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Research Article

THE SEPARATION AND QUANTITATIVE DETERMINATION OF CIPROFLOXACIN IN A PHARMACEUTICAL FORMULATION BY ULTRA PERFORMANCE LIQUID CHROMATOGRAPH

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ABSTRACT

Objective: A rapid, selective and sensitive ultra performance liquid chromatography method for quantitative determination of ciprofloxacin in pharmaceutical formulation has been developed and validated.

Methods: The assay of ciprofloxacin was carried out by using an octadecyl (C18) with particle size 1.8µm column connected with Waters Acquity UPLC system. A buffer solution was prepared by diluting 2.9ml of phosphoric acid (85%) with 1000ml of water and adjusted to pH 3.0 with triethylamine. The mobile phase was prepared by mixing the buffer and acetonitrile in the ratio of 88:12 v/v respectively. The flow rate was set as 0.3ml minute^{-1}, column oven temperature at 50°C and detection was made at wavelength 278nm.

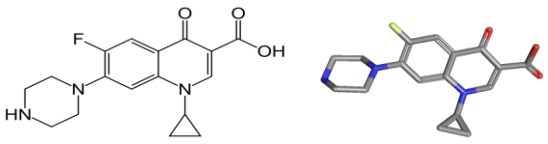
Results: The % RSD of peak areas from five replicates of ciprofloxacin was found to be 0.18, the tailing factor and theoretical plates for ciprofloxacin were found to be 1.20, 17341 respectively. The Resolution between ethylenediamine and ciprofloxacin was found to be 10.41. The linearity of detector response was noticed in the concentration range 6.33µg mL^{-1} to 50.69µg mL^{-1} of ciprofloxacin and the coefficient of variation was obtained as 0.9992.

Conclusion: The obtained results evident that the method is precise and accurate and hence the proposed method is suitable and can be employed for assay of ciprofloxacin in pharmaceutical formulations.

INTRODUCTION

Ciprofloxacin is in a class of antibiotics called fluoroquinolones and is used to treat or prevent certain infections caused by bacteria. In the event of biological warfare, ciprofloxacin may be used to treat and prevent dangerous illnesses that are deliberately spread such as plague, tularemia, and anthrax of the skin or mouth. Ciprofloxacin is available as the monohydrochloride monohydrate salt of 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid and belongs to the group of synthetic fluoroquinolone antibiotics with broad antimicrobial activity [1]. It is believed that the mode of action of this family of drugs is through binding DNA-gyrase enzyme [2]. Fluoroquinolones are considered to be the most effective gram-positive-gram-negative pathogens to combat infections caused by micro organisms that are resistant to other microbial, such as tetracycline. Ciprofloxacin attains therapeutic concentrations in most tissues and body fluids. The results of clinical trials with ciprofloxacin have confirmed its clinical efficacy and low potential for adverse effects. The empirical formula of monohydrochloride monohydrate salt of Ciprofloxacin is $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$ and the molecular weight is 331.346 g/mol. The structure of Ciprofloxacin was shown in Figure 1.

Assay of Ciprofloxacin in pharmaceuticals has previously been achieved by several analytical techniques such as HPLC [3-7], Spectrophotometry [8-10], HPTLC [11], Capillary Zone Electrophoresis (CZE) [12, 13], Chemiluminometry [14-16], enzymatic system [17], Potentiometric titration [18-20] and Voltammetry [21, 22]. However, many of these techniques requires more analysis time. Literature survey revealed that there was no methods were reported for the assay determination of Ciprofloxacin by using Ultra performance liquid chromatographic (UPLC) technique with less chromatographic run time in pharmaceutical dosage forms.



MATERIALS AND METHODS

Chemicals and Reagents

Analytical reagent grade of phosphoric acid was used for buffer preparation and diluted Triethylamine was used for pH adjustment of the buffer to 3.0. The water used for the buffer, standard and sample preparations was purified by Milli Q water purification system (Millipore, Bedford, MA, USA) which was equivalent to highly pure HPLC grade water. The HPLC grade solvent of Acetonitrile was used for the mobile phase preparation. Membrane filter with 0.22 µm size (millipore, Barcelona) was used for the mobile phase filtration. The Ciprofloxacin standard material used for the Assay determination of Ciprofloxacin in pharmaceutical formulation and Ethylenediamine compound was used to ensure system suitability requirement of chromatographic analysis. HPLC grade Methanol, Acetonitrile and water were used for seal wash and needle wash for an ultra performance liquid chromatograph system. The complete analysis was carried out by using "Class A" volumetric glassware for accuracy.

