 benzoxacine-6-carboxylic acid [1]. Tinidazole (TNZ) is a 1-[2-(ethyl sulphonyl) ethyl] – 2-methyl – 5- nitro – 1H- imidazole, derivative used as antiprotozoal/antibiotic and antibacterial [2]. The literature survey revealed that few methods have been reported for the estimation of Ofloxacin and Tinidazole. So far, no method has been reported [3-8] for estimation of OFL and TNZ in combined dosage forms, hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of OFL and TNZ in combination, using 2mM phosphate buffer and Acetonitrile with pH 3.0 adjusted with phosphoric acid in the ratio of 75: 25%v/v.. The column used was Phenomenex C_{18}, (250 mm x 4.6 mm i.d, 5μm) with flow rate of 1 ml / min using PDA detection at 303 nm.

EXPERIMENTAL

Chemicals, reagents and Instrumental Conditions

Standard bulk drug sample Ofloxacin and Tinidazole and Ambroxal were provided by Micro Laboratories Ltd., Bangalore. Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC (Double pump) with Rheodyne 7725i type injector with 20μl loop capacity and SPD M20A, Prominence Diode Array Detector. The wavelength of detection chosen was 303 nm. A reverse phase Phenomenex C18 column (250 mm × 4.6 mm, 5 μm) was used for the analysis. The mobile phase comprising of a mixture of 2mM phosphate buffer and Acetonitrile with pH 3.0 adjusted with phosphoric acid in the ratio of 75: 25%v/v with a flow rate of 1ml/min. The injection volume was 20 μL.

Preparation of stock, working standard solutions, and sample solution

A stock solution of OFL and TNZ (100 μg/mL) was prepared, by taking 10 mg of each drug, accurately weighed, in separate 100-mL volumetric flasks. They were dissolved in 25 mL of mobile phase and then the volume was made up to the mark to get 100 μg/mL. The internal standard solution was prepared by taking 10 mg of Ambroxol in a 100 ml standard flask. It is dissolved by adding 25 ml of mobile phase, shaken for few minutes to get a clear solution and the final volume was made up to 100 ml. For each drug, appropriate aliquots were pipetted out from the standard stock solution into a series of 10-mL volumetric flasks. For each drug, appropriate aliquots were pipette out from the standard stock solution into a series of 10 ml volumetric flasks to get a concentration of 5,10,20,30,40 and 50 μg/ml of Ofloxacin, 15, 30, 60, 90, 120 and 150 μg/ml Tinidazole and 50 μg/ml of Ambroxol (Internal Standard).

Method validation

Every 20 µL of the working standard solution of OFL in the mass concentration range of 5 to 50 µg/mL, and that for TNZ in the mass concentration range of 15 to 150 µg/mL, was injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each concentration of the drug solution. Calibration curves of OFL and TNZ were obtained by plotting the peak area ratio versus the applied concentrations of RP and IH. The linear regression coefficients were found to be 0.9997 and 0.9995 for OFL and TNZ respectively. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3.3 ∂/*S* and 10 ∂/*S*, respectively as per ICH guidelines [9], where ∂ is the standard deviation of the response (*y*-intercept) and *S* is the slope of the calibration plot.

Repeatability of the method was checked by injecting replicate injections of the combined solution (10 µg/mL and 30 µg/mL of OFL and TNZ respectively). Variability of the method was studied by analyzing the solution on the same day (intra-day precision) and on three different days (inter- day precision). The results obtained for intra-day precision (RSDs) were 0.684%& 0.482% respectively, at *n* = 6, for both OFL and TNZ. The inter-day precisions (RSDs) were 0.524% and 0.289%, respectively, at *n* = 6, for both OFL and TNZ.

Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, which are listed in **Table 1.**

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both OFL and TNZ from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific.

To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed. System suitability parameters for the method are listed in **Table 2.**

Analysis of formulation

Twenty tablets of OFL and TNZ in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 15 mg of OFL and 45 mg of TNZ was weighed and transferred to a 100 mL volumetric flask, first dissolved in 50 mL of mobile phase, and then the volume was made up to the mark with the

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mobile phase. The content was ultrasonicated for 30 min for complete dissolution. The solution was then whatman filter paper No-41. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The mixed sample solution of 10 µg/mL of OFL and 30 µg/mL of TNZ , which was falling in the Beer's-Lamberts range with 50 µg/mL internal standard , showed good results and was selected for the entire analysis. The results of tablet analysis (*n* = 6) were found to be 99.86% with ±0.25% standard deviation (SD) and 99.62% with ± 0.36% SD for OFL and TNZ respectively. From the typical chromatogram of OFL, TNZ and Ambroxol (Internal standard) (Fig. 3), it was found that the retention time of OFL was 2.3 min, TNZ was 4.1 min and Ambroxol was 5.1 min, which were well-resolved peaks with a resolution factor of 7.3 and 8.3.**Fig- 1**. The results analysis was shown in **Table – 3.**

