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Research Article

Formulation And Development Of Hplc Validation For The Simultaneous Estimation Of Antibacterial Agents

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ABSTRACT

A very sensitive, precise, accurate, robust, and repeatable RPHPLC technique was developed for the detection of cefpodoxime proxeti. This approach does not incorporate any sample excipients or degradants whatsoever. The method was developed by employing a mobile phase composed of a buffer containing 20 mM potassium dihydrogenphosphate, methanol, and acetonitrile. The separation was performed using a Hypersil keystone RP C18 column at a flow rate of 1.2 ml/min with a wavelength of 235 nm. During a 25-minute run, the R and S isomers of cefpodoxime proxetil exhibited peaks at 13.11 and 14.12 minutes, respectively, while the peak for ofloxacin occurred at 5.01 minutes. Based on this approach, the levels of cefpodoxime proxetil in Zedocef O tablets are 99.8% and 99.2%, respectively. The RSDs (Relative Standard Deviations) of 0.0708 and 0.596 for Cefpodoxime Proxetil and Ofloxacin, respectively, were deemed acceptable. The suggested method was validated by adhering to the ICH standards. The cefpodoxime proxetil and levofloxacin standards were found to be 80%, 100%, and 120% of the specified quantity. This contributed to assessing the precision of the testing procedure. The mean percentage recoveries for Cefpodoxime Proxetil and Levofloxacin were 99.67% and 99.69% respectively at the 80% concentration level, 99.65% and 99.75% respectively at the 100% concentration level, and 99.69% and 99.65% respectively at the 120% concentration level. All of the relative standard deviation (RSD) percentages observed in investigations on effective recovery fell within acceptable bounds, with no values exceeding 2 percent for both RSD and percent assay deviation. This approach demonstrated a linear connection between cefpodoxime proxetil concentrations ranging from 2 to 24 ng/ml and levofloxacin concentrations ranging from 2.5 to 30 ng/ml (r 2 = 0.9999). Linear correlations were observed between levofloxacin concentrations ranging from 2.5 to 30 ng/ml.

Keywords: HPLC, Cefpodoxime, Levofloxacin, Ftir, In-Vitro

Introduction

In light of the fact that the chemical stability of a pharmaceutical molecule has a direct impact on the efficacy and safety of the medical product, this is a key issue that has to be addressed. In order to evaluate how various environmental elements impact the quality of a drug ingredient or drug product over time, the United States Food and Drug Administration (USFDA) and the International Council for Harmonisation

(ICH) have both underlined how crucial it is to have data from stability testing. When choosing the ideal formulation, container, and storage technique for a chemical, as well as the appropriate shelf life, the molecular stability of the substance is a vital issue to take into consideration. This information is necessary for the documentation of regulatory requirements. Forcible degradation is a procedure that involves the breakdown of medication products and substances under conditions that are more severe than those of accelerated conditions. This approach is also known as the conditional degradation process. As a consequence of this, forced degradation results in the production of degradation products that can be studied to ascertain the level of stability possessed by the molecule. The objective of stress testing, as defined in the ICH standard, is to identify potential degradation products. This, in turn, assists in determining the intrinsic stability of the molecule, as well as the degradation pathways that it follows, and it also validates the stability indicators that have been utilised. [1] For both regulatory and scientific reasons, it is necessary to do research on the phenomenon of forced degradation. Prior to the filing of registration dossiers, it is now mandatory for a new pharma business to do stability tests on the goods that they manufacture. Both investigations into the immediate and long-term stability of a substance's qualities can benefit from the use of forced degradation tests to build a process for demonstrating the substance's stability. This is because the tests are designed to demonstrate the substance's stability. Throughout the entirety of the process of analysing the stability of the sample, various methods such as titrimetry, spectrophotometry, and chromatography were employed extensively. [2]

Material & Method

Cefpodoxime Proxetil with Levofloxacin: In vitro spectral investigations preliminary research

In order to verify the chemical in question, the FT-IR spectra of both medications were obtained with a Brukers alpha FT-IR instrument. An investigation was performed in order to assess the solubility of the two medicines. For the purpose of determining the maximum absorbance of the various medications, the absorbance spectra of these pharmaceuticals were acquired with a Shimadzu 2203 UV Visible Spectrophotometer. In addition, the stacked spectra were collected in order to assist in selecting the appropriate wavelength for this investigation. It was necessary to employ solutions in diluent with a concentration of 10 micrograms per millilitre in order to obtain the UV spectra. The open capillary method was utilised in order to ascertain the melting point of the widely used medications levofloxacin and cefpodoxime proxetil. After carrying out the procedure three times, the average result was obtained based on the results.

Obtaining able to complete the mobile phase the diluent

First, the mobile phase, which consisted of a buffer containing 10 mM potassium dihydrogen phosphate, methanol, and acetonitrile with a pH of 3.2 that was adjusted with orthophosphoric acid, was pumped out of the solvent reservoir at a flow rate of 1.2 ml/min. The volume/volume ratio of the mobile phase was 60:30:10, and the volume/volume ratio was modified with orthophosphoric acid. Before the mobile phase was degassed, it was first filtered through a membrane filter with a 0.45-micron micron pore size. Twenty-five minutes was the duration of the run, and 230 nanometers was the wavelength that was used to monitor the detection. The injection loop had a capacity of ten microliters across its entirety. It was necessary to enable the column to get equilibrated for a minimum of fifteen minutes prior to injecting the drug solution into the column. This was done while the mobile phase was being circulated through the apparatus. Diluent was the function that the mobile phase played in the inquiry that is now being conducted.

Performing Preparations for Standard Solutions

Approximately 250 mg of levofloxacin and 200 mg of cefpodoxime proxetil were each placed into a separate volumetric flask that was 100 ml in volume and contained 25 ml of mobile phase. This information was obtained after careful weighing. Sonication was subsequently applied to the flask for a period of thirty minutes, during which time the two antibiotics were totally dissolved. It was necessary to add diluent in order to bring the total volume up to 100 millilitres (Stock A contains 2500 micrograms of levofloxacin per millilitre and 2000 micrograms of cefpodoxime proxetil per millilitre). Additionally, the solutions were diluted with diluent until they reached a concentration of 10 g/ml for cefpodoxime proxetil and 12.5 g/ml for levofloxacin. This was done in order to achieve the desired concentrations.

The preparation of the sample solution (containing 200 mg of cefpodoxime and 250 mg of levofloxacin).

Twenty Glevopod tablets were broken up into a powdery form after a precise weight check was performed on the individual. The powdered sample was then placed into a volumetric flask that had a capacity of 100 millilitres. The powdered sample was carefully weighed out to be equivalent to 200 milligrammes of cefpodoxime (250 milligrammes of levofloxacin). After the sample had been sonicated for thirty minutes in fifty millilitres of diluent while being vigorously shaken intermittently, it was allowed to cool to room temperature, and then in order to bring the volume up to one hundred millilitres, additional diluent was added. Following the filtration of the solution via a Teflon filter syringe with a 0.45-micron pore size, the solution was further diluted with diluent in order to obtain a concentration of 10 micrograms per millilitre of

cefpodoxime proxetil. Following this, the solution was combined with 12.5 micrograms per millilitre of levofloxacin.

The Development of Methods and the Optimization of Chromatographic Conditions

For each individual medication, multiple chromatographic runs were carried out, and the combinations of those drugs were analysed using a wide range of different mobile phase configurations. To ensure that the appropriate process is chosen, it is important to take into consideration the characteristics of the sample, such as whether it is an ionic, ionizable, or neutral molecule, as well as its molecular weight and solubility. Because of its user-friendliness, adaptability, durability, and wide range of applications, the high-performance liquid chromatography (HPLC) technology with reversed phase was selected for the initial separation in this particular instance. Among the mobile phases that we tested, we tried a variety of various combinations, such as acetonitrile: water, methanol: water, methanol: buffer (OPA, KH_2PO_4 buffer), and buffer: methanol: acetonitrile. After everything was said and done, the chromatographic conditions that were followed in order to detect Cefpodoxime Proxetil and Levofloxacin were as follows: The buffer consists of methanol and acetonitrile in the proportions of 60:30:10, with a pH of 3.2, a wavelength of 230 nm, and a flow rate of 1.2 ml/min. As a result of this work, it is anticipated that the R and S isomers of cefpodoxime proxetil will each contribute one peak to the findings. Validation of the approaches that were developed was carried out with respect to the various criteria that were outlined in the ICH guidelines Q2 (R1). In order to demonstrate that the method can be successfully utilised for assay and stability examinations of tablets containing cefpodoxime proxetil and levofloxacin, the goal of this validation research is to demonstrate that the method can be employed successfully. Testing for system suitability, specificity, forced degradation, precision, linearity, accuracy, and stability in analytical solution were some of the criteria that were utilised in the process of validating the approach that was suggested.