Instruments / Equipments

An acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, Sample manager, column heating compartment was used for assay determination of Ciprofloxacin. UPLC instrument was controlled by Waters Empower chromatographic software. A Waters acquity HSS C18, 100 x 2.1mm, column with particle size of 1.8µm was used as stationary phase for chromatographic separation and determination of Ciprofloxacin. Sartorius semi micro analytical balance was used for all weighing, Thermo orion pH meter was used for buffer pH adjustment, Rotary shaker used to dissolve the standard, sample and were filtered about 2ml through 0.22µm.

Standard, System suitability and Sample Solution Preparation

The Ciprofloxacin standard stock solution was prepared by dissolving an accurately weighed amount of Ciprofloxacin Hydrochloride standard in water to achieve 0.3 mg mL^{-1} concentration, then the standard stock solution further diluted with water to achieve final concentration of 0.03 mg mL^{-1}. The

system suitability solution was prepared by using Ciprofloxacin Hydrochloride and Ciprofloxacin Ethylenediamine compound to achieve final concentration of 0.025 mg mL^{-1} and 0.025 mg mL^{-1} of Ciprofloxacin and Ethylenediamine compound respectively in same solution. The sample preparation of Ciprofloxacin in pharmaceutical formulations was a critical step as it involves the extraction of Ciprofloxacin from other formulated compositions. The Ciprofloxacin pharmaceutical formulation needs to be grinded in to powder form and was diluted with water to achieve a concentration of 1mg mL^{-1} and kept on rotary shaker till the grinded powder dissolves completely with an intermediate shaking to ensure extraction of ciprofloxacin from its pharmaceutical formulation. After completion of rotary shaking of 1mg mL^{-1} sample solution, further diluted with water to achieve final concentration of ciprofloxacin as 0.025mg mL^{-1}.

Chromatographic Conditions

An Ultra Performance Liquid Chromatograph (UPLC) was used to carry out the chromatographic analysis. The Ciprofloxacin was separated from its process impurities and formulated impurities by using an Acquity HSS C18 column, 100mm length, 2.1mm internal diameter and packed with particle size of 1.8µm. An isocratic pumping program with a flow rate of 0.3 ml $minute^{-1}$ at a column temperature of 50°C and the absorbance of Ciprofloxacin was measured at 278nm of UV wavelength. The injection volume set as 2µL. For strong needle wash, acetonitrile and water in the ratio of 88:12 v/v, weak needle wash, water and acetonitrile in the ratio of 88:12 v/v and for seal wash, water and methanol in the ratio of 70:30 v/v was used along with sample loop option as partial loop with needle overfill. Buffer prepared by diluting 2.9ml of phosphoric acid (85%) in 1000ml of water and adjusted to pH 3.0 with triethylamine. The mobile phase was prepared by mixing of buffer and acetonitrile in the ratio of 88:12 v/v respectively. The retention time of Ciprofloxacin found at about 3.1minutes and hence run time of chromatographic analysis was fixed as 4.0 minutes.

Method Validation

The method was verified to determine the system precision and system suitability parameters and validated for specificity, linearity of detector response, Precision of the method, accuracy, linearity of method, ruggedness and robustness as per the International Conference on Harmonization (ICH) guidelines [23, 24].

System Precision and System suitability

The standard solution was prepared by using Ciprofloxacin Hydrochloride standard as per test method and injected five times into the chromatographic system. Monitored the % Relative standard deviation of peak areas from five replicates of Ciprofloxacin, the tailing factor and theoretical plates for Ciprofloxacin peak, and the Resolution between Ethylenediamine compound and Ciprofloxacin peak.

