International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

# HPLC ASSAY OF MODEL TABLET FORMULATIONS CONTAINING METRONIDAZOLE AND CIPROFLOXACIN

**VANIA MASLARSKA[a*], BOYKA TSVETKOVA[b], LILY PEIKOVA[b], BISTRA KOSTOVA[c], DIMITAR RACHEV[c], STANISLAV BOZHANOV[a], NELINA ZAREVA[a]**

[a]Department of Chemistry, [b]Department of Pharmaceutical, [c]Department of Pharmaceutical Technology and Biopharmaceutics, Medical University of Sofia, Faculty of Pharmacy, 1000 Sofia, Bulgaria
Email: vmaslarska@mail.bg

*Received: 12 Feb 2016 Revised and Accepted: 30 Mar 2016*

**ABSTRACT**

**Objective:** This paper describes development and validation of a high-performance liquid chromatographic analytical procedure for simultaneously determination of metronidazole and ciprofloxacin in a model tablet formulations.

**Methods:** The separation was achieved with a LiChrosorb® RP-18 (250 x 4.6 mm) column, at 33 °C temperature with isocratic mode and a mobile phase containing triethylamine: o-phosphoric acid and acetonitrile (0.02:80:20 v/v/v). The flow rate was 1.0 ml/min and the eluent was monitored at 290 nm.

**Results:** The selected chromatographic conditions were found to separate effectively metronidazole and ciprofloxacin with a retention time of 3.46 min and 6.68 min, respectively. The method was validated for analytical parameters specificity, linearity, precision, accuracy, LOD and LOQ. The calibration curves were linear in the concentration range of 12.5-100.0 µg/ml for metronidazole and ciprofloxacin. The recovery for metronidazole and ciprofloxacin was 100.1 % and 100.2 %, respectively.

**Conclusion:** The analytical procedure was applied to quality control of model tablet formulations. It was established that the developed analytical procedure was successfully used for routine analysis of metronidazole and ciprofloxacin in model tablet dosage forms without any interference from included excipients.

**Keywords:** Metronidazole, Ciprofloxacin, RP-HPLC, Validation, Model tablet formulations, Quality control



## INTRODUCTION

Ciprofloxacin, (CIP) [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] is a second generation fluoroquinolone antibacterial agent. Its spectrum of activity includes most strains of Gram-positive and Gram-negative bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal and abdominal infections [1]. Because of its low toxicity, very wide spectrum of antibacterial effect and low ability to cause bacterial resistance, CIP has been widely used in clinical practice [2]. Metronidazole (MET) [2-methyl-5-nitroimidazole-1-ethanol] is an antiprotozoal, antiamebic and antibacterial drug [3]. CIP has a reduced activity against anaerobic pathogens. Therefore, a combination of CIP with an antimicrobial agent active against anaerobes, such as MET, seems to be interesting for the treatment of mixed aerobic/anaerobic infections [4].

A survey of literature revealed several analytical methods for the simultaneous determination of CIP and MET in tablet forms and intravenous admixtures. The reported procedures included reversed-phase high-performance liquid chromatography, thin-layer chromatography, UV-spectrophotometry, nuclear magnetic resonance spectrometry [5-17].

Literature survey shows that the most HPLC methods for determination of CIP and MET in the mixture are based on a separation with mobile phases including phosphate buffer (variety of pH values and concentration) and organic diluents in different ratios [8-11]. The use of phosphate salts should be avoided to ensure correct and trouble-free exploitation of HPLC system. In our study, the separation was achieved without the usage of any phosphate buffer salts, which we appreciate as an advantage of the so presented analytical method.

In this article, a rapid, specific and accurate RP-HPLC method have been described for the simultaneous determination of both drugs in model tablet formulations.

## MATERIALS AND METHODS

### Materials and chemicals

MET and CIP reference standards were obtained from Sigma-Aldrich. HPLC grade acetonitrile was procured from Merck Ltd. Different model tablet formulations containing MET 250 mg and CIP 250 mg was prepared. Microcrystalline cellulose (type Vivapur 101®, JRS Pharma-Germany), maize starch (Roquette Pharma-France), lactose monohydrate (Meggle Pharma-Germany), carboxymethylcellulose sodium, cross-linked (Vivasol®, JRS Pharma-Germany), povidone (Kollidone® K30, BASF–Germany), magnesium stearate and silica colloidal anhydrous (Aerosil® 200, Evonik Ind.) were used as excipients. All other chemical reagents were of analytical grade.

