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RS Method Development and Validation for Quantification of Ciprofloxacin Lactate and its Impurities by RP-UPLC

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ABSTRACT:

A stability indicating UPLC method has been developed for determination of ciprofloxacin lactate and its related substances. Optimum separation of drug and impurities was achieved in 5 minutes using ACQUITY UPLC BEH C18 (100x2.1mm, 1.8µm) column. The analytes were eluted by using 0.025M Ortho phosphoric acid and ACN (87:13) pH adjusted to 3.0 with TEA at flow rate of 0.3ml/min at wavelength of 278nm by using PDA detector. The retention times were found to be 1.13, 2.00, 2.30, 3.34min for Decarboxy, Desfluoro, Ethylenediamine, CFX respectively. The method is validated according to ICH guidelines. Linearity was found in the range of LOQ-150% and shows a correlation coefficient of 0.9999, 0.9997, 0.9999, 0.9998 for Decarboxy, Desfloro, Ethylenediamie impurities and CFX. For stability studies drug was subjected to acid, alkali, oxidation, photolytic and thermal degradation. Degradation was observed and their peaks are well separated from drug peak. The method can be employed for routine analysis of ciprofloxacin lactate and its related substances.

Keywords: Ciprofloxacin lactate, Decarboxy, Desfloro, Ethylenediamie, RP-UPLC, Validation, Stability Indicating.

1. Introduction

Ciprofloxacin is a second generation fluoroquinolone that has spawned many derivative antibiotics [1-8]. It is formulated for oral, intravenous, intratympanic, ophthalmic, and otic administration for a number of bacterial infections. Ciprofloxacin injection is in a class of antibiotics called fluoroquinolones. It works by killing bacteria that cause infections. Antibiotics such as ciprofloxacin injection will not work for colds, flu, or other viral infections. Ciprofloxacin is used to treat a wide variety of infections, including infections of bones and joints, endocarditis, gastroenteritis, malignant otitis externa, respiratory tract infections, cellulitis, urinary tract infections, prostatitis, anthrax, and chancroid.

Extensive literature review was conducted and an attempt was made to develop an unambiguous, valid method for the simultaneous estimation of ciprofloxacin lactate with its impurities. Few of spectroscopic, chromatographic, and other analytical methods [8-15] have been reported for the estimation of ciprofloxacin lactate individually and or along with drug combinations in pharmaceutical preparations. The aim of this study is to develop and validate a new simple, accurate and economic stability-indicating RP-UPLC method with less runtime, which would be able to separate and quantify ciprofloxacin lactate with its impurities in a single run. The developed method was validated as per ICH guidelines [16-17] and can be applied lucratively to quality control purposes.

The newly developed analytical method finds their importance in various fields like Research institutions, Quality control department in industries, Approved testing laboratories, Bio-pharmaceutics and Bio-equivalence studies, and Clinical pharmacokinetic studies.

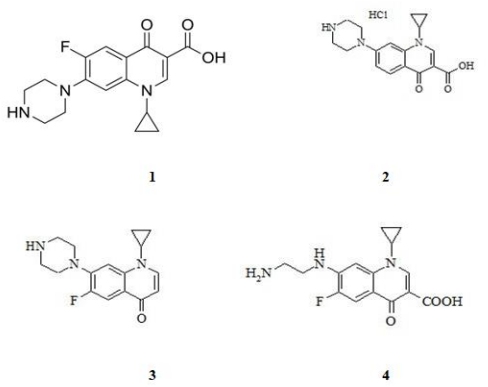


Figure 1. Chemical structures of (1) Ciprofloxacin (2) Desfluoro impurity (3) Decarboxy impurity (4) Ethylene diamine impurity

2. Materials and Methods

Equipment

The Method development and validation was carried out using ACQUITY UPLC-H Class system equipped with binary pump, auto sampler and photo diode array (PDA) detector. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents

Ciprofloxacin lactate and impurities working standards were kindly given as gift samples by Mylan Laboratories ltd, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on ACQUITY UPLC-H Class system equipped with binary pump, auto sampler and photo diode array (PDA) detector and the data was acquired by Empower® version 2. Separation was achieved using C18 100x2.1mmx1.8µm as a column with mobile phase of 0.025M ortho phosphoric acid, pH 3.0 buffer and Acetonitrile in the ratio 87:13. The samples were analyzed using 1µL injection volume, Flow rate was maintained at 0.3 mL/min with runtime of 5 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 278 nm wavelength.

