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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CIPROFLOXACIN AND TINIDAZOLE FOR CONTENT UNIFORMITY IN TABLET BLEND USING RP-HPLC METHOD

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ABSTRACT

An accurate and precise HPLC method was developed for the simultaneous determination of Ciprofloxacin and Tinidazole. Separation of the drug was achieved on a reverse phase ZORBAX SB C8 column using a mobile phase consisting of buffer and acetonitrile in the ratio of 75:25v/v. The flow rate was 0.8mL/min and the detection wavelength was 317 nm. The linearity was observed in the range of 8.13-73.19 ppm and 9.48-85.36 ppm with a correlation coefficient of 0.9999 and 1.0 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of Ciprofloxacin and Tinidazole tablet blend.

Key Words:- Ciprofloxacin, Tinidazole, HPLC, Validation.

INTRODUCTION

Ciprofloxacin (CPX) is a fluorinated quinolone antibacterial which is chemically 1-cyclopropyl-6-fluoro-4-oxo-7- (piperazin-1-yl) -quinoline- 3- carboxy-licacid. Cipro floxacin is a broad spectrum antibiotic active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. It is also used as antibacterial. Tinidazole is chemically 1 -(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole.

It is an antiprotozoal agent. Numerous analytical methods, such as high performance liquid chromatography (HPLC) have been reported for either Ciprofloxacin or Tinidazole in single or combined dosage forms[1-7]. From the literature survey revealed that a novel method for the determination of fluoroquinolones such as Ciprofloxacin hydrochloride (CPL) and Ofloxacin (OFX) by Isocratic reverse phase-high performance liquid chromatography (RP-HPLC) coupled with UV detection (Neetu Sachan *et a1.*, 2010), the development and validation of an RP-HPLC method for the determination of Ciprofloxacin hydrochloride in ophthalmic solutions (Edith *et a1.*, 2009), the development HPLC method for analysis of

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Ciprofloxacin hydrochloride in raw materials (Lacroix *et a1.*, 1996), The related substances of Ciprofloxacin hydrochloride and its complete monograph analysis (European pharmacopoeia, 2005), HPLC method for estimation of Tinidazole in pharmaceutical dosage forms utilizing reverse phase gradient system (Satyanarayana V *et a1.*, 2001), HPLC method for the determination of Tinidazole in human serum using Metronidazole as internal standard (Rajnarayana *et a1.*, 2009), UV-Spectrophotometric method for the estimation of Tinidazole in bulk and pharmaceutical dosage forms (Umadevi Kothapalli *et a1.*, 2009). However, no methods have been reported for the simultaneous determination of Ciprofloxacin and Tinidazole in blend form. Hence present attempt made to develop an RP-HPLC method with PDA detection for Quantitation of content uniformity of Ciprofloxacin and Tinidazole in tablet blend. The present RP-HPLC method was validated following ICH guidelines.

MATERIALS AND METHODS

The reference sample of Ciprofloxacin and Tinidazole was supplied by Alphamed Formulations Pvt Ltd., Hyderabad. HPLC grade water and Acetonitrile were purchased from E. Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and Triethylamine, orthophosphoric acid of AR Grade were obtained from S.D. Fine Chemicals Ltd., Mumbai. Tablet Blend with each tablet containing 500 mg of Ciprofloxacin and 600 mg of Tinidazole were procured from Alphamed Formulations Pvt ltd.

Chromatographic Condition

Chromatographic analysis was performed on a ZORBAX SB C-8 column with 250 x 4.6 mm i.d. and 5 µm particle size. The mobile phase consisted of Buffer: Acetonitrile (75: 25 v/v) and that was set at a flow rate of 0.8 ml/min. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system. The eluent was monitored by PDA detection at 317 nm.

Procedure

A mixture of buffer and Acetonitrile in the ratio of 75:25v/v was found to be the most suitable mobile phase for ideal separation of Ciprofloxacin and Tinidazole. It was pumped through the column at a flow rate of 0.8mL/min. The column was maintained at ambient temperature. The column was equilibrated by pumping the mobile phase through the column for at least 30 min prior to the injection of the drug solution. The detection of the drug was monitored at 317 nm. The run time was set at 12 min. Under these optimized chromatographic conditions the retention time obtained for the drug was 4.428 and 7.916 mins respectively. A typical chromatogram showing the separation of the drug is given in Fig 1.

Preparation of Ciprofloxacin and Tinidazole standard and sample Solutions

Preparation of Standard Solution

Weigh accurately about 115 mg of Ciprofloxacin HCL working standard and transfer into a 100 ml volumetric flask. Dissolve in 50 ml of diluents and sonicate for about 10 minutes to dissolve the material. Weigh accurately about 115mg of Tinidazole working standard into the same 100ml volumetric flask. Dissolve, dilute to volume with diluent and mix well. Pipette 5 ml of this solution into a 100ml volumetric flask, dilute to volume with mobile phase and mix.