### Instrumentation and chromatographic conditions

HPLC analysis was performed by isocratic elution with a flow rate 1.0 ml/min. A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD quaternary pump) with an autosampler, Shimadzu DGU-20A5 vacuum degasser, and a Shimadzu SPD-20A UV/VIS detector was used for analysis.

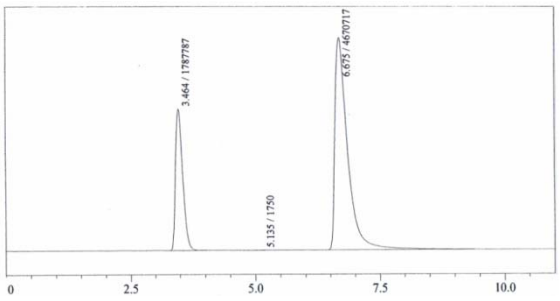


**Fig. 1: Typical chromatogram of MET and CIP**

The data was recorded using Lab Solutions Software. Separation was carried out at 33 °C, using LiChrosorb®RP-18 (250 x 4.6 mm) column packed with octadecylsilyl silica gel 5 µm. The detector was set at 290 nm. The mobile phase was prepared by mixing of triethylamine: o-phosphoric acid and acetonitrile (0.02:80:20 v/v/v). The mobile phase was sonicated for 10 min and then it was filtered through a 0.45 µm filter paper. The chromatographic conditions were found to yield good separation with a retention time of 3.46 min for MET and 6.68 min for CIP with sharp symmetrical peak. The chromatogram is shown in fig. 1.

**Preparation of standard solution**

50 mg of MET and 50 mg CIP working standards (accurately weighed) were transferred into a 100 ml volumetric flask. After addition of about 70 ml solvent A (prepared by mixing of 80 ml 0.3% o-phosphoric acid solution and 20 ml acetonitrile), the mixture was sonicated for about 2 min and after made up to the volume. The stock solution was suitably diluted to produce a concentration of 0.05 mg/ml of MET and 0.05 mg/ml of CIP respectively.

**Sample preparation**

Twenty tablets were weighed, finely powdered and the average weight was determined. A portion of powder equivalent to 250 mg MET and 250 mg CIP is transferred into 50 ml volumetric flask and 25 ml of solvent A was added and sonicated for 10 min to effect complete dissolution of both substances. The suspension was then made up to volume with solvent A and after filtered. The aliquot portion of the filtrate was further diluted to get a final concentration of 50 µg/ml of MET and 50 µg/ml of CIP. 20 µl of the test solution were injected, chromatogram was recorded, and the amounts of the drugs were calculated.

**Preparation of model tablets**

Model tablets containing 250 mg MET and 250 mg CIP were prepared by compression after wet granulation with a single punch tablet press (EK 0, Korsch, Berlin, Germany) with a set of 13 mm diameter standard concave tooling.

**Determination of mechanical strength of the tablets**

It was performed by the method of the progressive loading according to Eur. Ph. 8.0, apparatus-Erweka type TBH 30, Germany.

**Determination of friability**

It was performed according to Eur. Ph. 8.0 in friabilitor Erweka TAR 20, Germany.

**Disintegration time**

It was performed by the method described in Eur. Ph. 8.0 in water, using apparatus Erweka ZT 3, Germany.

***In vitro* drug dissolution studies**

Drug release profiles were evaluated using a dissolution test apparatus (Erweka DT 600, Hensenstmm, Germany). The test was carried out at a paddle rotation speed of 50 rpm, maintained at 37±0.5 °C, in 900 ml dissolution medium at 1.2 pH value. The dissolution progress was monitored by withdrawing 5 ml filtered samples (0.45 µm filter) at preselected intervals (up to 60 min). The quantity of MET and CIP in sample solutions was analyzed by described method. The cumulative percentage of drug release was calculated, and the average of six determinations was used in the data analysis.

**RESULTS AND DISCUSSION**

To optimize the RP-HPLC parameters, several mobile phase combinations were studied. Mobile phases containing acetonitrile and o-phosphoric acid solution (40:60; 30:70; 20:80; 10:90 v/v) were examined. In addition, the effects of the flow rate of the mobile phase (0.5–1.0 ml/min) and column temperature (25-40 °C) were checked. A satisfactory separation in suitable run time and good peak symmetry were found in a mixture of triethylamine: o-phosphoric acid and acetonitrile (0.02:80:20 v/v/v) at flow rate 1 ml/min proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 290 nm at which detector responses obtained were much better for both drugs. As shown in fig. 1, the retention times were 3.46 min for MET and 6.68 min for CIP. The developed method for determination of MET and CIP was further validated according to ICH guidelines as follows [18].