Preparation of standard solution

0.5 mg of ciprofloxacin drug is weighed accurately and transferred to a 10 mL clean dry volumetric flask, 5 mL of diluent was added and sonicated for 10 minutes to dissolve. The final volume was made up with the diluent and filtered through 0.45 µ nylon filter to obtain the solution with a concentration of 50 ppm. From the above stock solution 0.2 mL was pipetted out in to a 10 mL volumetric flask and then made up to the final volume with diluent to obtain a concentration of 1ppm solution.

Preparation of impurities standard solutions

0.5 mg of Impurities working standards A, B, and C were accurately weighed and transferred to a 10 mL clean dry volumetric flask, 5 mL of diluent was added and sonicated for 10 minutes to dissolve. The final volume was made up with the diluent and filtered through 0.45 µ nylon filter to obtain a concentration of 50 ppm solutions.

Method Validation

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

System suitability

System suitability parameters were evaluated to verify system performance. standard solutions of ciprofloxacin and its impurities were injected five times into the chromatograph, and the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined.

Specificity

The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solutions individually to investigate interference from the representative peaks.

Precision

Repeatability/ method precision was performed by injecting six replicates of standard solutions of ciprofloxacin and its impurities, calculated % assay and %RSD for each compound. Reproducibility/ Ruggedness/ Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (50%, 100% and 150%) of the working standard solutions of ciprofloxacin and its impurities to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity

Linearity was evaluated by analysing different concentrations of the standard solutions of ciprofloxacin and its impurities. Working standard solutions ranging between 0.001µg/mL-0.015µg/mL for ciprofloxacin, 0.0001µg/mL – 0.0005µg/mL for all three impurities respectively were prepared and

injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and Limit of quantification

Limit of detection (LoD) and limit of quantification (LoQ) of ciprofloxacin and its impurities were determined by calibration curve method. Standard solutions of ciprofloxacin and its impurities were prepared in linearity range and injected (n = 3).

Robustness

To examine the robustness of the developed method, experimental conditions were deliberately changed, resolution, tailing factor, and theoretical plates of peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.02mL/minute. The effect of column temperature on the developed method was studied at ± 2°C and the mobile phase composition was changed ±5% from the initial composition of the organic phase. In all the above varied conditions, the aqueous component of the mobile phase was held constant.

Forced Degradation Studies

Stress studies were performed by injecting working standard solutions of ciprofloxacin and its impurities to provide the stability-indicating property and specificity of the proposed method. Intended degradation was attempted by the stress conditions of exposure to photolytic stress by exposing to UV light and white fluorescent light (1.2 million lux hours followed by 200 Watt hours), heat (exposed at 60°C for 3,6,12,24 hours), acid (treating with 1 N HCl at 60°C for 3,6,12,24 hours), base (treating with 1 N NaOH at 60°C for 3,6,12,24 hours), oxidation (treating with 5% peroxide for 3 hours at 60°C). The solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

3. Results and discussion

System Suitability

From the results in table 1, the column efficiency for ciprofloxacin and its impurities peaks were identified from the theoretical plate count which is more than 3000, tailing factor less than 2.0, %RSD was found to be less than 2.0%. The resolution of the peaks was also found to be within the limits.

Table 1. System suitability data

Parameter	Ciprofloxacin	Decarboxy impurity	Desflouro impurity	Ethylene diamine impurity	Acceptance criteria
RT	3.34	1.13	2.00	2.30	--
USP Plate count	4538	3724	3805	4302	NLT 3000
Peak Tailing	1.3	1.3	1.2	1.3	NMT 2.0
Resolution	5.37	--	5.02	3.80	NMT 2.0

Specificity:

From the obtained chromatograms in figures 5 to 10 it can be inferred that there were no co-eluting peaks at the retention time of ciprofloxacin and its impurities which shows that peak of analyte was pure and the excipients in the formulation did not interfere with the analyte of interest.