System Suitability

In each of the five replicates, ten litres of standard preparations were injected. These preparations were made in the same way as the solutions described in the previous section. An examination of the chromatograms and peak responses of both 10 micrograms per millilitre of cefpodoxime proxetil and 12.5 micrograms per millilitre of levofloxacin was carried out. Analysis was performed on a number of factors, including resolution, capacity factor, theoretical plate, HETP, and asymmetry factor, in order to ascertain whether or not the technique was suitable for the system.

Specificity

A comparison was made between chromatograms that were created from blank (mobile phase) samples, chromatograms of a single drug, and chromatograms of a drug combination. This was done in order to determine the specificity of the approach. After doing an analysis on the peak purity of both Cefpodoxime Proxetil and Levofloxacin, it was established that there should not be any interference at the retention time of the major peaks.

Examination of the Composition

Evaluation of the Current Formulation

Accuracy

The investigation into the correctness of the method was carried out by incorporating the standard drug into the samples that had been evaluated in the past at three different levels, which are 80 percent, 100 percent, and 120 percent, respectively, and then measuring the percentage recovery after each of these additions. This was done in order to determine whether or not the method was accurate. We were able to produce three different volumetric flasks, each of which had a capacity of one hundred millilitres, after the procedure of weighing and transferring the sample powder, which was comparable to two hundred milligrammes of cefpodoxime proxetil and two hundred and fifty milligrammes of levofloxacin. This standard was supplemented with Cefpodoxime Proxetil and Levofloxacin, each of which was added at a concentration that was eighty percent, one hundred percent, and one hundred twenty percent of the promise that was stated on the label. After that, each of them was dissolved in fifty millilitres of diluent by sonicating the mixture for thirty minutes while also rapidly shaking the mixture often. This process was repeated several times. It was repeated a number of times through this method. It was decided that additional dilutions with the diluent were carried out, and the equation 6.1.2 was utilised in order to quantify the percentage of recovery that was achieved. Both the overall percentage of recovery and the overall percentage of RSD should not be higher than 2.0 percent. This guideline applies to both instances. It is expected that the entire percentage of recovery will fall somewhere in the range of 98 thousand to 102 thousand percent.

Linearity, as well as Range

In order to ascertain whether or not the procedure is linear, five different concentration levels were taken into consideration and implemented. Through the utilisation of the standard stock solutions, it was feasible to create standard solutions at a wide range of concentrations. Standard solutions had concentrations that ranged from 2 to 24 g/ml for cefpodoxime proxetil and from 5 to 30 g/ml for levofloxacin. Both of these

quantities were found in normal solutions. Immediately following the injection of ten microliters of each solution into the high-performance liquid chromatography (HPLC) apparatus, the peak area of the chromatogram that was obtained was measured and recorded. There were a total of six duplicates of each level that were investigated in accordance with the method that was suggested. The findings that were computed included the mean area, the standard deviation of that area, and the percent relative standard deviation of peak areas at each level. Furthermore, the normal distribution was also incorporated into the analysis. Following the development of the calibration curve, a plot was made of the response factor in relation to the concentration of the medicines. In order to determine the equation of the curve and the coefficients of correlation, the calibration curves were used as a basis for the calculation.

Sustained Stability in the Analytical Solution

To determine whether or not Cefpodoxime Proxetil and Levofloxacin in analytical solution are stable, an evaluation was performed on the sample both before and after it had been stored for a period of twenty-four hours in either a refrigerator or at room temperature. This was done in order to determine whether or not the results of the evaluation were consistent. Cefpodoxime Proxetil and Levofloxacin were both found to be present in the sample at a quantity of 10 grammes per millilitre for each of them. This was discovered from the analysis of the sample. The peak regions were an essential component of the calculation that was carried out in order to determine the percentage of the assay.

Both the Limit of Detection (LOD) and the Limit of Quantitation (LOQ) are important (LOQ)

The slopes (S) and standard deviations () that were generated from the response curve were used to calculate the LOD and LOQ values for Cefpodoxime Proxetil and Levofloxacin, respectively. Equations 1.3 and 1.4 were utilized in order to compute the LOD and LOQ, respectively.

Investigation of Forced Degradation

Controlled Sample

The sample powder (1015 mg) was carefully weighed, and then it was put into a 100 ml volumetric flask that contained 50 ml of diluent. This amount of Cefpodoxime Proxetil is comparable to 250 mg of Levofloxacin. With the help of the diluent, the volume was brought up to 100 milliliter's. then diluted with the diluent to reach the concentration of 10 g/ml of cefpodoxime proxetil and 12.5 g/ml of levofloxacin, and then tested according to the method specified in the test protocol. After that, we computed the percentage of the assay. This particul In order to ensure that the formulation contains Cefpodoxime Proxetil and Levofloxacin, which are effectively separated from any degradation products that may have been created by them, this analysis was carried out. For the purpose of determining the stability indicating features and the amount of specificity that the technique possesses, studies of forced degradation were carried out. The sample powder, as well as the standard pharmaceuticals Cefpodoxime, Proxetil, and Levofloxacin, were put through the identical stress conditions both individually and collectively. This was done in order to demonstrate that the suggested analytical test method is reliable for determining stability. In order to facilitate the comparison of the results, this was carried out (ICH, 2003). One can gain an idea of the source of the degradation by comparing the chromatograms that were obtained for the sample, the individual medications, and their mixing under stress conditions. This can shed light on the reason why the degradation occurred. This provides further evidence that the optimised approach is capable of identifying the stability of pharmaceutical ingredients as well as pharmaceutical products. For the purpose of carrying out the peak purity investigation, both the purity angle and the purity threshold parameters were utilised. as a result of the fact that the peak purity of cefpodoxime proxetil and levofloxacin was discovered to be well within the acceptable limits for samples that were under stress. Therefore, the strategy offers a sign of stability as a result of its implementation. During the course of the investigation into the forced degradation that was carried out, the sample was put through the following set of circumstances.

Preparing standard solutions in preparation for stability investigations

Both of the individual standards, cefpodoxime proxetil 200 mg and levofloxacin 250 mg, were first measured out, then weighed, and finally placed into volumetric flasks of 100 ml each (S1 and S2). In addition, the standard combination was created by dissolving 200 milligrammes of cefpodoxime proxetil and 250 milligrammes of levofloxacin in separate volumetric flasks (S3) that were each 100 millilitres in volume and contained 50 millilitres of diluent. This was done using sonication. These flasks were identified by the letter S3 on their labels. It was continued to add diluent until the capacity reached one hundred millilitres. The solutions were diluted even more until they reached a concentration of 10 g/ml of cefpodoxime proxetil and 12.5 g/ml of levofloxacin. After that, the solutions were tested by using the diluent in line with the testing technique. The solutions in question were regarded as a controlled sample due to the fact that they were never put through any of the demanding conditions.

Acid Degradation

Based on the accurate weighing of the sample powder (1014 mg) and its subsequent placement in a volumetric flask with a capacity of 100 ml and containing 50 ml of diluent, it was established that the sample

powder was equivalent to 200 mg of Cefpodoxime Proxetil (250 mg of Levofloxacin). In addition, the standard solutions S1, S2, and S3 were developed in accordance with the method that was explained earlier in this paragraph. Each of these solutions was subjected to refluxing for a total of thirty minutes at a temperature of eighty degrees Celsius after ten millilitres of 0.1 N hydrochloric acid had been added to individual solutions. After a period of thirty minutes, the flasks were removed from the refrigerator and allowed to recover to room temperature. Next, 10.0 millilitres of sodium hydroxide with a concentration of 0.1 N was utilised in order to neutralise the solutions. Using the diluent, the volume was raised up to the desired level, and after that, the contents were allowed to settle down. Following its passage through a membrane filter with a pore size of 0.45 microns, the solution was filtered. The solutions were diluted even more until they reached a concentration of 10 g/ml of cefpodoxime proxetil and 12.5 g/ml of levofloxacin. After that, the solutions were tested by using the diluent in line with the testing technique.

