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SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND CIPROFLOXACIN BY RP-HPLC METHOD IN BULK DRUG AND SUSPENSION

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ABSTRACT

A simple, accurate and precise reverse phase high performance liquid chromatographic method has been developed and validated for the estimation of metronidazole and ciprofloxacin simultaneous determination in combined dosage forms. A Lichrospher 100 RP-180, C_{18} column was used as stationary phase and mobile phase contain phosphate buffer : acetonitrile (80 : 20, v/v) and final pH adjusted to 5.45 ± 0.02 with 10 % v/v *o*-phosphoric acid. Measurements were made at the effluent flow rate of 1.0 mL/min with injection volume 20 µL and ultraviolet (UV) detection at 290 nm, as both components show reasonable good response at this wavelength. The retention times of metronidazole and ciprofloxacin were 3.51 min and 6.84 min, respectively. The method was validated in terms of linearity, accuracy, precision, robustness and specificity. Linearity of metronidazole and ciprofloxacin was in the range of 1-70 µg/mL for both, respectively. Average percentage recoveries obtained for metronidazole and ciprofloxacin were 100.03 % and 99.13 %, respectively. The limit of detection and limit of quantification were found to be 0.5 and 1.0 µg/mL for metronidazole, respectively and for ciprofloxacin were 0.4 and 1.0 µg/mL, respectively. The method is useful in the quality control of bulk manufacturing and pharmaceutical dosage forms.

Key words : Metronidazole, Ciprofloxacin, RP-HPLC, Simultaneous estimation

INTRODUCTION

Metronidazole (MAT), is chemically described as 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole or 2 - methyl - 5 - nitroimidazole - 1 - ethanol[1] and chemically ciprofloxacin (CIP) is - cyclopropyl - 6 - fluoro-1, 4 - dihydro - 4 - oxo - 7 - (1 - piperazinyl) - 3 - quinoline carboxylic acid[2]. Literature survey reveals that few HPLC and spectrophotometry methods are reported for the estimation of metronidazole, nalidixic acid and ciprofloxacin in biological samples such as plasma[3-10]. So far no HPLC method has been reported for the

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simultaneous estimation of metronidazole and ciprofloxacin in combined dosage forms. In the present investigation, an attempt has been made to develop accurate and precise HPLC method for the simultaneous estimation of metronidazole and ciprofloxacin in combined dosage forms.

EXPERIMENTAL

Materials and methods

Metranidazole and ciprofloxacin standards were procured as a gift sample from Excel Laboratories, Mehsana, India. Acetonitrile and water used were of HPLC grade and were purchased from Rankem, India. o-phosphoric acid was of AR grade from S. D. Fine Chemicals Ltd., Mumbai. Suspension containing metranidazole (100 mg) and ciprofloxacin (125 mg) were purchased from local market (Avilox-M, Avalanche Pharma. and Ocmix, Panacea biotech Ltd.). A Merck - Hitachi Isocratic High Performance Liquid Chromatography with a Lichrospher 100 RP-180, C-18, 5 μm column having 250 x 4.0 mm internal diameter and equipped with Hitachi pump L – 7110, Rheodyne universal injector 77251 with injection volume 20 μL and Hitachi L - 7420 UV - Visible detector and monitored by Merck - Hitachi HSM software, was used.

Preparation of standard and sample solutions

Accurately weighed MAT (25.0 mg) and CIP (25.0 mg) was transferred to a 25 mL volumetric flask, dissolved in and diluted up to the mark with water. Ten mL aliquots each from stock solutions of MAT and CIP were transferred and mixed in 100 mL volumetric flask and volume was made up with mobile phase up to mark to get 100 μg/mL mixed standard stock solution.

Accurately weighed MAT (10 mg) and CIP (12.5 mg) was transferred to a 100 mL volumetric flask and dissolved in and diluted to mark with water. The solution (4.0 mL) was transferred to a 10 mL volumetric flask and diluted to the mark with mobile phase to obtain final solution with MAT (40 μg/mL) and CIP (50μg/mL).

