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A VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METRONIDAZOLE AND CIPROFLOXACIN HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

S.S. Khadabadi * and Meenakshi G. Devkar

Department of Pharmaceutical Chemistry, Government college of Pharmacy, Osmanpura, Aurangabad-431005, Maharashtra, India

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Correspondence to Author:

Dr. S. S. Khadabadi

Principal, Government college of Pharmacy, Osmanpura, Aurangabad-431005, Maharashtra, India

E-mail: khadabadi@yahoo.com

ABSTRACT: A reversed-phase-liquid chromatographic (RP-HPLC) method was developed for the determination of Metronidazole (MTZ) and Ciprofloxacin HCl (CPX) in their bulk drug and marketed formulations. The separation was carried out using a mobile phase of 25mM Potassium dihydrgen phosphate buffer of pH 3.0 consisting of 0.15%v/v Triethylamine, 0.1% Phosphoric acid and Methanol in the ratio of(60:40v/v). The column used was Zorbax C18 column (250mm x 4.6mm, 5 µm) with flow rate of 1 ml / min using UVD 17 Detector. Detection carried out at 320 nm. The retention times of Metronidazole and Ciprofloxacin Hydrochloride were found to be 3.7 min and 5.6 min, respectively. Developed methods were validated according to ICH guidelines. Linearity was observed at concentration range of 50-250 µg/ml for Metronidazole and 62.5-312.5µg/ml for Ciprofloxacin Hydrochloride followed by Beer's law. The percentage RSD for the method precision was found to be less than 2%. The proposed method is precise, accurate, selective and rapid for simultaneous determination of Metronidazole and Ciprofloxacin Hydrochloride.

INTRODUCTION: Metronidazole is used as an anti-protozoal, chemically it is 2-(2-methyl-5-nitro-1*H*- imidazol-1-yl) ethanol. Ciprofloxacin HCL (CPX) is used as an anti- bacterial agent; chemically it is a 1-cyclopropyl-6-fluoro-4-oxo-7-(piperzin-1-yl)-quinoline-3-carboxylic acid [1,2].

Objective of Study: Literature Survey revealed that numbers of method have been reported in literature for the individual analysis of Metronidazole and Ciprofloxacin Hydrochloride by UV spectrophotometric and RP-HPLC method [3, 4].

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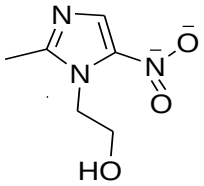

FIG. 1: METRONIDAZOLE

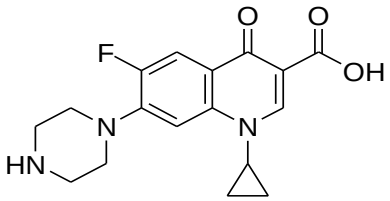

FIG. 2: CIPROFLOXACIN HYDROCHLORIDE

RP-HPLC and UV methods are available in literature for simultaneous determination of Metronidazole with other drugs [5, 6, 7, 8, 9]. RP-HPLC and UV Spectrophotometric methods are available in literature for determination of Ciprofloxacin HCl with other drugs [10, 11, 12, 13, 14].

However, there is no reported RP-HPLC method available for simultaneous estimation of Metronidazole and Ciprofloxacin HCl.

The aim of the present work was to develop simple, economic, accurate, specific and precise RP-HPLC methods for simultaneous estimation of Metronidazole and Ciprofloxacin HCL in bulk drugs and combined pharmaceutical formulations and validation of newly developed analytical methods.

MATERIALS AND METHODS:

Apparatus and Software: A Shimadzu HPLC instrument (Dionex system, with Chromeleon chromatographic software) equipped with UVD17 detector, Autosampler injector system, Zorbax C18 column (250mm x 4.6mm, 5 µm) were used. Other equipments used were Digital pH meter (Global DPH 500), Analytical Balance, Sonicator (Frontline ultrasonication FS-2) and Milipore filtration assembly.

Reagents and Chemical: Standard bulk drug sample Ciprofloxacin HCL and Metronidazole were provided by Wockhardt Pharmaceuticals, Aurangabad. Potassium Dihydrogen Phosphate, Methanol and Triehylamine (Dodal chemicals, Aurangabad).

Year of Experiment: 2013

Site: Government College of Pharmacy, Opposite Government Polytecnic, Hotel Vedant Road, Osmanpura, Aurangabad 431005, India.

