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STABILITY INDICATING HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND LEVOFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS.

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Abstract

An HPLC technique was devised and validated for the concurrent analysis of Cefpodoxime Proxetil and Levofloxacin in tablet dosage form. The drugs were separated using chromatography on a Hypersil BDS C8 column (250 mm x 4.6 mm, 5 µ) as the stationary phase. The mobile phase consisted of a mixture of phosphate buffer (pH adjusted to 4.5 with orthophosphoric acid), methanol, and acetonitrile in a ratio of 55:25:20 (v/v/v). The separation was performed at a flow rate of 1.0 mL/min, and the detection of the drugs was carried out using UV detection at a wavelength of 222 nm. The retention time for Cefpodoxime Proxetil was seen to be 4.07 minutes, while for Levofloxacin it was 6.13 minutes. The methodology employed in this study was determined to be highly selective, as evidenced by the clear separation of the peaks corresponding to Cefpodoxime Proxetil and Levofloxacin. The resolution achieved between these peaks was measured to be 9.82. The approach suggested in this study demonstrates linearity, as seen by the high coefficient of determination (R2 = 0.999) obtained for both Cefpodoxime Proxetil and Levofloxacin. Furthermore, the method exhibits accuracy, with recovery rates ranging from 99.45% to 100.08% for Cefpodoxime Proxetil and Levofloxacin. Additionally, the method demonstrates precision, as indicated by the low relative standard deviation (%RSD < 2%). The aforementioned methodology has been employed to ascertain the efficacy of the commercial product, resulting in the determination that its potency falls within the acceptable range. The present methodology is applicable for the quantitative analysis of Cefpodoxime Proxetil and Levofloxacin in tablet formulations.

Keywords: RP-HPLC, GIT, Cefpodoxime and Levofloxacin

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Introduction

The oral route of drug administration offers numerous benefits, such as its user-friendly nature, capacity to promote patient adherence, flexibility in formulation, portability, and elimination of the requirement for specialised staff training, among others. According to existing literature, a significant proportion of pharmaceutical medications now available necessitate oral administration for their intended therapeutic effects. While the majority of oral drug delivery systems have demonstrated the ability to enhance the bioavailability of the active pharmaceutical ingredient (API) and improve the clinical effectiveness of the drug, certain physiological factors such as pH levels, variations in absorption across different segments of the gastrointestinal tract (GIT), variances in surface area and enzymatic activity within different sections of the GIT, transit time through the absorptive regions of the GIT, and the rate of metabolism can potentially diminish these desired outcomes. Two key strategic areas for improving oral dosage forms are targeted dispersion and regulated release of active pharmaceutical ingredients (API). These effects have a substantial impact on the efficacy of numerous drugs. Gastroretentive delivery systems exemplify the aforementioned technique by facilitating the extended and regulated release of pharmaceutical substances within the upper gastrointestinal region. The integration of the aforementioned characteristics guarantees reduced fluctuation in the concentration of active pharmaceutical ingredients (APIs) in the bloodstream, hence enhancing the effectiveness of treatments that exert localised effects within the stomach [1]. This is particularly beneficial for drugs that exhibit optimal absorption in an acidic milieu or possess a limited range of absorption. Gastroretentive systems offer several clinical benefits, including enhanced maintenance of therapeutic concentrations for drugs with time-dependent pharmacodynamics, reduced activation of unfavourable counterregulatory mechanisms, rebound effects, and tolerance, as well as minimised fluctuations in therapeutic effects for drugs with concentration-dependent effects. To date, a variety of gastroretentive systems have been found. The systems encompass raft-forming, high-density, magnetic, mucoadhesive, floating, and expanding properties. The variations that received significant attention in research papers were those that were favourably distinct from intraspecies differences, such as the size of the pyloric sphincter and the condition of the gastrointestinal tract's mucous membranes. Additionally, these variations were relatively easy to prepare, suggesting the utilisation of standard technological processes. In order to develop gastroretentive delivery systems, particularly those with floating properties, it is customary to employ a diverse range of polymeric materials that facilitate stomach retention. Therefore, it is imperative to meticulously select the constituents of the pharmaceutical formulation to regulate the technological, therapeutic, and pharmacokinetic aspects of drug formulation [2].

The primary aims of this article are to enumerate the predominant categories of polymers employed in the production of FDF (Fixed Dose Formulations), examine the characteristics and roles of these polymers within various technological methodologies and strategies for modifying release, and propose potential avenues for future research and material selection to drive advancements in this domain.

Materials

The methodology was developed and verified utilising high-performance liquid chromatography (HPLC) with a Waters 2996 instrument equipped with a photodiode array (PDA) detector. The separation procedure involved the utilisation of a column (Intersil C18 250 to 4.6 mm, 5µm) that was maintained at a temperature of 40°C. Data collection was conducted via the Empower 2 programme. The additional instruments employed were identical to those used in the previous operation, known as Chemicals and Reagents.

Chromatographic Equipments and Conditions

The chemicals and reagents employed in this study were identical to those utilised in the preceding procedure, as stated.

Methodology

Mobile Phase

The mobile phase, consisting of a mixture of acetonitrile and buffer, has been prepared. The buffer solution was prepared by dissolving 1 ml of Orthophosphoric Acid in one litre of water. A mixture consisting of methanol, acetonitrile, and water at a ratio of 50:30:20 was prepared and employed as a diluent. Throughout the chromatographic run, the gradient elution mode was implemented at various time intervals.

The procedure for the preparation of standard solutions for Cefpodoxime Proxetil and Levofloxacin is outlined below.

Approximately 1000 mg of Cefpodoxime

Proxetil and 3000 mg of Levofloxacin were precisely weighed and placed into individual 100 ml volumetric bottles. These bottles were then supplemented with 70 ml of diluent and subjected to dissolution by the process of sonication. The solutions were cooled to ambient temperature, then mixed with the diluent to achieve a final volume of 100 ml, resulting in the creation of a stock solution. An additional 1 mL of standard stock solutions of Cefpodoxime Proxetil and Levofloxacin at concentrations of 30 µg/ml were separately transferred to individual 1000 ml flasks. The remaining volume in each flask was then filled with a diluent solution, resulting in final concentrations of 10 µg/ml for Cefpodoxime Proxetil and 30 µg/ml for Levofloxacin.

Preparation of Sample Solution (Cefpodoxime Proxetil & Levofloxacin 10mg+30mg) [3]

The components were carefully measured and mixed with a total of 20 capsules, each labelled to contain 10 mg of one substance and 30 mg of another substance. In a 100 ml volumetric flask, 70 ml of diluent was added. The contents of five capsules were then transferred into the flask and subjected to sonication for a duration of 30 minutes, with intermittent vigorous shaking. The solution was subjected to cooling until it reached the ambient temperature and thereafter underwent dilution to a final volume of 100 ml [4]. The solution underwent filtration using a syringe equipped with a teflon filter having a pore size of 0.45 micrometres. A volume of 1 millilitre of the given solution was subjected to additional dilution by blending it with 50 millilitres of diluent. The resulting mixture had a concentration of 10 micrograms per millilitre combined with a concentration of 30 micrograms per millilitre.

The optimisation of chromatographic conditions and the development of methods.

Numerous chromatographic cycles have been conducted to analyse specific pharmaceutical compounds and their combinations using diverse mobile phase compositions. Several mobile phases have been investigated, including acetonitrile and water, acetonitrile and buffer (KH2PO4, OPA buffer), and methanol buffer. In the present study, buffer (0.1% v/v OPA in water) and acetonitrile were employed as the mobile phase for further chromatographic investigations using gradient elution [5].

Method Validation

The method validation was performed according to ICH parameter requirements, such as specificities, forced degradation, precision, precision, linearity, LOD, LOQ and analysis solution stability (ICH 1996, ICH 2003, ICH 2005).

