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Development and optimization of RP-HPLC method for analysis of cefpodoxime proxetil impurity in pharmaceutical formulation

Dev Prakash Dahiya
School of Pharmacy, Manglayatan University, Aligarh
Email: devprakash936@yahoo.com

Geetanjali Saini
School of Pharmacy Abhilashi University, Chail Chowk, Mandi H.P
Corresponding author email: geetanjalisaini81@gmail.com

Bhupendra Singh
Abhilashi College of Pharmacy, Ner Chowk, Mandi HP
Email: bhupendratomar81@gmail.com

Amit Chaudhary
School of Pharmacy, Abhilashi University, Chail Chowk, Mandi HP
Email: deanpharmacy.au@gmail.com

Praveen Chaudhary
Department of Pharmaceutical Sciences Quantum University, Roorkee UK
Email: pkqa1981@gmail.com

Meenu Chaudhary
Sri Guru Ram Rai University Dehradoon UK
Email: 1979mchaudhary@gmail.com

***Abstract*---**The purpose of this research is to design and validate a unique, simple, and effective RP-HPLC analytical method using the QbD methodology. The QbD methodology not only confirms the method's robustness but also aids in the development of repeatable and reliable data. The goal of this work is to develop and validate a simple analytical RP-HPLC method for determining Cefpodoxime Impurity (Cefpodoxime Acid) in pharmaceutical formulations using the Analytical Quality by Design methodology (AQbd) in accordance with ICH Q8 guidelines. The Analytical Quality by Design technique was used in the proposed study. Cefpodoxime impurity (Cefpodoxime Acid) was chromatographically evaluated using an Inertsil C18 (5 m) column. The mobile phase was phosphate buffer and methanol pH 4.0

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in the ratio of 60:40, which was driven onto the column at 0.8 ml/min using an isocratic elution protocol. 222 nm was discovered to be the detection wavelength for impurity estimation. Design expert 11 (Trial edition) software was used to investigate the effects of the mobile phase composition, flow rate, and pH. The analysis of cefpodoxime acid took 20 minutes to complete & at 15.6 minutes, the impurity was eluted. The accuracy, precision, linearity, LOD, and LOQ of the analytical method were determined according to ICH Q2(R1) criteria during validation. Over a range of 0.4 µg/ml / to 2.4 µg/ml, the calibration curve exhibits high linearity. The limits of detection (LOD) and quantitation (LOQ) were found to be 0.070 µg/ml and 0.212 µg/ml, respectively. According to the findings, applying the QbD methodology to the analytical method development process results in a more robust technique in the hands that is capable of generating consistent, dependable data of standard level and quality throughout the process' life cycle, saving time and money.

***Keywords*---**cefpodoxime acid, RP-HPLC, optimization, pharmaceutical formulation.

Introduction

Cefpodoxime proxetil is an oral, broad-spectrum third-generation cephalosporin antibiotic. It is an ester-modified prodrug(Mathew et al., 2013). It possesses in vitro efficacy against a wide range of Gram-positive and Gram-negative microorganisms associated with frequent pediatric illnesses, making it an effective empirical treatment option. To evaluate contaminants and degradation products, different analysis methods have been developed and compared(Malathi et al., 2009). Using high-performance liquid chromatography-hyphenated methods, Fukutsu et al.found three degradation products of cefpodoxime proxetil(Fukutsu et al., 2006). However, according to ICH recommendations Q3A, all contaminants (from processing and degradation) must be recognized if they exceed a specified level(Council et al., 2006). The Cefpodoxime Acid is the major metabolic impurity of the Cefpodoxime Proxetil EP. It is a pale yellow solid amorphous solid having a molecular weight of 427.46

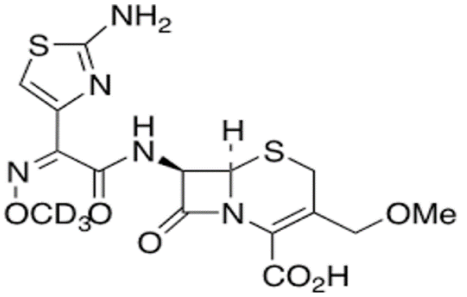

Fig.1. Chemical structure of Cefpodoxime impurity EP (Cefpodoxime Acid)

Currently, there is no systematic study for identifying cefpodoxime proxetil impurities. As a result, we employed a chromatographic technique to discover unknown process contaminants and degradation products in cefpodoxime proxetil(Jain et al., 2012).

According to the International Council of Harmonization (ICH), Quality by design is defined as a systematic approach that begins with predefined objectives and highlights product and process understanding and process control based on sound science and quality risk management.

Unlike QbD the outcome of the analytical quality by design (AQbD) is well understood and fit for the intended use. The analytical quality by design has different parameters in its life cycle like

- Analytical Target Profile (ATP)
- Critical Quality Attribute (CQA)
- Risk Assessment
- Optimization and development of the method
- Design Space
- Control strategy
- Method Validation
- Monitoring

The Critical Quality attributes (CQA) in analytical method developments include methods parameters. Each analytical method has different quality attributes(Singh et. al, 2019). HPLC analytical techniques have mobile phase composition, pH, diluent, selection of column, column temperature & organic modifier as critical quality attributes (CQA)

Materials and Methods

Characterization

Infrared spectroscopy

The FTIR spectra for the impurity were recorded to confirm the identity of the impurity. A small quantity of each powdered sample was taken and embedded between the KBr discs for the analysis(Tomar et al., 2021). IR Spectra for the impurity (Cefpodoxime Acid) are shown in Fig. no. The interpretation of the spectra for the impurity Cefpodoxime Acid is shown in table number 2

Determination of λ max for Cefpodoxime Proxetil and its impurity A (Cefpodoxime Acid) in mobile Phase

Preparation of mobile phase

The mobile phase was prepared by mixing methanol and phosphate buffer of pH 4.0 adjusted with orthophosphoric acid (prepared with 5.04 g of disodium hydrogen phosphate and 3.01 g potassium dihydrogen phosphate dissolved in 1000 mL of triple distilled water and the pH was adjusted to 4.0 using

orthophosphoric acid) in the ratio of 60: 40 v/v. Then it was filtered through 0.45 m membrane filter paper using a filtering assembly before being sonicated for 10 minutes in an ultrasonic water bath(Gandhi et al., 2009).

A Cefpodoxime Acid standard stock solution was made by dissolving 10 mg of impurity in 10 ml of methanol to obtain a concentration of 1000 µg/ml. Further dilutions were made from the standard stock solution.

