Simultaneous Estimation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole and Ornidazole by Reverse Phase – High Performance Liquid Chromatography

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Abstract

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay has been developed for simultaneous estimation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole and Ornidazole in tablet formulations. The separation was achieved by using C-18 column (Phenomenax, 250 x 4.6mm i.d.) coupled with a guard column of same material, in mobile phase Acetonitrile: Water: Tri ethylamine (25:75). The pH of mobile phase was adjusted to 6.0 ± 0.1 with 50% ortho phosphoric acid. The flow rate was 1.0 mL.min^{-1} and the separated drugs were detected using UV detector at the wavelength of 300 nm. The retention time of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole, and Ornidazole was noted to be 2.7, 3.5, 4.5 and 5.8 min, respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent. It was successfully applied for the analysis of these drugs in marketed formulations and could be effectively used for the routine analysis of formulations containing any one of the above drugs, or a combination, without any alteration in the chromatographic conditions.

1. Introduction

Ciprofloxacin Hydrochloride (1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) and Ofloxacin (9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid) are fluoroquinolones and antimicrobials with potent activity against a broad spectrum of bacteria.Tinidazole (1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole) and Ornidazole (1(3-chloro-2- hydroxypropyl)-2-methyl-5-nitro imidazole) are antiprotozoal and antibacterial drugs [1, 2, 3]. The structures of these four drugs are shown in Fig. 1.These drugs are being used either alone or in combination for the treatment of diarrhoea and dysentery of amoebic, bacterial or mixed origin [4].
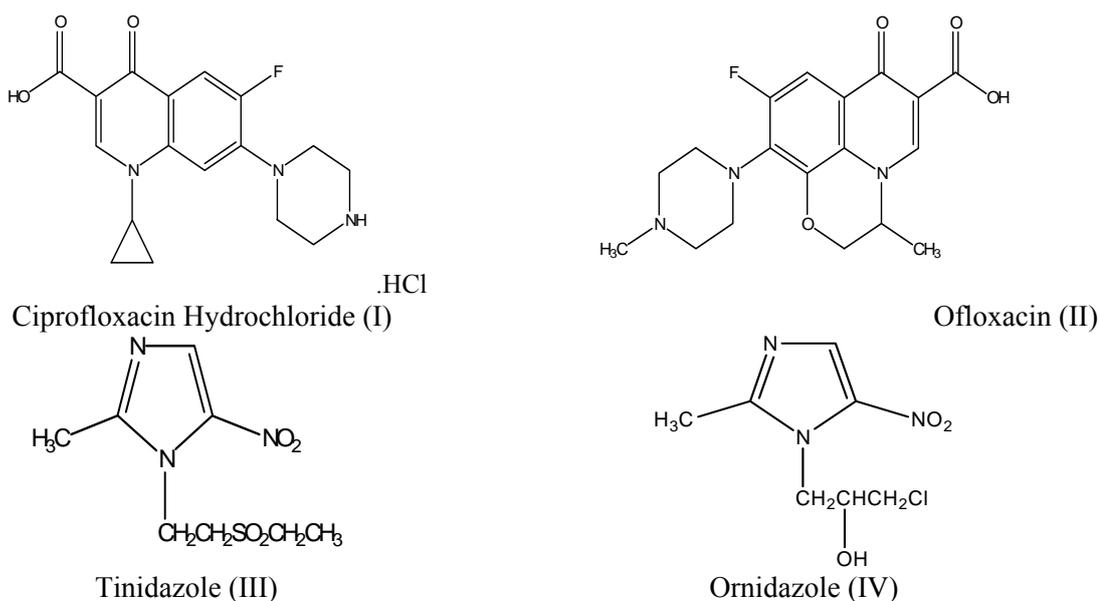
Fig.1. The structures of ciprofloxacin hydrochloride, ofloxacin, tinidazole and ornidazole

Literature survey revealed a few HPLC methods for the estimation of Ciprofloxacin Hydrochloride and Tinidazole [5-9]. Also some HPLC methods are available for the determination of Ofloxacin and Ornidazole [10-11]. Similarly some HPLC methods are reported for the estimation of Ofloxacin and Tinidazole [12-15]. No HPLC method for simultaneous estimation of these four drugs has been reported till date.

In the present study, an attempt has been made to develop a method for the simultaneous estimation of all four drugs- Ciprofloxacin Hydrochloride (I), Ofloxacin (II), Tinidazole (III) and Ornidazole (IV). It can also be applied for routine analysis of either one or of any combinations of in these drugs dosage forms.