System Suitability Study

The chromatograms and peak responses of Cefpodoxime Proxetil and Levofloxacin were quantified. The system appropriateness requirements encompass parameters such as retention time (Rt), peak area, tail factor, theoretical plate, and resolution.

Specificity

The method's specificity was assessed through the comparison of chromatograms obtained from blank solutions containing 10 µg/ml of Cefpodoxime Proxetil and 30 µg/mL of Levofloxacin, with those obtained from a mixed standard solution. Additionally, individual injections of Cefpodoxime Proxetil (10 µg/ml) and Levofloxacin (30 µg/ml) were performed to further evaluate specificity. The comparison of pitch purity was conducted, ensuring that the retention times of the primary peaks do not cause any interference.

Assay of the Formulation

The peak responses and percentage test calculation for Cefpodoxime Proxetil and Levofloxacin were determined by injecting 10 µl of sample solutions into duplicates.

Precision

System Precision

In the high-performance liquid chromatography (HPLC) system, a total of six replicate injections were performed. Each injection consisted of a mixture containing 10 µg/ml of Cefpodoxime Proxetil and 30 µg/ml of Levofloxacin. The mean, standard deviation, and relative standard deviation (RSD) percentage were calculated.

Method Precision

A total of six samples were analysed in accordance with the prescribed test technique, each containing a predetermined quantity of Cefpodoxime Proxetil and Domeperidone (10 µg/ml and 30 µg/ml, respectively). The percentage test and relative deviation of the standard were calculated.

Intraday and Interday Precision

The intraday precision of the experiment was assessed using a series of examinations conducted over several time intervals, namely two hours, twelve hours, and daily. These examinations were carried out using three

separate concentration levels of Cefpodoxime Proxetil (7.5 µg/ml, 10 µg/ml, and 12.5 µg/ml) and Levofloxacin (30 µg/ml and 37.5 µg/ml). The experiment on interday precision was carried out over the course of three distinct days, namely day 1, day 2, and day 3, with three different levels of intraday concentration.

Accuracy (Recovery study)

The procedure was meticulously examined by introducing the standard drug into the preanalyzed sample at three different concentrations: 80%, 100%, and 120%. The average recoveries were afterwards determined. A precise quantity of powdered substance weighing 614.31 mg was accurately measured and added to a volumetric flask with a capacity of 100 mL. This quantity corresponds to 10 mg of Cefpodoxime Proxetil and 30 mg of Levofloxacin. In accordance with the established criteria, the inclusion of Cefpodoxime Proxetil and Levofloxacin in the designated assertion was made at percentages of 80%, 100%, and 120% correspondingly. Subsequently, each specimen was subjected to sonication for a duration of 25 minutes in 50 ml of diluent, with intermittent vigorous shaking. The solutions were subsequently equilibrated to ambient temperature, and a maximum volume of 100 ml of diluent was generated. The solution underwent filtration using a Teflon filter syringe with a pore size of 0.45µ, followed by subsequent dilution and further blending.

Linearity and Range

The linearity of the process was assessed by doing measurements at nine different levels of concentration. Standard stock solutions were prepared with standard solutions of Cefpodoxime Proxetil with concentrations ranging from 0.1 to 15 µg/ml, and Levofloxacin with concentrations ranging from 0.3 to 45 µg/ml. The High Performance Liquid Chromatography (HPLC) instrument introduced 10 µl of every solution and recorded the peak area of the resulting chromatogram. A total of six replicates were examined at each level, following the specified methodology. The mean area, together with its corresponding standard deviation and relative standard deviation of the percentages of peak areas, were calculated for each level. The construction of the calibration curve involved plotting the medium area on the curve against the corresponding drug concentration. The equation for the curve and the correlation coefficients were computed based on the calibration curves.

Stability in Analytical Solution

The stability of Cefpodoxime Proxetil and Levofloxacin in an analytical solution was assessed by storage at two different temperatures, namely a refrigerator set at 8°C and room temperature. The samples were evaluated before and after a 24-hour period. The concentration of Cefpodoxime Proxetil was maintained at 10µg/ml, while the concentration of Domperidon Maleate was maintained at 30 µg/mL. The percentage test has been established based on the analysis of the peak regions corresponding to Cefpodoxime Proxetil and Levofloxacin.

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

The limits of detection (LOD) and limits of quantification (LOQ) were calculated based on the slope (S) and standard deviation (Ś) of the reactions observed for Cefpodoxime Proxetil and Levofloxacin.

Robustness

Samples of Cefpodoxime Proxetil at a concentration of 10 µg/ml and levofloxacin at a concentration of 30 µg/ml were prepared using a sample stock solution. These samples were then subjected to analysis using the suggested technique. Minor yet deliberate modifications were implemented in order to evaluate its resilience, including:

Column temperature – The temperatures were adjusted to 35°C and 45°C, while keeping the column temperature constant at 40°C.

Flow rate – The effect of flow rate change was observed at two different values, specifically 0.8 ml/min and 1.2 ml/min. It should be noted that the real column flow rate was 1.0 ml/min.

Forced Degradation Study

The purpose of the inquiry was to establish an efficient separation method for Cefpodoxime Proxetil, Levofloxacin, and their respective degradation products. The assessment of stability indicating features and specificity of the approach was conducted using forced degradation studies. In order to ascertain stability, the sample powder and standard pharmaceuticals Cefpodoxime Proxetil and levofloxacin, along with their combination, were subjected to identical stress conditions to determine the recommended analytical testing method (ICH, 2003). The determination of the cause of degradation can be achieved by the comparative analysis of samples, individual drugs, and their combined chromatograms under stressful conditions. This analysis provides

evidence for the reliability and robustness of the enhanced methodology in the field of pharmaceuticals [6]. The assessment of peak purity was conducted by employing criteria related to the purity angle and purity threshold. The stressed samples were able to meet acceptable requirements effectively due to the high level of purification of Cefpodoxime Proxetil and Levofloxacin. Hence, the method demonstrates stability. The contents of the capsule were accurately combined to transfer samples of Cefpodoxime Proxetil, weighing 625 mg, which is equivalent to 10 mg of Levofloxacin (30 mg). These samples were transferred into a 100 ml volumetric flask containing 70 ml of diluent. The substance has been submerged for a duration of approximately 30 minutes, experiencing periodic agitation, thereafter returned to ambient temperature, and mixed with a diluent volume of no more than 100 ml (as a sample stock solution)[7]. Comparable remedies were designed for Cefpodoxime Proxetil at a concentration of 10mg, Levofloxacin at a concentration of 30mg, and a combination of the two in a standard stock solution. The sample was subjected to forced degradation experiments under the following conditions:

Acidic Degradation
In 50 ml volumetric flask with 30 ml of diluent, 5.0 ml of the above-mentioned stock solution was transferred. Added 5.0 ml 5 N hydrochloric acid to it and refluxated at 80°C for 30 minutes. The flasks were removed and refrigerated to room temperature after 30 minutes. With 5.0 ml of 5 N sodium hydroxide, the resultant solutions were neutralised. The volume was diluted to the mark and the components were adjusted. Filtered using 0.45µ membrane filter, the solution was analysed using the optimal technique.

Alkaline Degradation
In the aforementioned instance, the 5 ml volumetric flask, which held 30 ml of diluent, was afterwards put into a 50 ml flask. Furthermore, a volume of 5.0 ml of a 5 N solution of sodium hydroxide was introduced, and the resulting mixture was subjected to reflux at a temperature of 80°C for a duration of approximately 30 minutes. After a duration of 30 minutes, the flasks were extracted and thereafter subjected to refrigeration until they reached the ambient temperature. The solutions were neutralised using 5.0 mL of 5 N hydrochloric acid. The volume was diluted up to the designated mark, and the contents have the potential to be modified. The solution was subjected to analysis using the optimum approach after being filtered using a 0.45µ membrane filter.