Result & Discussion

Studies of Cefpodoxime Proxetil and Levofloxacin on a spectrum level, in addition to preliminary studies

The initial step in the preliminary identification process consisted of recording the FTIR spectrum of cefpodoxime proxetil and levofloxacin. This can be seen in figures 1 and 2. It was found that every drug had the groups that were expected to be present, and a tabular depiction of the group frequencies that were observed can be found in table 1. When the solubility was measured, it was found that Cefpodoxime Proxetil had a very low degree of solubility in water, but Levofloxacin had a very high degree of solubility in water. This was observed during the process of testing the solubility. The fact that Cefpodoxime Proxetil is easily soluble in solvents like methanol and acetonitrile was a discovery that was made. The antibiotic levofloxacin was found to be easily soluble in glacial acetic acid, as well as in methanol and acetonitrile. This was a discovery that was made. On the basis of the overlapping spectra of the medicines, the wavelength of 230 nm was selected for the current strategy (Fig. 2). The melting point of levofloxacin was found to be in the range of 214 degrees Celsius to 217 degrees Celsius, whereas the melting point of cefpodoxime proxetil was found to be in the range of 110 degrees Celsius to 114 degrees Celsius to 214 degrees Celsius.

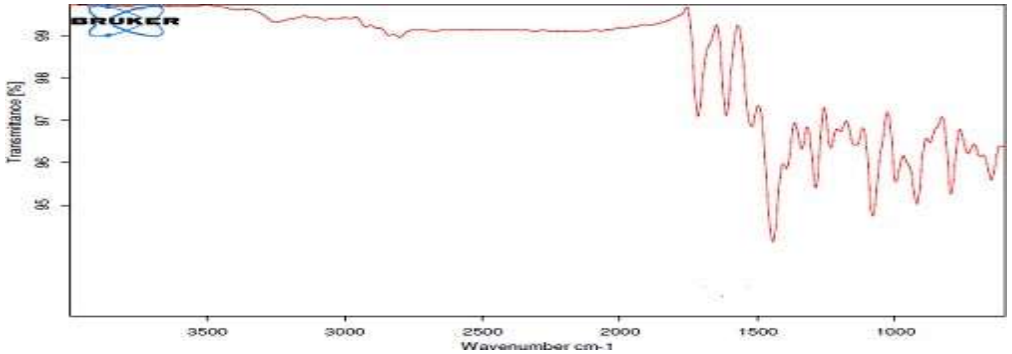


Fig 1 Cefpodoxime Proxetil's Fourier Transform Infrared Spectrum

Table 1: Observed Group Frequencies by FT-IR

Name of Drug	Expected group	Group Frequency
Cefpodoxime Proxetil	N-H	3324 cm^{1}
	S=O	1078 cm^{1}
	C=O	1636 cm^{1}
	C–C aromatic	1407 cm^{1}
Levofloxacin	N-H	3214 cm^{1}
	C=O	1716 cm^{1}
	C–H aromatic	1488 cm^{1}

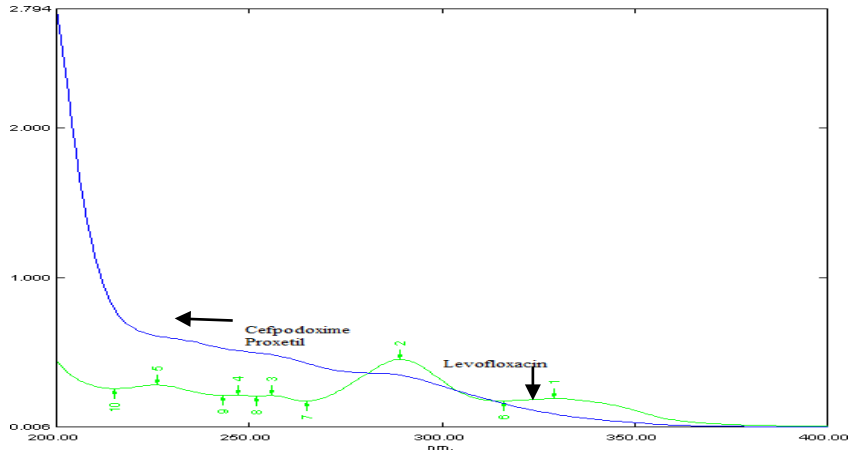


Fig 2: Cefpodoxime Proxetil and Levofloxacin's UV Spectrums Superimposed on One Another

The Development of Methods and the Optimization of Chromatographic Conditions

During each trial, one or two parameters were altered in order to achieve the best chromatographic conditions that were required for the successful separation and quantification of cefpodoxime proxetil and levofloxacin. After that, chromatograms were recorded with each and every one of the chromatographic conditions that were provided. It was necessary to conduct a lot of trials in order to arrive at a conclusion on the chromatographic parameters that were most suitable. In Table 2, just a few of them were recognised as being significant. In the experiments, the chromatographic conditions were rejected because they lacked appropriate resolution, which resulted in large peaks, merging of peaks, and inaccurate retention.

Table 2: Chromatographic Conditions Were Characterized by a Number of Experiments and Optimized

Mobile phase	Ratio	Flow rate	Conclusion	Remarks
Buffer: Methanol	50:50	0.7ml/min	Poor resolution and long retention time for Cefpodoxime and very short retention time for Levofloxacin with tailedpeak	Rejected
Acetonitrile :Buffer	50:50	1.0ml/min	Peak Broadening in Levofloxacin and asymmetric cefpodoxime peaks	Rejected
Phosphate Buffer: Methanol	20:80	1.5ml/min	Very small retention time and peak broadening of Levofloxacin, but shorterretention time for Cefpodoxime	Rejected
Methanol: Phosphate Buffer	90:10	1.2 ml/min	Poor resolution in cefpodoxime, more tailing in Levofloxacin peak	Rejected
Buffer: Methanol: Acetonitrile	65:25:10	1.2 ml/min	Good resolution and retention time, but cefpodoxime has more asymmetric peaks	Can be accepted
Buffer: Methanol: Acetonitrile	60:30:10	1.2 ml/min	Better resolution andretention time	**Accepted**

Method Validation

System Suitability Study

The chromatograms of blank, conventional pharmaceuticals are shown in Figures 3–6. These chromatograms show the pharmaceuticals both by themselves and in mixes with other substances. At 13.103 and 14.201 minutes, the chromatogram of the standard combination showed two peaks that were caused by the presence of cefpodoxime proxetil. The R and S isomers, which are respectively present in the racemic mixture, are the ones responsible for these peaks. It was at 4.91 minutes that the standard Levofloxacin was observed to have made its appearance. In Table 3, a tabular representation of the system's applicability is presented. This representation contains the retention duration, resolution, tailing factor, and the number of theoretical plates. High-performance liquid chromatography (HPLC) is the method that has been developed for the purpose of determining the percentage test of cefpodoxime proxetil and levofloxacin in its tablet dosage forms. This technique was developed for the purpose of determining that percentage.