Selection of Detection Wavelength

From the standard stock solution, further dilutions were made using the mobile phase and scanned over the UV range and the spectrum was obtained. It was observed that Cefpodoxime Proxetil and its major impurity Cefpodoxime Acid showed considerable absorbance at 222 nm(Gedawy et al., 2019)

Optimization of Analytical Conditions using Design of experiments (DOE Software)

The Taguchi screening method has opted for initial screening for the identification of some initial critical factors. During the preliminary screening the column temperature, mobile phase ratio, pH of the buffer, flow (Isocratic/ Gradient), and injection volume were investigated(Jadhav & Tambe, 2013). The Central Composite (CCD) model was chosen to optimize the separation of the Cefpodoxime Acid, with one replicate at the center point. The low, medium, and high levels are specified in Table 1 for the investigation of the independent variable. Mobile Phase composition (X1) and pH (X2) have been taken as the independent variable and their effects on the dependent variables were recorded.

Table 1 Translation of factor levels into units

Levels	Mobile phase ratio (Methanol: Phosphate buffer:)	pH	Flow rate
-1	40:60	1	0.5
0	50:50	3	0.7
+1	70:30	5	1.0

The Retention Time (Y1) Number of theoretical plates (Y2) Assay (Y2) and Recovery (Y3) were the dependent variables (Evaluated response). The optimum composition prediction is done by using an overlay plot and desirability function. By using numerical optimization techniques with the help of Design-Expert Software the anticipated analytical conditions were determined(Acharya & Patel, 2013).

Statistical assessment of Method characteristics

Design-Expert software version 13.0.9.0 was used for statistical assessment of the results of the experimental design. Statistical validation involved assessing statistical parameters of F-value, correlation coefficient (R2), adjusted R-squared (R2 Adj), predicted R-squared (R2 Pred), predicted residual error sum of squares (PRESS), and adequate precision (AP) generated by ANOVA provision to ascertain

model sufficiency and adequacy. An F-value with $p<0.05$ implied the significance of the model. A difference of less than 0.2 between R2 Adj and R2 Pred would prove that both values were in reasonable agreement with each other. The measure of fit was provided by PRESS statistics with a PRESS statistic of smaller value being preferred.

Diagnostic analysis of method characteristics

Design-Expert software developed diagnostic plots like externally studentized residuals vs. predicted plot, predicted vs. actual plot, normal probability plot, and externally studentized residuals vs. run number plot. These plots were analyzed to check if the points fell on the diagonal in a normal probability plot and if they lay within the described limits or not

HPLC method development and validation of Cefpodoxime Proxetil and impurity (Cefpodoxime Acid)

Selection of mobile phase and chromatographic conditions

The working standard solution of Cefpodoxime Proxetil (200 µg/ml) was used for chromatographic separation investigations. To get the desired system suitability characteristics, eleven trial runs were carried out using methanol and buffer in various proportions as per the design of experiments (DoE) runs, along with a buffer of varying pH. The different combinations and feasible solutions were suggested by the software after optimization(Bashir et al., 2017). Out of these combinations the 60:40 of methanol and phosphate buffer of pH 4.0 which was the most desirable from the point of retention time, Theoretical Plates and Recovery was selected for a robust process(Bala et al., 2019)(Jhanwar et al., 2017). The flow rate was selected to 0.8 ml/min after different trials

Preparation of mobile phase

The mobile phase was made by combining 60:40 v/v methanol and phosphate buffer adjusted to pH 4.0 with orthophosphoric acid (prepared by dissolving 5.04 g disodium hydrogen phosphate and 3.01 g potassium dihydrogen phosphate in 1000 mL triple distilled water and adjusting the pH to 4.0 with orthophosphoric acid). Then it was filtered through 0.45 m membrane filter paper using a filtering assembly before being sonicated for 10 minutes in an ultrasonic water bath.

Preparation of Standard stock solution

Cefpodoxime Proxetil standard stock solution was made by dissolving 10 mg of the medication in 10 ml of methanol to achieve a concentration of 1000 µg/ml. Further dilutions were made from the standard stock solution.

Chromatogram and system suitability parameters of drug & Impurity

The mobile phase had saturated the column (indicated by constant backpressure at desired flow rate). Cefpodoxime Proxetil (200 µg/ml) and Cefpodoxime Acid (0.8 µg/ml) were administered into the system as a working standard solution.

Validation of Analytical Method

Linearity

Further dilutions with the mobile phase were made from the standard stock solution (1000 µg/ml) of Cefpodoxime Proxetil to achieve the range of solution containing six distinct concentrations. For each concentration, six replicates were injected. The linearity (relationship between peak area and concentration) of Cefpodoxime Proxetil was investigated throughout a concentration range of 100-600 µg/ml(Vadera et al., 2007). Further dilutions with the mobile phase were made from the standard stock solution (1000 µg/ml) of Cefpodoxime Acid to obtain a range of solutions containing six different concentrations. The linearity (relationship between peak area and concentration) of Cefpodoxime Acid was studied throughout a concentration range of 0.4-2.4 µg/ml.

Precision

Intra-day and inter-day variation experiments proved the method's precision. Three replicates of three different concentrations of Cefpodoxime Proxetil (200, 400, 600 µg/ml) were evaluated in a day for the Intraday investigations, and the percentage RSD was computed. Three replicates of different concentrations were examined on three consecutive days for the inter-day variation investigations, and the percentage RSD was computed. The intraday and intraday results were obtained(Jain et al., 2012).

Three replicates of Cefpodoxime Acid at three different concentrations (0.8, 1.6, and 2.4 µg/ml) were evaluated in one day, and the percentage RSD was calculated. Three replicates of different concentrations were examined on three consecutive days for the inter-day variation investigations, and the percentage RSD was computed.

Range

In the Analytical method, the range is defined as the upper and lower and lower concentration of the analyte in the sample for which the analytical procedure has been proved to have adequate precision, accuracy, and linearity.

Accuracy

Recovery studies were carried out to assess the method's accuracy by adding the standard drug to the sample at three distinct levels: 50, 100, and 150 percent. The sample chosen had a basic concentration of 200 µg/ml of Cefpodoxime Proxetil from tablet solution. For accuracy content detection of Cefpodoxime Acid from the spiked sample, the sample solutions were also spiked with 0.4, 0.8, and 1.2 µg/ml(Jhanwar et al., 2017). Cefpodoxime Acid (approximate levels 50, 100, and 150 percent). To obtain the chromatograms, these solutions were injected in triplicate in stabilized chromatographic conditions. The impurity concentration was determined using the Cefpodoxime Acid linearity equation.

Limit of Detection (LOD)

LOD is calculated from the formula: -

$$LOD = 3.3 \times \sigma/S$$

Where,
σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.

Limit of Quantification (LOQ)

The quantitation limit is expressed as:

$$LOQ = 10 \times \sigma/S$$

Where,
σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.

Specificity

The specificity of the method was ascertained by peak purity profiling studies(Ansari et al., 2005).