Peroxide Degradation
A 5 mL volumetric flask was utilised to transfer 30 mL of diluent from the aforementioned stock solution. A volume of 5.0 ml of hydrogen peroxide, with a concentration of 30 percent, was introduced into the solution, which was then allowed to react for a duration of 30 minutes. The volume was diluted up to the designated mark, and the contents can be afterwards modified if necessary. The solution was subjected to analysis using the optimum approach after being filtered using a 0.45µ membrane filter.

Reduction
A 5 mL volumetric flask was utilised to transfer 30 mL of diluent from the aforementioned stock solution. A volume of 5.0 mL of a 1N solution of sodium bisulphate was added and sonicated for a duration of 30 minutes. The volume was diluted up to the designated mark, and the contents have the potential to be modified as necessary. The solution underwent filtration through a membrane with a pore size of 0.45µ and was thereafter subjected to analysis using the most suitable methodology.

Thermal Degradation
A powder sample weighing 621.4 mg, which is equivalent to 10 mg of Cefpodoxime Proxetil, was placed into a 100 ml volumetric flask. The flask was then sealed and subjected to cooling in a hot air oven at a temperature of 60 °C for a duration of 24 hours. The volume was diluted up to the designated mark, and the contents can be further modified if necessary. The fluid underwent filtration through a membrane with a pore size of 0.45µ and was thereafter subjected to analysis using the most effective methodology available.

Photolytic Degradation
A quantity of powder (620.6 mg) representing 10 mg of Cefpodoxime Proxetil was placed into a 100 ml volumetric flask and subjected to photolytic conditions for a duration of 24 hours, with an exposure of 1.2 million lux hours. The volume was diluted up to the designated mark, and the components were afterwards adjusted. The solution underwent filtration through a membrane with a pore size of 0.45µ and was thereafter subjected to analysis using the most effective methodology.

Humidity

A quantity of powder weighing 619.9 mg, which is equivalent to 10 mg of Cefpodoxime Proxetil, was placed in a volumetric flask with a capacity of 100 ml. The flask was then exposed to a temperature of 40°C and a relative humidity of 75 percent for approximately 24 hours. The volume was diluted up to the designated mark, and the contents can be further modified if necessary. The solution underwent filtration using a 0.45µ membrane, and the most effective technique was evaluated.

Hydrolysis

A powder sample weighing 618.9 mg of Cefpodoxime Proxetil, equivalent to a dosage of 10 mg, was put into a 100 ml volumetric flask along with 50 ml of water. The mixture was then left undisturbed for a duration of 24 hours. The volume was diluted up to the designated mark, and the contents can be further modified if necessary. The volume was diluted up to the designated mark, and the components were afterwards adjusted. The fluid underwent filtration through a membrane with a pore size of 0.45µ and was thereafter subjected to analysis using the most effective methodology available. The experiment involved subjecting a 5 ml sample of each standard stock solution to forced deterioration using acid, alkaline, peroxide, and reduction. The solutions underwent filtration using a 0.45µ membrane, subsequent dilution, and were then assessed following the recommended protocol. The thermal, photolytic, humidity, and hydrolysis tests were conducted on individual volumetric flasks containing the standard solid samples. Forced degradation was applied to each flask according to the prescribed conditions for the given sample, which consisted of 10 mg of Cefpodoxime Proxetil, 30 mg of Levofloxacin, and a combination of 10 mg and 30 mg of the two substances. After being diluted with a diluent, the standard drug was filtered through a 0.45µ membrane and evaluated using the best process following its deterioration. In a similar manner, the samples and standards were subjected to dilution using a diluent, ensuring that no degradation occurred, and afterwards analysed using the improved methodology.

Preliminary studies and spectral studies of Cefpodoxime Proxetil & Levofloxacin

The preliminary identification was carried out by recording the FTIR spectrum (fig1&2) for Cefpodoxime Proxetil & Levofloxacin. The expected groups were found to be present in each drug and the observed group frequencies are tabulated in table 1. While determining the solubility it was found that Cefpodoxime Proxetil was slightly soluble and Levofloxacin was sparingly soluble in water. Cefpodoxime Proxetil was found to be freely soluble in the solvents such as methanol, acetonitrile. Levofloxacin was found be freely soluble in glacial acetic acid, methanol and acetonitrile. From the overlain spectrum of drugs, 230 nm was selected as wavelength for the present method (Fig 3). The melting point was found in the range of 110 ºC to 114 ºC for Cefpodoxime Proxetil and 214 ºC – 217 ºC for Levofloxacin[8].

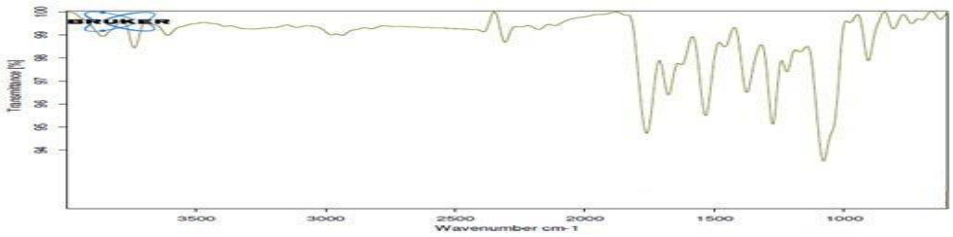


Figure 1. FT-IR Spectrum of Cefpodoxime Proxetil

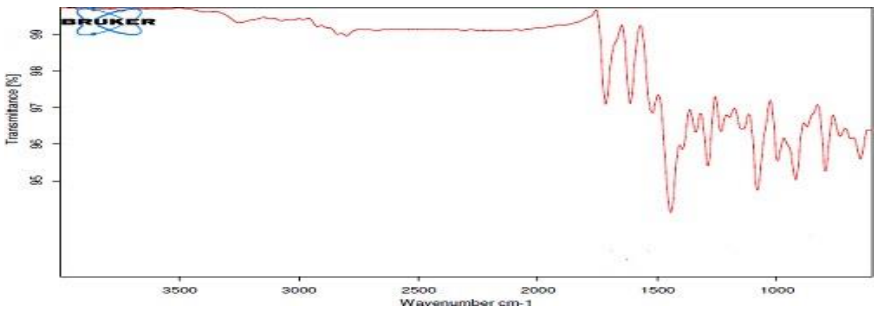


Figure 2. FT-IR Spectrum of Levofloxacin

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}

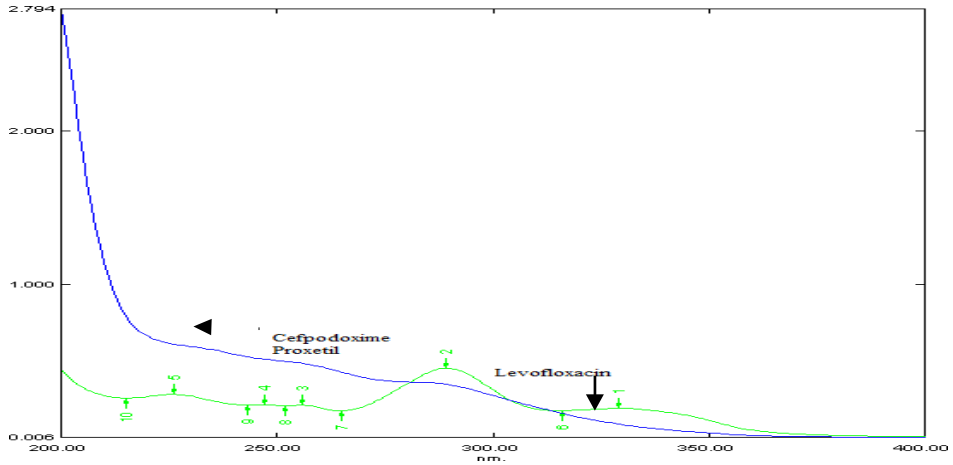


Figure 3. Overlain UV Spectrum of Cefpodoxime Proxetil and Levofloxacin

Chromatographic condition optimisation and method development

To attain the optimised chromatographic conditions for the separation and quantification of Cefpodoxime Proxetil and Levofloxacin, one or two parameters were adjusted during each trial. Subsequently, chromatograms were obtained under the stated chromatographic circumstances. Several experiments were conducted in order to determine the most effective chromatographic settings [9]. A limited number of them were referenced in table 6.87. The rejection of chromatographic conditions in trials was attributed to several factors, including poor resolution, broad peaks, merging of peaks, and incorrect retention.