Preparation of Sample Solution

Transfer whole amount of powder (1289.59mg) into 500ml volumetric flask and add 250ml of diluent, sonicate for 30 minutes with intermediate shaking and shake for 30 minutes mechanically and bring to volume with diluent. Filter a portion of solution through 0.45µm filter. Transfer 4ml of this solution into 250ml volumetric flask, dilute to volume with mobile phase and mix.

VALIDATION

The method validation was carried out according to ICH Guidelines to the recommendations for analytical method validation.

System Suitability

System-suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (tR), number of theoretical plates (N) and tailing factor (T) were evaluated for six replicate injections of the drug at a concentration of 50 µg/ml. The results which are given in Table.1 and 2 were within acceptable limits.

Linearity

Prepare a series of standard solutions (not less than 5 is recommended) in the range of 50% to 150% of the target assay concentration. A plot of average peak area versus the concentration in µg/ml or mg/ml is made and from this the correlation coefficient, y-intercept (const. of regression) and slope (coefficient of regression) of the regression line were calculated. The calibration data of Ciprofloxacin and Tinidazole is given in Table.3 and the calibration curve of linearity of is shown in Fig 2 and 3.

Precision

The precision of the test procedure was evaluated for Ciprofloxacin and Tinidazole in Ciprolet A blend by performing CU as per the test method with six samples for 1X and 3X respectively. The %assay of Ciprofloxacin and Tinidazole and Relative Standard Deviation and Confidence interval of assay results were calculated. The result of Precision studies is given in Table 4.

Specificity

Specificity is the ability of a method to discriminate between the analyte(s) of interest and other components that are present in the sample. A study of placebo interference from excipients was conducted. Equivalent weight of placebo taken as per the test method and placebo interference was conducted in duplicate.

Accuracy

To validate the test method can accurately quantify Ciprofloxacin and Tinidazole, prepare samples in six times for higher and lower levels, in triplicate for other levels by using Ciprolet-A blend or by spiking Ciprofloxacin and Tinidazole active material with equivalent amount of placebo and perform assay as per test procedure. Prepare samples at levels 50%, 75%, 100%, 125% and 150% of the target assay concentration i.e. 50% of the lowest strength initial concentration to 150% of the highest strength initial concentration level. Table 5 and Table 6 shows the results for accuracy of Ciprofloxacin and Tinidazole.

Ruggedness

The ruggedness test of analytical assay method is defined as degree of reproducibility of assay results obtained by the successful applications of the assay over time and among multiple laboratories and analyst. The result of ruggedness testing is reported in Table 7.

Robustness

Robustness of the method is determined by using different filters. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust. The result of robustness testing is reported in Table.8 shows the results for accuracy of Ciprofloxacin and Tinidazole.

RESULTS AND DISCUSSION

System suitability

The %Relative standard deviation of five replicate injections for Ciprofloxacin and Tinidazole standard should not be more than 2.0%.The %Relative standard deviation of five replicate injections for Ciprofloxacin and Tinidazole standard were found to be within limits. The tailing factor for Ciprofloxacin and Tinidazole peaks should be not more than 2.0. The tailing factor for Ciprofloxacin and Tinidazole peaks were found to be within limits.

Linearity

The Correlation coefficient should not be more than 0.999.The Correlation Coefficient of Ciprofloxacin and Tinidazole was found to be 0.9999 and 0.9999 respectively. Bias at 100% should not be more than 2%. Bias at 100% for Ciprofloxacin and Tinidazole were found to be 0.40% and 0.08% respectively.

Precision

The Relative standard deviation of individual % assay of Ciprofloxacin and Tinidazole from six sample preparations should be not more than 5.0%. The Relative standard deviation of individual % assay of Ciprofloxacin and Tinidazole were found to be 0.52 and 0.37 for 1X and 0.46 and 0.49 for 3X respectively. The method is considered "PRECISE" if the assay results of Ciprofloxacin and Tinidazole should be not less than 95.0% and not more than 105.0%. Assay results of Ciprofloxacin and Tinidazole were found to be within limits.

Specificity

Placebo chromatogram should not show any peak at the retention time of Ciprofloxacin and Tinidazole. Placebo interference from excipients was not found. (No peak was found).

