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Liquid Chromatography With UV Detection For Simultaneous Determination Of Ciprofloxacin And Metronidazole

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Abstract

The aim of this study was to develop HPLC method capable of facilitating the simultaneous determination of ciprofloxacin hydrochloride (CIP.HCl) and metronidazole (MDZ). The analytes were separated with Lichrospher 100 RP-18 C_{18} column (100 x 4.6 mm, 5 µm). The mobile phase consisted of monobasic potassium phosphate (50 mM, pH 3.5) and acetonitrile (80: 20, v/v) containing triethylamine (7.5 mM) delivered isocratically with flow rate of 1.0 mL/min. The UV detection was set 298 nm. The developed method was validated in terms of precision, accuracy, linearity, selectivity and sensitivity. The precision of the method was evaluated using repeatability assay which RSD values of 0.37 – 1.72 % and 0.10 – 1.90 % for CIP.HCl and MDZ, respectively. The mean recoveries of CIP.HCl and MDZ were 99.83 – 100.77% and 99.80 – 101.14%, respectively. The dynamic linear response exhibited good correlation (r > 0.99) within the concentration range of 30 – 90 µg/mL for both drugs. The proposed method has been successfully applied for the simultaneous determination of CIP.HCl and MDZ in tablet dosage forms.

Keywords: Ciprofloxacin HCl, metronidazole, tablet, triethylamine, validation

1.0 INTRODUCTION

Ciprofloxacin hydrochloride (CIP.HCl), 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid is a relatively new, second-generation fluoroquinolone antibiotic with antibacterial activity against both gram-positive and gram-negative [1]. CIP.HCl has been reported to have a reduced activity against anaerobics pathogens. Meanwhile, Metronidazole (MDZ), 2-methyl-5-nitroimida-zole-1-ethanol has acted against protozoa and anaerobic bacteria [2]. Therefore, a combination of CIP.HCl with MDZ is currently used as a combined therapy for the treatment of mixed aerobic and anaerobic infections [3]. The chemical structure of CIP.HCl and MDZ was shown in Figure 1.

Several analytical methods have been deveoped for the determination of CIP.HCl and MDZ in some pharmaceutical formulations. Such methods are visible spectrophotometry [4-5], atomic absorption spectrometry and conductometry [6], and liquid chromatography [7-8]. While, MDZ can be analyzed using spectrophotometry [9, 10], near infrared spectrophotometry [11], and high performance liquid chromatography [12]. However, there is a few report regarding the use of analytical methods for simultaneous determination of CIP.HCl and MDZ. Reinscheid [13] has used NMR spectroscopy for direct analysis of admixture of ciprofloxacin with metronidazol and ampicillin.

Vega *et al.* [14] validated a reversed phase liquid chromatography method for quantitative analysis of intravenous admixtures of CIP.HCl and MDZ. Elkady and Mahrouse [3] developed reversed phase ion pair HPLC for the simultaneous determination of CIP.HCl and MDZ in tablet dosage form. In this study, simple HPLC with UV detection has been developed for simultaneous analysis of CIP.HCl and MDZ in tablet dosage form.

2.0 MATERIALS AND METHOD

2.1 Apparatus

High performance liquid Chromatograph of Jasco LC-Net II/ADC equipped with detector of UV-2070 Plus, Agilent EZChrom Elit Chromatography Data System, and Rheodyne Loop Injector 20 µL, spectrophotometer UV-Vis (Shimadzu UV-1700), pH meter (Schott pH 11), ultrasonic bath (Branson 1510), analytical balance (Ohaus AR 2140, sensitivity 0.1 mg).

2.2 Materials

The standards of Ciprofloxacin HCl anhydrate and Metronidazole EP were kindly obtained from Zhejiang Guobang Pharmaceutical Co., Ltd., and Wuhan Wuyao Pharmaceutical Co., Ltd., China, respectively. Triethylamine,

orthophosporic acid, dihydrogen sodium phosphate, acetonitrile and methanol of HPLC grade were purchased from E. Merck (Darmstat, Germany). Aquadest was bought from PT. Otsuka (Indonesia). The tablet dosage form was prepared in our laboratory, each contained 500 mg CIP and 500 mg MDZ.