Table 3: System Suitability Parameters

Sr. No.	Parameters	Cefpodoxime Proxetil		Levofloxacin
		1	2	
1.	Resolution (Rs)	6.7243	6.3458	9.4352
2.	Capacity Factor (k')	4.567	4.348	5.0782
3.	Theoretical Plate	385416.4571	445516.6657	120503.3583
4.	HETP	0.13202	0.1131	0.0544
5.	Tailing Factor	1.0672	1.0719	1.0688
6.	Retention time	13.103	14.201	4.909
7.	Asymmetry	1.041	1.105	1.4124

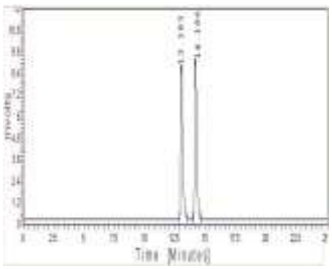


Fig 3: Chromatogram of Blank

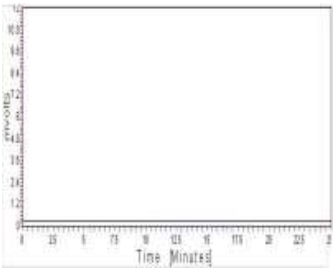


Fig.4: Chromatogram of Cefpodoxime

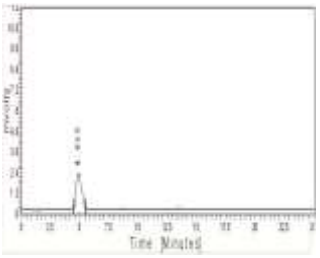


Fig 5: Chromatogram of Levofloxacin

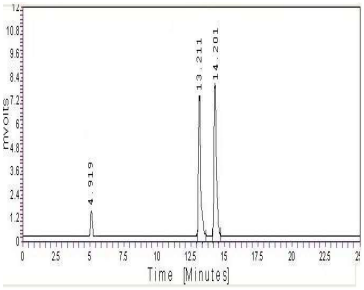


Fig 6 Chromatogram of Mixture

Specificity

There was no interference produced by the blank during the time that the analytical peaks were being retained. There was no interference at any point during the retention duration of the reference drugs, as indicated by the peak purity data, which demonstrates that both Cefpodoxime Proxetil and Levofloxacin were homogeneous. An exhaustive summary of the findings is presented in Table 4, which may be found here.

Table 4: Results of Specificity Study

Sr. No.	Peak name	Retention Time
1	Diluent	No peaks are observed at retention time of main peak
2	Main Peak Cefpodoxime Proxetil	13.103 min, 14.201 min
3	Main Peak Levofloxacin	4.901 min

Assay of Marketed Formulations

There was no interference produced by the blank during the time that the analytical peaks were being retained. There was no interference at any point during the retention duration of the reference drugs, as indicated by the peak purity data, which demonstrates that both Cefpodoxime Proxetil and Levofloxacin were homogeneous. An exhaustive summary of the findings is presented in Table 4, which may be found here.

Table 5: Assay of Tablet Formulation (Cefpodoxime Proxetil and Levofloxacin)

Brand Name	Cefpodoxime Proxetil		Levofloxacin	
	Label Claim (mg)	% Assay	Label Claim (mg)	% Assay
Glevopod	200	99.9	250	99.7
	200	99.8	250	98.9
	200	99.7	250	99.6
	200	99.9	250	98.4
	200	99.8	250	99.8
Mean		99.82		99.28
SD		0.0836		0.60580
%RSD		0.08381		0.6101

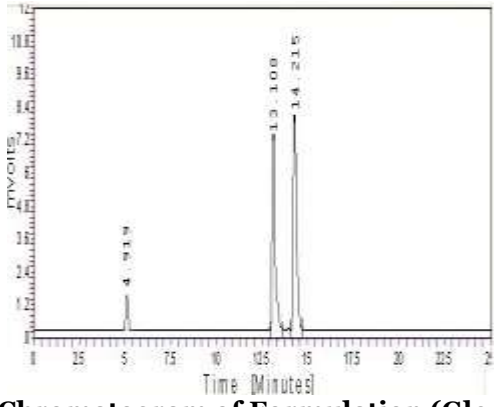


Fig 7: Chromatogram of Formulation (Glevopod)

Precision

System Precision

It was necessary to measure the peak responses of standard medication solutions in six different duplicates in order to determine the degree of precision that the system possessed. A comparison of the peak responses for Cefpodoxime Proxetil and Levofloxacin is presented in Table 6.6. This table also includes the mean, standard deviation, and percent relative standard deviation (RSD). According to the findings, these numbers are well within the parameters of what is considered acceptable. According to the findings, the relative

standard deviation, also known as RSD, for Cefpodoxime Proxetil was found to be 0.3144 percent, whereas the RSD for Levofloxacin was found to be 1.3721 percent.

Table 6. System Precision Data

Sr. No.	Peak areas of Cefpodoxime Proxetil		Total area	Peak areas of Levofloxacin
	1	2		
1.	237432.58	255342.32	492774.90	49870.9
2.	235237.17	257984.56	493221.73	50341.3
3.	236654.09	253867.38	490521.47	49076.3
4.	235274.41	256190.17	491464.58	49492.9
5.	236307.85	255887.29	492195.14	50823.9
6.	236993.63	258021.13	495014.76	50669.2
Mean	236316.52	256215.48	492532.10	50045.74
SD (□)	902.12	1598.40	1548.8267	686.6841
RSD (%)	0.38174	0.623848	0.3144	1.3721
Acceptance criteria			% RSD should not be more than 2	

i.Method Precision

In order to ascertain the degree of precision possessed by the procedure, we measured the peak response for sample solutions by employing six distinct duplicates. The results of the calculations that were performed to calculate the percent RSD and percent assays for Cefpodoxime Proxetil and Levofloxacin for each of the six samples that were tested are presented in Table 7. In order to illustrate that the approach that was used to generate the numbers is accurate, the numbers that were obtained for the % RSD.

Table 7: Method Precision Data

Sample No.	% Assay of Cefpodoxime Proxetil	% Assay of Levofloxacin
1.	99.84	98.23
2.	100.2	99.17
3.	99.74	100.63
4.	98.92	99.31
5.	97.56	99.07
6.	100.6	99.84
Mean	99.48	99.38
SD	1.092697	0.805177
RSD (%)	1.098446	0.810241

ii. Intraday and Interday Precision

Intraday and intraday testing disclosed a % RSD that was found to be well within the permissible range. This was the case for both types of testing. For your convenience, the results that were obtained are shown in tables 8 and 9, respectively.

iii.Accuracy (Recovery Study)

To conduct the research on accuracy, the extra standards of cefpodoxime proxetil and levofloxacin were recovered at 80 percent, 100 percent, and 120 percent of the level of the labelled claim, respectively. This was done in order to ensure that the research was accurate. The percentage of recovery was found to be between 99.37 and 99.98 percent for both of the drugs at all of the levels, which was deemed to be well within the parameters of acceptance requirements. This was determined to be the case. Calculations were made to determine the % recovery, as well as its standard deviation and its percent relative standard deviation. The results of these calculations are provided in table 10 below. In order to determine the levels of Cefpodoxime, Proxetil, and Levofloxacin, the approach that was utilised is a reliable and accurate method, as demonstrated by the percentage of medicine that was recovered.

iv. Linearity and Range

In order to determine whether or not the technique was linear, measurements were taken at nine distinct concentration levels. Following the construction of the calibration curves, we proceeded to graph the response factor versus the concentration of the drugs in order to get the desired results. In the case of Cefpodoxime Proxetil, linearity was observed throughout the entire concentration range of 2-24 g/ml (r2 = 0.999), while in the case of Levofloxacin, linearity was observed across the entire concentration range of 2.5-30 g/ml (r2 = 0.999). The results of the study showed that there is a significant connection between the areas that were investigated and the amount of drugs that were present. The results are summarised in table 11, which may be found below. Figure 8 and figure 9 show the calibration curves, and figure 10 shows the chromatograms for each medicine at each of the five distinct concentration levels. Both of these pictures may be accessed in the same document.