**Selectivity**

Selectivity of the current method was demonstrated by good separation of both active ingredients (MET and CIP). Furthermore, matrix components, e. g. excipients, do not interfere with the two analytes.

**Linearity**

Standard solutions containing MET (12.5-100 µg/ml) and CIP (12.5-100 µg/ml) were prepared in the solvent A. Triplicate 20 µl injections were made for each standard solution to estimate the reproducibility of the detector response at each concentration level and chromatographed under the conditions described above. The area of each peak was plotted against the concentration to obtain the calibration graphs (fig.2 and 3).

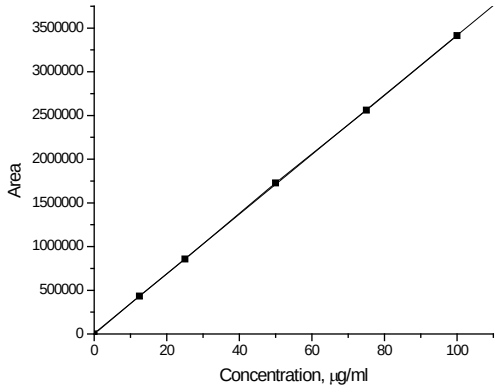


**Fig. 2: Linearity graph of MET**

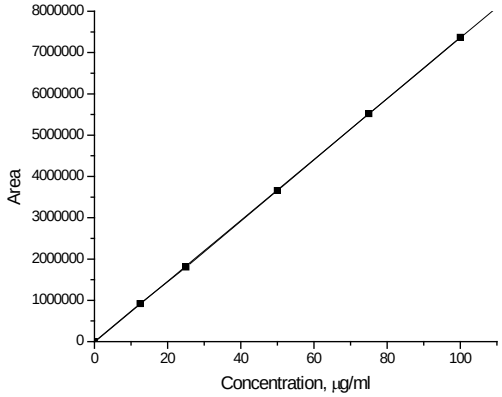


**Fig. 3: Linearity graph of CIP**

The five concentrations of each compound were subjected to regression analysis to calculate the calibration equation and correlation coefficients. The results obtained are shown in the tables 1-2. The described method was linear for the two analytes in the range specified above with a correlation coefficients better than 0.999.

**Limit of detection (LOD) and limit of quantitation (LOQ)**

LOD and LOQ were experimentally verified by six injections of MET and CIP at the appropriate concentrations. The LOD was calculated to be 0.012 and 0.04 µg/ml and the LOQ was calculated to be 0.125 and 0.4 µg/ml for MET and CIP, respectively.

**Precision**

The system precision of this method was evaluated by calculating the %RSD of the peak areas of six replicate injections of the standard solution, which were found to be 0.39% for MET and 0.37% for CIP. For method precision evaluated with six sample replicate injections were found to be 0.41% and 0.39% for MET and CIP respectively and it was found to be less than 1.0% shown in the table 3.

**Table 1: Linearity data for MET**

| Linearity level | Concentartion (µg/ml) | Average area (n=3) |
|---|---|---|
| 1 | 12.5 | 433347 |
| 2 | 25.0 | 858222 |
| 3 | 50.0 | 1727487 |
| 4 | 75.0 | 2560635 |
| 5 | 100.0 | 3413392 |
| Slope | | 34048.1 |
| Y-intercept | | 11089.4 |
| Correlation coefficient | | 0.9999 |

N=5

**Table 2: Linearity data for CIP**

| Linearity level | Concentration (µg/ml) | Average area (n=3) |
|---|---|---|
| 1 | 12.5 | 921318 |
| 2 | 25.0 | 1804911 |
| 3 | 50.0 | 3656745 |
| 4 | 75.0 | 5520841 |
| 5 | 100.0 | 7364964 |
| Slope | | 73839.0 |
| Y-intercept | | -22793.8 |
| Correlation coefficient | | 0.9997 |

N=5

**Table 3: Results of precision for MET and CIP**

| | MET | CIP |
|---|---|---|
| System precision % RSD | 0.39 | 0.37 |
| Method precision %RSD | 0.41 | 0.39 |