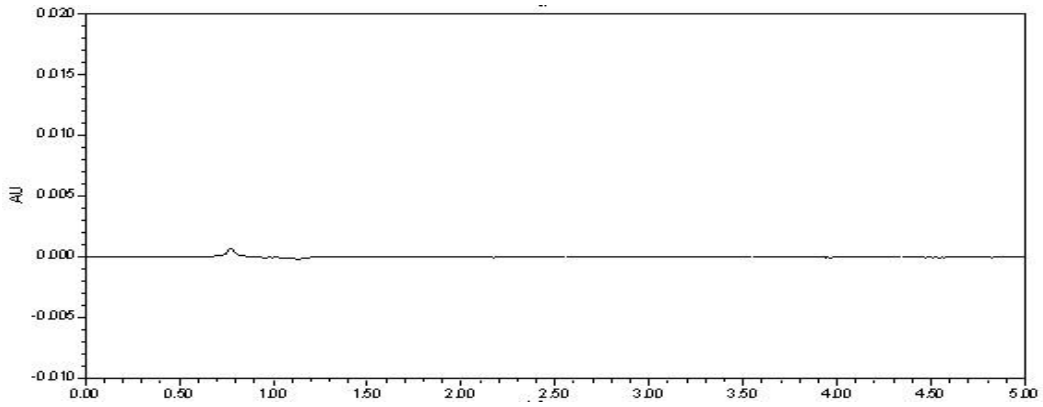


Figure 5. Chromatogram of blank

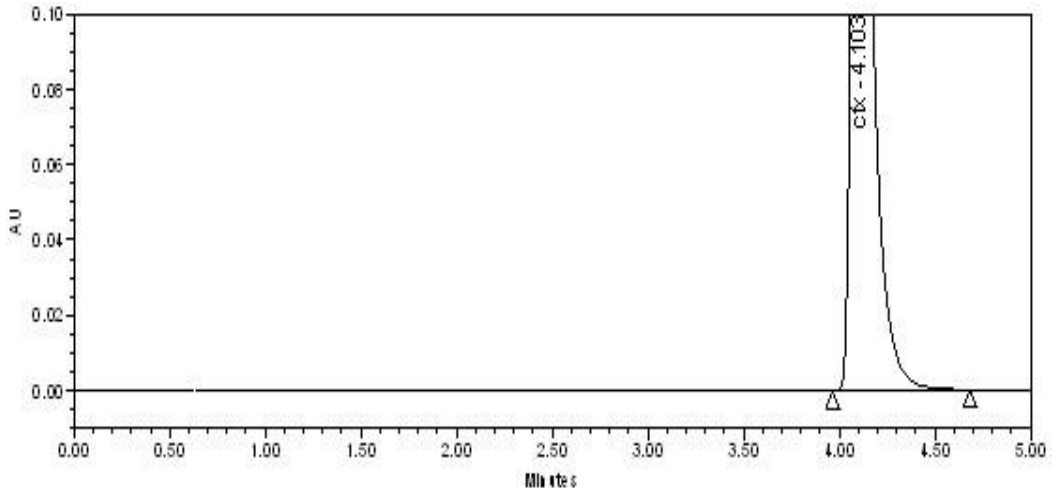


Figure 6. Chromatogram of Ciprofloxacin lactate

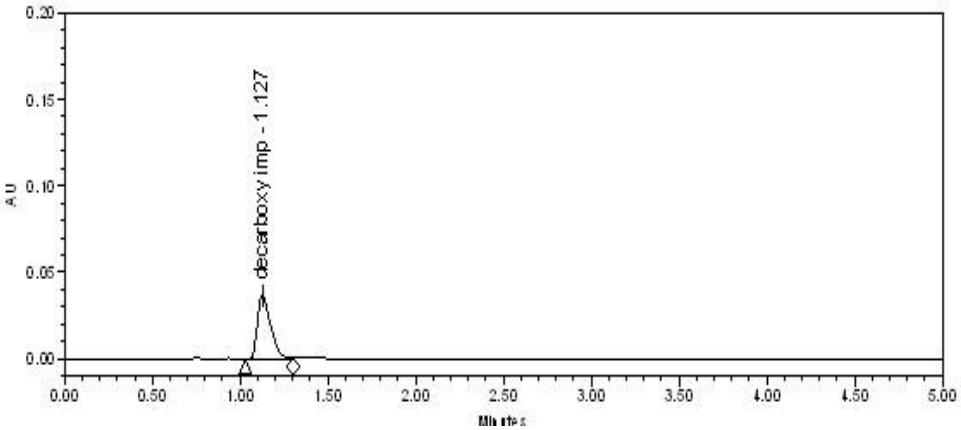


Figure 7. Chromatogram of decarboxy impurity

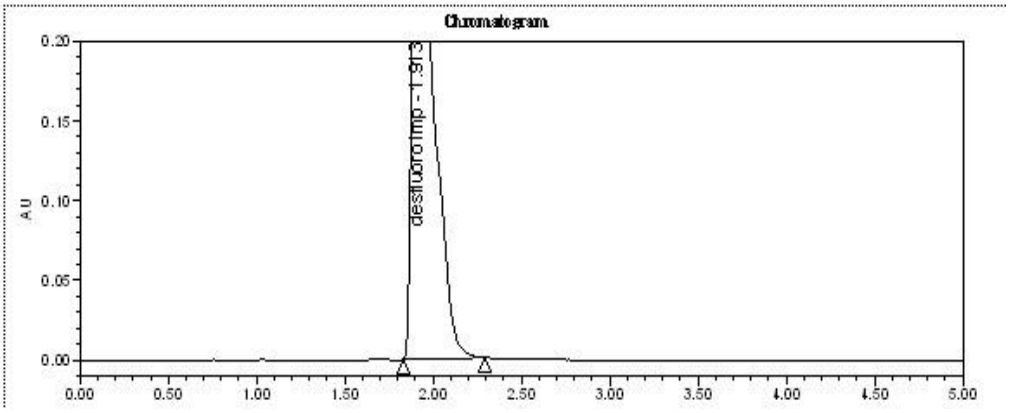


Figure 8. Chromatogram of desflouro impurity

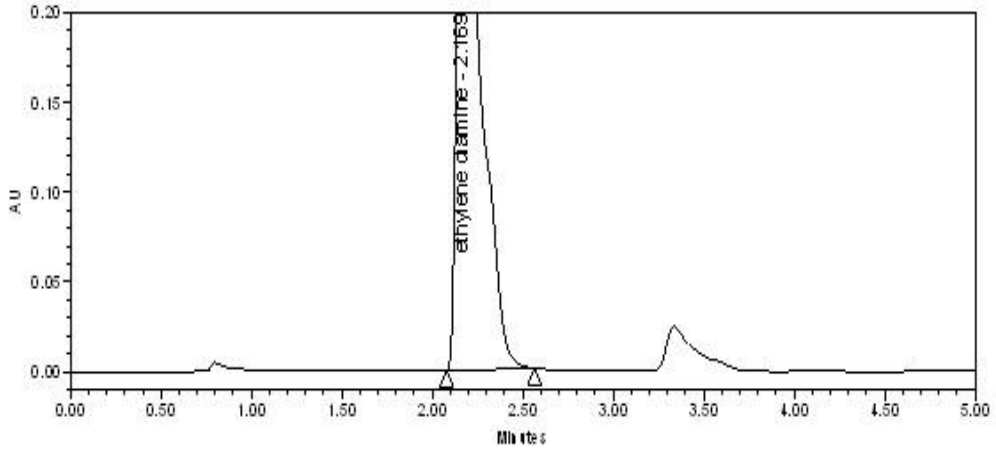


Figure 9. Chromatogram of Ethylene diamine impurity

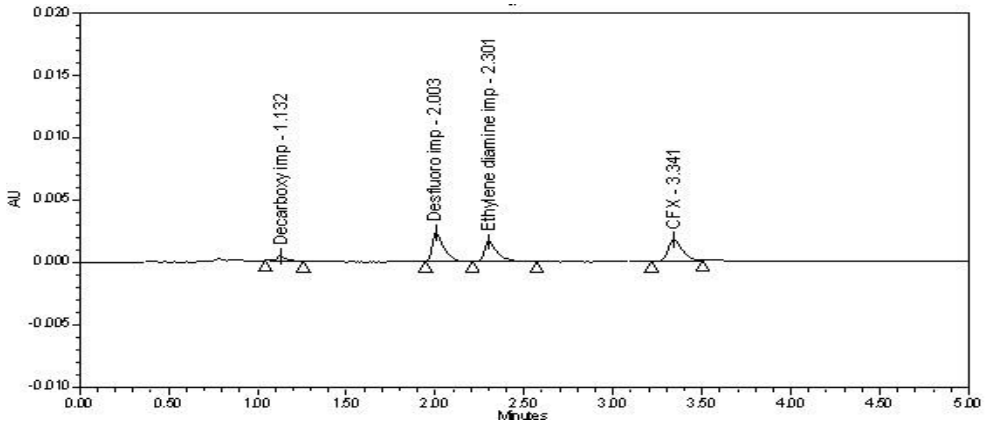


Figure 10. Chromatogram of ciprofloxacin with its impurities

Precision

From the results in table2, % RSD for ciprofloxacin and its impurities was found to be within 2% and from the results tabulated from table 3, % Assay of ciprofloxacin and its impurities was found to be in the range of 98 – 102%. Hence the method is said to be precise, reproducible and rugged for 48 hours' study.

Table 2. System Precision data

No of Injections	**Peak Area**			