Preparation of Mobile Phase and Stock Solution: 25 mM Potassium dihydrgen phosphate buffer of pH 3.0 consisting of 0.15%v/v Triethylamine, 0.1% Phosphoric acid and Methanol in the ratio of(60:40v/v), used as mobile phase. Standard stock solution of CPX and MTZ were prepared by weighing about 10 mg of drug, dissolved in 10 ml mobile phase to get 1000µg/ml of MZT and CPX.

Preparation of working standards: From stock solution of metronidazole and Ciprofloxacin HCL 0.5 to 2.5 mL and 0.62 to 3.12 ml respectively were transferred to 10 ml volumetric flask. The volume was made up to the mark with mobile phase to get a set of solutions for Metronidazole having concentration range 50, 100, 150, 200, 250µg/ml and for Ciprofloxacin 62.5, 125, 187.5, 250, 312.5 µg/ml.

Preparation of sample solution of mixture of MTZ and CPX: Mixture was prepared by weighing accurately 1ml of suspension, and dissolved it in mobile phase volume made upto 30 ml then kept in ultrasonicator for 30 min .The solution was filtered through 0.45 micron membrane filter paper. This solution was further diluted with mobile phase to obtain mixed sample solution containing 55, 110, 220 ug/ml of MTZ and 69, 138, 276 ug/ml of CPX respectively. Each sample solution was injected into sample injector of HPLC three times (n=3) under chromatographic condition as described above.

Area of each peak was measured at 320 nm .The amount of drug present in the sample was determined from peak area of MTZ and CPX present in the pure mixture respectively. The chromatographic conditions were found to yield good separation with satisfactory retention time of about 3.7 min for MTZ and 5.6 min for CPX with sharp symmetrical peak **(Figure 3)** and analysis of marketed formulations shows in **Table 1**.

TABLE 1: ASSAY OF SUSPENSION FORMULATION

Parameter	Metronidazole	Ciprofloxacin hydrochloride
% Estimated	100.19 ± 0.058	100.34 ± 0.147
%RSD	1.2	0.80

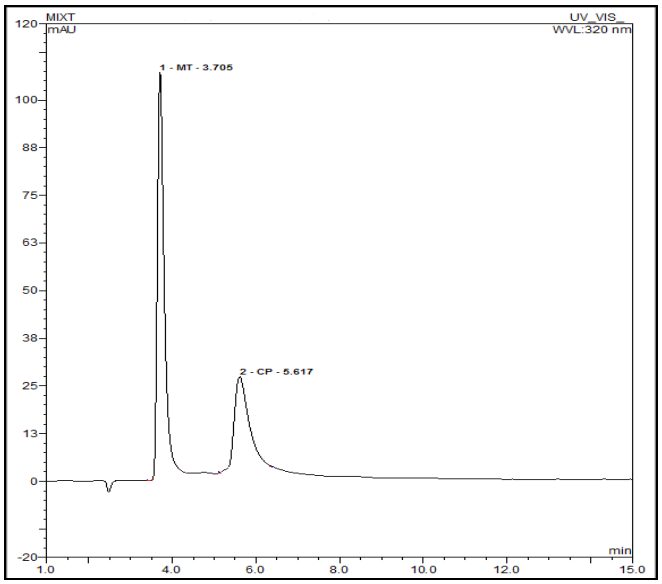

FIG. 3: CHROMATOGRAM OF MTZ AND CPX

Validation of developed analytical method: Validation was done with respect to various parameters, as required under ICH guideline Q2 (R1).

Linearity: Several aliquots of standard solution of MTZ and CPX were taken in different 10 ml volumetric flasks and diluted upto the mark such as final concentration of MTZ and CPX were 50-250 µg/ml and 62.5-312.5µg/ml respectively. These standards were tested three times replicates. Calibration curves were constructed and the proposed method was evaluated by its correlation coefficient and intercept value, calculated in the corresponding statistical study.

Precision: The precision is a measure of the ability of the method to generate reproducible results. The precision of the assay was determined by repeatability (intraday) and intermediate precision (inter-day), system precision and method precision reported as %RSD. For this, 100µg/mL and125 µg/ml of the solution were measured three times in a day, the same was repeated in next three days, same was repeated for bulk drug and marketed formulation.

Accuracy: Accuracy indicates the deviation between the mean value found and the true value. Accuracy was determined by means of recovery experiments, by the addition of active drugs to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyte recovered by the assay.

Robustness: To verify the robustness of the method, the analysis was done under variables pH, mobile phase ratio and flow rate. Sample solution were injected and run under set chromatographic condition.