Robustness

The robustness of the method was determined by carrying out the analysis under conditions during which flow rate, wavelengths, and pH of the mobile phase were altered and the effects on the area were noted.

Results and Discussion

FTIR Characterization

The FTIR spectra for the impurity were recorded to confirm the identity of the impurity. A small quantity of each powdered sample was taken and embedded between the KBr discs for the analysis. IR Spectra for the impurity (Cefpodoxime Acid) are shown in Fig. no 2. The interpretation of the spectra for the impurity Cefpodoxime Acid is shown in table number 2

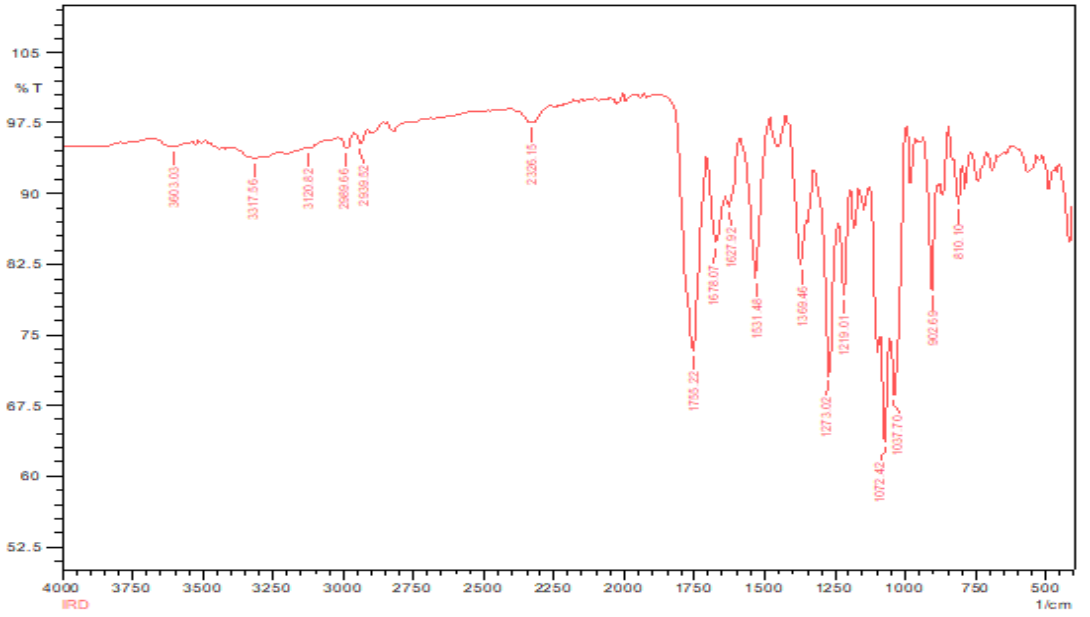

Fig. 2. IR Spectra of Cefpodoxime Impurity (Cefpodoxime Acid)

Table 2. Interpretation of FTIR spectra of Cefpodoxime Impurity EP (Cefpodoxime Acid)

Sr. No	Peak observed (cm-1)	Functional group
1.	3300	N-H Stretching
1	2900-3000	N-H Stretching
	2100	C-H Stretching (Aromatic)
2.	1750	C= O Stretching
3.	1680	Amide C=O stretch
4.	1620	C=C stretch
5.	1600	C=C Stretching
6	1450	C-H Bending
7.	1200	C-O Stretching
8.	1100	C-O Stretching
9.	1085	C-O Streching
10.	1050	C-O Streching
11.	990	C=C Bending
12.	960	C=C Bending
13.	850	C-H Bending

Determination of λmax

The wavelength of the cefpodoxime impurity (Cefpodoxime Acid) was determined in mobile phase Methanol: Phosphate buffer in 60:40 v/v. The pH of the mobile phase was adjusted to 4.0 using orthophosphoric acid. Cefpodoxime Proxetil standard stock solution was made by dissolving 10 mg of the drug in 10 ml of methanol to achieve a concentration of 1000 µg/ml. Further dilutions were made

from the normal stock solution Cefpodoxime Proxetil and its main impurity Cefpodoxime Acid was found to have a high absorbance at 222 nm (Fig 3.)

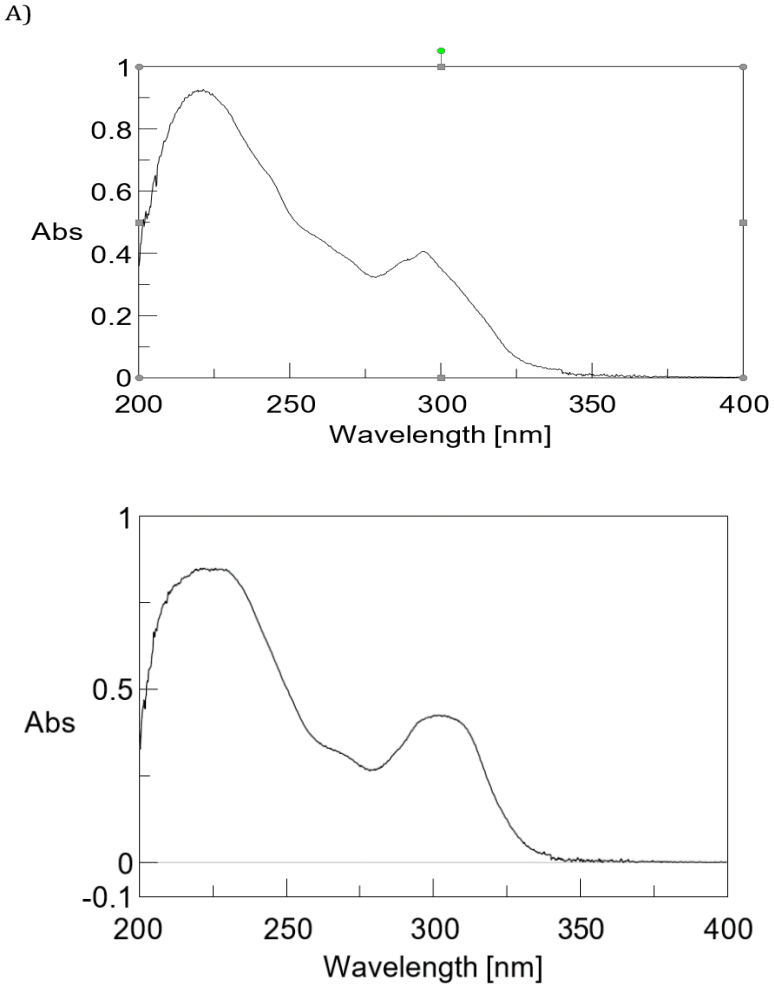

Fig. 3 UV Spectra of A) Cefpodoxime Proxetil and its major impurity B) Cefpodoxime Acid (10 µg/ml)

Optimization of analytical conditions for RP-HPLC Method Development of Cefpodoxime Proxetil and its impurity A (Cefpodoxime Acid)

Experimental design

The Retention Time, Theoretical Plates, and Recovery were the responses evaluated during optimization. Three components at three levels were used to create the design matrix, resulting in a total of 11 trial runs (Table 3). It was feasible to explain the mathematical models by assessing the acquired findings after experimental runs.