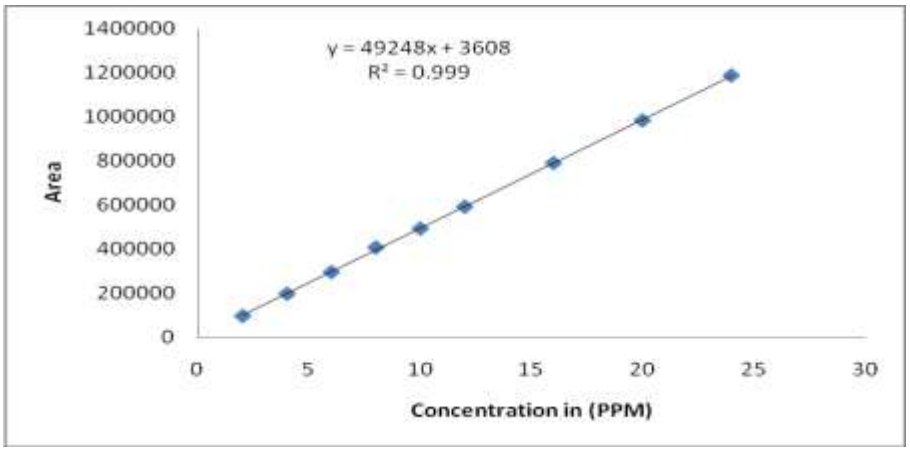


Fig. 8: Calibration Curve of Cefpodoxime Proxeti

Table 8: Intraday Precision

			Time Interval	Cefpodoxime Proxetil				Levofloxacin			
Sr. no.	Conc of CFP (µg/ml)	Conc of Levoflox (µg/ml)		% Assay	Mean % Assay	SD(±)	%RSD	% Assay	Mean % Assay	SD(±)	%RSD
1	8.0	10	After 2hr	99.5	99.45	0.918	0.923	100.3	99.35	0.739	0.744
			After4hr	100.2				99.5			
			After6hr	98.3				98.8			
			After8hr	99.8				99.6			
			After10hr	98.4				99.7			
			After12hr	100.5				98.2			
2	10	12.5	After 2hr	99.6	99.42	0.487	0.490	99.8	99.37	0.539	0.542
			After4hr	99.8				98.6			
			After6hr	98.7				99.9			
			After8hr	98.9				98.8			
			After10hr	99.7				99.5			
			After12hr	99.8				99.6			
3	12	15	After 2hr	99.5	99.32	0.617	0.622	99.4	99.52	0.708	0.711
			After4hr	100.3				100.5			
			After6hr	99.4				99.6			
			After8hr	98.6				99.7			
			After10hr	98.7				98.3			
			After12hr	99.4				99.6			

Table 9: Interday Precision

		Cefpodoxime Proxetil					Levofloxacin				
Sr. no.	Day	Conc (µg/ml)	% Assay	Mean % Assay	SD(±)	%RSD	Conc (µg/ml)	% Assay	Mean % Assay	SD(±)	%RSD
1	Day 1	8	99.5	99.43	0.802	0.806	10	100.3	99.57	0.702	0.703
	Day 2		100.2					99.5			
	Day 3		98.6					98.9			
2	Day 1	10	99.8	99.1	0.624	0.630	12.5	99.7	99.73	0.152	0.153
	Day 2		98.9					99.6			
	Day 3		98.6					99.9			
3	Day 1	12	99.5	99.17	0.351	0.354	15	99.4	99.20	0.624	0.629
	Day 2		99.2					99.7			
	Day 3		98.8					98.5			

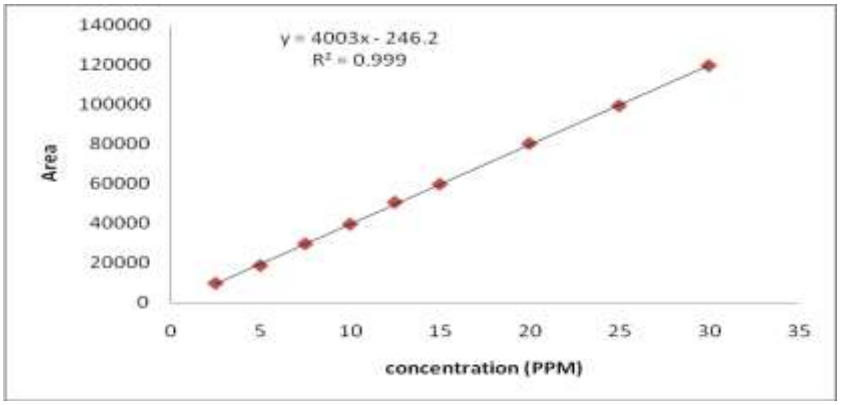


Fig. 9: Calibration Curve of Levofloxacin

Table 10 Linearity and Range

Sr. No.	Conc. (µg/ml) of LEVOFLOX	Area*(± SD)	Conc. (µg/ml) of CFP	Area*(± SD)
1	2.5	9912.93 (±91.64)	2	98824.34(±623.54)
2	5	18857.7(±192.99)	4	199052.32(±3.303)
3	7.5	29804.15(±287.42)	6	297563.64(±5.07)
4	10	39660.84(±173.02)	8	409073.91(±7.58)
5	12.5	50770.72(±476.31)	10	494586.41(±3.84)
6	15	59841.76(±294.9)	12	593450.2(±288.99)
7	20	80311.5 (±623.41)	16	791491.9(±724.56)
8	25	99374. 1 (±376.70)	20	984148.65(±7.913)
9	30	119637.44 (±898.01)	24	1187582(±816.3)
	Equation of line	**y = 4003x - 246.2**		**y = 49248x + 3608**
	Slope	**4003**		**49248**
	y-intercept	**-246.2**		**3608**
	r^2	**0.999**		**0.999**

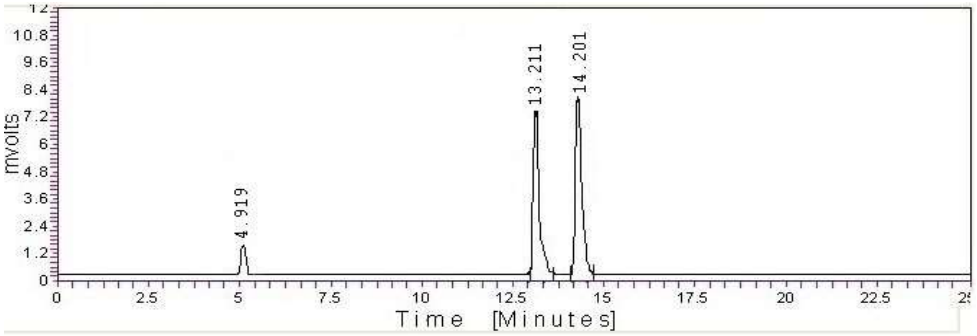


Fig 10: Representative Chromatogram of Linearity

Table 11: Recovery Study

Recovery Levels	80%		100%		120%	
Recovery (%)	Cefp	Levoflox	Cefp	Levoflox	Cefp	Levoflox
Amount Present (mg)	200	250	200	250	200	250
	200	250	200	250	200	250
	200	250	200	250	200	250
Amount of Std. Added (mg)	159.5	200.4	200.1	250.5	240.0	299.7
	160.3	200.6	200.2	249.8	240.3	300.5
	159.7	199.3	199.98	249.6	240.5	300.0
Amount Recovered (mg)	159.35	199.53	199.08	250.04	239.71	297.99
	159.29	200.10	200.17	248.53	238.94	300.28
	159.29	198.83	198.94	249.46	239.88	298.78
% Recovery	99.91	99.57	99.49	99.82	99.88	99.43
	99.37	99.75	99.99	99.49	99.43	99.93
	99.74	99.76	99.48	99.94	99.74	99.59
Mean Recovery	99.67	99.69	99.65	99.75	99.69	99.65
SD	0.28	0.11	0.29	0.23	0.23	0.25
%RSD	0.28	0.11	0.29	0.23	0.23	0.25

v. Stability in Analytical Solution

It was determined that the stability of Cefpodoxime Proxetil and Levofloxacin in analytical solution was verified by conducting an analysis on the sample both before and after it had been stored for twenty-four hours at room temperature (25 degrees Celsius) and in a refrigerator (at eight degrees Celsius). The percentages of Cefpodoxime, Proxetil, and Levofloxacin that were found to have test values that were within the permissible range are laid out in the following table. In addition to the percentage deviations, the percentage assay is tabulated in Table 12, which also includes the percentages.