2. Experimental

2.1. Chemicals and Reagents

Pharmaceutical grade (>99%) drugs (I), (II), (III) and (IV) were obtained from PEGASUS FARMACO INDIA (P) LTD (Roorkee, Uttarakhand State, India). Water and Acetonitrile (HPLC grade) were obtained from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard volumetric glassware.

2.2. Instrumentation and Chromatographic Conditions

The instrument used was a Shimadzu chromatographic system (Japan), equipped with an LC-10 AT vp solvent delivery module, SPD-10A UV-Visible detector, and a Rheodyne model (7725i) injector valve fitted with 20μL volume sample loop. The samples were injected through a Hamilton, Bonodaz AG microliter syringe. Chromatographic separation was performed on C-18 column (Phenomenax, 250 x 4.6mm i.d.) coupled with a guard column of the same material. The mobile phase was composed of Acetonitrile: Water: Tri ethylamine (25:75:1.0 v/v) and pH of mobile phase was adjusted to 6.0 ± 0.1 with 50% ortho phosphoric acid. The flow rate was maintained at 1.0 mL.min^{-1}. The column effluent was monitored on UV detector set at 300 nm.

2.3. Preparation of Stock and Working Standard Solution

Individual stock solutions of the four drugs (600 µg.mL^{-1}) were prepared by dissolving 6 mg of individual drug in 10 ml of mobile phase. The mobile phase standards containing mixture of (I), (II), (III) and (IV) were prepared by appropriately diluting the stocks in the range of 15–150 µg.mL^{-1} using mobile phase.

2.4. Preparation of Sample Solution

Mixture of sample solutions were prepared from formulations containing:

(A) Ciprofloxacin Hydrochloride (555mg) and Tinidazole (600mg)

(B) Ofloxacin (200 mg) and Ornidazole (500mg)

Twenty tablets of both the formulations A (Ciplox –TZ; CIPLA LTD) and B (Oflox – OZ; CIPLA LTD) were weighed and crushed seperately to a fine homogenous powder. Quantity equivalent to 25 mg each of both formulations was accurately weighed and taken individually in a 25 mL volumetric flask. The powdered mixtures were dissolved in the mobile phase and volume was made up.

The supernatants of both the solutions were taken, mixed thoroughly and diluted with the mobile phase (final concentration, 73.75, 37.80, 79.73 and 94.50 µg.mL^{-1} for Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole and Ornidazole, respectively) and 100 µL of this solution was injected for HPLC analysis.

2.5. Quality Control Standards

The quality control (QC) standards for four drugs were prepared from stock solutions by dissolving 6mg each of individual drugs in 10 mL of mobile phase. The working solutions of the mixture of four drugs were prepared in the concentration ranges of low (30 µg.mL^{-1}), medium (60 µg.mL^{-1}), and high (120 µg.mL^{-1}) concentrations using mobile phase as a solvent.
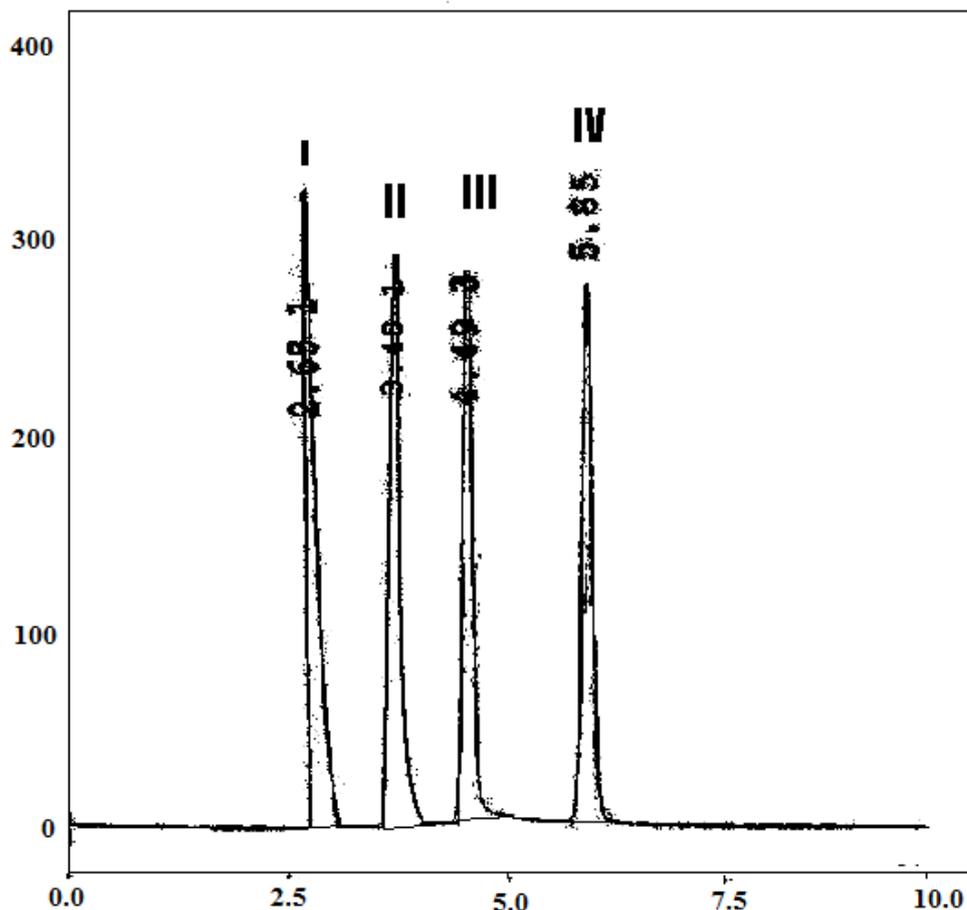
2.6. Method Validation