Accuracy

The mean % Recovery of the Ciprofloxacin and Tinidazole at each level should be not less than 95.0% and not more than 105.0%. The mean % Recovery of the Ciprofloxacin and Tinidazole were found to be within limits at each level .The %RSD of recovery of Ciprofloxacin and Tinidazole from the six sample preparations at 50% and 150% levels should not be more than 5.0%. The % RSD of recovery of Ciprofloxacin and Tinidazole from the six sample preparations were found to be 0.40 and 1.22 at 50% level and 0.39 and 0.54 at 150% level respectively.

Robustness

Similarity factor of test solution against unfiltered standard should be 0.98 to 1.02. Similarity factor. Similarity factor of test solution against unfiltered standard was found to be be within limits

Ruggedness

The RSD of individual %assay of Ciprofloxacin and Tinidazole from the six sample preparations at 50% and 150% levels should not be more than 5.0%. The RSD of individual %assay of Ciprofloxacin and Tinidazole were found to be 0.11% and 0.13% for 1X and 0.15% and 0.31% for 3X respectively.

The method is considered "PRECISE" if the assay results of Ciprofloxacin and Tinidazole should be not less than 95.0% and not more than 105.0%.

Assay results of Ciprofloxacin and Tinidazole were found to be within limits.

Fig 1.Chromatogram showing the separation of drugs

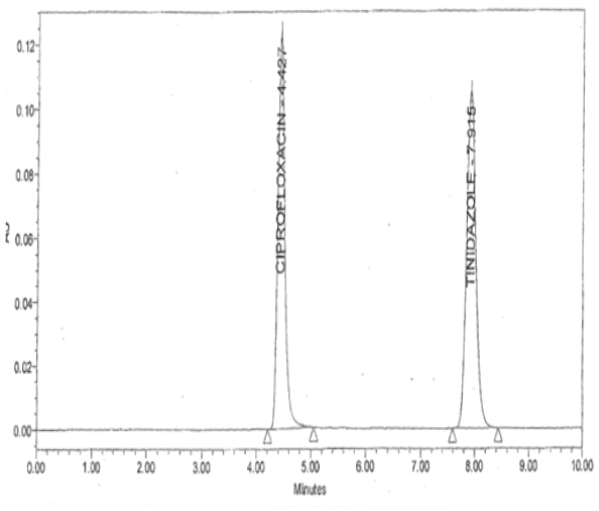

Fig 2. Linearity of Ciprofloxacin

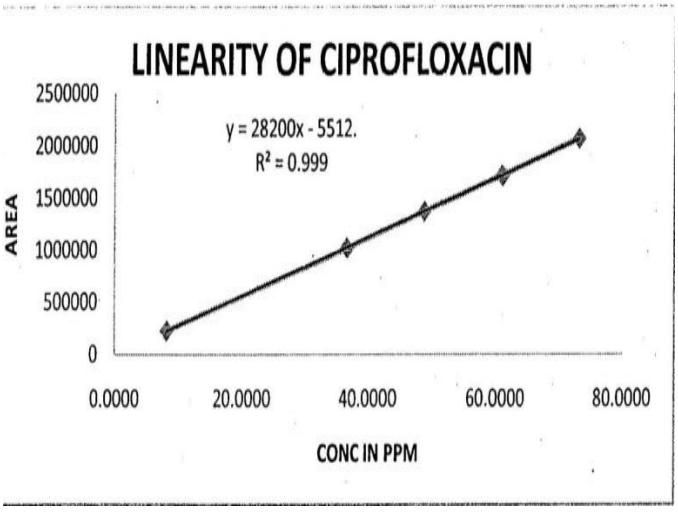

Fig 3. Linearity of Tinidazole

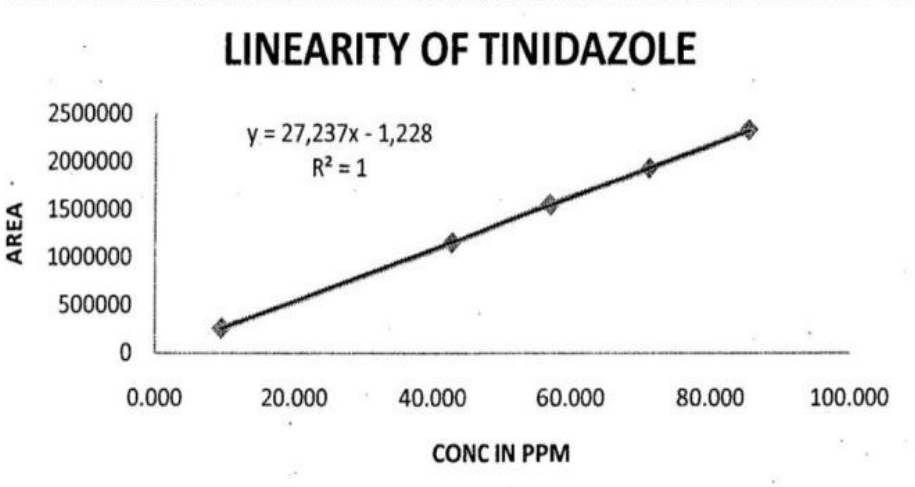

Table 1. Results from system suitability studies of ciprofloxacin

Compound name		Ciprofloxacin
S.No	Parameter	Std-2
1	Tailing factor	1.6
2	Theoretical plates	5435
3	%RSD of std area	0.2