	Ciprofloxacin	**Decarboxy Impurity**	**Desfluoro impurity**	**Ethylene diamine impurty**
Inj 1	8177	4045	10573	7486
Inj 2	8224	3999	10372	7674
Inj3	8164	4179	10514	7325
Inj 4	8503	4076	10318	7697
Inj 5	8161	4010	10497	7375
Inj 6	8390	4063	10212	7575
Average	8269.78	4062.19	10414.41	7521.93
Std. Dev	143.45	64.58	136.99	153.64
%RSD	**1.7**	**1.6**	**1.3**	**2.0**

Table 3. Method Precision data

No. of injections	**% Assay**			
	Ciprofloxacin	**Decarboxy impurity**	**desfluoro impurity**	**Ethylene diamine impurity**
Inj 1	100.2	99.8	97.4	100.8
Inj 2	100.5	98.8	98	102.2
Inj 3	101.1	99.2	99	100.8
Inj 4	100.9	99.4	98	101.6
Inj 5	100.8	100.6	100	100.1
Inj 6	101.3	99.8	98.2	101.6
Average	100.8	99.6	98.4	101.1
Std. Dev	68.5	54.8	75.6	65.2
%RSD	0.7	0.6	0.8	0.8

Linearity

Linearity was evaluated by analysing different concentrations of ciprofloxacin and its impurities. From the results tabulated in table 4, it is inferred that the correlation coefficient was greater than 0.999. The slope and y-intercept values were also provided, which confirmed good linearity between peak areas and concentration.

Table 4. Linearity data of ciprofloxacin and impurities

Conc.(mg/ml)	**Peak areas of impurities**			**Ciprofloxacin**	
	Decarboxy impurity	**desfluoro impurity**	**Ethylene diamine impurity**	**Conc.(mg/ml)**	**Peak areas**
0.0001	863	2443	1865	0.001	1958
0.0002	1548	4211	3763	0.006	3854
0.0003	2237	6309	5325	0.009	5684
0.0004	3018	8227	7354	0.012	7652
0.0005	3964	10105	8896	0.015	9521
Slope	8083844	18844485	17950617	--	17875331
Intercept	0.9968	-618.17	66.39	--	347.4
Correlation coefficient	0.9999	0.9997	0.9999	--	0.9998

Accuracy

From the results in table 5, the % recovery for ciprofloxacin and its impurities found to be in the range of 98 –102% and the % RSD for ciprofloxacin and its impurities is less than 2%. Hence the proposed method was accurate.