The method was validated in terms of stability, linearity, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) [16].

3. Results and Discussion

3.1. Optimization of Chromatographic Conditions

Spectroscopic analysis of compounds showed that (I), (II), (III) and (IV) have maximum UV absorbance (λ_{max}) at 272.2 nm, 292 nm, 316.8 nm, and 317.4 nm respectively. Therefore, the chromatographic detection was performed at 300 nm using a UV-Visible detector. It was observed that when a combination of all the four drugs was injected, Ciprofloxacin Hydrochloride and Ofloxacin together gave a single peak. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in the mobile phase. Different experiments were performed to optimize the mobile phase but adequate separation of drugs could not be achieved. By altering the pH of mobile phase a good separation was achieved. Tri-ethylamine was used as a modifier for better peak shape. The optimized mobile phase was determined as a mixture of Acetonitrile: Water: Tri-ethylamine (25:75:1.0) at a flow rate of 1.0 mL.min^{-1}. Under these conditions (I), (II), (III) and (IV) were eluted at 2.73, 3.59, 4.49, and 5.85 minutes respectively with a run time of 10 min.

A typical chromatogram for simultaneous estimation of (I), (II), (III) and (IV), obtained by using the aforementioned mobile phase from 100 µL of the assay preparation is illustrated in Fig. 2.

Fig. 2. HPLC chromatogram obtained during simultaneous determination of Ciprofloxacin Hydrochloride (I), Ofloxacin   (II), Tinidazole (III) and Ornidazole (IV)

3.2. Method Validation

3.2.1. Linearity and Calibration standards

Six different concentrations of a mixture of all four drugs were prepared for linearity studies. The response was measured as peak area .The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 15 to 150 µg.mL^{-1} for all drugs. The best fit for the calibration curve could be achieved by a linear regression equation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole, and Ornidazole found to be y = 12.835x − 18.032, y = 33.788x − 47.343, y = 14.233x + 32.164, and y = 15.602x + 35.822, respectively and the regression coefficient values (r^2) were found to be 0.9999, 0.9999, 0.9998 and 0.9999 respectively indicating a high degree of linearity for all drugs.

3.2.2. Specificity

The specificity studies revealed the absence of any other excipient interference, since none of the peaks appeared at the same retention time of (I), (II), (III) and (IV), as shown in Fig. 2.

The interaction study of all four drugs in standard solution was also carried out by comparing peak of each drug, individually Vs peaks of drug mixture. Interaction studies indicated that the analytes did not interact with each other and were well within the acceptance level of ± 2.0%.

3.2.3. Accuracy and precision

The accuracy of method was determined and calculated as % bias. The low value of % bias showed that the method is accurate within the acceptance limit of 2%. The results are shown in Table 1.

Table 1. Accuracy (% Bias) and Precision (%RSD) of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole, and Ornidazole