Table 2. Results from system suitability studies of Tinidazole

Compound name		Tinidazole
S.No	Parameter	Std-2(1st inj)
1	Tailing factor	1.4
2	Theoretical plates	7743
3	%RSD of std area	0.2

Table 3. Linearity for Ciprofloxacin and Tinidazole

Ciprofloxacin			Tinidazole		
S.No	**Concentration ppm**	**Peak Area**	**S.No**	**Concentration ppm**	**Peak Area**
1	8.1331	228454	1	9.4845	259909
2	36.5988	1019078	2	42.6803	1155383
3	48.7985	1370745	3	56.9070	1552589
4	60.9981	1712173	4	71.1338	1931320
5	73.1977	2063820	5	85.3606	2327969

Table 4. Precision for Ciprofloxacin and Tinidazole

Sample Name	Ciprolet A			
	1X Dose		3X Dose	
	%Ciprofloxacin	%Tinidazole	%Ciprofloxacin	%Tinidazole
Sample-1	98.54	99.98	100.02	99.87
Sample-2	97.72	100.37	100.83	99.60
Sample-3	99.19	100.42	100.77	99.21
Sample-4	98.85	99.83	100.33	100.21
Sample-5	98.24	100.87	101.31	100.21
Sample-6	98.73	100.46	100.99	99.04
Average	98.55	100.32	100.71	99.69
%RSD	0.52	0.37	0.46	0.49
Confidence Interval 95% to 105.0%	98.1% t 98.9%	100.0 to 100.6%	100.3 to101.1%	99.3 to100.1%

Table 5. Accuracy for Ciprofloxacin

Sample no	Spike level	Amount of Ciprofloxacin added(ppm)	Amount of Ciprofloxacin found(ppm)	Mean (%recovery)
1	50%	8.01	8.01	100.43
2	75%	36.01	36.10	100.33
3	100%	48.00	48.29	100.49
4	125%	60.01	60.36	100.81
5	150%	72.00	72.73	100.75

*average of six determinations

Table 6. Accuracy for Tinidazole

Sample no	Spike level	Amount of Tinidazole added(ppm)	Amount of Tinidazole found(ppm)	Mean (%recovery)
1	50%	9.61	9.67	100.75
2	75%	43.21	43.38	100.14
3	100%	57.61	57.63	100.08
4	125%	72.01	72.95	100.59
5	150%	86.40	87.06	101.15

*average of six determinations

Table 7. Ruggedness for Ciprofloxacin and Tinidazole

Sample Name	Analyst -1		Analyst -2	
	%Assay of Ciprofloxacin	%Assay of Tinidazole	%Assay of Ciprofloxacin	%Assay of Tinidazole
Sample-1	98.54	99.98	99.13	98.69
Sample-2	97.72	100.37	99.11	98.53
Sample-3	99.19	100.42	99.16	98.62
Sample-4	98.85	99.83	98.94	98.67
Sample-5	98.24	100.87	99.02	98.53
Sample-6	98.73	100.46	99.28	98.88
Average	98.55	100.32	99.11	98.65
%RSD	0.52	0.37	0.11	0.13

Table 8. Robustness by Changing the filters for Ciprofloxacin and Tinidazole

S.No	Name of solution	Average Area			Similarity factor	
1	Unfiltered standard	Ciprofloxacin		Tinidazole	Ciprofloxacin	Tinidazole
		1128771		1563816	-	-
2	Sample filtered through PVDF	Prep-1	1137944	1573809	1.01	1.01
		Prep-2	1130751	1564289	1.00	1.00
3	Sample filtered through NYLON	Prep-1	1563989	1563989	1.00	1.00
		Prep-2	1567315	1567315	1.01	1.00

CONCLUSION

A simple and specific HPLC method has been developed and validated for quantitation of content uniformity of Ciprofloxacin and Tinidazole in tablet blend. The HPLC assay uses a simple pre-treatment and meets commonly accepted criteria for precision, accuracy, robustness, ruggedness and recovery. The sensitivity of this method is within the acceptable range. Hence this method can be employed for routine quality control analysis of Ciprofloxacin and Tinidazole tablet blend.

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