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SIMULTANEOUS ESTIMATION OF OFLOXACIN AND TINADAZOLE BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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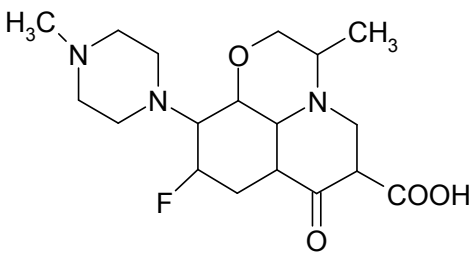
ABSTRACT

An HPLC method for simultaneous estimation of ofloxacin and tinidazole was developed using SS wakosil II. C18, 250 x 4.6 mm, 5 μm column. With mobile phase composition of acetonitrile and phosphate buffer 3 : 1 (pH 5), flow rate of 1.0 mL/min and UV detection at 295 nm linearity was observed over concentration range of 10-50 μg/ mL for ofloxacin and 10-80 μg/ mL for tinidazole. The accuracy of the proposed method was determined by recovery studies and found to be 95-105% for ofloxacin and 101-103% for tinidazole, the proposed method was validated and results conformed with the ICH parameters.

Key words: Ofloxacin, Tinidazole, RP-HPLC

INTRODUCTION



Ofloxacin is chemically ± 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-

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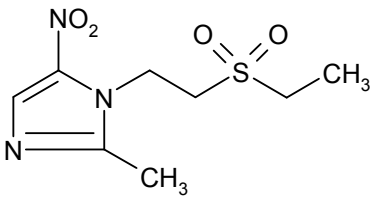
pipiraziynl)-7-oxo-7H-pyridol (1,2,3,-de)-1,4 benzoxazine-6-carboxylic acid.



Which is used for the treatment of urinary tract prostrate and skin or soft tissue infections caused by susceptible bacteria. Tinidazole is chemically 1-(2-ethylsulfonylethyl)-2-methyl-5-nitroimidazole.

It is indicated for the treatment of abdominal abscess and brain abscess. A tablet formulation containing 200 mg of ofloxacin and 600 mg of tinidazole is available (Oflox-TZ, protec division of Cipla Ltd). A survey of literature revealed that no official method is available for the estimation of ofloxacin and tinidazole as combination. However, one reported method was found[1]. Individually ofloxacin has been estimated by HPLC[2-4], spectrophotometry[5,6] and tinidazole has been estimated by HPLC [7-10] and specrtophotometry[11,12]. Present work describes the development of a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of ofloxacin and tinidazole in tablets.

The drug sample of oflaxacin and tinidazole were obtained as a gift sample from Cipla Ltd.

EXPERIMENTAL

Materials and methods

Chemical and reagents

Water of HPLC grade was collected from a milli-Q system. Potassium dihydrogen phosphate AR (Ranbaxy) and ortho-phosphoric acid AR (Ranbaxy) mobile phase were purchased from the market.

Apparatus and chromatographic conditions

A gradient high pressure liquid chromatograph Shimadzu 10AT, SPD 10A detector was used for study. The column used was a reverse phase SS Wakosil II, C18, 250 x 4.6 mm, 5 mm i.d and particle size 5 µm. The flow rate of mobile phase was maintained at

1 mL/min and detection was carried out at 295 nm at the room temp.

Preparation of mobil phase

A mixture of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer (adjusted to pH 5.0 using orthophosphoric acid) in the ratio of 75 : 25 v/v was filtered through 0.45 µ membrane filter and then used as mobile phase and sonicated for 10 min.

Preparation of standard solution

Standard stock solution of ofloxacin was prepared in mobile phase of concentration 500 µg/ mL. Standard stock solution of tinidazole was prepared in mobile phase of concentration 500 µg/ mL. The stock solutions were diluted separately to obtain working standard solution of concentration of 10 µg/ mL to 50 µg/ mL. The resulting solutions were sonicated for 10 min and 100 µL was injected. The retention time for ofloxacin was found to be 2.32 min and for tinidazole 3.04 min. The linearity range for ofloxacin was found to be 10-50 µg/ mL and for tinidazole 10-80 µg/ mL.