Table 1: Recovery Studies of OFL and TNZ (n=6)

Drug	Concentration of Std Solution used (µg/mL)	Concentration of Sample Solution Added (µg/mL)	Amount Found (µg/mL)	% Recovery	% RSD
OFL	10	5	14.90	99.33	0.263
	10	10	20.14	100.70	0.734
	10	15	24.87	99.48	0.474
TNZ	30	15	45.69	100.53	0. 597
	30	30	59.22	98.70	0.501
	30	60	89.43	99.36	0. 243

Table 2

Validation Parameters	OFL	TNZ
Linearity range (µg / ml)	5-50	15-150
r	0.9982	0.9987
LOD (ng /ml)	5	10
LOQ (ng /ml)	10	30
Intra day (% RSD)*	0.684	0.482
Inter day (% RSD)*	0.524	0.289
Repeatability (% RSD)*	0.3251	0.4250
Accuracy	99.83±1.342	99.53±0.524
Peak purity index	1.0000	1.0000
Resolution factor (R_s)	-	6.218
Asymmetry factor (A_s)	0.95	
No. of theoretical plates (N)	6452	6957
Capacity factor (K')	-	0.330
High equivalent to theoretical plates (HETP)	21.075	23.475
Tailing factor	1.320	1.443
Seletivity factor (α)	3.959	

- Each value is a mean of six observations.

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Table 3: Analysis of formulation

Drugs	Labelled Amount (mg)	Amount taken for assay (µg/ml)	*Amount found(mg)	% label claim	*Precision# (%RSD)	
					Inter day	Intra day
Ofloxacin	15	15	14.98±0.679	99.86	0.524	0.684
Tinidazole	45	45	44.86±1.140	99.62	0.289	0.482

*Each value is a mean of six observations.