Table 3. Experimental runs and response variables for Cefpodoxime impurity A (Cefpodoxime Acid)

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	Run	A: Mobile Phase Ratio	B: pH	Retention Time	Theoretical Plates	Recovery
		%		Min	Numbers	%
2	1	1	3	15.94	11019	98.96
5	2	-1.414213562	4	16.21	11026	100
9	3	0	4	16.14	11020	99.98
6	4	1.414213562	4	16.13	11000	99.96
10	5	0	4	16.14	11025	99.16
3	6	-1	5	15.41	11038	100.05
7	7	0	2.5857864	16.97	11010	98.01
11	8	0	4	16.12	11022	99.95
1	9	-1	3	16.11	11020	98.88
8	10	0	5.4142136	15.32	11045	100.06
4	11	1	5	15.3	11040	100.08

The relationship between the independent variables and the replies was analyzed. The quadratic polynomials for each response, as well as the ANOVA statistical parameters, were determined. The generated models were found to be highly significant (p.0001) for all three response variables. The residual mean square and mean square of regression were greater than F indicating the excellent fit model to the responses (p<.0001in all cases)

Table 4. Experimental results and predicted values of response variables Retention Time, Theoretical Plates, and Recovery

Run	Retention Time			Theoretical Plates			Recovery		
	Actual Value	Predicted Value	% Error*	Actual Value	Predicted Value	% Error*	Actual Value	Predicted Value	% Error*
1	15.94	16.39	-2.745	11019.0	11008.56	0.09483	98.96	98.87	0.09102
2	16.21	16.05	0.9968	11026.0	11030.41	-0.0399	100.0	99.96	0.04001
3	16.14	15.98	1.0012	11020.0	11024.09	-0.0371	99.98	99.70	0.28084
4	16.13	15.91	1.3827	11000.0	11017.77	-0.1612	99.96	99.98	-0.02
5	16.14	15.98	1.0012	11025.0	11024.09	0.00825	99.16	99.70	-0.5416
6	15.41	15.57	-1.027	11038.0	11039.62	-0.0146	100.05	100.15	-0.0998
7	16.97	16.63	2.0444	11010.0	11008.45	0.01408	98.01	98.11	-0.1019
8	16.12	15.98	0.8760	11022.0	11024.09	-0.0189	99.95	99.70	0.25075
9	16.11	16.49	-2.304	11020.0	11017.50	0.022691	98.88	98.83	0.05059
10	15.32	15.33	-0.065	11045.0	11039.74	0.04764	100.06	99.94	0.1200
11	15.30	15.47	-1.098	11040.	11030.68	0.08449	100.08	100.14	-0.0599

%Error*= (Actual Value-Predicted Value) X 100/Predicted Value

The observed Retention Time, Theoretical Plates, and % Recovery have been put in DOE software and the predicted Retention Time, Theoretical Plates, and % Recovery obtained by the model using the above equation were compared with

observed values. A low % error of <5% ascertained that the model has good predictability (Table 4)