Table 12: Solution Stability of Sample

Time level	Refrigerator	Room Condition (25°C)
Time in hour	% Assay of Cefpodoxime Proxetil	% Assay of Levofloxacin
Initial	99.5(± 0.251)	99.6(± 0.178)
After 24 hour	99.1(± 0.467)	98.8(± 0.075)

Table 13: Robustness- Effect of pH on sample

S. No	Cefpodoxime Proxetil 1				Cefpodoxime Proxetil 2				Levofloxacin			
	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Platecount
3.0	13.105	251264.89	1.119	214179.6	14.184	239566.3	1.121	214178.994	4.92	50471.744	1.0691	125933.66
3.4	13.103	251272.67	1.126	214183.65	14.199	239581.7	1.109	214184.164	4.91	50481.294	1.0799	125945.554
Mean	13.104	251268.78	1.1225	21181.63	14.1915	239574.06	1.115	214181.579	4.91	50476.519	1.0745	125939.609
S.D.	0.0014142144	5.5006543	0.0049	2.856	0.0106	10.88	0.0084	3.655	0.004	6.752	0.0076	8.40
%RSD	0.01079	0.00218	0.440	0.0013	0.074	0.004	0.761011782	0.001706842	0.10065	0.0133	0.710	0.0066

Table 14: Robustness-Effect of temperature on sample

Temp. °C	Cefpodoxime Proxetil 1				Cefpodoxime Proxetil 2				Levofloxacin			
	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count
25°C	13.105	251255.4	1.109	214188.7	14.183	239553.8	1.112	214187.5	4.921	50441.08	1.0889	125920.2
35°C	13.103	251249.2	1.103	214179.6	14.18	239581.7	1.111	214181.8	5.012	50436.40	1.0871	125915.5
Mean	13.104	251252.3	1.106	214184.2	14.181	239567.8	1.1115	214184.7	4.967	50438.74	1.088	125917.9
S.D.	0.0014	4.38483	0.004	6.427600	0.00212	19.74334	0.00070	4.051721	0.064	3.30925	0.00127	3.30925
%RSD	0.0107	0.00174	0.384	0.0030	0.01495	0.008241	0.063617	0.001891	1.296	0.00656	0.11698	0.00262

Table 15: Robustness-Effect of Flow rate on sample

Flow rate ml/min	Cefpodoxime Proxetil 1				Cefpodoxime Proxetil 2				Levofloxacin			
	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count
1	13.10	251287.4	1.172	214181.6	14.07	239563.8	1.174	214188.6	4.80	50472.0	1.078	125922.6
1.3	13.11	251301.5	1.162	214176.2	14.09	239591.7	1.178	214166.2	4.93	50475.3	1.079	125919.2
Mean	13.10	251294.5	1.167	214178.9	14.085	239577.8	1.176	214177.4	4.86	50473.6	1.079	125920.9
S.D.	0.0056	9.95	0.00707	3.8466	0.0148	19.7	0.00282	15.8	0.092	2.298	0.00028	2.425
%RSD	0.0431	0.0039	0.605	0.00179	0.105	0.00824	0.240	0.0074	1.90	0.00455	0.0262	0.0019

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

It was determined that the stability of Cefpodoxime Proxetil and Levofloxacin in analytical solution was verified by conducting an analysis on the sample both before and after it had been stored for twenty-four hours at room temperature (25 degrees Celsius) and in a refrigerator (at eight degrees Celsius). The percentages of Cefpodoxime, Proxetil, and Levofloxacin that were found to have test values that were within the permissible range are laid out in the following table. In addition to the percentage deviations, the percentage assay is tabulated in Table 12, which also includes the percentages.

Robustness

In order to explore what occurred when the pH of the column was changed, the temperature of the column was changed, and the flow rate was changed, the sample solution was utilised. Both the system suitability parameters and the peak regions were analysed under each of the various conditions, and the results were

compared to the findings obtained from the technique precision analysis. This event demonstrates that the technique may be relied upon to get the outcome. You can find the tabular presentation of the findings in tables 16, which can be obtained here.

Ruggedness

On the sample solution, the effects of varying the flow rate, the temperature of the column, and the pH were investigated and examined. The characteristics of the system's appropriateness as well as the peak regions were examined in each circumstance, and the findings were compared with the results of the method's precision. The percent RSD was determined to be less than 2 across all conditions. This demonstrates that the procedure has a high degree of consistency. The tabulated results may be found in tables 16

Table 16: Ruggedness Data

Analyst	Label claim		Amount found*		Label claim	
	mg/tab		mg/tab		(%)	
	CFP	LFX	CFP	LFX	CFP	LFX
1	200	250	198.97	247.68	99.485	99.072
2	200	250	199.98	249.32	99.99	99.728
3	200	250	199.91	248.49	99.955	99.396
Mean	200	250	199.62	248.496	99.81	99.39
SD			0.564	0.82002	0.2820	0.3280
% RSD			0.2825	0.32999	0.2825	0.3299

Forced Degradation Study

Cefpodoxime Proxetil, when subjected to a forced degradation study, demonstrated approximately 91.19 percent degradation in 0.1N HCl and 91.45 percent degradation in 0.1 NaOH. On the other hand, it did not demonstrate any degradation when subjected to the stress conditions of hydrogen peroxide solution which consisted of 30 percent hydrogen peroxide, 10 percent sodium bisulphate, and photolytic condition. In a study that investigated the effects of heat on the degradation of cefpodoxime proxetil, the researchers found that the compound degraded by 24.57 percent. Levofloxacin, on the other hand, showed a decline of 49.02 percent and 24.35 percent in comparison to the control when it was subjected to 0.1N hydrochloric acid and 0.1N sodium hydroxide, respectively. The degradation of levofloxacin was observed at the rates of 19.71 percent, 29.75 percent, and 19.35 percent, respectively, when it was submitted to photolytic conditions, when it was treated with 30 percent hydrogen peroxide, and when it was kept in an oven at sixty degrees Celsius for twenty-four hours. On the other hand, levofloxacin did not exhibit any evidence of degradation after being treated with sodium bisulfate. There is a tabulation of the percentage assay, the percentage degradation at each condition, the purity angle, and the purity threshold for both Cefpodoxime Proxetil and Levofloxacin that can be found in table 16. In addition to the standard drug by itself, mixes of standard drugs and the formulation of those pharmaceuticals were also subjected to studies of forced deterioration. These studies were carried out on the standard drug. It was observed, following an analysis and comparison of the degradants products that were found in individual pharmaceuticals, standard mixtures, and formulations, that the degradation products that were found in formulations were identical to those that were found in individual pharmaceuticals. When the formulation and the pure medications were subjected to the identical stress conditions, it was clear that the same degradation products were formed in both of them. This was evident based on the fact that the retention periods were comparable. When looking at the peak purity, the purity angle, and the purity threshold, it is possible to verify that there was no interference during the time that the major peaks were being retained. Figure 11 is a representation of the chromatogram of the control sample, while figures 12 (A-E) and 13 are examples of the chromatograms for the various stress scenarios (A-E). The curves of peak purity that were obtained under a wide range of varied stress conditions are depicted in Figure 14, which ranges from A to K.

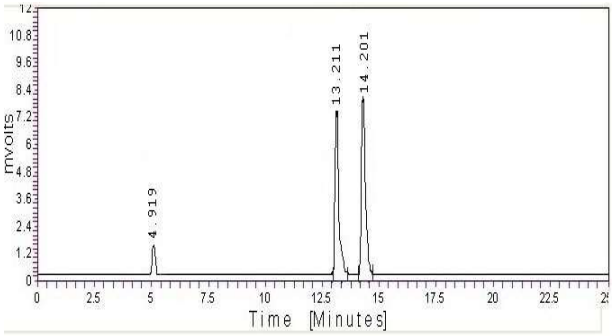
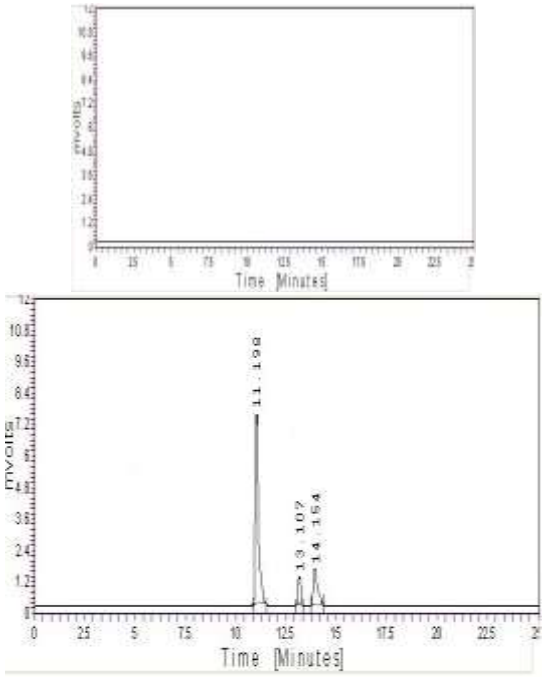