2.3 Preparation Of Stock And Working Solutions Of Cip Hcl And Mdz

Stock solution of CIP.HCl and MDZ with the concentration of 0.6 mg/mL was prepared by dissolving 150.0 mg of each CIP and MDZ into 250 mL-volumetric flask and diluted until mark with methanol. The working solution was obtained by appropriate dilution to give the concentration of both drugs at 30, 45, 60, 75 and 90 µg/Ml

2.4 Preparation of Buffer Solution

Solution of phosphate buffer 50 mM was prepared by dissolving 3450 mg sodium dihydrogen phosphate with 300 mL water, and pH was adjusted to 3.0 with orthophosporic acid. The solution was transferred into 500 mL-volumetric flask and diluted to volume with water. Phosphate buffers with pH 3.5 and 4.0 were also made.

2.5 Chromatography

Separation of CIP and MDZ was performed on reversed phase Lichrospher 100 RP-18 C_{18} column from E. Merck. (100 mm X 4.6 mm ID, 5 µm) at ambient temperature. The optimization of mobile phase was carried out by varying monobasic potassium phosphate 50 mM (pH 3.0, 3.5, and 4.0) and acetonitrile, with ratio of buffer-acetonitrile (75: 25, v/v; 80: 20, v/v 83: 17, v/v and 85: 15, v/v). Flow rate was optimized at 1.0; 1.2 and 1.4 mL/minute. The addition of triethylamine into mobile phase (monobasic potassium phosphate 50 mM, pH 3.5: acetonitrile, 80: 20, v/v) varies with the concentration of 2.5; 5.0; 7.5 and 10 mM. The UV detection was set 298 nm. The injecton volume was 20 µL.

2.6 System Suitability Test

A-20 µL working solution containing CIP.HCl 30 µg/mL was injected into HPLC system under the optimized condition in six replicates. The similar procedure was also subjected to working solution with MDZ 90 µg/mL. The RSD values of retention time, peak area, and tailing factor were calculated.

2.7 Analytical Method Validation

The developed HPLC method was validated according to guideline in International Conference Harmonization [15] by assessing several parameters namely linearity, sensitivity expressed with limit of detection (LOD) and limit of quantification (LOQ), precision, and accuracy.

2.8 Sample Preparation

Ten tablets were weighed, crushed, and the powdered tablet equivalent to 500 mg of the CIP.HCl and MDZ was transferred to a 50-mL volumetric flask and dissolved with methanol until mark. After sonification for 30 minute, the solution was filtered with Whatman paper No. 41. A-60 µL clear supernatant was accurately pippetted and transferred to a 10-mL volumetric flask and made to volume with methanol. This final solution contained approximately 60 µg/mL CIP.HCl and MDZ. The solution was filtered using membrane filter 0.20 µm, and 20 µL of filtered solution was injected into the HPLC system. Quantification of CIP.HCl and MDZ was performed using an external standard technique.

3.0 RESULTS AND DISCUSSION

CIP.HCl is a polar and an amphoteric compound [16]. It is cationic under acidic condition and it will interact with acidic surface of silanols on the silica packing which causes peak tailing in CIP.HCl chromatogram. The use of the pairing ion approach was, therefore, is an alternative HPLC method in order to reduce peak tailing and to improve peak resolution.

In this study, the analytical method for determination of CIP.HCl and MDZ was developed by the optimization of mobile phase and triethylamine concentration as an ion pairing reagent. The wavelength for UV detection was selected by scanning the solution of CIP.HCl and MDZ using UV-Vis spectrophotometer over the wide range of 200 - 340 nm. The result showed that the maximum wavelength of CIP.HCl and MDZ were of about 279.5 nm and 319.8 nm, respectively (Figure 2), with the isosbestic point at 298 nm. This wavelength (298 nm) was further used for detection of analytes. Mobile phase was optimized in terms of pH, flow rate, and its composition in order to get good resolution and keep the life time of column. Using these combinations, the chromatogram obtained revealed poor peak shape with obvious tailing for CIP.HCl. In order to resolve this problem, the use of triethylamine as an ion pair reagent mixed with mobile phase is an alternative approach. Finally, the optimum condition of mobile phase consisted of monobasic potassium phosphate (50 mM, pH 3.5) and acetonitrile (80: 20, v/v) containing triethylamine (7.5 mM) and was delivered isocratically at flow rate 1.0 mL/minute. Using the optimized condition, both drugs revealed good separation with acceptable tailing factor, as shown in Figure 3.