System suitability parameter: The system was evaluated by analyzing repeatability, retention time, tailing factor and theoretical plats of the column.

RESULTS AND DISCUSSION: All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines.

Linearity: The linearity of this method was determined at ranging from 50-250 µg/ml for MTZ and 62.5-312.5 µg/ml for CPX. The regression equation were found to be Y = 0.117x+0.696 and Y = 0.130x+0.182 the correlation coefficient (r2) 0.999 and 0.997 for MTZ and CPX respectively shown in **figure 4 and 5.**

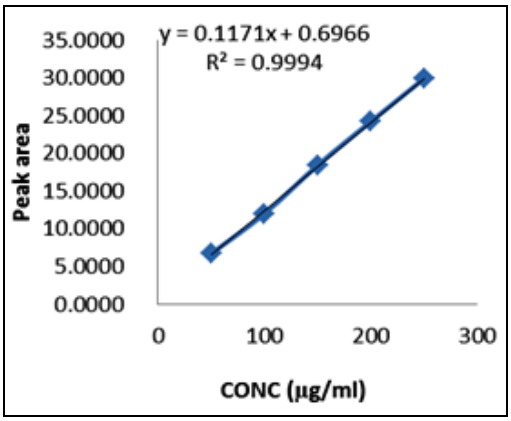

FIG. 4: CALIBRATION CURVE OF MTZ

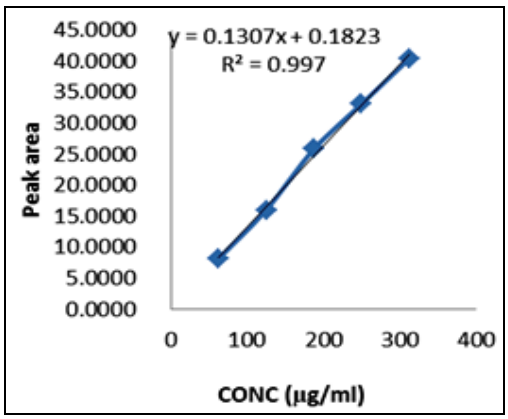

FIG. 5: CALIBRATION CURVE OF CPX

Precision: The precision (measurements of intraday, inter day, system precision and method precision) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0% shown in **Table 2**. This indicated that method was highly precise.

TABLE 2: DATA OF PRECISION

Precision	Metronidazole* (%RSD±S.D)	Ciprofloxacin Hydrochloride*(%RSD±S.D)
Intraday	0.96 ± 0.35	0.05 ± 0.35
Interday	0.76 ± 0.37	0.12 ± 0.87
System precision	0.32 ±1.34	0.10 ± 1.27
Method precision	0.45 ±1.45	0.15 ± 1.32

*Mean value of three determinations

Accuracy: Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to the pre-analyzed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. Recovery study was carried out at three levels 80%, 100% and 120% for the formulation concentration of 100µg/ml for MTZ and 125µg/ml for CPX shown in **Table 3**

TABLE 3: DATA OF ACCURACY

Level of Addition %	Metronidazole*		Ciprofloxacin Hydrochloride*	
	Addition of pure drug	% recovery of pure drug	Addition of pure drug	% recovery of pure drug
80	80	99.44	100	99.58
100	100	99.5	125	99.64
120	120	99.31	150	99.89

*Mean value of three determinations

System Suitability Test: The parameters of system suitability study were presented in table 4. It was found that the average retention time for MTZ and CPX were found to be 3.7 min and 5.6 min for five replicate injections respectively. The asymmetry factor was found to be 1.1 and 1.12 for MTZ and CPX respectively, which indicated asymmetric nature of the peak. The numbers of theoretical plates were found to be 7545 and 5670 for MTZ and CPX respectively, which suggested an efficient performance of the column. The resolution was found to be for both drug. These parameter shows in **Table 4.**

TABLE 4: DATA OF SYSTEM SUITABLE PARAMETER

Parameters	Metronidazole*	Ciprofloxacin hydrochloride*
Retention time	3.7	5.6
Asymmetry factor	1.1	1.2
Theoretical plates	7545	5670
Resolution	7.6	10.2

*Mean value of three determinations

Robustness: Robustness was performed by small but deliberate variation in the chromatographic conditions and was found to be unaffected by small variations like ±2% in volume of mobile phase composition, ±0.2 ml/min in flow rate of mobile phase and ±2% change in pH. It was observed that there were no marked changes in the criteria, which demonstrated that the proposed method was robust. These parameter shows in **Table 5.**