Finalized Chromatographic Conditions

The chromatographic conditions were determined based on the factors of system appropriateness.

Column : Hypersil-keystone RP18

Wave length : 235nm.

Column Temp : 30ºC. Injection Volume : 10 µL. Run Time : 25 min

Flow Rate : 1.2 ml / min

Pump Mode : Isocratic

Retention time : About 5.0 to 6.0 min (for Levofloxacin)

About 12.5 to 14.5 min (for

Cefpodoxime Proxetil) Mobile Phase : Buffer: methanol: acetonitrile (pH 3.2 OPA)

(10 mM potassium dihydrogenphosphate)

Diluent : Mobile phase

Table 2. Various Trials and Optimization of Chromatographic Conditions

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 tailed peak	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 shorter retention 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	Cefpodoxime has superior resolution and retention time, however with a higher occurrence of asymmetric peaks.	Can be accepted
Buffer: Methanol: Acetonitrile	60:30:10	1.2 ml/min	Better resolution and retention time	**Accepted**

Method Validation

System Suitability Study

The chromatograms of blank, standard drugs alone and in their mixture are shown in fig 4-7. In the chromatogram of standard mixture Cefpodoxime Proxetil showed two peaks at 13.103 and 14.201 min which are due to *R* and *S* isomers respectively present in the recimic mixture. Similarly standard Levofloxacin was appeared at 4.91 min. Table 3 presents the tabulated data for system suitability parameters, including retention time, resolution, tailing factor, and number of theoretical plates. The HPLC method was devised to determine the % assay of Cefpodoxime Proxetil and Levofloxacin in tablet dosage forms.

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

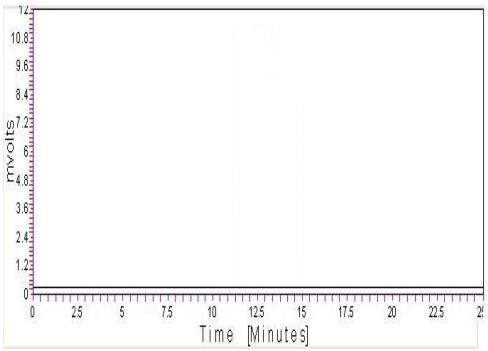


Figure 4. Chromatogram of Blank

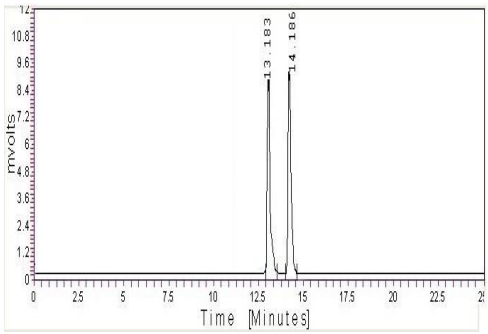


Figure 5. Chromatogram of Cefpodoxime

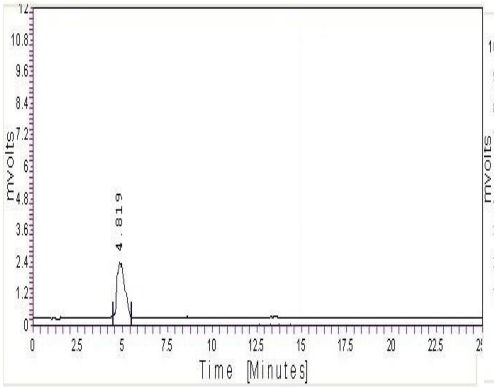


Figure 6. Chromatogram of Levofloxacin

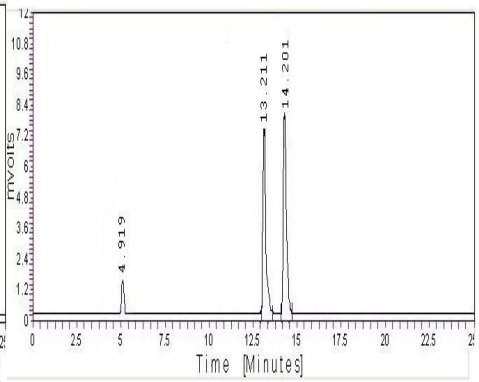


Figure 7. Chromatogram of Mixture

Specificity

No interference was seen at the retention time of the analytic peaks. The peak purity data analysis indicates that both Cefpodoxime Proxetil and Levofloxacin exhibited homogeneity, with no observed interference at the retention time of the

standard medicines. The findings are succinctly presented in Table 4.

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

The tablets Glevopod were subjected to analysis, wherein the % assay for Cefpodoxime Proxetil and Levofloxacin was determined. The experiment was conducted with five repetitions, and the average assay percentages for Cefpodoxime Proxetil and Levofloxacin in Glevopod were determined to be 99.82% and 99.28% respectively [10]. The relative standard deviation (RSD) values for Cefpodoxime Proxetil and Levofloxacin were determined to be 0.0836% and 0.6101% respectively. These values were found to be within the permitted limit. The corresponding data have been presented in Table 5. Figure 8 displays the chromatogram for the sample.

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**

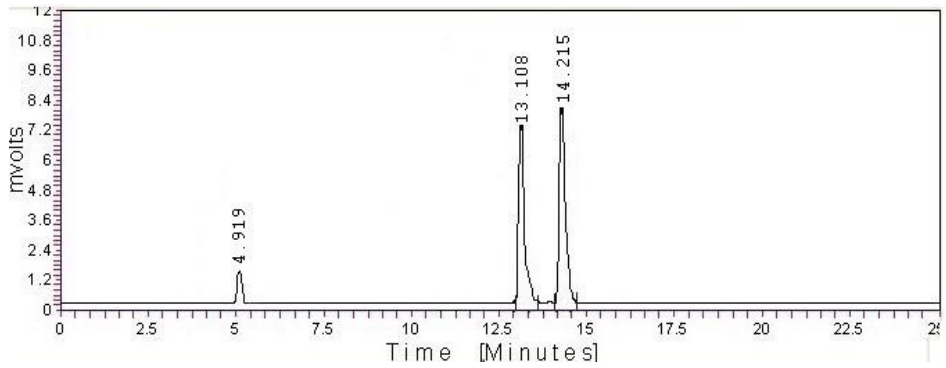


Figure 8. Chromatogram of Formulation (Glevopod)

Precision

System Precision

The determination of system precision was conducted by assessing the peak response of standard drug solutions in six replicates. Table 6 displays the peak responses, mean, standard deviation, and percentage relative standard deviation (RSD) for Cefpodoxime Proxetil and Levofloxacin. It is observed that these values fall comfortably within the acceptable criteria [11-13]. The relative standard deviation (RSD) for Cefpodoxime Proxetil was determined to be 0.3144%, while for Levofloxacin it was found to be 1.3721%.

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**	

Method Precision

The precision of the approach was assessed by measuring the peak response of sample solutions in six duplicates. The percentages of Cefpodoxime Proxetil and Levofloxacin tests in six samples were determined and the relative standard deviations (%RSD) were calculated. The results are presented in Table 7. The obtained relative standard deviation (RSD) figures provide confirmation that the approach employed is precise for the determination of the desired parameter [14].