Fig 6.11: Chromatogram of Control Sample

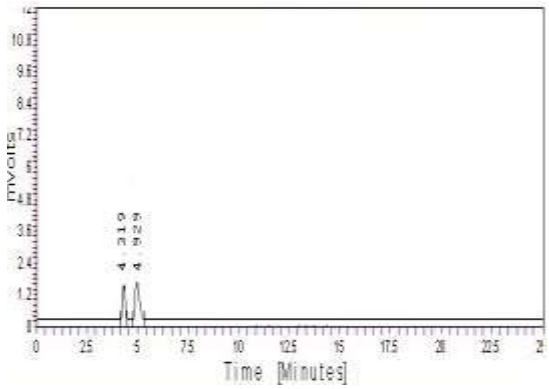
Table 17: Forced Degradation Study

Sr. No.	Condition	% Assay Of Cef	% Degradation w. r. t. control sample of Cef	% Assay Of Levof lox	% Degradation w. r. t. control sample of Levoflo	Peak Purity for CEF PEAK1		Peak Purity for Levofloxacin peak		Peak Purity for CEF PEAK2	
						Peak Purity Angle	Peak Purity Threshold	Purity Angle	Purity Threshold	PEAK2 Purity Angle	PEAK2 Purity Thresh Old
1	**Control Sample**	100.04		100.10		0.212	1.32	0.178	1.092	0.185	1.045
2	**Acid degradation**	8.81	91.19	51.08	49.02	0.332	1.347	0.196	1.078	0.305	1.409
3	**Alkali degradation**	8.58	91.45	75.75	24.35	0.325	1.422	0.187	1.083	0.298	1.377
4	**Peroxide degradation**	100.04	0.000	80.39	19.71	0.335	1.326	0.173	1.11	0.308	1.423
5	**Reduction**	100.04	0.000	100.10	0.00	0.316	1.338	0.177	1.079	0.289	1.335
6	**Thermal degradation**	75.46	24.576	70.35	29.75	0.344	1.253	0.159	1.087	0.317	1.465
7	**Photolytic degradation**	100.04	0.000	80.75	19.35	0.363	1.334	0.183	1.075	0.336	1.553

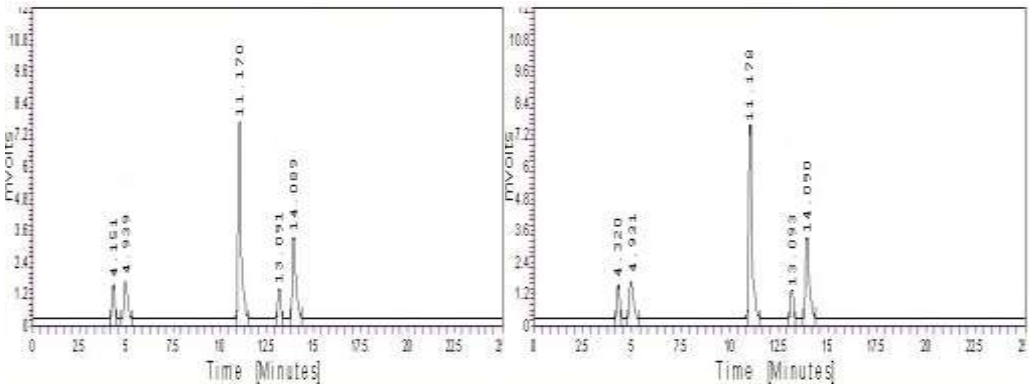
Acidic Degradation of Cefpodoxime Proxetil and Levofloxacin



A) Acidic degradation Blank



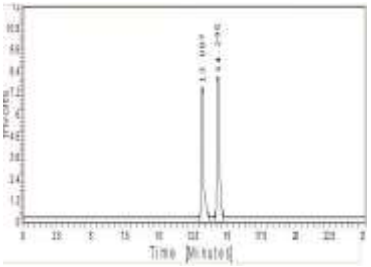
(B) CFP acidic degradation



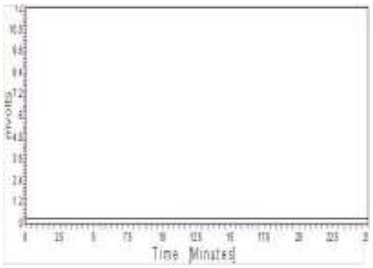
(C) Levoflox acidic degradation **(D) Std. mixture acidic degradation**

Fig 12 (A-D): Chromatograms of Acidic Degradation

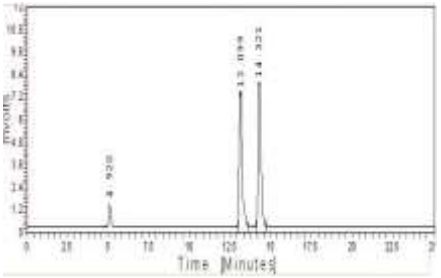
Peroxide Degradation (A-E) of Cefpodoxime Proxetil and Levofloxacin



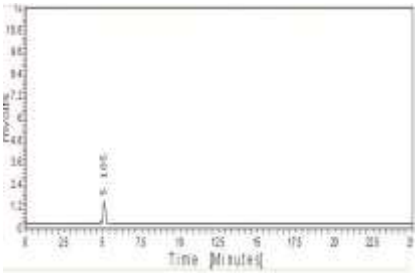
(A) Peroxide blank



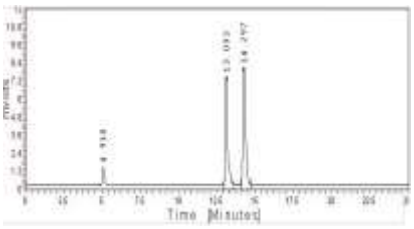
(B) CFP std peroxide degradation



(C) Levoflox in hydrogen peroxide degradation



(D) Std mixture peroxide degradation

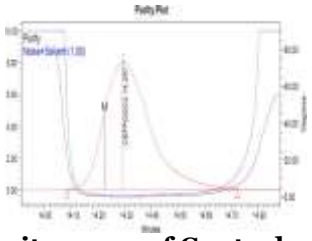


(E) Formulation peroxide degradation

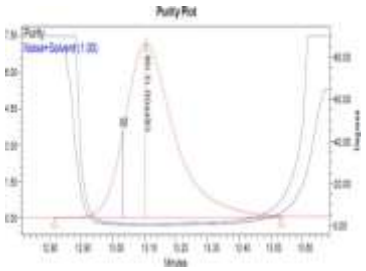
Fig 13 (A-E): Chromatograms of Peroxide Degradation

Peak purity plots for Cefpodoxime Proxetil and Levofloxacin at various stressconditions Peak

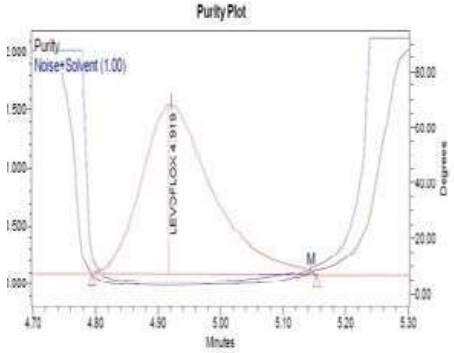
purity curve of Control sample CFP peak 1& 2



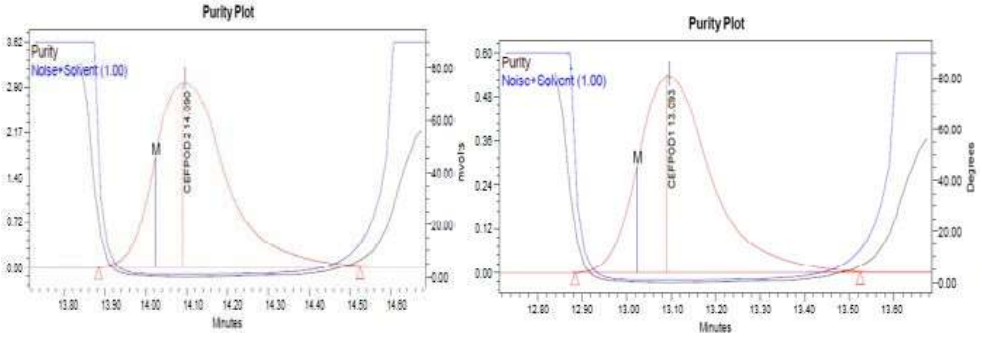
(A) Peak purity curve of Control sample Levoflox