Preparation of sample solution

Oflox-TZ, tablets five in number were weighed. An amount equivalent to 5 mg of ofloxacin was transferred into 10 mL volumetric flask. The powder was first dissolved with a few drops of mobile phase and the volume then made upto 10 mL with mobile phase. The solution was filtered through membrane filter with pore size of 0.45 micron. The sample stock solution was adequately diluted to obtain ofloxacin concentration of 10 µg/ mL. The resulting solution was sonicated for 10 min and 100 µL of the sample was injected. The peak area from the chromatogram was tabulated and the amount of ofloxacin and tinidazole present in the tablet formulation was determined from the linearity curve.

Table 1. Recovery studies

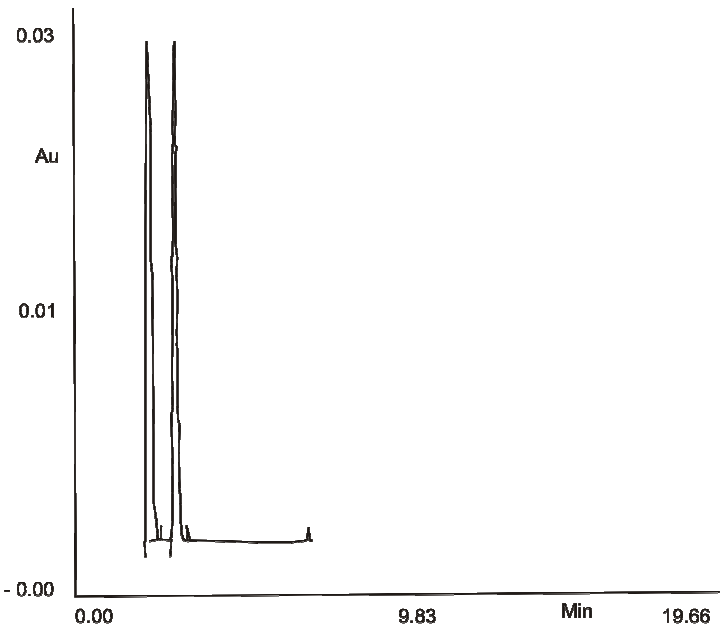
Drug	Amount added	Amount recovered	Average recovery (%)
Ofloxacin	20	9.5	95
	30	15.9	103
	40	21.0	105

Cont…

Drug	Amount added	Amount recovered	Average recovery (%)
Tinidazole	30	15.24	101.6
	40	20.52	102.6
	50	25.93	103.7

Table 2. System suitability parameter

Parameter	Ofloxacin	Tinidazole
Theoretical plates	3925	10647
Tailing factor	0.3	0.3
Resolution	0.872	
Calibration range	10-50 μg/mL	10-80 μg/mL



No.	Retn. Time	Height	Area	Height %	Area %	Peak Type
1	2.27	8701	1732685	55.4098	54.2380	BB
2	3.04	7002	1461869	44.5902	45.7620	BB
		2e+04	3194504			

Fig. 1 : Chromatogram of sample solution

The proposed method was validated as per ICH parameters. Precision of the proposed HPLC method was carried out by injecting replicate of six of concentration 10 μg/mL and the precision of the proposed HPLC method was found to be 0.4% for ofloxacin and 1.58 % RSD for tinidazole. The low RSD values indicated that the proposed method had good precision. The precision of instrument was carried out by injecting replicate of six of concentration 10 μg/mL, which was found to be 0.12 for ofloxacin and 0.3% RSD for tinidazole. Accuracy of the method was also determined. The average recovery of ofloxacin were 95-105% and for tinidazole 101-103%, respectively. The sample recovery in the formulation was in good agreement with the label claim. High percentage recovery showed that the method was free from interferences of the excipients used in the formulations. Ruggedness of the method was determined by carrying out the assay by different analysts on different days. The test results were found to be satisfactory with RSD for set of analysis on the same date being less than 0.8 % and RSD between set of analysis on different days being less than 1.6 % for both; ofloxacin and tinidazole. The percentage area on calculation was found to be 101-102 % for ofloxacin and 99-101 % for tinidazole. This shows that the result are reproducible. Robustness of the method was determined by carrying out the assay during which the mobile phase ratio and pH of mobile phase were altered slightly. The percentage recovery was found to be 95-102 % for ofloxacin and 72-102% for tinidazole, when mobile phase was alter slightly. System suitability parameters of ofloxacin and tinidazole are given in the Table 2. Assay of the combination in tablet dosage form was found to be 94.4% of ofloxacin and 105.7% of the tinidazole

CONCLUSIONS

The method was simple and had short runtime of 4 min, which makes the method rapid. The results of the study indicate that the proposed HPLC method was simple, precise, highly accurate, specific and less time consuming.

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