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**

Intraday and Interday Precision

% RSD in intraday and interday studies were found well within the acceptable limits. The results obtained were mentioned in the table 8 & 9.

Accuracy (Recovery Study)

Accuracy study was performed by the recovery of the added standards of Cefpodoxime Proxetil and Levofloxacin at three different levels of the labeled claim that are 80%, 100%, 120% level of the labeled claim. The percentage recovery for both medications was observed to fall within the range of 99.37-99.98% at all tested levels, which was determined to be within the acceptable ranges of the acceptance criteria. The calculation of the percentage recovery, together with its corresponding standard deviation and relative standard deviation (% RSD), has been performed and the results are presented in Table 10[15]. The percentage of recovery serves as validation that the analytical approach is precise and reliable for quantifying the concentrations of Cefpodoxime Proxetil and Levofloxacin.

Linearity and Range

The linearity of the approach was assessed by conducting experiments at nine different concentration levels. The calibration curves were generated through the graphing of the response factor versus the concentration of medicines. The linearity of Cefpodoxime Proxetil was observed within the concentration range of 2-24 µg/ml (r2 = 0.999), while for Levofloxacin, linearity was observed within the concentration range of 2.5-30 µg/ml (r2 = 0.999). The findings indicate a strong link between the spatial distribution of medicines and their concentration levels. The findings are presented in Table 11. The calibration curves can be observed in Figure 10 and 11, whereas Figure 12 displays the chromatograms of the five concentration levels for each medication[16].

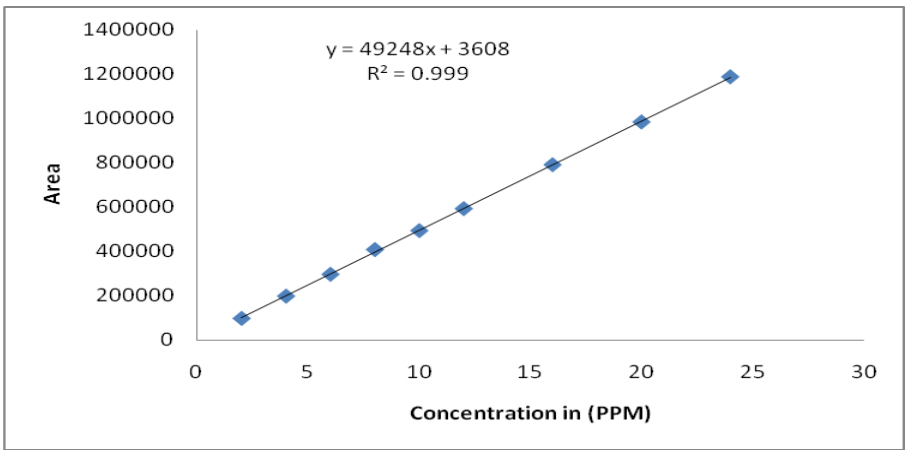


Figure 9. Calibration Curve of Cefpodoxime Proxetil

Table 8. Intraday Precision

			Time Interval	Cefpodoxime Proxetil				Levofloxacin			
Sr. no.	**Concentration of CFP (µg/ml)**	**Concentration 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**	**Concentration (µg/ml)**	**% Assay**	**Mean % Assay**	**SD(±)**	**%RSD**	**Concentration (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			

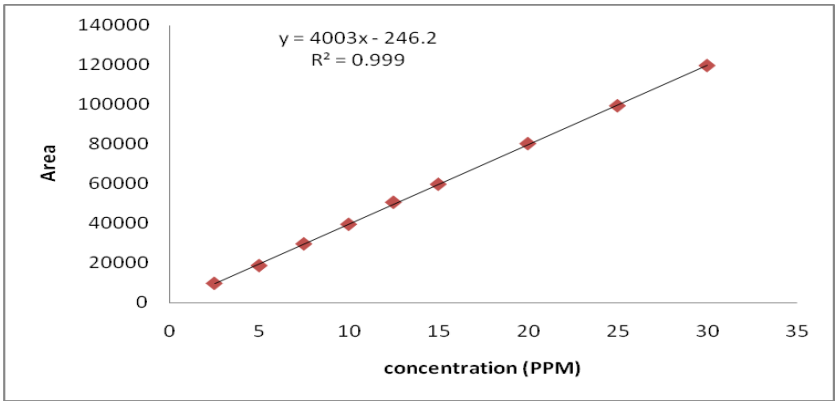


Figure 10. Calibration Curve of Levofloxacin

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**

**Average of six determination*

Figure 11. Representative Chromatogram of Linearity

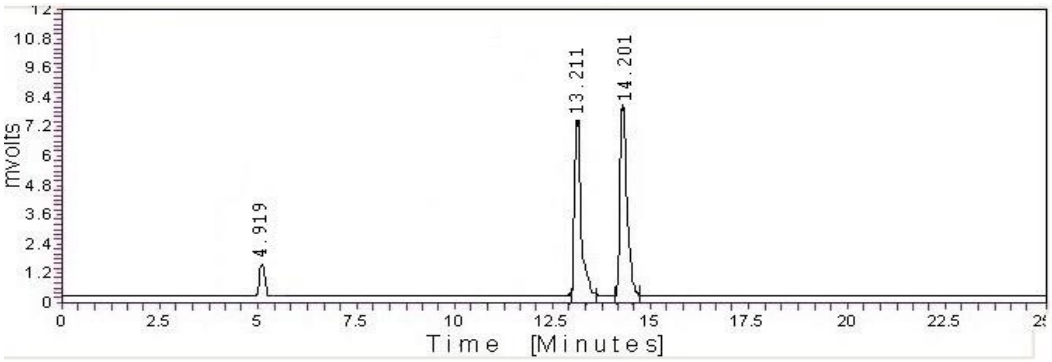


Table 11. Recovery Study

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

Stability in Analytical Solution

The stability of Cefpodoxime Proxetil and Levofloxacin in an analytical solution was assessed by analysing the sample both before and after a 24-hour storage period. The storage conditions included refrigeration at 8 °C and room temperature. The assay percentages of Cefpodoxime Proxetil and Levofloxacin were found to be within the acceptable range. The % assay and percentage variations were recorded and organised in Table 12[17].

Table 12. Solution Stability of Sample

Time level	Refrigerator	Room Condition (25°C)
Time in Hrs	% Assay of Cefpodoxime Proxetil	% Assay of Levofloxacin
Initial	99.5(± 0.251)	99.6(± 0.178)
After 24 hrs	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	Tailin g	Plate count	Rt	Area	Tailing	Plate count
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.	0.001414214	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 13. Robustness-Effect of temperature on sample

Temp. ℃	Cefpodoxime Proxetil 1				Cefpodoxime Proxetil 2				Levofloxacin			
	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count	Rt	Area	Tailing	Plate count
25℃	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℃	13.10	251249.2	1.103	214179.6	14.18	239581.7	1.111	214181.8	5.012	50436.40	1.0871	125915.5

	3											
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.	0.00144	4.38483	0.004	6.427600	0.00212	19.74334	0.00070	4.051721	0.064	3.309259	0.00127	3.30925
%RSD	0.01007	0.00174	0.384	0.0030	0.01495	0.008241	0.063617	0.001891	1.296	0.006560	0.11698	0.00262

Table 14. 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)

The determined limit of detection and limit of quantitation for Levofloxacin were 0.0064 µg/ml and 0.00211 µg/ml, respectively. The study determined the limit of detection (LOD) and limit of quantitation (LOQ) for Cefpodoxime Proxetil to be 0.0011µg/ml and 0.0003µg/ml, respectively.