Table 5. Accuracy data

	% RSD & Recovery at each level			
Name of The Impurity	**At LOQ level**	**At 40 % level**	**At 100 % level**	**At 150% level**
Ethylene diamine impurity	98.8	99.8	102.8	99.3
	99.0	99.7	102.2	99.6
	99.1	100.1	103.1	99.1
% RSD	**0.2**	**0.2**	**0.5**	**0.6**
Desfluoro impurity	99.5	100.1	98.2	99.7
	99.4	100.5	98.3	100.1
	99.2	99.4	99.5	101.1
%RSD	**0.2**	**0.4**	**0.8**	**0.2**
Decarboxy impurity	96.5	100.2	98.7	96.4
	97.3	99.2	99.2	95.9
	97.5	99.4	99.9	97.1
%RSD	**0.5**	**0.5**	**0.6**	**0.6**

LoD and LoQ

The Limit of Detection and Limit of Quantification were calculated by using following equations (ICH, Q2 (R1)) and the LoD values are reported in table 6 and LoQ values are reported in table 7. These LOD = 3.3 × σ/S and LOQ = 10 × σ/S

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

Table 6. LOD data

Impurity Name	**Decarboxy impurity**	**Ethylene diamine impurity**	**Desfluoro impurity**	**Ciprofloxacin lactate**
LOD Concentration	0.00003	0.00003	0.00006	0.00006
LOD (%) Concentration with respect to test	0.003	0.003	0.006	0.006

Table 7. LOQ data

Impurity Name	**Decarboxy impurity**	**Ethylene diamine impurity**	**Desfluoro impurity**	**Ciprofloxacin lactate**
LOQ Concentration	0.00010	0.00010	0.00020	0.00020
LOQ (%) Concentration with respect to test	0.01	0.01	0.02	0.02

Robustness:

From the results in table 8, it is evident that the system suitability parameters such as resolution, RSD, tailing factor, and the theoretical plate count of ciprofloxacin and its impurities remained unaffected by deliberate changes. The results were presented along with the system suitability parameters of optimized conditions. Thus, the method was found to be robust with respect to variability in applied conditions.

Table 8. Robustness data

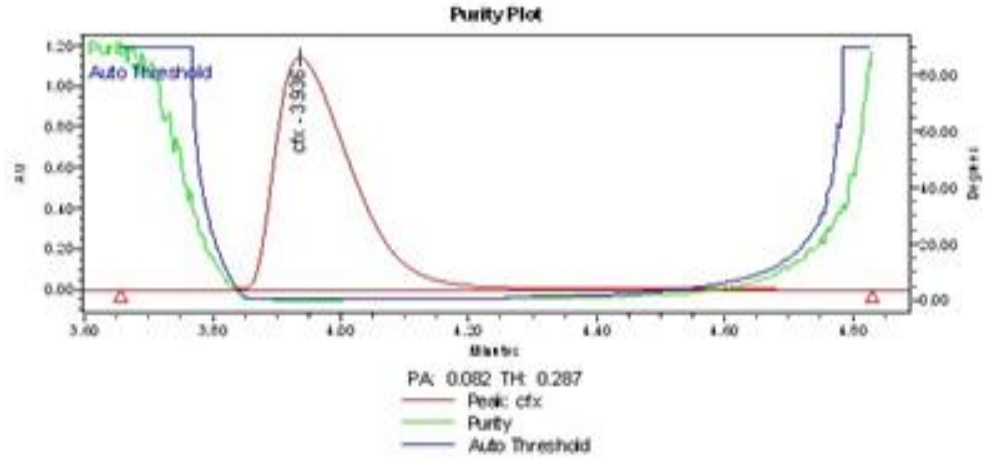
S.NO	**Peak name**	**RT**	**Area**	**%Area**	**USP Tailing**	**USP Resolution**
Low flow rate						
1	CFX-Pharma	3.26	8308056	99.60	1.36	8.08
2	Decarboxy impurity	1.26	7590	0.1	1.05	--
3	Desfluoro impurity	2.24	10256	0.12	1.08	2.62
4	Ethylene diamine impurity	2.58	15842	0.19	1.14	6.08
High flow rate						
1	CFX-Pharma	3.11	13255	40.79	1.3	7.34
2	Decarboxy imp	1.08	5119	15.75	1.95	--
3	Ethylene diamine impurity	2.16	6345	19.53	1.92	6.37
4	Desfluoro imp	1.88	7779	23.54	1.71	2.76
Low temperature						
1	Decarboxy imp	1.25	3678	10.70	1.7	--
2	Desfluoro imp	2.22	12614	35.83	1.8	6.08
3	Ethylene diamine	2.56	9016	25.61	1.4	2.73
4	CFX	3.71	9807	27.86	1.2	8.72
High temperature						
1	Decarboxy imp	1.26	6612	16.32	1.91	--
2	Desfluoro imp	2.24	13260	32.61	1.72	6.08
3	Ethylene diamine	2.56	10987	27.02	1.8	2.62
4	CFX	3.76	9776	24.05	1.32	8.08

Forced degradation studies

The results obtained in the solid state stability study indicate that CFX is stable upon exposure to white fluorescent light. The results obtained in the force degradation study CFX were stable at different stress conditions. The specificity of the method was confirmed and the method is stability-indicating.