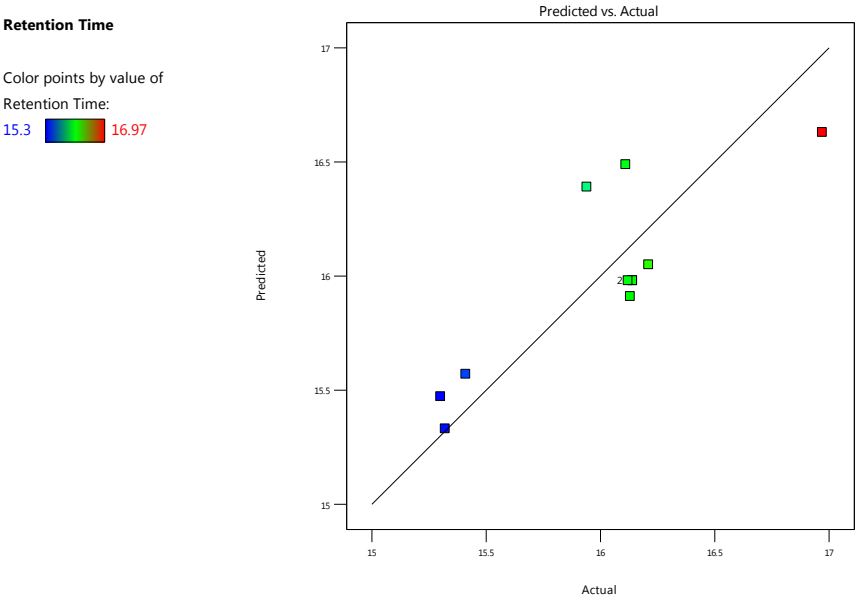

Fig 4. Plot Predicted Vs Actual Retention Time of Cefpodoxime Impurity A

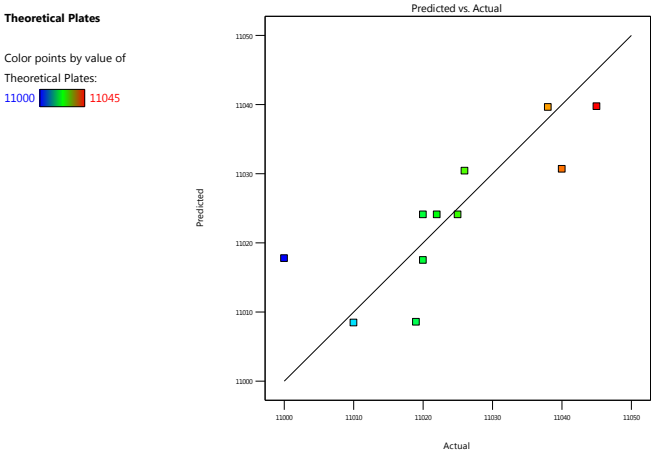

Fig 5. Plot Predicted Vs Actual Theoretical Plates of Cefpodoxime Impurity A

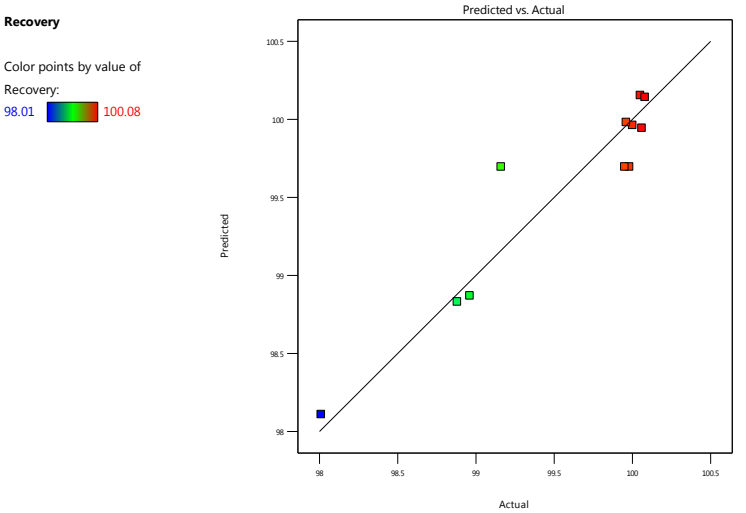

Fig 6. Plot Predicted Vs Actual Recovery of Cefpodoxime Impurity A

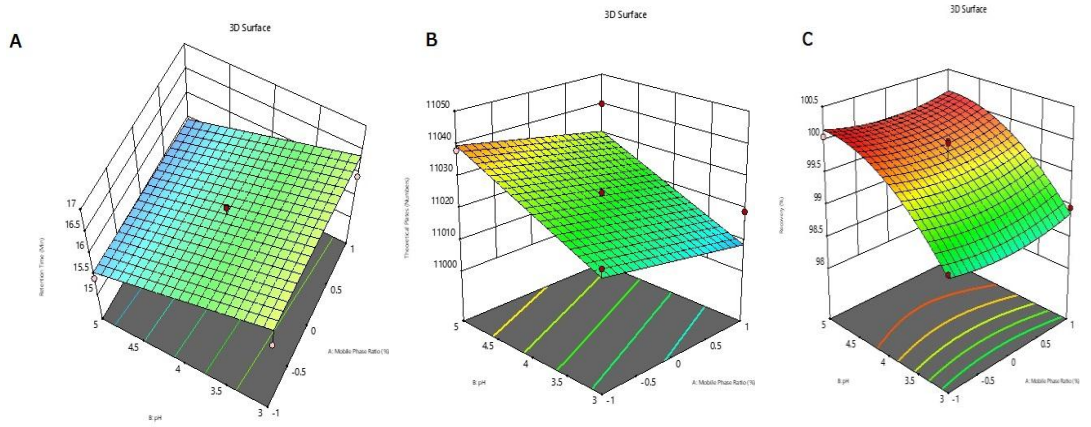

Fig 7. 3D Response surface graph showing the consequence of mobile phase ratio & pH on (A) retention time, (B) Theoretical Plates, and (C) recovery of Cefpodoxime impurity A

Effect of the Mobile Phase ratio and pH on Retention Time

The retention time of the Cefpodoxime Impurity A (Cefpodoxime Acid) increases with decreasing pH it may be suggested that the impurity is a weak acid in nature and partly ionized at low pH however the retention time significantly decreases when the pH of the mobile phase increases beyond 3.0. The ratio of the buffer in mobile shows the effect on the retention time of the impurity. The retention time decreases with increasing the ratio of the buffer in the mobile phase. The number s of theoretical plates increases with increment in the pH value and shows a sharp peak in the chromatogram. However, the mobile phase ratio and pH have the least influence on the recovery of the impurity (Fig 7)

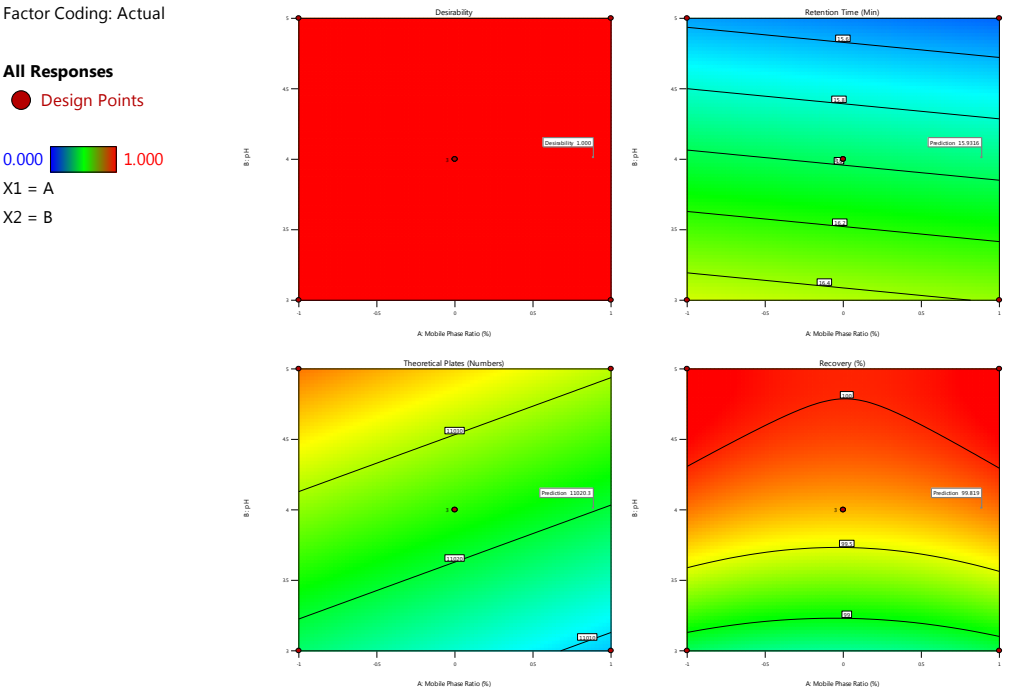

Fig 8. Optimized analytical conditions with design space

Optimization of the method

There could be different combinations that may provide several feasible solutions for the robust process by varying the composition of the mobile phase and pH of the mobile phase. Out of the number of combinations whichever is the most desirable in terms of retention time, theoretical plates, and recovery was selected. (Fig 8)

Based on the optimized mobile phase ratio of 0.886 & pH 4.012 which provided the retention time of 15.931 min, Theoretical plates 11020.26, and the % recovery of 99.81% at a 95% confidence interval with a desirability value of 1. Based on the coded level value of the mobile phase ratio the composition of 40:60 (Methanol: Phosphate Buffer) was selected for the analysis. The pH of the mobile phase was adjusted to 4 with the help of orthophosphoric acid. The flow rate was selected to 0.8ml/min.