An accurately measured quantity of 5 mL suspension containing 100 mg metronidazole and 125 mg ciprofloxacin was transferred to a 100 mL of volumetric flask and mixed with water (50 mL) and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with 10 mL water. The filtrate and washings were combined in a 100 mL volumetric flask and diluted to the mark with water. 0.4 mL of the above solution was further diluted to 10 mL with

mobile phase to obtain final solution with MAT (40 µg/mL) and CIP (50 µg/mL).

HPLC method and chromatographic conditions

The chromatographic separations were performed using LiChrospher® 100 C18, 5 µm, 250 × 4.0 mm i. d. column, at ambient temperature. The mobile phase comprised of phosphate buffer : acetonitrile (80 : 20, v/v) and final pH adjusted to 5.45 ± 0.02 with 10 % v/v *o*-phosphoric acid and was pumped at a flow rate of 1.0 mL/min. The mobile phase was filtered through nylon 0.45 µm - 47 mm membrane filter and was degassed before use. The elution was monitored at 290 nm. The injection volume was 20 µL.

Calibration curve

Appropriate aliquots from standard stock solution of mixed drugs were suitably diluted with mobile phase in such a way to get concentrations in a range of 1-100 µg/mL for both the drugs. These solutions (n = 5) were injected into the universal injector 77251 (Rheodyne) with injection volume 20 µL. Evaluation of two drugs was performed with UV/Visible detector at 290 nm. Peak areas were recorded for all the peaks. The plots of peak area versus the respective concentration of MAT and CIP were found to be linear in the range of 1-70 µg/mL for both the drugs. Calibration curves were constructed by plotting peak areas versus concentrations of MAT and CIP and the regression equations were calculated.

Validation of the method[11]

The developed method was validated in terms of linearity, accuracy, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement as well as repeatability of sample application.

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RESULTS AND DISCUSSION

The published literature for the estimation of other antibacterial drug in combination and knowledge of the molecule suggest that reverse phase liquid chromatography (RPLC) is suitable for the simultaneous analysis of MAT and CIP. In

RPLC, lichrospher® 100 rp-180, c_{18}, column having 250 mm length, 4.0 mm internal diameter and 5 μm particle size was used. Resolution is the most important criteria for the method and is imperative to achieve good resolution among the both compounds. As per the value of Ka and solubility of both the compounds, various compositions of mobile phase with different pH ranges (2.75 to 7.0) were tried and best resolution was obtained with mobile phase consisting of phosphate buffer and acetonitrile in the proportion of 80 : 20 with final pH adjusted to 5.45 ± 0.02 with *o*-phosphoric acid.