Table 9. Degradation studies data

S.NO	Peak name	RT	Area	%Area	Purity angle	Purity threshold	Purity test
Acid							
1	CFX-Pharma	4.07	1888966	99.98	0.609	1.093	Pass
2	Ethylene diamine impurity	2.75	327	0.01	14.25	18.15	Pass
3	Decarboxy impurity	1.71	354	0.01	28.699	90.00	Pass
Base							
1	CFX-Pharma	4.07	1875621	99.94	0.262	1.026	Pass
2	Ethylene diamine impurity	2.75	542	0.03	2.56	7.54	Pass
3	Decarboxy impurity	1.71	483	0.02	0.025	0.32	pass
4	Degradent	1.17	284	0.01	0.889	1.290	Pass
Oxidation							
1	CFX	3.96	1856324	98.89	0.58	1.12	Pass
2	Ethylene diamine impurity	2.70	382	0.02	6.24	10..25	Pass
3	Degradent	3.52	456	0.03	3.21	6.57	Pass
4	Decarboxy impurity	1.71	865	0.06	1.25	5.16	Pass
UV (Photolytic)							
1	CFX-Pharma	4.10	1884175	99.96	30.57	48.46	Pass
2	Ethylene diamine impurity	2.75	354	0.02	25.31	28.52	Pass
3	Decarboxy impurity	1.74	312	0.02	0.035	0.25	Pass



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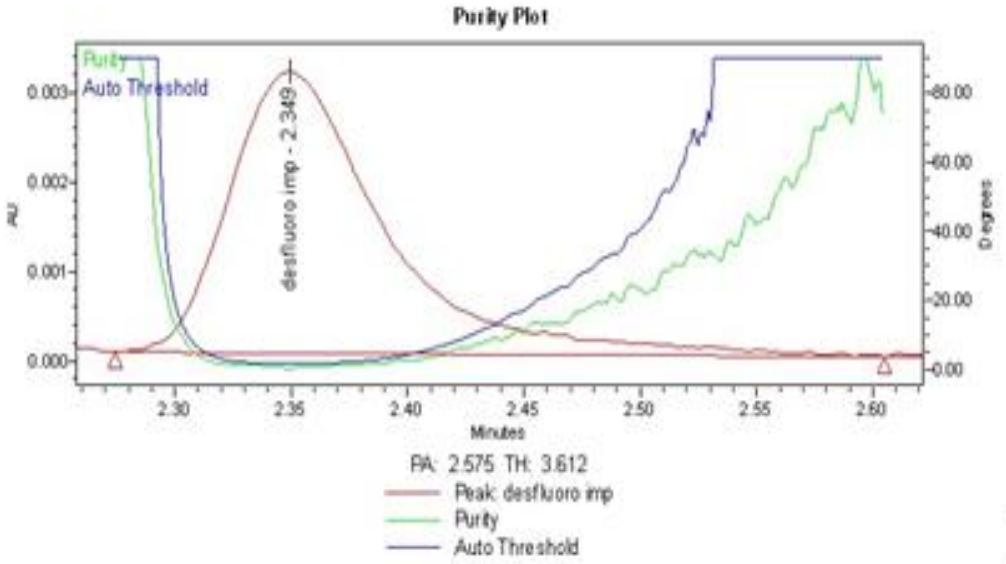