The following conditions were implemented after optimization for the development of the analytical method for the determination of Cefpodoxime Impurity A (Cefpodoxime Acid) (Table 5)

Tab 5. Summary of optimized chromatographic Conditions

Sr. No.	Parameter	Conditions used for Analysis
1.	Mobile phase	Methanol and phosphate buffer of pH 4.0 adjusted with orthophosphoric acid (40: 60

1.		v/v)
2.	Flow rate	0.8 ml/min
3.	Detection Wavelength	222 nm
4.	Sample injector	20 µl loop
5.	Column	Grace C18 column (250 x 4.6 mm, 5µm)
6.	Column temperature	Ambient

Validation of developed Analytical Method By RP-HPLC of Cefpodoxime Proxetil & impurity (Cefpodoxime Acid)

Chromatogram and system suitability parameters of the drug

The mobile phase had saturated the column (indicated by constant backpressure at desired flow rate). Cefpodoxime Proxetil (200 µg/ml) and Cefpodoxime Acid (0.8 µg/ml) were injected into the system as a working standard solution. The retention times of repeated injections were found to be 18.25, 20.06 & 15.96 for Cefpodoxime Proxetil peak 1, Cefpodoxime Proxetil Peak 2 & impurity (Cefpodoxime Acid) respectively (Table 6)

Table 6. Retention times Cefpodoxime Proxetil & its impurity A (Cefpodoxime Acid)

Sr. No.	Cefpodoxime Proxetil RT (Peak 1)	Cefpodoxime Proxetil RT (Peak 2)	Cefpodoxime Acid RT
1	18.26	20.04	15.98
2	18.24	20.15	15.97
3	18.23	20.09	15.79
4	18.25	20.01	15.95
5	18.27	20.11	16.01
6	18.23	19.98	16.07
AVG	18.25	20.06	15.96
STDEV	0.02	0.06	0.09
% RSD	0.09	0.31	0.58

Chromatogram of mixture of Cefpodoxime Proxetil (200 µg/ml) and Cefpodoxime Acid (0.8 µg/ml) are shown in Figure 9

Table 7. System suitability parameter

Name	RT (Min)	Concentration (µg/ml)	Area (µV.Sec)	Plates	Asymmetry
Cefpodoxime Acid	15.963 ± 0.579	0.8	11026	6849	1.08
Cefpodoxime Proxetil (Peak 1)	18.248 ± 0.092	2	2059274	10542	0.98

Cefpodoxime Proxetil (Peak 2)	20.064 ± 0.307	0 0	2246580	11248	0.99

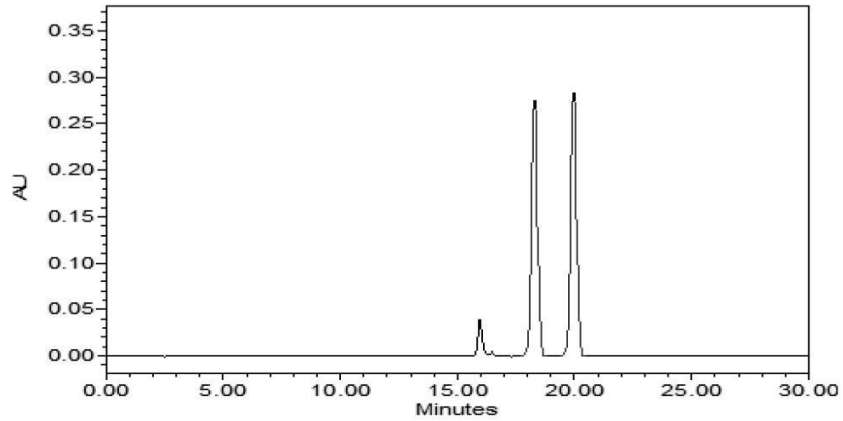

Fig 9. Chromatogram of Standard mixture of Cefpodoxime Proxetil (200µg/ml) and Cefpodoxime Acid (0.8 µg/ml)

Linearity

From the standard stock solution (1000 µg/ml) of Cefpodoxime Proxetil and Cefpodoxime Impurity A (Cefpodoxime acid), With mobile phase, further dilutions were made to obtain a range of solutions having six different concentrations. For each concentration, six replicates were injected. The linearity (relationship between peak area and concentration) of Cefpodoxime Acid was measured at concentrations of 600-1000 µg/ml and 0.4-2.4 µg/ml, respectively. Tables 8 and 9 illustrate the results that were achieved. The calibration curve was created by plotting the peak area against the corresponding concentrations as shown in Fig 10 and Fig 11

Table 8. Linearity study of Cefpodoxime Proxetil

Replicates	Concentrations of Cefpodoxime Proxetil					
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	600 µg/ml
	Peak Area					
1	2466846	4305854	5587134	7355435	8916467	9985152
2	2451069	4456377	5442993	7463660	8924973	9896212
3	2464079	4305732	5591724	7359827	9015642	9964314
4	2458079	4319795	5575127	7356794	8908724	9985783
5	2471970	4313942	5586715	7337219	8915593	9984699
6	2466345	4301582	5584243	7357420	8912319	9984672
Mean	2463064.67	4333880.33	5561322.67	7371725.83	8932286.33	9966805.33
Std. Dev.	7404.671	10352.89	58965.89	45775.98	41193.32	35569.03
%RSD	0.301	0.239	1.060	0.621	0.461	0.357