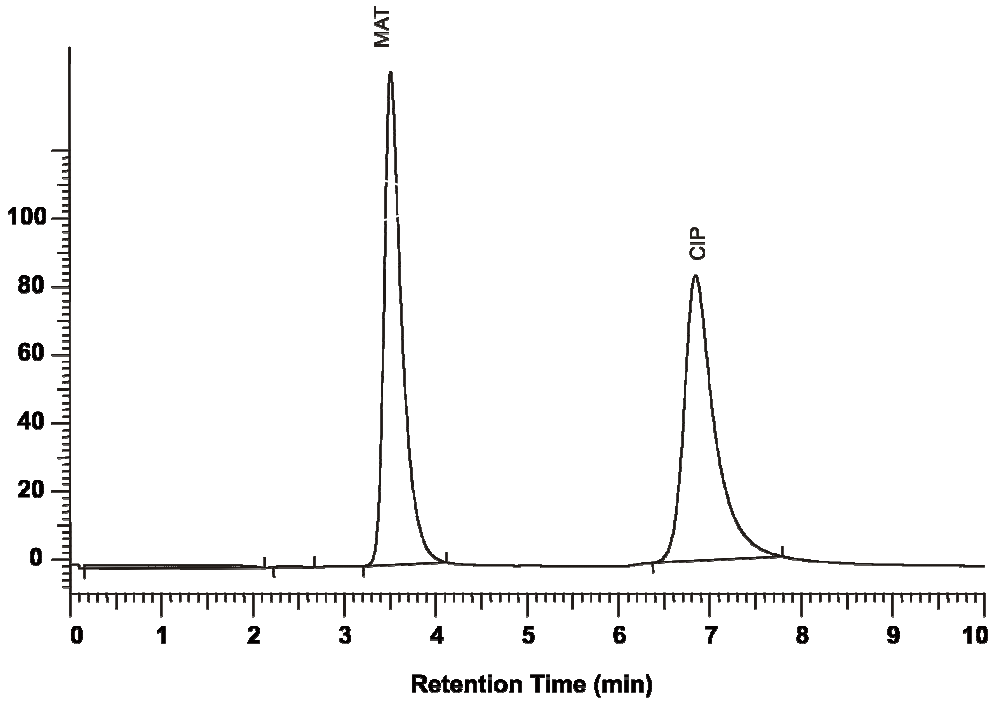

Fig. 1 : A typical RPLC chromatogram of MAT and CIP

Quantification was achieved with UV detection at 290 nm based on peak area. Better resolution of the peaks with clear base line separation was found. Retention time for MAT and CIP was 3.51 min and 6.84 min, respectively (Fig. 1).

System suitability tests were carried out on freshly prepared standard stock solutions of MAT and CIP and parameters obtained are summarized (Table 1).

Table 1 : Validation and system suitability parameters

Parameters	Values	
	MAT	**CIP**
Retention time (min)	3.51	6.84
Tailing factor (n = 5)	1.694	1.774
Asymmetry (n = 5)	1.742	1.731
Theoretical plates (n = 5)	2361.71	2531.17
Linearity range (μg/mL)	1-70 μg/mL	1-70 μg/mL
Limit of detection	0.5 μg/mL	0.4 μg/mL
Limit of quantification	1.0 μg/mL	1.0 μg/mL

Intra- and Inter-day precision studies were carried out and results show that the method is reproducible. Limit of detection and limit of quantification were found to be 0.5 and 1.0 μg/mL for MAT, respectively and 0.4 and 1.0 μg/mL for CIP, respectively (Table 1). The results obtained by the proposed method were close to the label claim of both the drugs (Table 2).

Table 2 : Analysis of mat and cip

Formulation	Drug	Label claim mg/tablet	Amount found (mg)	% Amount found ± S. D. (n = 5)
Bulk powder	Metronidazole	10	10.06	100.63 ± 1.05
Avilox-M	Ciprofloxacin	12.5	12.23	97.86 ± 1.16
(Suspension)	Metronidazole	100	99.45	99.45 ± 1.42
Ocmix	Ciprofloxacin	125	128.85	103.08 ± 0.23
(Suspension)	Metronidazole	100	97.69	97.69 ± 1.62
	Ciprofloxacin	125	128.35	102.68 ± 1.09

The low value of standard deviation indicates that the method is accurate. To study the accuracy of the proposed method, recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at three different concentrations. The values of percentage recovery show that the proposed method is accurate (Table 3).

Table 3 : Recovery study of MAT and CIP

Drug	Amount taken (µg/mL)	Amount added (µg/mL)	% Recovery ± S. D. (n = 5)
Metronidazole	20	10	100.33 ± 1.29
	20	20	101.10 ± 1.09
	20	30	98.66 ± 1.24
Ciprofloxacin	25	10	99.46 ± 1.46
	25	25	99.64 ± 1.24
	25	35	98.30 ± 1.36

The proposed method is accurate, precise, repeatable and reproducible and can be used for routine analysis of MAT and CIP in combination.

CONCLUSION

The developed RPLC technique is simple, precise, specific and accurate and the statistical analysis proved that method is reproducible and selective for the analysis of MAT and CIP in bulk drug and tablet formulations.

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