Robustness

The effects of change in pH, change of column temperature, change in flow rate were studied on the sample solution. The system suitability parameters and peak areas were evaluated in each condition and the results were compared with method precision results. % RSD at each condition was found less than 2. This indicates the robustness of the method. The results are tabulated in table12-13[18].

Ruggedness

The % assay for both Cefpodoxime Proxetil and Levofloxacin was calculated at same level in triplicate. The average % assay for Cefpodoxime Proxetil was found to be 99.81% and for Levofloxacin was 99.39% with % RSD 0.282 and 0.329% respectively. The relative standard deviation (RSD) was determined to be within an acceptable range, with the RSD not exceeding 2%. This observation demonstrates the strength and reliability of the methodology. The outcomes pertaining to the toughness are presented in Table 14.

Table 14. 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

The forced degradation study of Cefpodoxime Proxetil revealed a degradation of approximately 91.19% in 0.1N HCl and 91.45% in 0.1 NaOH when compared to the control. However, no degradation was observed when subjecting the compound to stress conditions such as a solution of hydrogen peroxide (30% hydrogen peroxide), 10% sodium bisulphate, and photolytic conditions. The thermal degradation research of Cefpodoxime Proxetil revealed a degradation rate of 24.57%. On the contrary, Levofloxacin exhibited degradation percentages of 49.02% and 24.35% in 0.1N hydrochloric acid (HCl) and 0.1N sodium hydroxide (NaOH) solutions, respectively, as compared to the control. Levofloxacin exhibited degradation rates of 19.71%, 29.75%, and 19.35% when subjected to treatment with a 30% concentration of hydrogen peroxide, maintained in an oven at a temperature of 60 ºC for a duration of 24 hours, and exposed to photolytic conditions, respectively. No degradation of levofloxacin was seen upon treatment with sodium bisulphate. Table 6.101 presents the % assay, percentage degradation for each condition, as well as the purity angle and purity threshold for Cefpodoxime Proxetil and Levofloxacin. Forced degradation experiments were conducted on the individual standard medication, as well as a combination of standard pharmaceuticals and their corresponding formulation[19]. A comparison was conducted between the degradation products in individual medications, standard mixture, and formulation. The results revealed that the degradation products in the formulation were identical to those identified in the individual drugs. It is evident from the equal retention duration that the formulation and pure medicines undergo the formation of similar degradation products when subjected to comparable stress conditions[20]. The assessment of peak purity, purity angle, and purity threshold provides confirmation that there are no interferences present at the retention time of the primary peaks. As depicted in Figure 12.

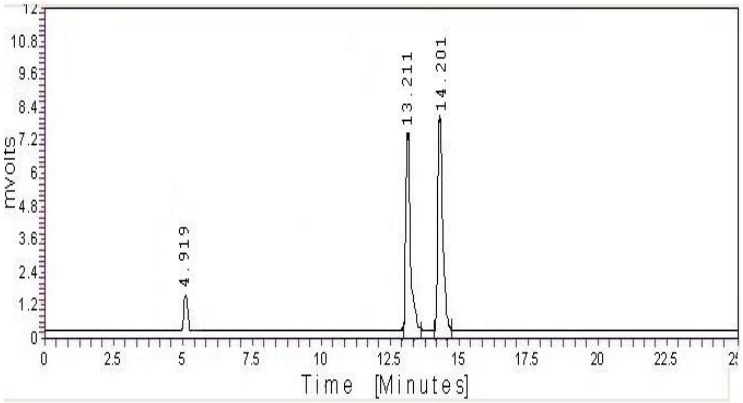


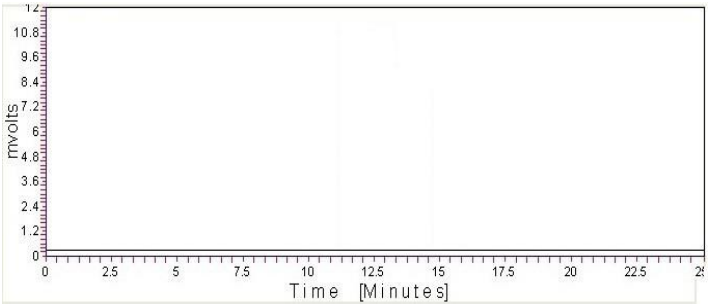
Figure 12. Chromatogram of Control Sample

Table 15. Forced Degradation Study

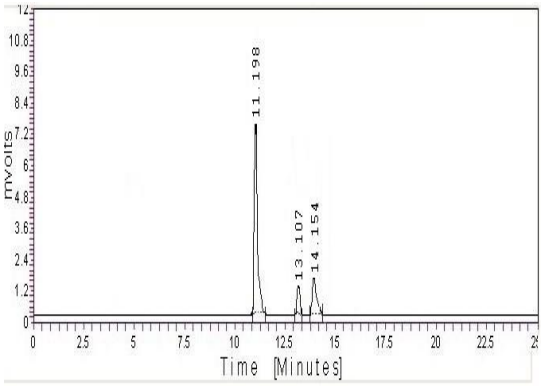
Sr. No.	Condition	%Assay Of Cef	% Degradation w. r. t. control sample of Cef	% Assay of Levof lox	% Degrad ation w. r. t. control sample of Levof lo	Peak Purity for CEF PEAK1		Peak Purity for Levofloxacin peak		Peak Purity for CEF PEAK2	
						Peak Purity Angle	Peak Purity Threshold	Purity Angle	Purity Threshold	PEAK2 Purity Angle	PEAK 2 Purity Threshold
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

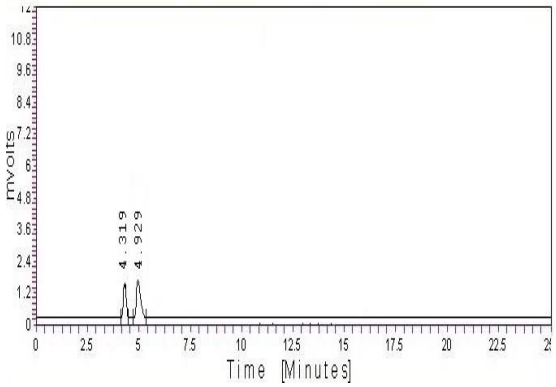
Acidic Degradation of Cefpodoxime Proxetil and Levofloxacin