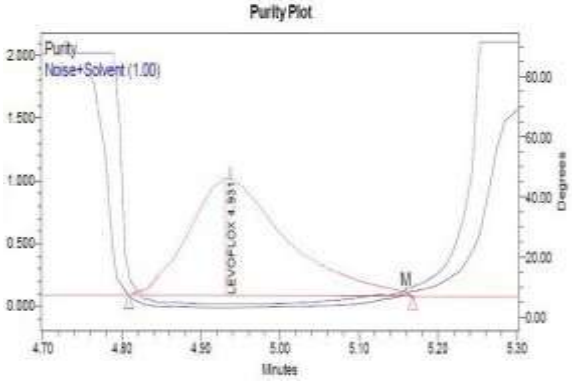
(B) Peak purity curve of CFP 1&2 in acidic condition



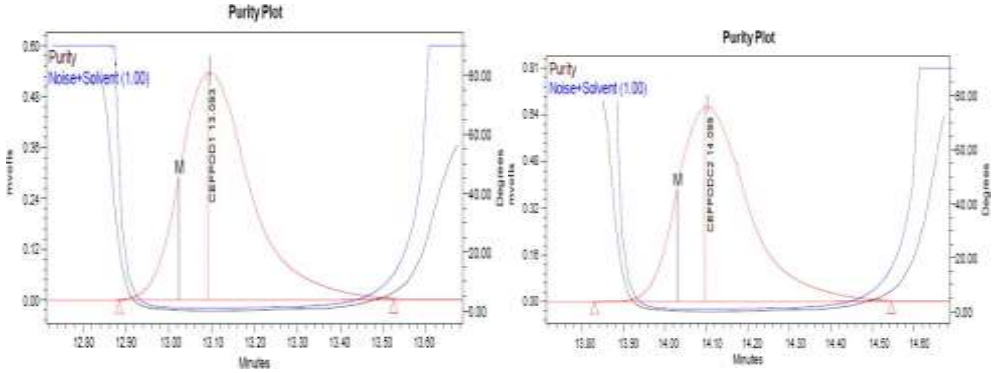
(C) Peak purity curve of Levoflox in acidic condition



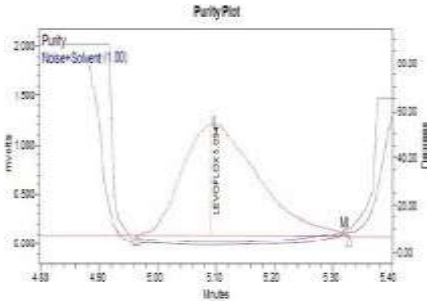
(D) Peak purity curve of CFP 1 & 2 in alkaline condition



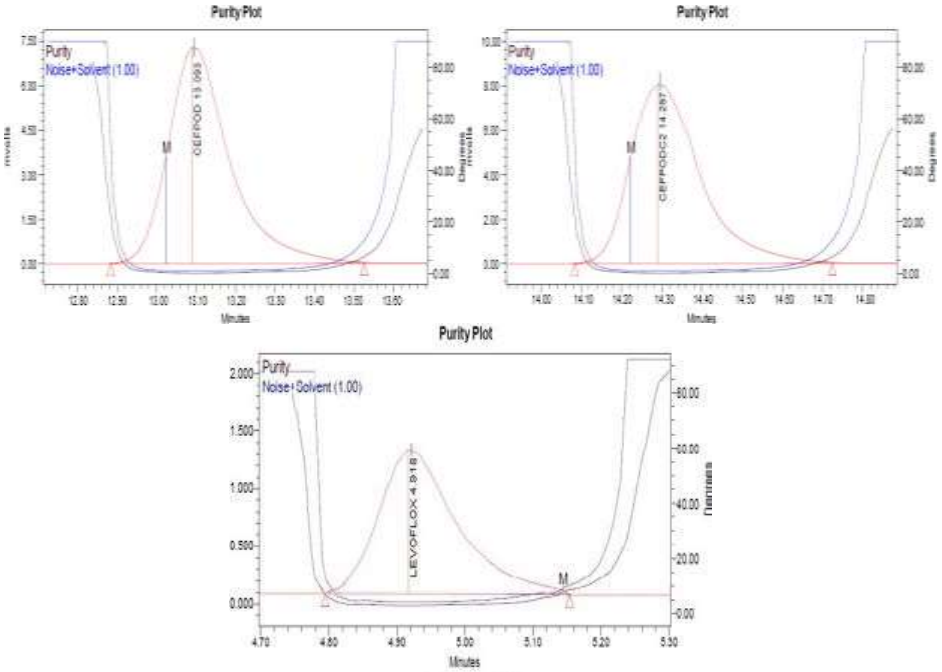
(E) Peak purity curve of Levoflox in alkaline condition



(F) Peak purity curve of CFP 1 & 2 in peroxide condition



(G) Peak purity curve of CFP 1 & 2 in peroxide condition



(H) Peak purity curve of Levoflox in peroxide condition

Fig. 14 (A–H): Peak Purity Plots at Various Stress Conditions

Conclusion

The detection of cefpodoxime proxeti was accomplished through the development of an RPHPLC method that is sensitive, precise, accurate, robust, and reproducible. This method does not include any sample excipients or degradants in its formulation. When developing the method, a buffer containing 20 mM potassium dihydrogen phosphate, methanol, and acetonitrile mobile phase were utilised. The column used was a Hypersil keystone RP C18 column with a flow rate of 1.2 ml/min and a wavelength of 235 nm. Over the course of a 25-minute run, the R and S isomers of cefpodoxime proxetil generated peaks at 13.11 and 14.12 minutes, respectively, while the peak of ofloxacin occurred at 5.01 minutes. When this method is utilised, the concentrations of Cefpodoxime Proxetil in Zedocef O tablets are 99.8 and 99.2%, respectively. RSDs of 0.0708 and 0.596 were found to be acceptable for both cefpodoxime proxetil and ofloxacin, respectively. The proposed method was validated by adhering to the requirements established by the ICH. It was determined that the standards for cefpodoxime, proxetil, and levofloxacin were recovered at 80%, 100%, and 120% of the

indicated claim, respectively. The accuracy of the testing process was improved as a result of this. Cefpodoxime Proxetil and Levofloxacin had mean percent recoveries of 99.67 and 99.69 at the 80% level, 99.65 and 99.75 at the 100% level, and 99.69 and 99.65 at the 120% level. All relative standard deviation (RSD) percentages in successful recovery experiments were within acceptable limits (either not exceeding 2 percent or not exceeding 2 percent in terms of assay deviation). With the use of this method, linear connections were discovered between concentrations of cefpodoxime proxetil ranging from 2 to 24 ng/ml and concentrations of levofloxacin ranging from 2.5 to 30 ng/ml (r 2 = 0.9999). The assay results for either medicine did not alter after being stored for twenty-four hours, regardless of whether they were kept at room temperature or in the refrigerator. This demonstrates that the solution is stable. On the other hand, the LOD and LOQ for Cefpodoxime Proxetil were 0.0064 and 0.00211 g/ml, but the LOD and LOQ for Levofloxacin were 0.0011 and 0.0003 g/ml. By systematically adjusting the pH of the mobile phase, the temperature of the column, and the flow velocity, we were able to assess the stability of the technique. The percent relative standard deviation (RSD) for system suitability metrics ranged from 0.0013% to 1.90% when these factors were taken into consideration, which is within the acceptable ranges. In light of this, the new approach appears to be sound. It was necessary for each analyst to do the percentage assays for Cefpodoxime Proxetil and Levofloxacin in duplicate in order to demonstrate that the technique that was suggested was successful. Despite the fact that both Cefpodoxime Proxetil and Levofloxacin had average percent tests of 99.81, their relative standard deviations were 0.282 and 0.329 each. It was appropriate for the RSD percentage.

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