**Table 4: Results of % recovery studies for MET and CIP**

| Sample | Recovery | Amount present, mg | Amount recovered, mg | % recovered | SD* | %RSD |
|---|---|---|---|---|---|---|
| MET | 50% | 125 | 125.3 | 100.2 | 0.878 | 0.881 |
| | 100% | 250 | 249.7 | 99.88 | 1.022 | 1.025 |
| | 150% | 375 | 374.8 | 99.95 | 1.012 | 1.015 |
| CIP | 50% | 125 | 124.8 | 99.84 | 1.628 | 1.631 |
| | 100% | 250 | 251.3 | 100.5 | 0.643 | 0.645 |
| | 150% | 375 | 375.6 | 100.2 | 0.541 | 0.543 |

*average value of three determinations

**Table 5: Model tablet compositions**

| Composition | Model MC1 (g) | Model MC2 (g) |
|---|---|---|
| MET | 0.250 | 0.250 |
| CIP | 0.250 | 0.250 |
| Microcrystalline cellulose | 0.050 | 0.050 |
| Maize starch | 0.100 | - |
| Lactose monohydrate | - | 0.070 |
| Carboxymethylcellulose sodium, cross-linked | - | 0.030 |
| Magnesium stearate | 0.007 | 0.007 |
| Silica colloidal anhydrous | 0.003 | 0.003 |
| Povidone | 0.006 | 0.005 |
| Model tablet characteristics | | |
| Uniformity of mass of tablets, g | 0.666±5% | 0.665±5% |
| Mechanical strength, N | 50-60 | 50-55 |
| Friability, % | 0.5 | 0.4 |
| Disintegration time, min | 1 | 3 |

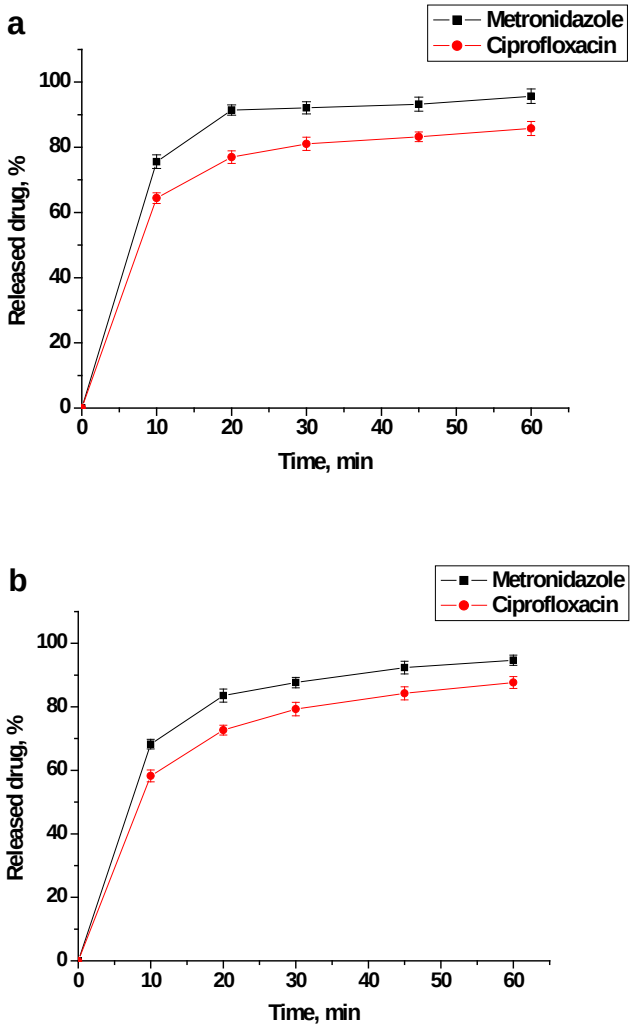


**Fig. 4: *In vitro* MET and CIP release profiles from developed model tablet formulations (n=6): a-model MC 1 and b-model MC 2**

**Accuracy**

The accuracy of the method was calculated by recovery studies. It is carried out by preparing the samples of 50%, 100% and 150% of target concentration. The samples were prepared in triplicate in each level. The results of studies along with its evaluation are given in the table 4.

**Obtaining of model tablet compositions**

Model tablet formulations and characteristics of tablets are presented in table 5.