TABLE 5: DATA OF ROBUSTNESS

Parameters	Retention time	MTZ		CPX		
		Peak area	% RSD	Retention time	Peak area	% RSD
1. Change in mobile phase composition(v/v)						
60:40	3.7	14.653		5.6	16.5365	
62:38	3.2	14.653	0.169	5.8	16.5361	0.975
58:42	2.9	14.663		5.4	16.5563	
2. Change in mobile phase flow rate (ml/ min)						
1 ml/min	3.7	14.6535		5.6	16.566	
1.2 ml/min	3.2	14.659	0.169	5.8	16.555	0.975
0.8 ml/min	2.9	14.663		5.3	16.568	
3. Change in pH of mobile phase						
pH (3)	3.7	14.6867		5.6	16.668	
pH (3.2)	3.1	14.6845	0.168	5.8	16.665	0.978
pH (2.8)	3.2	14.6857		5.4	16.682	

*Mean value of three determinations

CONCLUSION: The RP-HPLC method has been developed for the simultaneous estimation of Metronidazole and Ciprofloxacin Hydrochloride in their combined marketed formulation and bulk drugs. The method gave good resolution for both the drugs with a short analysis time below 8 minutes which enables rapid quantification of many sample in routine and quality control analysis. The developed method was validated. It was found to be simple, precise, accurate and robust. The proposed method can be used for routine analysis of Metronidazole and Ciprofloxacin Hydrochloride in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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REFERENCES:

1. Indian pharmacopoeia, The Indian Pharmacopoeia Commission, Ghaziabad, 2010, Vol.-2, 1090-1094 , 1682-1686.
1. 2.British pharmacopoeia, The Stationery Office on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA), London, 2004, Vol.-1-2, 2504-2505 , 2896-2898.
2. United State Pharmacopoeia United State Pharmacopoeial Convention, , 2009, 1939-1945 , 2971-2973.
3. ICH, Q2 (R1), Validation of analytical procedures: text and methodology International Conference on Harmonization, Geneva, 2005, 1- 13.
4. Minal R Ghant:Development and Validation of A RP-HPLC method for Simultaneous Estimation of Metronidazole and Norfloxacin in Bulk and Tablet Dosage form.International Journal of Pharmacy and Pharmaceutical Sciences.2012;Vol.4,Suppl 4, 241-245.
5. Amit J Kasabe : Simultaneous Estimation of Ofloxacin and Metronidazole in combined dosage form by RP-HPLC method . International Journal of Chem Tech Research. Oct-Dec 2009; Vol.1, No.4, 1244 - 1250.
6. Danao K.R: RP-HPLC simultaneous estimation of metronidazole and diloxanide furoate in combination.International Journal of Pharmacy & life sciences. June 2010, IJPLS, 1(2):82-85.
7. Jadhav G P, More H N, Mahadik K R : Simultaneous UV-Spectrophotometric estimation of Nalidixic acid and Metronidazole. Indian Journal of Pharm.sci. 1998 ; 246-248.
8. Natesh.G : A new analytical method development and validation for estimation of ciprofloxacin and metronidazole by iso absorption method by using UV spectrophotometer. Journal of Chemical, Biological and Physical Sciences. May 2013- July 2013, Vol. 3, No. 3, 1663-1670.
9. Satish A Patel: Development and validation of RP-HPLC method for Simultaneous determination of Ciprofloxacin and Ornidazole in Tablets. International Journal of Current Pharmaceutical Research. 2011; Vol 3, Issue 4, 72-75.
10. Parag Patel: Development and validation of stability indicating method for simultaneous estimation of ciprofloxacin hcl and tinidazole using rp-uplc method. Journal of Pharmacy. Sep-Oct. 2012; Volume 2, Issue 5, 12-19.
11. Argekar A P, Kapadia S P,Raj S V: Determination of Ofloxacin., Norfloxacin and Lomefloxacin, Ofloxacin, Perfloxacin and Ciprofloxacin by RP-HPLC. Indian Drugs,1996, 33, 261-266.
12. Sani A. Ali: High Performance Liquid Chromatography (HPLC) Method Development and Validation Indicating Assay for Ciprofloxacin Hydrochloride. Journal of Applied Pharmaceutical Science. 2011; 01 (08); 239-243.
13. Dhavanikanikanti,Karunapriyachitra: Development and validation of RP-HPLC method for Simultaneous determination of Ciprofloxacin and Tinidazole in Tablets. International Journal of Advances in Pharmaceutical Sciences.2012; Volume 3, Issue 3-4,196-203.

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