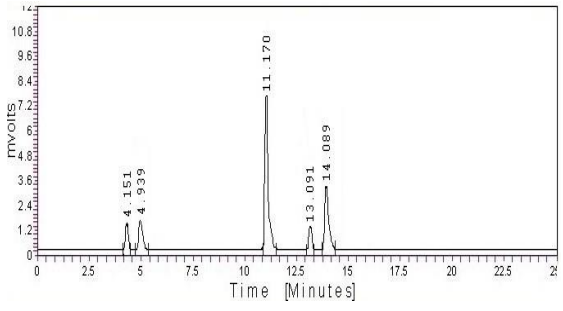
A) Acidic degradation Blank



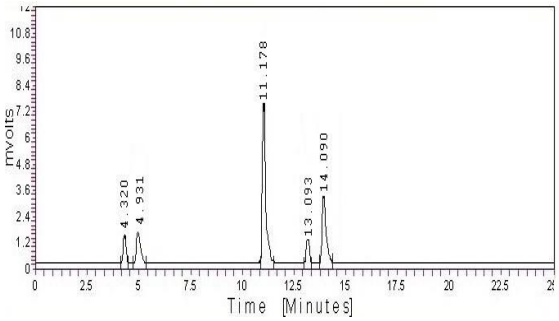
(B) CFP acidic degradation



(C) Levoflox acidic



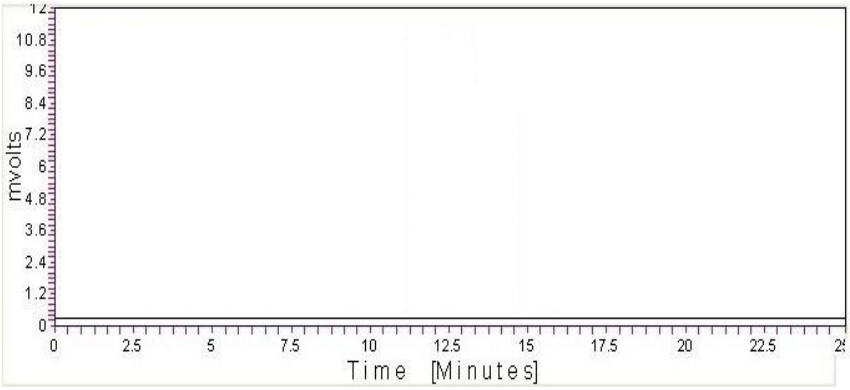
(D) **Std. mixture acidic degradation**



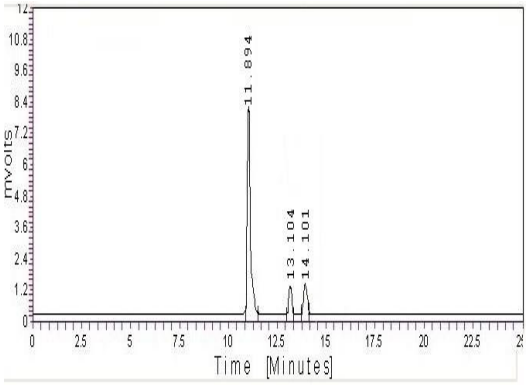
(E) Formulation acidic degradation

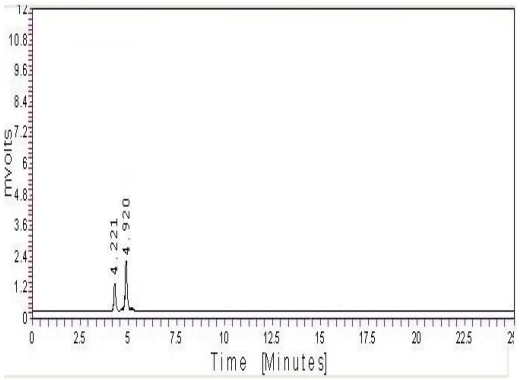
Figure 13 (A-E). Chromatograms of Acidic Degradation

Alkaline Degradation of Cefpodoxime Proxetil and Levofloxacin

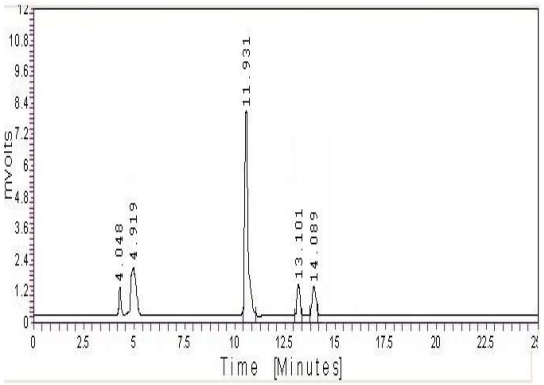


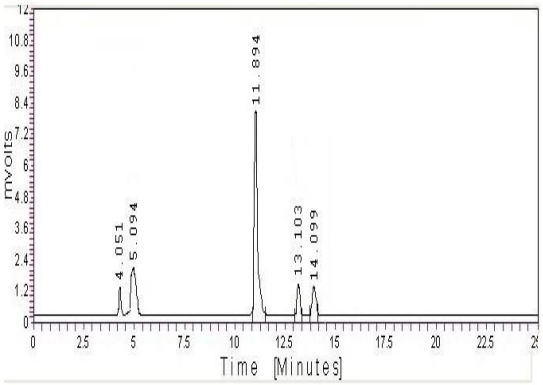
(A) Alkaline blank





(B) CFP alkaline degradation **(C) Levoflox alkaline**

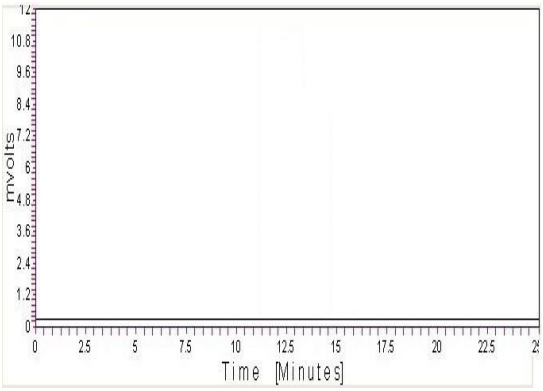




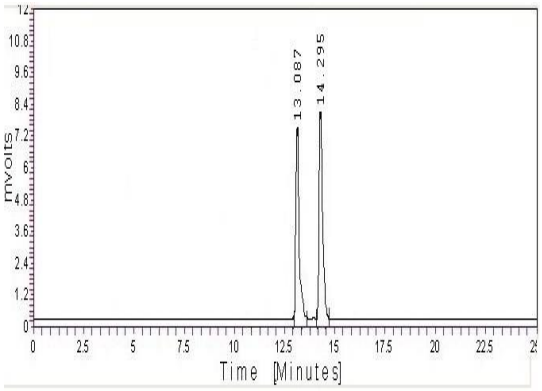
(D) degradation **Std mixture alkaline (E) Formulation of alkaline**

Figure 14 (A-E). Chromatograms of Alkaline Degradation

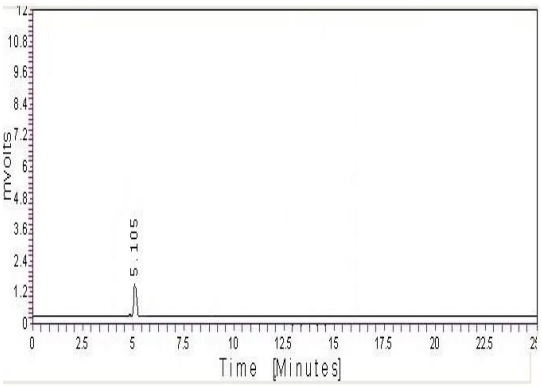
Peroxide Degradation (A-E) of Cefpodoxime Proxetil and Levofloxacin



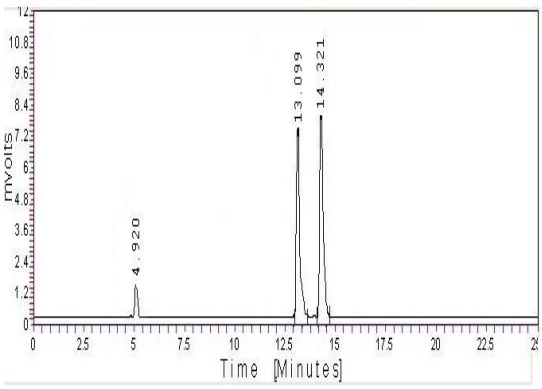
(A) Peroxide blank



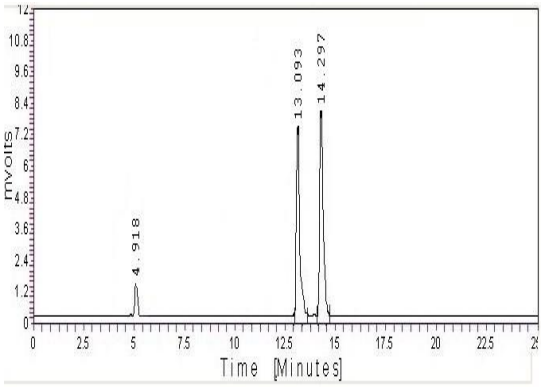
(B) CFP std peroxide degradation degradation



