Research Article

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Estimation of Metronidazole and Norfloxacin in Formulations by Reverse Phase HPLC Method

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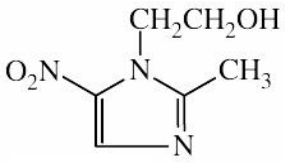
An accurate, sensitive and specific Reverse phase HPLC method for the estimation of Metronidazole and Norfloxacin in combined dosage forms. Chromatography was carried out on zorbax c8 column by gradients elution with mobile phase (Acetonitrile phosphate buffer). Mobile phase was pumped at flow rate of 1.5 ml/min, and UV response was monitored at 250 nm. The average retention times for amoxicillin (Internal standard), Metronidazole and Norfloxacin was found to be 2.37, 3.51 and 5.5 respectively. The calibration was showed linearity in the range of 10-50µg/ml for all these three drugs. The method is accurate and precise with recoveries of Metronidazole and Norfloxacin in the range of 100.37 ± 0.62 and 99.99 ± 0.74. The recovery values were also satisfactory with showed reliability and suitability of the method.

Keywords: Reverse phase HPLC, Metronidazole, Norfloxacin , combined dosage form.

INTRODUCTION

Administration of two or more drugs at a time becomes imperative for several therapeutic reasons and there exist a number of drugs combinations which have proved to be effective due to the combined mode of action on the body. Special effects in combined dosage forms include 1. Prolong the duration of the action of the drugs, 2.Potentiate the activity of the drugs, 3.Masks the adverse effects of the drugs.

In the present study Metronidazole and Norfloxacin drug in combined dosage form was selected for the estimation.



Metronidazole

Norfloxacin

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Literature survey reveals several methods that have been used for the quantitative determination of Metronidazole and Norfloxacin alone and combined dosage forms such as UV spectrophotometric method (1), Colorimetric method (2), and spectrofluorimetric method (3), TLC method (4), GLC method (5), HPLC method (6), HPTLC method (7), reverse phase HPLC method with UV detection for the determination of Metronidazole and Misonidazole and their metabolites (8), reverse phase HPLC method with UV detection for the determination of Theophylline, Fluroquinolones ciprofloxacin, Norfloxacin in rat plasma (9), HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially those containing more than one active component. Therefore, the aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of Metronidazole and Norfloxacin. This paper describes the development and validation of reliable, simple, time and money saving reversed phase HPLC method, using UV detection, for the simultaneous determination of Metronidazole and Norfloxacin drug combination in formulation. The reverse phase HPLC method was found to be simple and convenient for the simultaneous determination of the two drugs and results indicate high accuracy and precision.

MATERIALS AND METHODS

Materials

Metronidazole and Norfloxacin were obtained as with WATERS 501 solvent delivery system(Pump), Rheodyne 7125 injector with 20µL loop, Analytical Column (Zorbax C8, 5µ, 25cm X 4.6mm id), WATERS 486 tunable absorbance detector. All the chemicals, reagents and solvents used were of AR grade and HPLC grade.

Methods

Chromatographic conditions

The HPLC system used consisted of the 'Waters 501 pump and Waters 486 tunable wavelength uv-visible detector. The data station was computer controlled using Baseline 810 software, which includes an integrator and a recorder. The injector device was "Rheodyne 7125" with a 20µL fixed volume loop. The flow rate was kept at 1.5ml/min with an average operating pressure of 3000 psi and the UV response was monitored at 250nm. The mobile phase was filtered through a 0.45 micron nylon 66 membrane. The analytical column used was Zorbax C8, (5µ 25cm x 4.6mm id).

Reagents and Solvents

Acetonitrile HPLC grade, Glacial acetic acid HPLC grade, Disodium hydrogen ortho phosphate buffer (pH 3.0), Phosphoric acid (1%v/v in water), Water (HPLC grade from Milli-Q system).

Mobile Phase

Acetonitrile: phosphate buffer, pH adjusted to 3.0 (15:85) was used as the mobile phase.

Preparation of the standard solution

50mg of each of metronidazole and norfioxacin was weighed accurately and transferred to a 50m1 standard flask, dissolved using dilute glacial acetic acid and phosphate buffer solution and made up the volume up to the mark. This solution was further diluted to get five mixed standard concentrations ranging from 10-50µg/ml of both metronidazole and norfloxacin and 100µg/ml of amoxycillin as internal standard in each.

Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity equivalent to 1/10th of the gift samples from Cipla Ltd, Bangalore. Instrument used Shimadzu UV 160A recording Spectrophotometer, WATERS Gradient HPLC average weight of a tablet is weighed accurately and dissolved in dilute glacial acetic acid and buffer then filtered. Finally made up the volume with phosphate buffer, and further dilutions were made to get 32µg/m1 of norfioxacin and 40µg/m1 of metronidazole.

Procedure

The chromatographic system was set at the conditions mentioned earlier and a steady baseline was recorded, after stabilisation of the baseline, the standard solution containing 10 µg/ml each of metronidazole and norfloxacin was injected with 100 µg/ml of amoxycillin (IS). The above procedure was repeated in the same way with the remaining 4 standard concentrations with 100 µg/ml of amoxyciilin (IS) in each. The chromatograms were obtained for all the concentrations. This procedure was done in triplicate to ensure that repeatability was obtained. The retention time of amoxycillin, metronidazole and norfloxacin was found to be 2.37, 3.51 and 5.55 respectively. The chromatograms are shown in Figure 1 to 5. The calibration curve of concentration Vs peak area of metronidazole and norfloxacin are given in Figure 6 & 7 respectively. The sample solution obtained from the formulation was then injected and the procedure as described for the standard solution was followed and the chromatogram obtained was recorded and integrated. (Figure 8). The concentration of metronidazole and norfloxacin was obtained from the data station and the content of the drugs as well as the % claim in the tablet formulation were calculated. Results are shown in Table. 1

Table. 1. Analysis & Recovery studies of formulations by HPLC method

Drug	Amount (mg/tablet)		% Label claim Mean±SD*	% Recovery Mean±SD*
	Labelled	Found Mean±SD*		
Metronidazole	500	501.51 ± 2.93	100.3 ± 0.59	100.37 ± 0.62
Norfloxacin	400	401.88 ± 2.29	100.47 ± 0.57	99.99 ± 0.74

* Mean ± S.D. of 6 observations

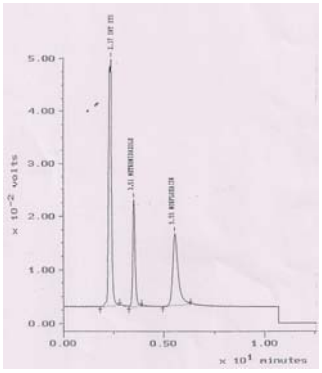

Figure 1.Chromatogram of metronidazole and norfloxacin (10µg/ml each)

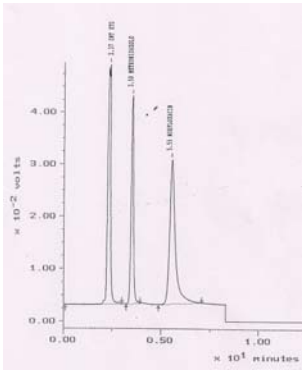

Figure 2.Chromatogram of metronidazole and norfloxacin (20µg/ml each)

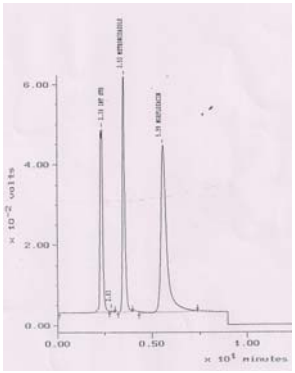

Figure 3. Chromatogram of metronidazole and norfloxacin (30µg/ml each)

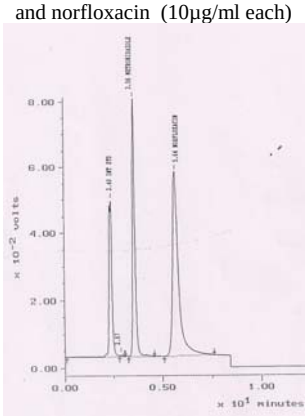

Figure 4 Chromatogram of metronidazole and norfloxacin (40µg/ml each)