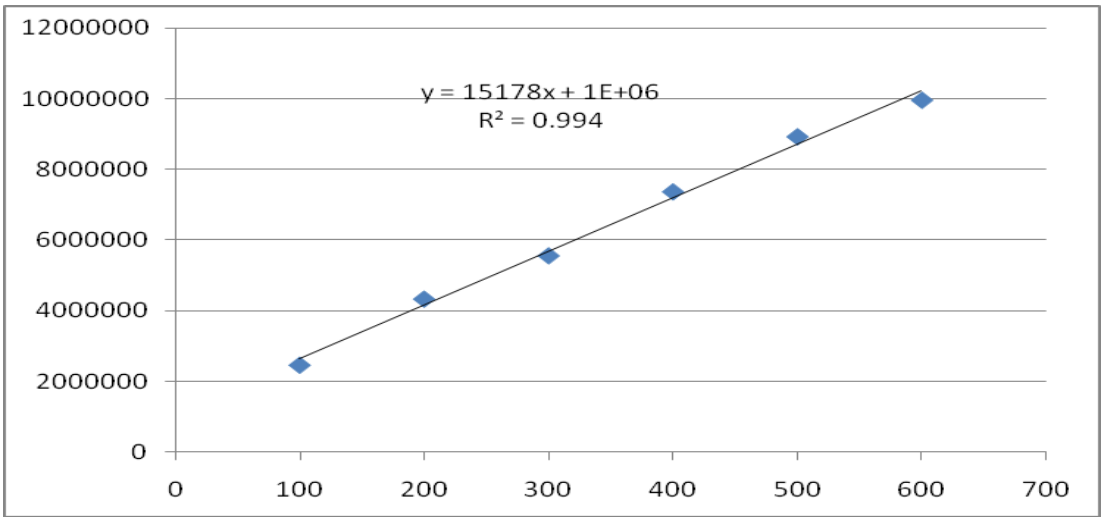

Fig 10. Calibration curve for Cefpodoxime Proxetil

Table 9. Linearity study of Cefpodoxime Acid

Replicates	Concentrations of Cefpodoxime Acid					
	0.4 µg/ml	0.8 µg/ml	1.2 µg/ml	1.6 µg/ml	2 µg/ml	2.4 µg/ml
	Peak Area					
1	7405	11026	14491	17632	21561	24732
2	7251	11105	14472	17605	21505	25696
3	7168	11057	14502	17610	21553	24748
4	7357	11099	14634	18153	22245	24624
5	7213	10810	14321	17587	21607	24707
7	7261	11040	14799	17819	21455	24555
Mean	7275.83	11022.8	14536.5	17734.3	21654.3	24843.6
Std. Dev.	81.327	99.434	148.478	202.820	268.372	386.944
%RSD	1.118	0.902	1.021	1.144	1.239	1.558

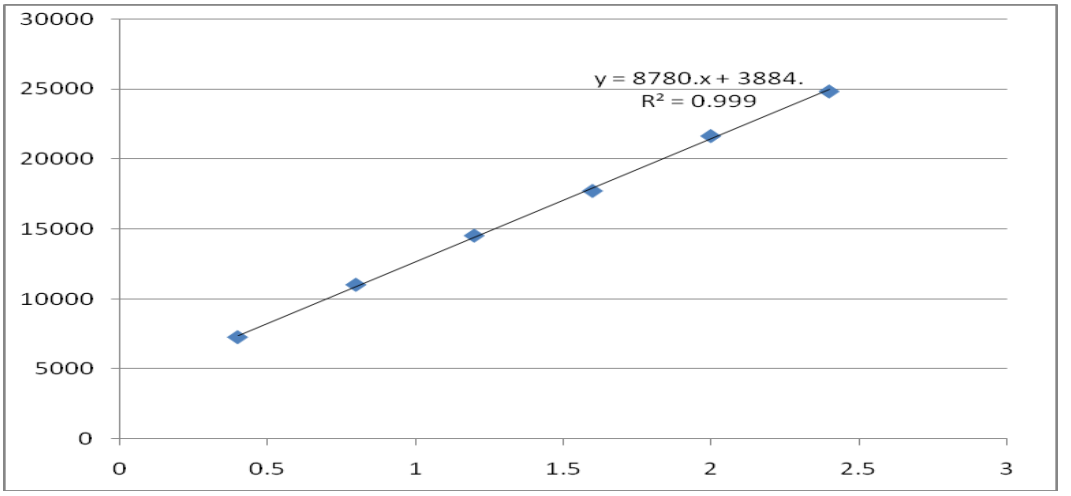

Fig. 11. Calibration curve for Cefpodoxime Proxetil impurity A (Cefpodoxime Acid)

Range

Based on the linearity study it was found that the method follows Beer's Law with a concentration range of 100 – 600 µg/ml and 0.4 – 2.4 µg/ml for Cefpodoxime Proxetil and Cefpodoxime Acid respectively.

Precision

Three replicates of Cefpodoxime Proxetil at three different concentrations (200, 400, and 600 µg/ml) and Cefpodoxime Acid at three different concentrations (0.8, 1.6, and 2.4 µg/ml) were chosen. The percent RSD of the chosen concentration was obtained after intraday and interday analysis. Tables 10- 13 and Fig 12-13 show the results for intraday and interday variability.

Table 10. Intra-day precision study Cefpodoxime Proxetil

Concentration (µg/ml)	Area (µV. Sec)	% Recovery ±SD	Mean % Recovery* ± SD	% RSD*
200	4127099			
200	4175781			
200	4157277	99.74 ± 0.81		
400	7214032			
400	7172962			
400	7154448	99.73 ± 0.50		
600	10118400			
600	10158146			
600	10216783	99.25 ± 0.54		
			99.57 ± 0.60	0.60

*Average of three determinations

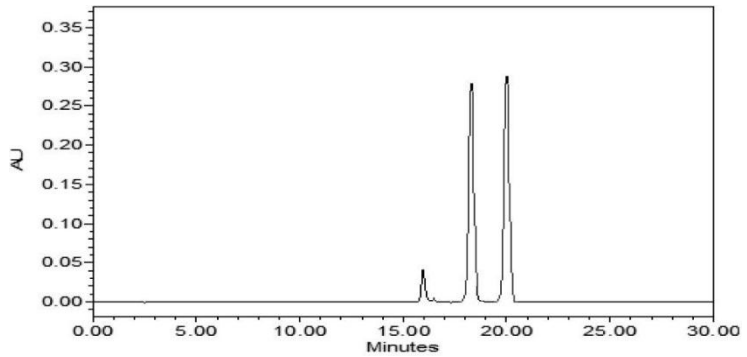

Fig 12. Chromatogram of inter-day precision of Cefpodoxime Proxetil & Impurity A (Cefpodoxime Acid)

Table 11. Inter-day precision of Cefpodoxime Proxetil

Concentration ((µg/ml)	Area (µV. Sec)	% Recovery ± SD	Mean % Recovery* ± SD	% RSD*
200	4151267			
200	4189235			
200	4153989	100.11 ± 0.70		
400	7172835			
400	7195942			
400	7215017	99.96 ± 0.35		
600	10215706			
600	10226999			
600	10325144	100.25 ± 0.66	100.11 ± 0.53	0.53

*Average of three determinations

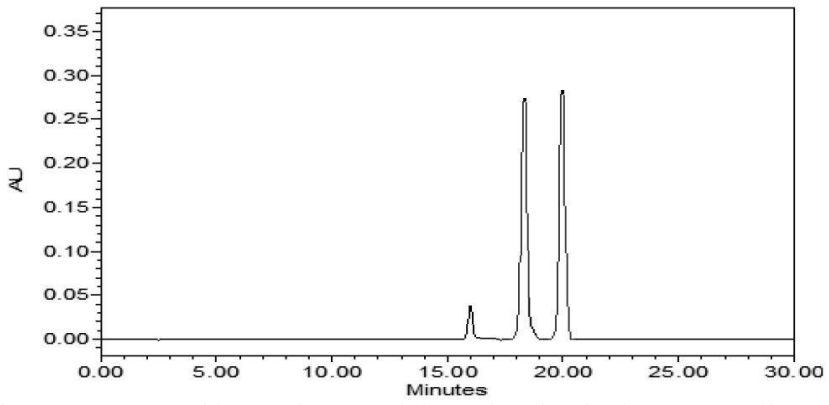

Fig 13. Chromatogram of inter-day precision of Cefpodoxime Proxetil & Impurity A (Cefpodoxime Acid)