Specificity

A study to establish the interference of placebo was conducted. Assay test was performed on Granules placebo equivalent the amount present in the test preparation in triplicate as per test method and injected into system. Review the chromatograms for the placebo interference at the retention time of Ciprofloxacin.

Linearity of Detector Response

A study to establish the linearity of detector response of standard solution was conducted from 6.33 µg mL^{-1} to 50.64 µg mL^{-1} Ciprofloxacin of the standard concentrations. The above study was established that the detector linearity is from 25% to 200% of the target standard concentration (25µg mL^{-1}) and calculated the coefficient of variation.

Precision of Method

The precision of test procedure was evaluated for Ciprofloxacin by performing Ciprofloxacin Granules assay on six sample units as per the test method. The % assay of Ciprofloxacin, the % Relative standard deviation of individual % assay of six Ciprofloxacin sample units was calculated.

Accuracy

A study of Accuracy was conducted. Assay test was performed by analyzing six samples each at 50% and 150% levels and three samples each at 75%, 100% and 125%levels from the target concentration (25 µg mL^{-1}). Calculated the recovery of Ciprofloxacin obtained from the chromatographic analysis at each level of 50%, 75%, 100%, 125% and 150% of the labeled amount of Ciprofloxacin present in the test preparation as per the test method. The mean % assay recovery of Ciprofloxacin, the % relative standard deviation of individual % assay of Ciprofloxacin was calculated.

Linearity of Method

A graph was plotted to the "mg/ml added," versus "mg/ml found" from Accuracy section and calculated the correlation coefficient of Ciprofloxacin. The linearity study was established from 50% to 150% of the labeled amount of Ciprofloxacin present in the test sample (25 µg mL^{-1}).

Ruggedness

To demonstrate ruggedness of test method, System to System, Analyst to Analyst and Column to Column variability study was conducted on Ciprofloxacin by performing Ciprofloxacin Granules assay on six units using two different Systems, Columns and with different Analysts on different days, under similar conditions as per the test method. The bench top stability of Ciprofloxacin standard and test preparation was established over a period of 2 days. Both standard preparation and test preparation (in duplicate) were stored on bench top and analyzed at initial, day-1 and day-2 intervals against a freshly prepared standard each time. The difference in % assay of test preparation from initial to day-1 and the similarity factor for standard preparation were calculated. The refrigerator stability of Ciprofloxacin standard and test preparation was established over a period of 2 days. Both standard and test preparation (in duplicate) were stored in refrigerator and analyzed at initial, day-1 and day-2 intervals against a freshly prepared standard each time. The difference in % assay of test preparation from initial to day-1 and the similarity factor for standard preparation were calculated. The bench top stability of mobile phase was established over a period of 2 days. Mobile phase was prepared as per the test method and stored on bench top and analyzed for stability at Initial, day-1 and day-2 by injecting a freshly prepared standard, system suitability solution and test preparation. The standard and system suitability solution preparation was evaluated for system suitability parameters and test preparations were evaluated for % assay each time as per the test method.

Robusness

Robustness for mobile phase composition was evaluated by varying the organic phase composition from 90% to 110% of the actual composition of mobile phase. Injected standard and system suitability solution preparation with 90% and 110% of the actual composition of the mobile phase and evaluated the system suitability parameters. Robustness for flow rate was evaluated by varying the flow rate from 0.27 ml/min to 0.33 ml/min. Injected standard and system suitability solution preparation with 0.27 ml/min to 0.33 ml/min flow rate and evaluated the system suitability parameters. Robustness for column temperature was evaluated by varying the column temperature from 45°C to 55°C. Injected standard and system suitability solution preparation with 45°C and 55°C column temperature and evaluated the system suitability parameters. Robustness for pH of mobile phase was evaluated by varying the pH from 2.8 to 3.2 Injected standard and system suitability solution preparation with pH 2.8 to 3.2 and evaluated the system suitability parameters. Robustness for filter

validation was evaluated by varying two different filters namely, 0.45µm SY25NH syringe filters and 0.45µm SY25VF syringe filters. Prepared test (duplicate) by spiking standard stock solution on placebo (equivalent to test concentration) as per test method and filter different portions of the test preparation through different filters, injected in to the system along with unfiltered standard and evaluated the similarity factor.