The system suitability tests used to ensure the conditions of the chromatographic system were adequate for the analysis. These tests were assessed by analyzing a mixture of both drugs (CIP.HCl and MDZ) containing either of 30 µg/mL (low) or 90 µg/mL (high). The relative standard deviation (RSD) values of the retention time, peak area and peak height was calculated. The results fulfilled the requirement of the RSD values required by the Association of Official Analytical Chemistry, Peer Verified Methods (AOAC PVM), namely 11.3% for the level of analyte(s) of 30 µg/mL, and less than 8 % for level of analyte 90 µg/mL [17].

3.1 Analytical Method Validation

The developed method was validated in terms of precision, accuracy, linearity, selectivity and sensitivity. The linear dynamic range was evaluated at the concentration of CIP.HCl and MDZ at 30, 45, 60, 75, and 90 µg/mL for both drugs. A good correlation was observed for the relationship between concentration of both drugs (CIP.HCl and MDZ) (x-axis) and detector response (y-axis) with coefficient of correlation (r) higher than 0.99. The equation obtained is y = Y = 56.93 X – 7.07 (CIP.HCl) and Y = 58.61 X – 128.17 (MDZ). Limit of detection (LOD) and limit of quantification (LOQ) were computed based on the regression equation, as in Miller and Miller [18]. The LOD values, as determined by yOD = 3Sb in which Sb is standard deviation of blank or blank spiked with the lowest level of analyte detected by instrument, were of 8.84 µg/mL and 2.65 µg/mL, for CIP.HCl and MDZ, respectively. While, LOQ values, as determined as 3.33 x LOD, are 29.47µg/mL (CIP.HCl) and 8.84 µg/mL (MDZ). This suggested that HPLC is sensitive enough for analysis of CIP.HCl and MDZ.

The precision of the method was evaluated using repeatability assay based on the RSD values of the retention time, peak area and peak height. Six repeated injections of standard solution of 45; 60; and 75µg/mL for each compound were performed. The results showed that RSD values were in the range of 0.37 – 1.72% (CIP.HCl) and 0.10 – 1.90% (MDZ), as shown in Table 1. These results suggested that the proposed method indicates good precision with RSD values < 8% [17].

Accuracy was performed by determining the recovery percentage using standard addition method by spiking 80; 100; and 120% of the target analytes into tablet formulation. The mean recoveries obtained for CIP.HCl were 99.83 – 100.77% those for MDZ were 99.80 – 101.14%. These values were in agreement with those required by AOAC PVM. It can be stated that the developed method is accurate enough for simultaneous determination of CIP.HCl and MDZ.

The selectivity of developed method was checked by investigating the appearance of any extra peaks in the chromatogram. In the present work, there was no interference from other materials in the pharmaceutical formulation at the retention times of the examined drugs. In addition, the peak of CIP.HCl and MDZ was perfectly separated with resolution (Rs) > 1.5. These results confirmed the selectivity of the method. Table 2 summarizes the assay parameters during the method validation of HPLC for simultaneous determination of both drugs.

The developed method was further used for determination of the content uniformity test of the tablet. The results showed that HPLC method is able to determine both drugs, with the average contents of both drugs are 95.37 – 102.90% (CIP.HCl) and 96.78 – 103.56% (MDZ). The USP XXX required that tablet dosage forms should contain CIP.HCl and MDZ in the range of 90 – 110% from the stated contents. Furthermore, HPLC method was also used for determination of both drugs in tablet samples. The evaluated samples contained 496.32 ± 4.77 mg (CIP.HCl) and 502.98 ± 4.15 (MDZ), respectively. The contents for both drugs are 500 mg.

■4.0 CONCLUSION

It can be concluded that HPLC using reversed phase column with a mobile phase consisting of monobasic potassium phosphate (50 mM, pH 3.5) and acetonitrile (80: 20, v/v) containing triethylamine (7.5 mM) with a flow rate of 1.0 mL/minute allows quantitative analysis of ciprofloxacin and metronidazole simultaneously. The developed method was valid to be applied for the analysis of binary mixture CIP.HCl and MDZ in tablet dosage forms.

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