Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 3, Issue 4, 2011

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF CIPROFLOXACIN AND ORNIDAZOLE IN TABLETS

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Received: 05 August 2011, Revised and Accepted: 21 September 2011

ABSTRACT

A simple, sensitive, specific, economic, accurate and precise reverse phase high performance liquid chromatographic (RP-HPLC) method was developed for simultaneous estimation of ciprofloxacin and ornidazole in pharmaceutical formulations. The separation was achieved on Phenomenex C_{18} column (250 mm i.d., 4.6 mm, 5 µm particle size) using water: acetonitrile: triethylamine (80:20:0.1, v/v/v) and final pH adjusted to 3.06 ± 0.02 with 5% v/v ortho-phosphoric acid as the mobile phase at a flow rate of 1.5 ml/min at an ambient temperature. The quantification was achieved with UV detection at 318 nm. The injection volume was 20 µl. The retention times of ciprofloxacin and ornidazole were 4.02 min and 9.13 min, respectively. The method was validated for linearity, precision, recovery, specificity, limit of detection, limit of quantification and robustness. The linearity was obtained in the concentration range of 1 - 16 µg/ml for each ciprofloxacin and ornidazole with mean recovery of 99.41 ± 1.30 and 99.87 ± 1.10 for ciprofloxacin and ornidazole, respectively. The limit of detection and quantification for ciprofloxacin were 0.169 and 0.512 µg/ml, respectively and for ornidazole were 0.283 and 0.858 µg/ml, respectively. The method was found to be simple and sensitive and can be useful for the routine quality control testing of ciprofloxacin and ornidazole combined pharmaceutical dosage forms.

Keywords: Ciprofloxacin; Ornidazole; RP-HPLC; Validation; Simultaneous; Pharmaceutical dosage form

INTRODUCTION

Ciprofloxacin (CPX), 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)- 3-quinolinecarboxilic acid[1], is a broad spectrum fluoroquinolone antibacterial agent used in the treatment of various bacterial infections caused by gram-positive and gram-negative microorganisms[2]. Its antibacterial spectrum is wider than that of aminoglycosides, third generation cephalosporins and other fluoroquinolones. Ornidazole (ORN), 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole[3], is used as an antiinfective agent. ORN is used in the treatment of susceptible protozoal infections and also in the treatment and prophylaxis of anaerobic bacterial infections[4]. ORN is used in combination with CPX in the treatment of intraabdominal infection[5]. CPX is official in IP, USP and BP. The IP[6] and USP[7] describe HPLC method and BP[8] describes non-aqueous titration method for estimation of CPX. Literature survey reveals HPLC[9-10], spectrophotometric[11-12] and spectrofluorimetric[13] methods for its determination in pharmaceutical dosage form as well as in biological fluids. Ornidazole is official in IP. The IP[14] describes non-aqueous titration method for estimation of ORN. Literature survey reveals HPLC[15], chemiluminescence[16] and spectrophotometric[17] methods for its determination in dosage forms and biological fluids. The combination of two drugs is not official in any pharmacopoeia; hence no official method is available for the estimation of CPX and ORN in their combined dosage form. Literature survey reveals spectrophotometric[24] and HPLC[25] methods for simultaneous estimation of CPX and ORN in combined dosage forms. Since no simple RP-HPLC method is reported for simultaneous estimation of these drugs in combined dosage form. The present communication describes simple, sensitive, accurate, precise and specific HPLC method for simultaneous estimation of CPX and ORN in combined tablet dosage form.

MATERIALS AND METHODS

Reagents and Materials

CPX and ORN pure powder were kindly gifted by Torrent Research Centre, Gandhinagar, Gujarat., India. The marketed formulations included in the study were procured from the local pharmacy. HPLC grade acetonitrile was purchased from S. D. Fine Chemicals Ltd., Mumbai, India. The water for HPLC was prepared by triple glass distillation and filtered through nylon 0.45 µm - 47 mm membrane filter (Gelman Laboratory, Mumbai, India). Triethylamine, ortho-phosphoric acid and methanol (AR Grade) were procured from S. D. Fine Chemicals Ltd., Mumbai, India.

Instrumentation

A Shimadzu HPLC instrument (LC-10AT vp) equipped with UV-Visible detector, manual injector of 20 µl loop, Phenomenex C_{18} (250 mm x 4.6 mm i.d., 5 µm particle size) column, analytical balance (Sartorius CP224S, Mumbai, India), ultrasonic bath (Frontline FS 4 ultrasonic cleaner, Mumbai, India) were used in the study.