RESULT AND DISCUSSION

An ultra performance reverse phase chromatographic technique was used to determine the % of Ciprofloxacin present in the pharmaceutical formulation. Based on the solubility study of Ciprofloxacin, water was chosen as diluents to prepare standard, system suitability and sample solutions. The phosphate buffer with pH 3.0 was found more appropriate for asymmetric peak shape, robust resolution and for better separation of Ciprofloxacin from its impurities and placebo. The buffer strength selected to reduce load on column and to achieve good symmetric peak. The presence of organic solvents in the mobile phase was studied. Acetonitrile was used as an organic modifier. A non polar C18 stationary phase was used for Ciprofloxacin separation as it shows polar nature. When Acquity HSS C18 column used as a stationary phase, the method is more selective, sensitive and achieved well separation of Ciprofloxacin from its impurities and degrades within a short run time and also more rugged with respect to the variations in the mobile phase compositions.

The concentration of Ciprofloxacin was established according to the absorbance at wavelength 278nm which was given good recovery through out its range of the analytical method. The utilization of advanced chromatographic technique such as Ultra Performance Liquid Chromatograph resulted precise, accurate method with less run time which benefits to quality control or routine testing of Ciprofloxacin. The required selectivity, separation and symmetry of Ciprofloxacin peak was achieved within the short run time of 4 minutes. The developed analytical method was subjected for further validation as per the current ICH guidelines to use for its intended purpose.

The observed values of system suitability parameters such as tailing factor for Ciprofloxacin peak was 1.2, theoretical plates were 17341, % relative standard deviation of the area response of Ciprofloxacin was 0.18% and the resolution between Ethylenediamine compound and Ciprofloxacin peaks were found 10.4 which are well within the prescribed acceptance criteria.

Specificity study of the analytical method was demonstrated the well separation of Ciprofloxacin peak from its impurities and placebo interference. As there were no peaks of impurity, placebo was eluted at the same retention time of Ciprofloxacin peak.

The observed coefficient of variation results (R^2 = 0.9992) from the plotted calibration curve with the Ciprofloxacin peak area versus concentration throughout the range of about 6.33µg mL^{-1} to 50.69µg mL^{-1} evidenced that the method was linear throughout its concentration range. The data of regression analysis of the Linearity plot was shown in Table 1 and Figure 2.

Table 1: Linearity results of ciprofloxacin concentration and peak area

S. No	Linearity Level	Concentration (µg/mL)	Peak Area
1	25%	6.3374	238656
2	50%	12.6747	558701
3	75%	19.0122	931017
4	100%	25.3495	1253301
5	125%	31.6869	1587482
6	150%	38.0243	1852165
7	175%	44.3617	2211321
8	200%	50.6991	2560164