***In vitro* drug dissolution studies**

Data from the MET and CIP release studies from models MC1 and MC2 at pH 1.2 are presented in fig. 4.As seen from the data presented in fig. 4 the rate of both drugs release is faster in model MC1 in comparison with Model MC2, especially in the beginning of the process. Model MC1 released 92% MET and 81% CIP for 30 min, while the model MC2 reaches 82% MET and 75% CIP for the same period. Over 80% CIP release from model MC2 is observed at 45 min.

**CONCLUSION**

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of MET and CIP was developed and validated for quality control analysis in combined tablets. This method is also applicable for the determination of the above drugs separately in other formulations. The proposed method is rapid, where the total analytical run time for both drugs are less than 8 min and shows a high degree of accuracy and precision with less than 2 % RSD. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

**ACKNOWLEDGMENT**

The present study was kindly supported by Medical University–Sofia, Medical Science Council–Grant № 34/2015.

**CONFLICT OF INTERESTS**

Declared none

**REFERENCES**

1. Fairclough PD, Silk DBA. Clinical medicine. In: Kumar P, Clark M. editors. Gastrointestinal disease. 7th ed. Amsterdam: Elsevier; 2005. p. 241-359.
2. Sweetman SC. Martindale the complete drug reference. 35th ed. London, Chicago: The Pharmaceutical Press; 2007.
3. Beale JM. Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry. In: Block JH, Beale JM. Editors. Antibacterial Antibiotics. 11th ed. Philadelphia: Lippincott Williams and Wilkins; 2004. p. 258-330.
4. Welk R, Schneider L. Ciprofloxacin in combination with metronidazole. Infection 1988;16:257-60.

5. Hafez H, Elshanawany A, Abdelaziz L, Mohram M. Design of experiment utilization to develop a simple and robust RP-UPLC technique for stability indicating the method of ciprofloxacin hydrochloride and metronidazole in tablets. Eur J Anal Chem 2015;10:84-105.
6. Elkady EF, Mahrouse MA. Reversed-phase ion-pair HPLC and TLC-densitometric methods for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in tablets. Chromatographia 2011;73:297–305.
7. Vega E, Dabbene V, Nasseta M, Sola N. Validation of a reversed-phase LC method for quantitative analysis of intravenous admixtures of ciprofloxacin and metronidazole. J Pharm Biomed 1999;21:1003–9.
8. Khadabadi SS, Devkar MG. A validated RP-HPLC method for simultaneous estimation of metronidazole and ciprofloxacin hydrochloride in pharmaceutical dosage form. Int J Pharm Sci Res 2013;4:4736-40.
9. Patel A, Shah N, Patel N. Simultaneous estimation of metronidazole and ciprofloxacin by RP-HPLC method in bulk drug and suspension. Int J Chem Sci 2009;7:2115-21.
10. Ramzia I, Asma A, Ehab E, Maha M, Asma M. Stability indicating HPLC method for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in the presence of ciprofloxacin acid degradation product. Asian J Biochem Pharm Res 2015;5:5-17.
11. Piponski M, Bakovska T, Naumoska M, Rusevska T, Serafimovska G, Andonovska H. Preliminary investigation of the possibility for implementation of modified pharmacopoeial HPLC methods for quality control of metronidazole and ciprofloxacin in medicinal products used in veterinary medicine. Maced Vet Rev 2015;38:31-42.
12. Mahrouse M, Elkady E. Validated spectrophotometric methods for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in tablets. Chem Pharm Bull 2011;59:1485-93.
13. Mahrouse M. Development and validation of a UV spectrophotometric method for the simultaneous determination of ciprofloxacin hydrochloride and metronidazole in a binary mixture. J Chem Pharm Res 2012;4:4710-5.
14. Natesh G. A new analytical method development and validation for estimation of ciprofloxacin and metronidazole by isoabsorption method by using UV spectrophotometer. J Chem Biol Phys Sci 2013;3:1663-70.
15. Chadha R, Aggarwal A, Jain DVS, Kapoor VK, Thakur D, Sharma A. Degradation kinetics of metronidazole and its mutual prodrug with ciprofloxacin: a calorimetric analysis. Int J Biol Chem Sci 2007;1:197-210.
16. Vega E, Sola N. Quantitative analysis of metronidazole in intravenous admixture with ciprofloxacin by first derivative spectrophotometry. J Pharm Biomed 2001;25:523-30.
17. Reinscheid U. Direct determination of ciprofloxacin in admixtures with metronidazole and ampicillin by NMR. J Pharm Biomed 2006;40:447-9.
18. International Conference on Harmonization (2005) ICH harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov; 2005.