Preparation of Mobile Phase

The mobile phase comprised of water: acetonitrile: triethylamine (80:20:0.1, v/v/v) and final pH adjusted to 3.06 ± 0.02 with 5% v/v ortho-phosphoric acid. The mobile phase was filtered through nylon 0.45 µm - 47 mm membrane filter and was degassed before use.

Preparation of CPX and ORN Standard Stock Solution

Accurately weighed CPX and ORN (5 mg) was transferred to a separate 100 ml volumetric flask, dissolved in and diluted to the mark with methanol to obtain a standard solution having concentration of CPX and ORN, 50 µg/ml.

Chromatographic Separation

The mobile phase containing water: acetonitrile: triethylamine (80:20:0.1, v/v/v) and final pH adjusted to 3.06 ± 0.02 with 5% v/v ortho-phosphoric acid was selected because it was found to ideally resolve the peaks of CPX (T_R = 4.02) and ORN (T_R = 9.13), respectively. Wavelength of maximum absorption was selected by scanning standard solutions of both the drugs over 200 nm to 400 nm region. Both components showed reasonable good response at 318 nm.

Calibration Curves for CPX and ORN

A calibration curve was plotted over a concentration range of 1-16 µg/ml for each CPX and ORN. Accurately measured standard stock solutions of CPX and ORN (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 3.2 ml) were transferred in to a series of 10 ml volumetric flasks and diluted to the mark with mobile phase. Twenty microlitres of each solution was injected under operating chromatographic conditions described above in triplicate. Calibration curves were constructed by plotting peak areas versus concentrations of CPX and ORN and the regression equations were calculated.

Preparation of Sample Solution

Twenty tablets were accurately weighed and powdered. A quantity of powder equivalent to 6 mg of CPX or ORN was transferred to a 100 ml volumetric flask and mixed with methanol (50 ml) and

sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the residue was washed thoroughly with water. The filtrate and washings were combined in a 100 ml volumetric flask and diluted to the mark with methanol. The solution (1.0 ml) was transferred to a 10 ml volumetric flask and diluted to the mark with mobile phase to obtain final solution with CPX (6 µg/ml) and ORN (6 µg/ml).

Validation of the Method

The developed method was validated in terms of linearity, accuracy, precision, specificity, limit of detection, limit of quantification and robustness in compliance with ICH guidelines[20].

Linearity

A calibration curve was plotted over a concentration range of 1-16 µg/ml for each CPX and ORN. Accurately measured standard stock solutions of CPX and ORN (0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 3.2 ml) were transferred in to a series of 10 ml volumetric flasks and diluted to the mark with mobile phase. Twenty microlitres of each solution was injected under operating chromatographic conditions described above in triplicate. Calibration curves were constructed by plotting peak areas versus concentrations of CPX and ORN and the regression equations were calculated.

Accuracy (Recovery Study)

The accuracy of the method was determined by calculating the recoveries of CPX and ORN by the standard addition method. Known amounts of standard solutions of CPX and ORN were at added at 50, 100 and 150 % level to prequantified sample solutions of CPX and ORN (6 µg/ml for both drug). The amounts of CPX and ORN were estimated by applying obtained values to the respective regression line equations.

Method Precision (Repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) solutions of CPX and ORN (6 µg/ml for both drugs) without changing the parameters for the method. The result was reported in terms of relative standard deviation (% RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precisions of the proposed method was determined by estimating the corresponding responses 3 times on the same day and on 3 different days over a period of one week for different concentrations of standard solutions of CPX and ORN (1 – 16 µg/ml). The results were reported in terms of relative standard deviation (% RSD).

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[20].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Specificity

The specificity of the method was ascertained by analyzing standard drugs and the sample. The retention time for CPX and ORN in the samples was confirmed by comparing with the retention time of the standards.

Robustness

The robustness was studied by analyzing the same samples of CPX and ORN by deliberate variations in the method parameters. The change in the responses of CPX and ORN were noted. Robustness of the method was studied by changing the extraction time of CPX and ORN from tablet dosage forms by ± 2 min, composition of mobile phase by ± 2 % of organic solvent, flow rate by ± 0.1 ml/min and column oven temperature by ± 2 °C. The parameters used in system suitability test were asymmetry of the chromatographic peak, peak resolution and theoretical plates, as RSD of peak area for replicate injections.