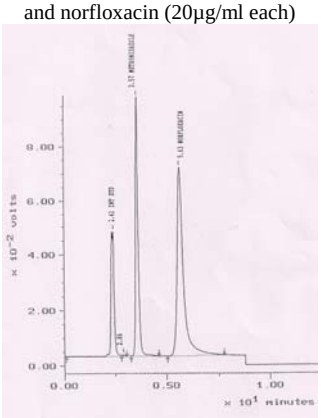

Figure 5 Chromatogram of metronidazole and norfloxacin (50µg/ml each)

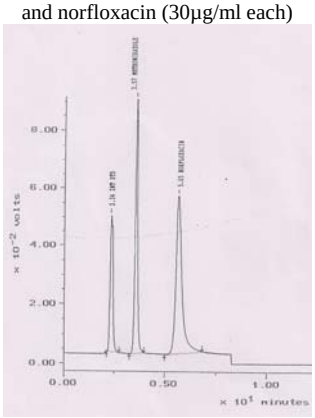

Figure 8 Chromatogram of Formulation

Recovery Studies

To confirm the suitability, reliability and precision of the proposed method, recovery studies were carried out. A known quantity of standard metronidazole and norfioxacin solution was added to the previously analyzed samples and the mixture was reanalyzed by the proposed method. An aliquot of 5m1 of the analysed sample was transferred to l0ml standard flask and 5m1 of standard solution which contains 15 µg/ml of metronidazole and l5 µg/ml of norfloxacin and 200 µg/ml of amoxycillin was added to it. The resulting solution was injected and the chromatogram was recorded and integrated. The results are shown in Table. 1.

RESULTS AND DISCUSSION

Chromatograms of mixed standard solutions which contained norfioxacin and metronidazole along with internal standard (amoxycillin) were recorded, A computer controlled data station with Baseline 810 software was used to plot the peak area Vs concentration in µg/ml. Calibration curves were obtained by using peak area ratios of standard and internal standard Vs concentration. All these three drugs showed linearity in the range of 10-50 µg/ml. From the marketed formulation, sample solutions were made and spiked with internal standard (ketoprofen) and response factor was used to calculate the concentration of each drug.

From the concentration, amount of each drug present in tablets were calculated. (Table no 1).

Analysis of the results of this method showed that the amount found was in good agreement with the label claim of the formulation. Further, there is no interference due to excipients and the recovery values were also satisfactory, which showed the reliability and suitability of the method. Thus the proposed HPLC method for the simultaneous estimation of norfioxacin and metronidazole is simple, rapid and accurate.

CONCLUSION

The development of a reverse phase HPLC method for the estimation of Metronidazole and

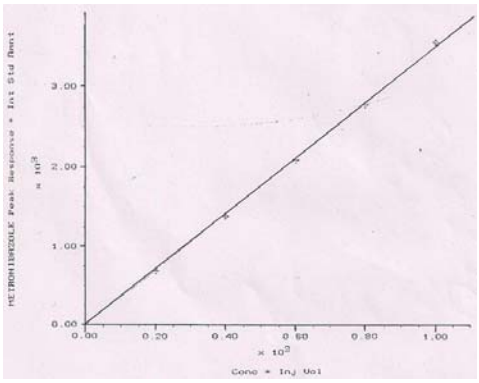

Fig 6 Calibration Curve for Metronidazole

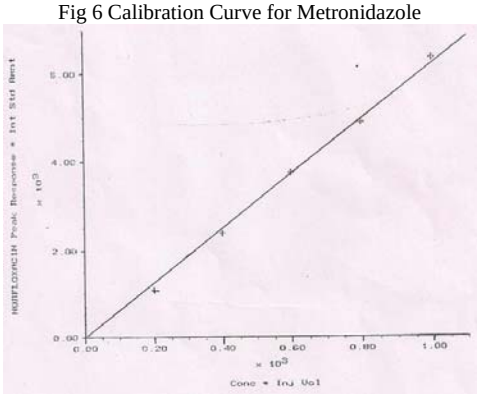

Fig 7 Calibration Curve for Norfloxacin

Norfloxacin in combined dosage forms was analysed. The presence of different excipients in the formulations has not interfered in big incidence on the measurement signal. The proposed method, due to the high separation power of reverse phase HPLC provides a useful tool for removing the contribution of these interferences. The reliability and suitability of the method could be seen from the recovery values. So, it can be the suitable method for the quality control of raw materials, pharmaceutical formulations and dissolution studies.

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