The resulted coefficient of variation from above data is 0.9992

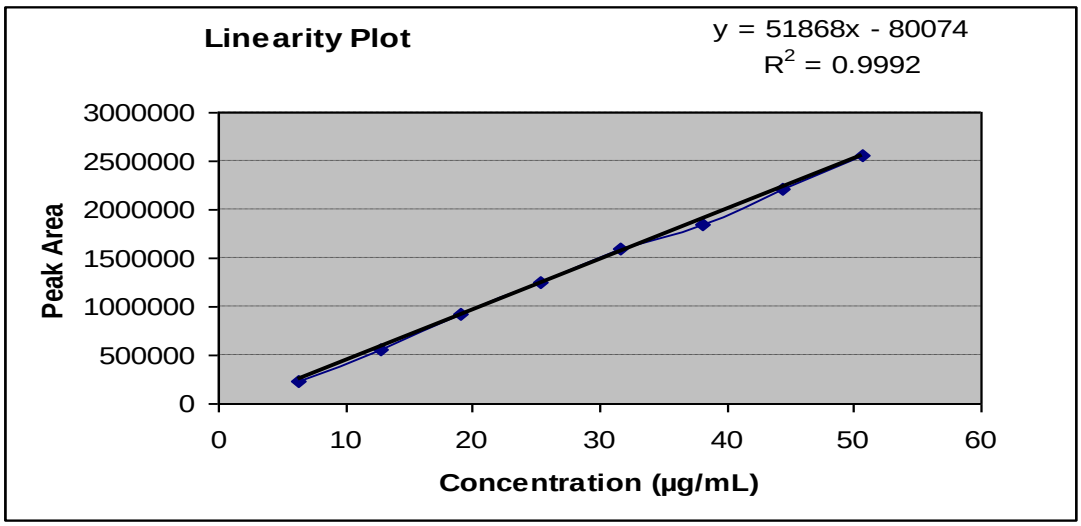


Fig. 2: Linearity Plot of Ciprofloxacin

The relative standard deviation of Ciprofloxacin content in six different preparations was found less than 2.0% during repeatability test and it was evidenced that the method is more precise as shown in Table 2.

Table 2: Results of Repeatability study of ciprofloxacin

S. No.	Preparation No.	% Assay of Ciprofloxacin
1	Preparation No - 1	100.54%
2	Preparation No - 2	100.45%
3	Preparation No - 3	99.65%
4	Preparation No - 4	100.39%
5	Preparation No - 5	100.78%
6	Preparation No - 6	99.65%
Average % Assay of Ciprofloxacin		100.24%
% Relative Standard Deviation		0.5%

The recovery results of method was found between 97.0% and 103.0% throughout its concentration levels of 50%, 75%, 100% 125% and 150% of target concentration (25µg/mL) which was demonstrated the accuracy of analytical method throughout its range as shown in Table 2.

Table 3: Results of Recovery (Accuracy) study of ciprofloxacin

S. No.	Level in %	"µg/mL" added	"µg/mL" found	% Recovery	% RSD
1	50%	12.4	12.2	98.39	
2	50%	12.5	12.6	100.80	
3	50%	12.5	12.2	97.60	
4	50%	12.4	12.2	98.39	
5	50%	12.5	12.5	100.00	
6	50%	12.4	12.2	98.39	1.22
1	75%	18.7	18.4	98.40	
2	75%	18.6	18.4	98.92	
3	75%	18.5	18.3	98.92	0.30
1	100%	25.1	25.2	100.40	
2	100%	24.9	24.5	98.39	
3	100%	25.0	24.8	99.20	1.01
1	125%	31.6	32.0	101.27	
2	125%	31.7	32.2	101.58	
3	125%	31.4	32.0	101.91	0.31
1	150%	38.0	38.3	100.79	
2	150%	38.0	38.3	100.79	
3	150%	37.9	38.6	101.85	
4	150%	37.8	38.6	102.12	
5	150%	38.1	39.0	102.36	
6	150%	37.9	38.8	102.37	0.73

From the above study it was established that the linearity of test method is from 50% to 150% of the labeled amount of Ciprofloxacin present in the test sample and the correlation coefficient of Ciprofloxacin was found to be 0.9998.

The comparison of the obtained results on two Systems, Analysts and Columns shows that the Assay method was rugged for system to System, Column to Column and Analyst to Analyst variation.

The results obtained from the bench top stability and refrigerator solution study of standard and test preparations, the difference in % assay of test preparation from initial to day-1 was not varied by 3.0% from the initial value. The similarity factor for standard preparation was found to be within 0.98 to 1.02 for a period of one day (24 hours) on bench top. From the above study it was established that the standard and test preparations are stable up to one day (24 hours) on bench top and refrigerator conditions.

The results obtained from the study on bench top stability of mobile phase evidences that the mobile phase was stable up to 2 days on bench top as there was no significant variation in system suitability parameters and % assay of sample between each day result of bench top stored mobile phase.