RESULTS AND DISCUSSION

Method Development

To develop a precise, accurate and suitable HPLC method for the simultaneous estimation of CPX and ORN, different mobile phases and stationary phases were employed and proposed chromatographic condition was found appropriate for the quantitative determination. Mobile phase consisting water: acetonitrile: triethylamine (80:20:0.1, v/v/v) and final pH adjusted to 3.06 ± 0.02 with 5% v/v ortho-phosphoric acid, at a flow rate of 1.5 ml/min, 250 mm x 4.6 mm C_{18} column of 5 µm particle size, detection wavelength of 318 nm, an injection volume of 20 µl and an ambient temperature for the HPLC system were found to best for analysis (Figure 1).

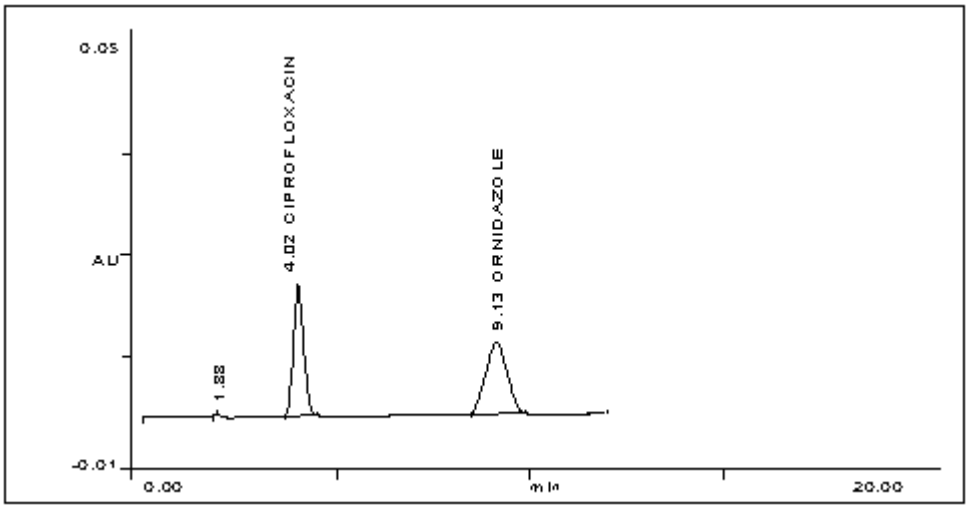

Fig. 1: HPLC chromatogram of CPX and ORN with corresponding retention time at 318 nm

System suitability results are as follows: Retention time (T_R) for CPX and ORN were 4.02 and 9.13, respectively, Tailing factor, T_{CPX} = 1.21 and T_{ORN} = 1.29, Theoretical plate numbers for CPX and ORN were 4945 and 6158, respectively, Asymmetry factor (As), for CPX and ORN were 1.27 and 1.38, respectively.

The linearity range was found in the concentration range of 1 - 16 µg/ml for each CPX and ORN. The regression equations for CPX and ORN were found to be y = 157477x + 37742 and y = 187529x – 8484.7 with co-efficient of correlation (r^2), 0.9980 and 0.9977, respectively.

Relative standard deviation for repeatability is less than 2% (0.11 - 0.98,), which indicates that the proposed method is repeatable (Table 1).

The low % RSD values of intra-day (0.43 - 1.68 for CPX and 0.53 - 1.89 for ORN) and inter-day (0.47 - 1.44 for CPX and 0.45 - 1.51 for ORN) precision reveal that the proposed method is precise (Table 2 and 3).

The average percent recovery obtained was 99.41 ± 1.30 and 99.87 ± 1.10 for CPX and ORN, respectively indicates that the proposed method is highly accurate (Table 4).

The limit of detection and quantification for CPX were 0.169 and 0.512 µg/ml, respectively and for ORN were 0.283 and 0.858 µg/ml, and respectively indicates sensitivity of the method.

The peak purity of CPX and ORN were assessed by comparing the retention time (T_R) of standard CPX and ORN. Good correlation was also found between the retention time of standards and samples of CPX and ORN reflects specificity of the method.

The assay results of CPX and ORN in tablet dosage forms were comparable with label claimed values (Table 5).