B

Figure 11. Purity plots of (A) Ciprofloxacin lactate (B) Desflouro impurity

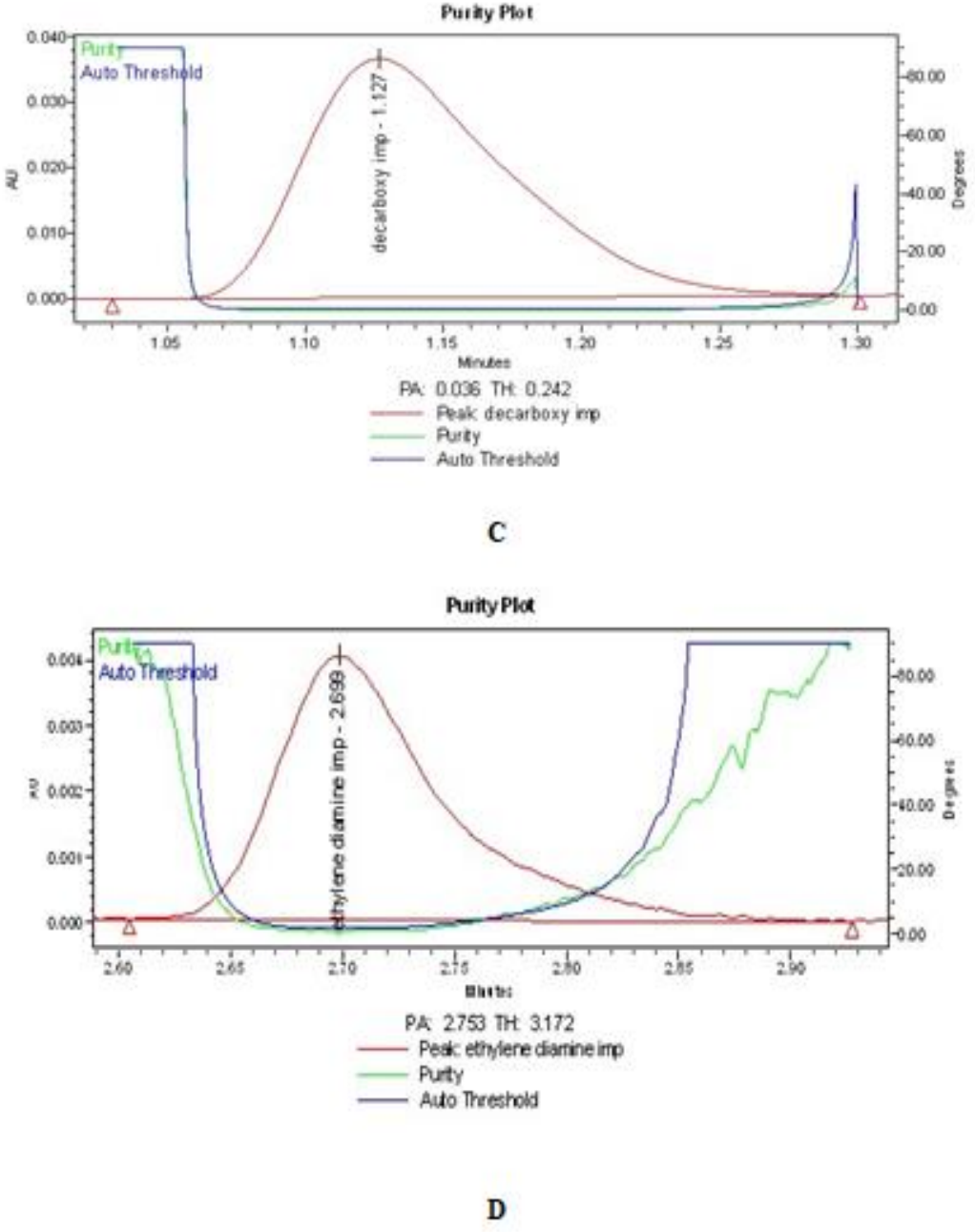

Figure 12. Purity plots of (C) Decarboxy impurity (D) Ethylene diamine impurity

4. Conclusions

A simple and robust RP-UPLC method has been developed for the simultaneous estimation of ciprofloxacin and its three impurities. The proposed method was validated in accordance with ICH guidelines considering all the parameters which include system suitability, specificity, precision, linearity, LOD, LOQ, accuracy and robustness. The method was found to be specific to separate the peaks of ciprofloxacin and its three impurities with better resolution. Thus, the obtained data prove the effectiveness of the proposed RP-HPLC method for the separation of three impurities with the ciprofloxacin, which can be adopted in routine analysis in pharmaceutical industries.

Conflict of interest

The authors declare no conflicts of interest.

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