Table 12. Intra-day precision study Cefpodoxime Acid

Concentration (ng/band)	Area (µV. Sec)	% Recovery ±SD	Mean % Recovery* ± SD	% RSD*
0.8	11016			
0.8	10905			
0.8	10987	100.85 ± 0.82		
1.6	17824			
1.6	18245			
1.6	17818	100.20 ± 1.74		
2.4	25032		100.49 ± 1.05	1.05
2.4	24924			
2.4	25187	100.42 ± 0.62		

*Average of three determinations

Table 13. Inter-day precision study Cefpodoxime Acid

Concentration ((ng/band)	Area (µV. Sec)	% Recovery ±SD	Mean % Recovery* ± SD	% RSD*
0.8	10955			
0.8	11039	100.86 ± 0.89		
0.8	10916			
1.6	17910			
1.6	17818	99.55 ± 0.34		
1.6	17884			
2.4	24917			
2.4	24857			
2.4	25147	100.07 ± 0.73	100.16 ± 0.83	0.83

*Average of three determinations

Accuracy

Recovery studies were carried out to assess the method's accuracy by adding a standard medication to the sample at three distinct levels: 50, 100, and 150 %. The sample chosen had a basic concentration of 200 µg/ml of Cefpodoxime Proxetil in the sample solution. Also spiked the sample solutions with 0.4, 0.8, and 1.2 µg/ml Cefpodoxime Acid (Approximate levels 50, 100, and 150 %) for accuracy content determination of Cefpodoxime Acid from the spiked sample. These solutions were injected in stabilized chromatographic conditions in triplicate to obtain the chromatograms. The drug concentration of Cefpodoxime Proxetil was calculated by using the linearity equation of Cefpodoxime Proxetil. The results obtained are shown in Tables 14 & 15

Table 14. Recovery study of Cefpodoxime Proxetil

Level	Conc. (µg/ml)		Area	% Recovery	Mean	% RSD
	Sample	Std				
50 %	200	100	5721368	100.925	100.473	1.494
			5624507	98.798		
			5756493	101.696		
100 %	200	200	7212812	100.259	100.287	0.703
			7172608	99.597		
			7258162	101.006		
150 %	200	300	8694865	99.736	99.899	0.288
			8732443	100.231		

*Average of three determinations

Table 15. Recovery study of Cefpodoxime Proxetil impurity A (Cefpodoxime Acid)

Approx Level	Conc. (µg/ml)		Area	% Recovery	Mean	% RSD
	Sample	Std				
50 %	0.781	0.4	14196	99.432	99.586	0.365
			14185	99.326		
			14255	100.001		
100 %	0.781	0.8	17918	101.086	100.397	0.844
			17691	99.451		
			17858	100.655		
150 %	0.781	1.2	21329	100.284	99.755	0.558
			21246	99.807		
			21136	99.175		

*Average of three determinations

Limit of Detection (LOD)

LOD is calculated from the formula: -

$$LOD = 3.3 \times \sigma / S$$

Where,
σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.
LOD of Cefpodoxime Proxetil = 5.217 µg/ml
LOD of Cefpodoxime Acid = 0.070 µg/ml

Limit of Quantification (LOQ)

The quantitation limit is expressed as:

$$LOQ = 10 \times \sigma/S$$

Where,
σ = standard deviation of response for the lowest conc. in the range
S = slope of the calibration curve.
LOQ of Cefpodoxime Proxetil = 15.810 µg/ml
LOQ of Cefpodoxime Acid = 0.212 µg/ml

Specificity

Peak purity profiling studies were used to determine the method's specificity. Peak purity values of more than 997 for Cefpodoxime Proxetil and more than 996 for Cefpodoxime Acid were reported, indicating that no other peak of a degradation product, impurity, or matrix interfered with the desired compound's retention time.

Robustness

The method's robustness was determined by running the analysis under different flow rates, wavelengths, and pH levels of the mobile phase, and observing the impact on the area. The results obtained are shown in Table 16

Table 16. Robustness study of Cefpodoxime acid and its impurity (Cefpodoxime Acid)

Drug/Impurity	% RSD Found for Robustness Study								
	pH			Flow Rate (1 ml/min)			Wavelength (nm)		
	3.9	4	4.1	0.78	0.8	0.82	221	222	223
Cefpodoxime Proxetil	0.8783	0.6060	0.4842	0.2298	1.5060	0.2599	0.3137	0.1068	0.5776
Cefpodoxime Acid	1.3110	1.0768	1.4665	0.8698	1.5046	1.0798	0.4622	0.7318	0.6617

*Average of three determinations

Application of the developed analytical method for analysis of drugs and impurity in pharmaceutical formulations

The developed analytical method for Cefpodoxime Proxetil and Cefpodoxime Acid was applied for the determination of Cefpodoxime Proxetil and Cefpodoxime Acid in the dosage forms like tablets and Suspensions and the stability analysis of API in different packaging materials like Blisters, Strips for tablet and Glass and Plastic Containers for liquid formulations were carried out.

Preparation of sample solution of Cefpodoxime Proxetil Tablets

Twenty tablets [Spodox 200; Saintroy Lifescience, Label Claim: Each film tablet containing Twenty pills was weighed and pulverized [Spodox 200; Saintroy Lifescience, Label Claim: Each film tablet contains Cefpodoxime Proxetil equivalent to Cefpodoxime 200 mg, B. No. CP5428; Mfg. 08/2020, Exp. 07/2022]

A tablet powder containing 10 mg of Cefpodoxime Proxetil was weighed and transferred to a 10 ml volumetric flask, where it was diluted with methanol to produce a 1000 µg/ml solution. It was sonicated for 10 minutes, filtered (the first few drops were discarded), and 2 ml of the filtrate was diluted with mobile phase to obtain a final concentration of Cefpodoxime Proxetil of 200 µg/ml. A chromatograph was created by injecting this solution. Six tests were performed on a homogeneous sample to assess the percent content of Cefpodoxime Proxetil and main impurity Cefpodoxime Acid from the linearity equation.

Conclusion

Using the quality by design technique (Central Composite design), a rapid, simple, sensitive, and unique RP-HPLC analytical method was successfully created, and the new analytical method was further validated according to ICH guidelines. The developed RP-HPLC analytical method for determining cefpodoxime proxetil and impurity EP (Cefpodoxime Acid) in pharmaceutical formulations is acceptable. Overall, the proposed RP-HPLC method for the estimation of Cefpodoxime Proxetil and impurity EP (Cefpodoxime Acid) in dosage forms is accurate, precise, linear, robust, simple, and rapid.

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