Table 1: Method precision data for analysis of CPX and ORN by HPLC method

CPX (6 µg/ml)/ ORN (6 µg/ml)	Retention Time (minutes)		Area		Asymmetry		Tailing Factor	
	CPX	ORN	CPX	ORN	CPX	ORN	CPX	ORN
1	4.02	9.12	994856	1118114	1.15	1.20	1.00	1.02
2	4.01	9.14	993208	1122623	1.16	1.21	1.01	1.04
3	4.01	9.12	995125	1115894	1.15	1.21	1.00	1.03
4	4.03	9.15	993948	1121652	1.17	1.19	1.02	1.03
5	4.01	9.14	994898	1115023	1.15	1.18	1.01	1.02
6	4.02	9.13	992365	1119458	1.17	1.20	1.01	1.04
Mean	4.017	9.133	994067	1118794	1.158	1.198	1.008	1.030
S. D.	0.0082	0.0121	1102.84	3045.10	0.0098	0.0117	0.0075	0.0089
% RSD	0.20	0.13	0.11	0.27	0.85	0.98	0.75	0.87

Table 2: Intra-day precision data for analysis of CPX and ORN by HPLC method

Concentration		Intra-Day Precision			
CPX	ORN	CPX		ORN	
(µg/ml)	(µg/ml)	Mean ± S. D. (n = 3)	% RSD	Mean ± S. D. (n = 3)	% RSD
1	1	185858 ± 2190.35	1.18	193103 ± 2297.54	1.19
2	2	364072 ± 2441.51	0.67	386629 ± 2060.94	0.53
4	4	698960 ± 3024.47	0.43	768990 ± 4449.78	0.58
6	6	990269 ± 8069.86	0.82	1127405 ± 16092.8	1.43
8	8	1282373 ± 15367.1	1.20	1434439 ± 27170.8	1.89
10	10	1526601 ± 18366.4	1.20	1769366 ± 22706.4	1.28
12	12	1924568 ± 18414.7	0.96	2223477 ± 17410.7	0.78
16	16	2603709 ± 43591.6	1.68	3070192 ± 53996.1	1.76

Table 3: Inter-day precision data for analysis of CPX and ORN by HPLC method

Concentration		Inter-Day Precision			
CPX	ORN	CPX		ORN	
(µg/ml)	(µg/ml)	Mean ± S. D. (n = 3)	% RSD	Mean ± S. D. (n = 3)	% RSD
1	1	180908 ± 1415.99	0.78	199663 ± 1718.44	0.86
2	2	357618 ± 4457.66	1.25	389155 ± 3056.24	0.79
4	4	690414 ± 4609.22	0.67	781581 ± 3515.47	0.45
6	6	985352 ± 4593.96	0.47	1096143 ± 6926.98	0.63
8	8	1205662 ± 7948.53	0.66	1447064 ± 18424.8	1.27
10	10	1504834 ± 7114.36	0.47	1802583 ± 10540.4	0.59
12	12	1978507 ± 28502.2	1.44	2157716 ± 32625.9	1.51
16	16	2542921 ± 25319.3	1.01	3096345 ± 22051.8	0.71

Table 4: Data of recovery study for cpx and orn by hplc method

Drug	Level	Amount Taken (µg/ml)	Amount Added (µg/ml)	% Recovery ± S. D. (n = 3)
CPX	I	6	3	100.8 ± 1.45
	II	6	6	99.34 ± 0.87
	III	6	9	98.08 ± 1.57
ORN	I	6	3	101.4 ± 1.18
	II	6	6	98.65 ± 0.97
	III	6	9	99.56 ± 1.14

Table 5: Results of analysis of marketed tablets formulation

Formulation	Drug	Labeled Amount (mg)	Amount Found (mg)	% Amount Found ± S. D. (n = 3)
Tablets	**Brand - I**			
	Ciprofloxacin	500	501.0	100.2 ± 0.98
	Ornidazole	500	497.3	99.45 ± 1.24
	Brand - II			
	Ciprofloxacin	500	506.5	101.3 ± 1.36
	Ornidazole	500	491.3	98.25 ± 1.04

CONCLUSION

The proposed method was found to be simple, sensitive, precise, accurate, repeatable, reproducible, specific and robust and can be used in routine for the simultaneous determination of CPX and ORN in pharmaceutical tablet formulations.

ACKNOWLEDGMENT

The author is thankful to Torrent Research Centre, Gandhinagar, and Gujarat., India for providing gift samples of ciprofloxacin and ornidazole and also to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University for providing all the facilities to carry out the research work.

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