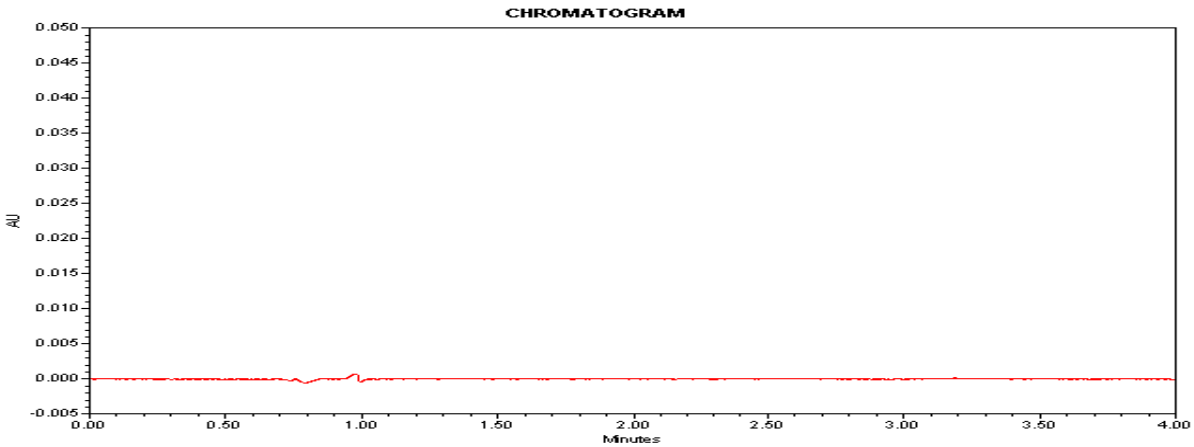


Fig. 3(a): Specimen Chromatogram – Ciprofloxacin Assay - Blank

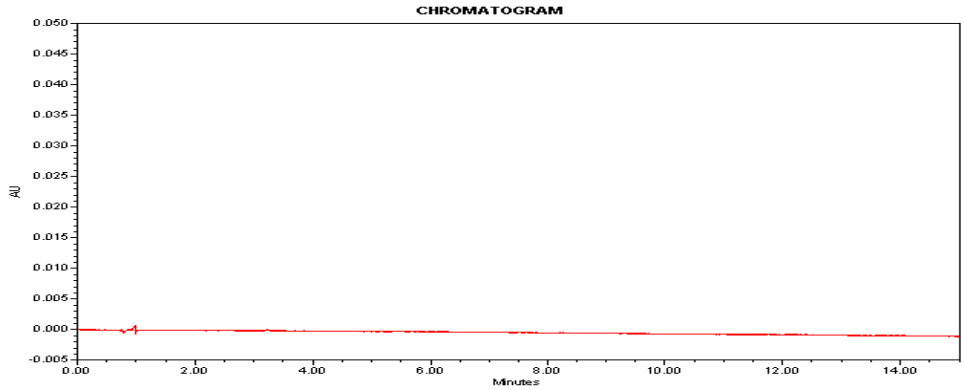


Fig. 3(b): Specimen Chromatogram – Ciprofloxacin Assay - Placebo

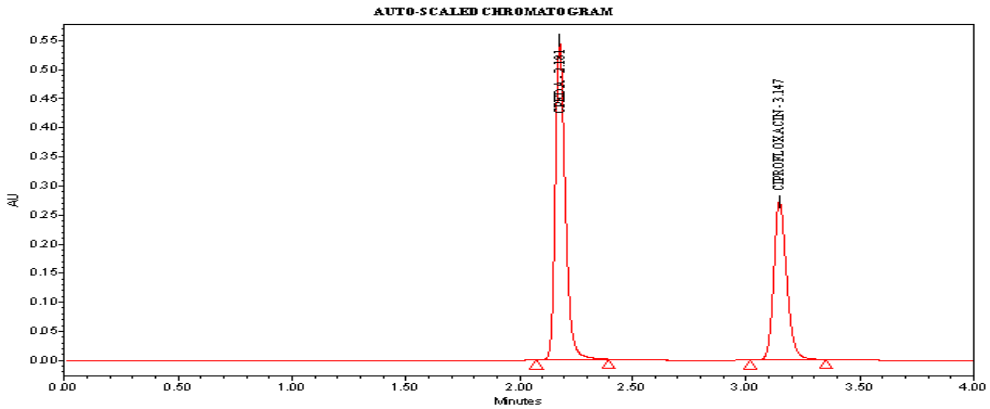


Fig. 3(c): Specimen Chromatogram – Ciprofloxacin Assay - Resolution

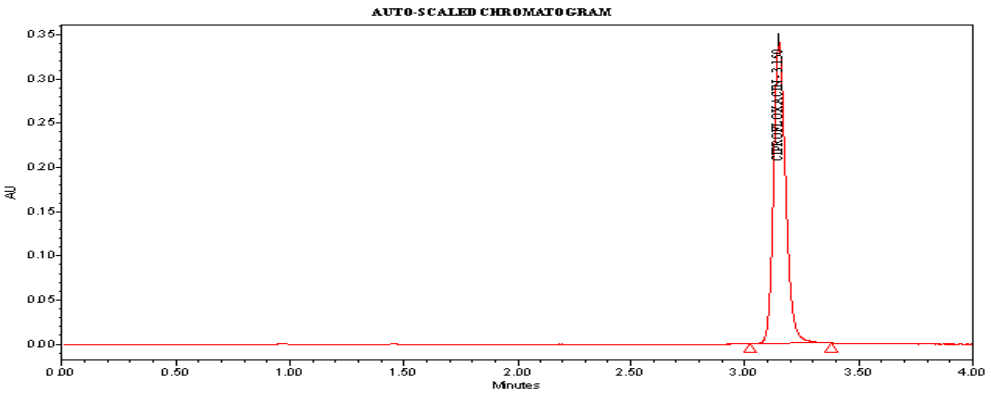


Fig. 3(d): Specimen Chromatogram – Ciprofloxacin Assay - Standard

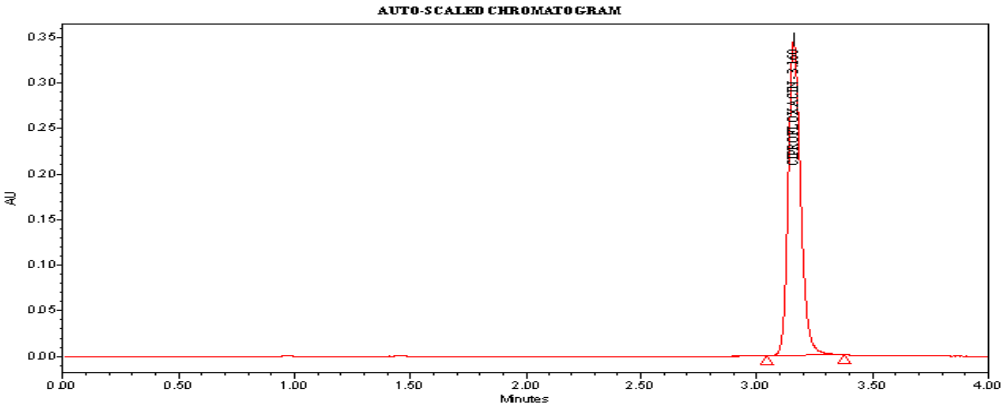


Fig. 3(e): Specimen Chromatogram – Ciprofloxacin Assay - Sample

The results obtained from effect of variation in mobile phase composition, effect of variation in flow rate, effect of variation in column temperature and effect of variation in mobile phase pH as part of robustness study were found no significant variation in system suitability parameters and also meeting with the prescribed acceptance criteria of system suitability. The similarity factor results observed within 0.98 to 1.02 from the filter validation study. These observations evidenced that the reported method was robust enough for the intended purpose.

The Chromatograms generated for blank, system suitability, standard and sample of Ciprofloxacin by using above validated method on an Ultra performance liquid chromatograph system were shown in Figure: 3.

Figure 3: Specimen Chromatograms of Ciprofloxacin Blank, Placebo, Standard, Sample, and system suitability solutions.

CONCLUSION

The above data proved that the analytical method developed and validated for assay determination of Ciprofloxacin in pharmaceutical formulations is more precise, accurate, robust and rugged throughout its range and can be more useful for commercial applications with unique advantage of less analysis time and cost effective. The reported method was rapid, simple and selective for routine testing of Ciprofloxacin for pharmaceutical quality control applications.

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