Concentration ($\mu g.mL^{-1}$)	% Bias		% RSD	
	Intra day	Inter day	Intra day	Inter day
Ciprofloxacin Hydrochloride				
15	+ 0.6	+ 0.9	+ 0.2	+ 0.5
30	+ 0.4	+ 0.7	+ 0.2	+ 0.4
60	- 0.4	- 0.1	+ 0.5	+ 0.7
90	+ 0.2	+ 0.5	+ 0.3	+ 0.5
120	+ 0.2	+ 0.6	+ 0.5	+ 0.6
150	+ 0.5	+ 0.8	+ 0.4	+ 0.8
Ofloxacin				
15	+ 0.9	+ 1.2	+ 0.9	+ 1.2
30	+ 1.1	+ 1.2	+ 1.1	+ 1.4
60	+ 0.9	+ 0.9	+ 0.6	+ 0.8
90	+ 1.2	+ 1.3	+ 0.5	+ 0.9
120	+ 1.4	+ 1.6	+ 1.2	+ 1.2
150	+ 1.0	+ 1.1	+ 0.6	+ 0.8
Tinidazole				
15	- 0.2	- 0.1	+ 0.1	+ 0.2
30	+ 0.3	+ 0.5	+ 0.1	+ 0.4
60	- 0.1	- 0.1	+ 0.3	+ 0.8
90	- 0.2	- 0.1	+ 0.2	+ 0.3
120	+ 0.2	+ 0.5	+ 0.6	+ 0.7
150	+ 0.3	+ 0.8	+ 0.7	+ 0.9
Ornidazole				
15	+ 0.1	+ 0.1	+ 0.4	+ 0.5
30	+ 0.4	+ 0.5	+ 0.3	+ 0.5
60	- 0.2	- 0.1	+ 0.5	+ 0.6
90	+ 0.2	+ 0.3	+ 0.2	+ 0.5
120	+ 0.1	+ 0.7	+ 0.4	+ 0.9
150	+ 0.4	+ 0.6	+ 0.4	+ 0.6

The precision (repeatability and intermediate precision) of the method was established by carrying out analysis of the analytes using proposed method. The low value of % CV showed that the method is precise within the acceptance limit of 2%. The results are shown in Table 1.

3.2.4. LOD and LOQ

For determining the limit of detection (LOD) and limit of quantitation (LOQ), the method based on the standard deviation and slope was adopted.

The limit of detection for (I), (II), (III) and (IV) was 0.22 µg.mL^{-1}, 0.40 µg.mL^{-1}, 0.19 µg.mL^{-1}, 0.37 µg.mL^{-1}, respectively and the limit of quantitation (LOQ) was 0.67 µg.mL^{-1}, 1.24 µg.mL^{-1}, 0.59 µg.mL^{-1}, 1.13 µg.mL^{-1}, respectively.

3.2.5. Stability studies

The stability of the analyte solutions was determined at intervals of 1st day, 3rd day, and 6th day. The stability of solutions was determined by comparing the analyte solutions at 3rd day and 6th day with that of the freshly prepared solution at 1st day. The differences determined on 3rd day were ±1.1, ±1.25, ±0.35, and ±0.50 for I, II, III, and IV respectively. The differences determined up to 6th day were ±1.4, ±1.6, ±0.40, and ±1.0 for I, II, III, and IV respectively.

3.2.6. Analysis of Tablets

The values of analysis of tablets obtained by the proposed method were between 98.35% and 101.62% (Table 2), which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%.

Table 2. Estimation of (I), (II), (III) and (IV) in mixture of tablet

Drug	Quantity claimed (mg/tablet)	Quantity found (mg/tablet)	% Quantity found ±SD
Ciprofloxacin Hydrochloride	555	545.89	98.35 (±1.02)
Ofloxacin	200	199.72	99.86 (±1.64)
Tinidazole	600	609.80	101.62 (±0.80)
Ornidazole	500	495.25	99.05 (±1.72)

3.3. System suitability parameters

For system suitability parameters, three replicate injections of mixed standard solution were injected and parameters such as the Resolution, Capacity factor, Tailing factor, Theoretical plate, Retention volume and Asymmetry factor of the peaks were calculated. The results are shown in Table 3.

Table 3. System suitability data of the method

Parameters	Ciprofloxacin Hydrochloride	Ofloxacin	Tinidazole	Ornidazole
Resolution	-	6.20	7.89	11.39
Capacity factor	0.100	0.45	0.89	1.46
Tailing Factor	1.0	1.15	1.05	1.0
Theoretical plates	7817	8339	25834	34928
Asymmetry factor	1.0	1.10	1.15	1.0

4. Conclusion

An RP-HPLC method for simultaneous estimation of Ciprofloxacin Hydrochloride, Ofloxacin, Tinidazole, and Ornidazole was developed and validated. Results of analysis of the formulations are tabulated in Table 1. The amounts obtained by the proposed method are between 98.35% and 101.62%, within the acceptance level of 95% to 105%.

The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 15 to 150 µg.mL^{-1} for all four drugs. The method has been successfully applied for the analysis of marketed tablets. It can be used for the routine analysis of formulations containing any one of the above drugs or their combinations without any alteration in the assay. The main advantage of the method is the common chromatographic conditions adopted for all formulations. Therefore, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing that are typically associated when different formulations are analyzed.

Acknowledgements

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