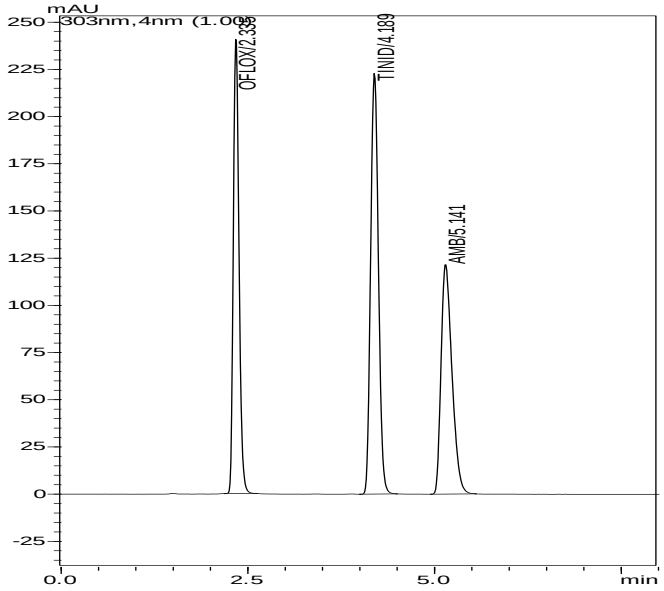

Fig-1 Typical Chromatogram of OFL, TNZ and Ambroxol in sample

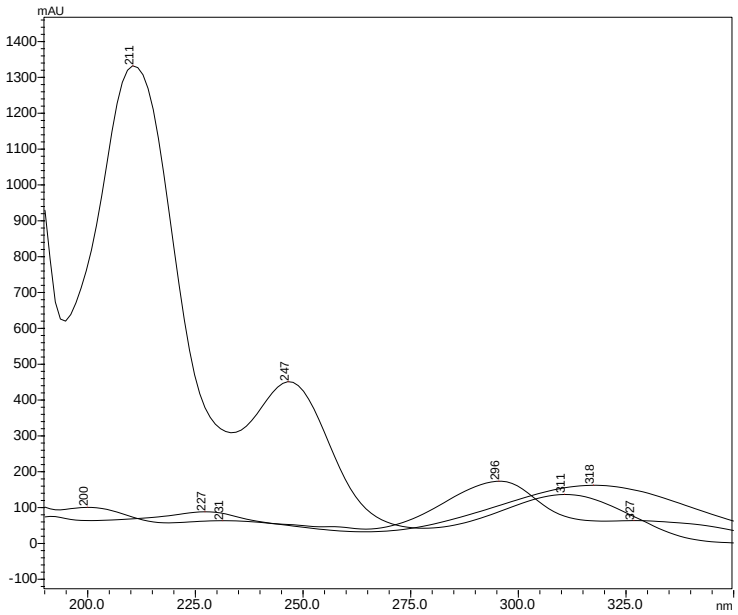

Fig-2 Overlay UV Spectrum of OFL, TNZ and Ambroxol

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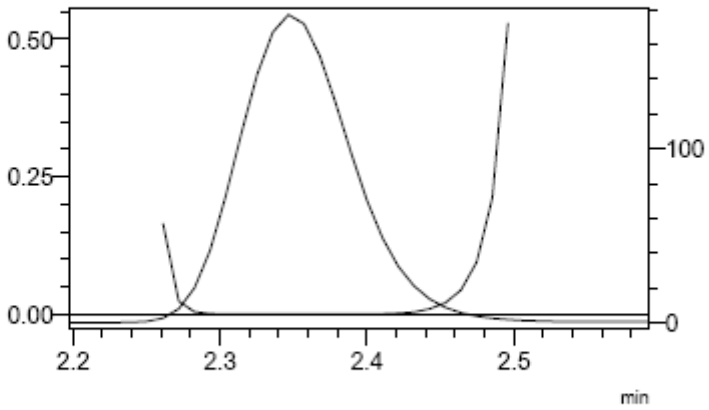
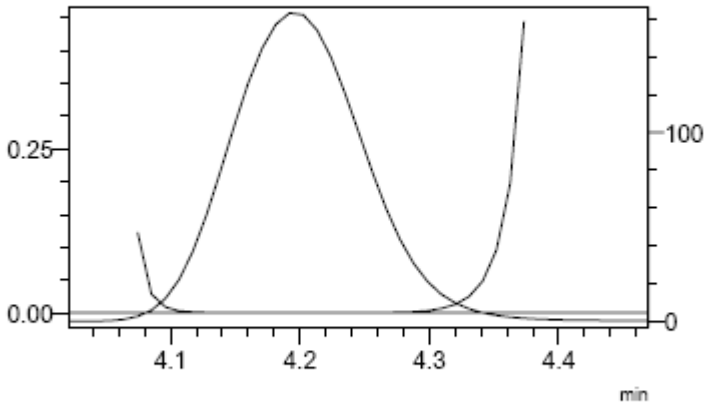
Purity

Retention Time : 2.343
Compound Name : OFLOX



Impurity :Not Detected
Peak purity index : 1.000000
Single point threshold : 0.999874

Retention Time : 4.190
Compound Name : TINID



Impurity :Not Detected
Peak purity index : 1.000000
Single point threshold : 0.999897

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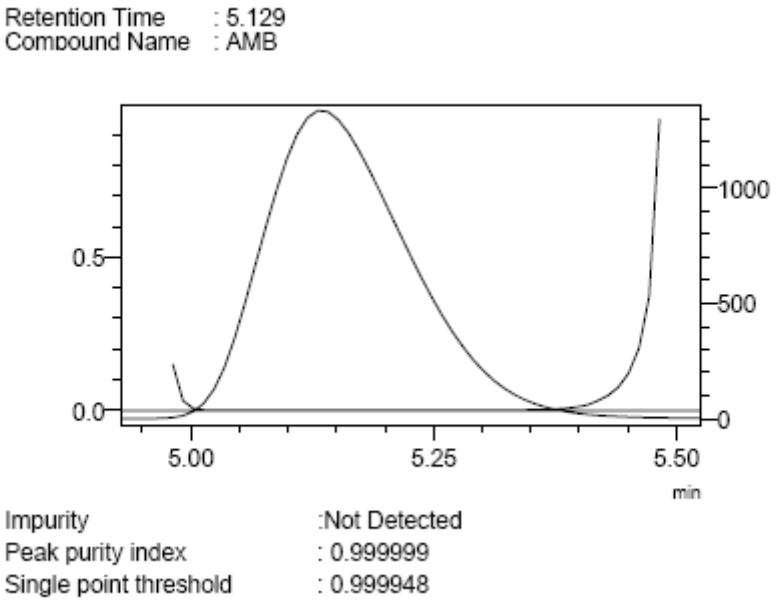

Fig-3 Peak Purity Curve of OFL, TNZ and Ambroxol

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