(C) Levoflox in hydrogen peroxide



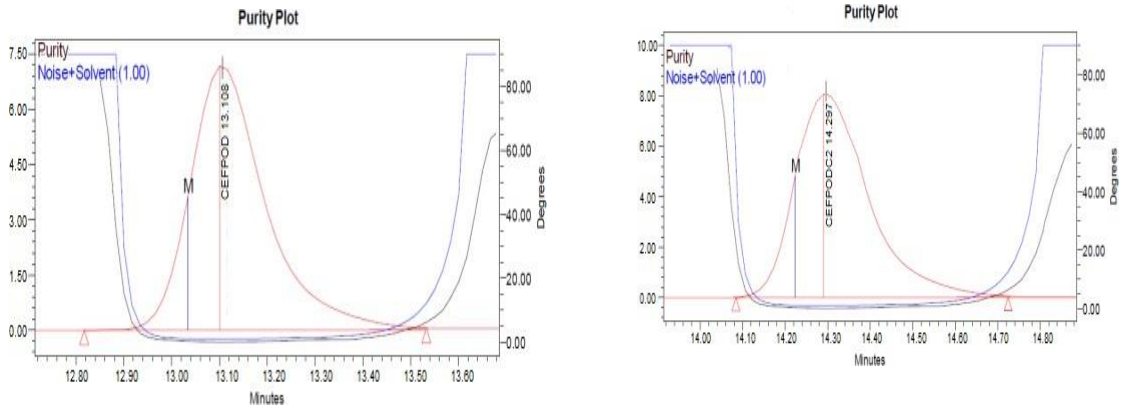
(D) Std mixture peroxide degradation



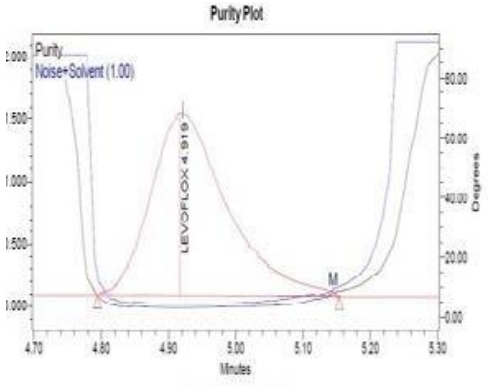
(E) Formulation peroxide degradation

Figure 15 (A-E). Chromatograms of Peroxide Degradation

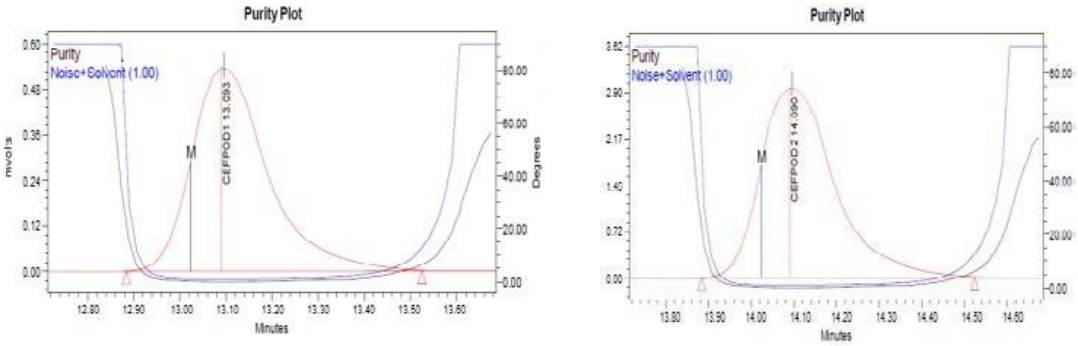
Peak purity plots for Cefpodoxime Proxetil and Levofloxacin at various stress conditions



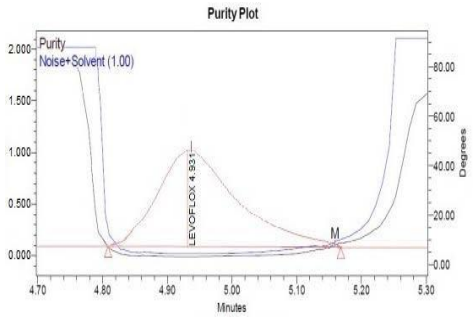
(A) **Peak purity curve of Control sample CFP peak 1& 2**



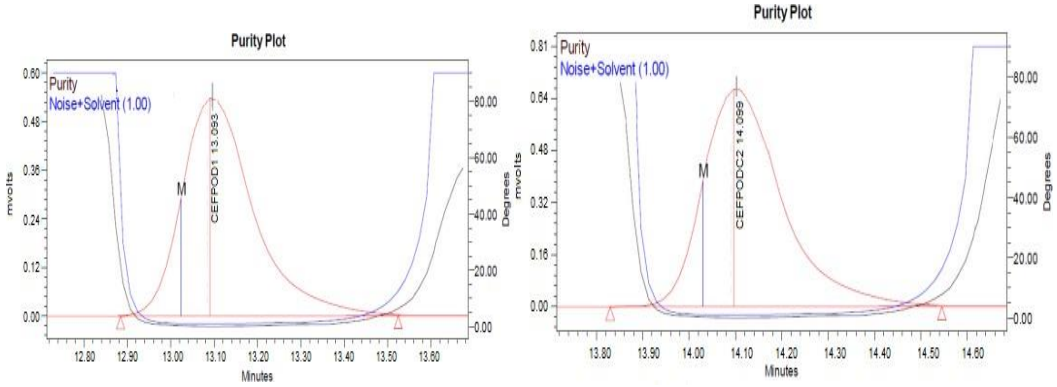
(B) **Peak purity curve of Control sample Levofloxacin**



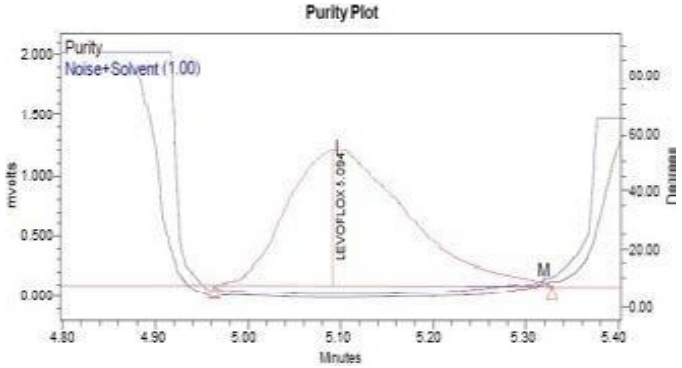
(C) **Peak purity curve of CFP 1&2 in acidic condition**



(D) Peak purity curve of Levoflox in acidic condition

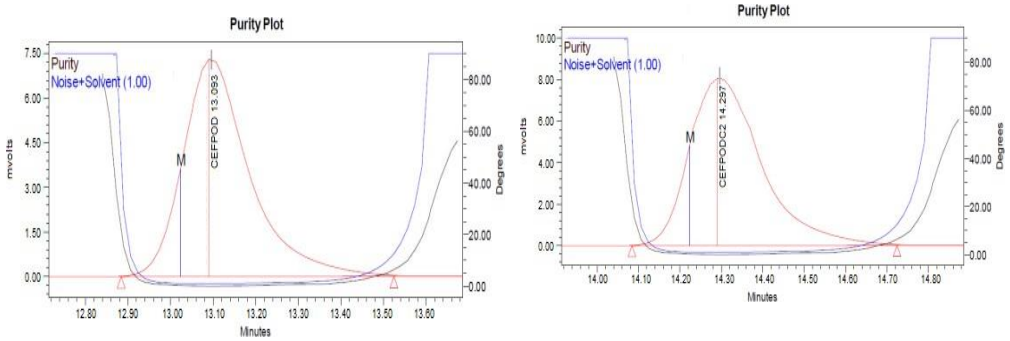


(E) Peak purity curve of CFP 1 & 2 in alkaline

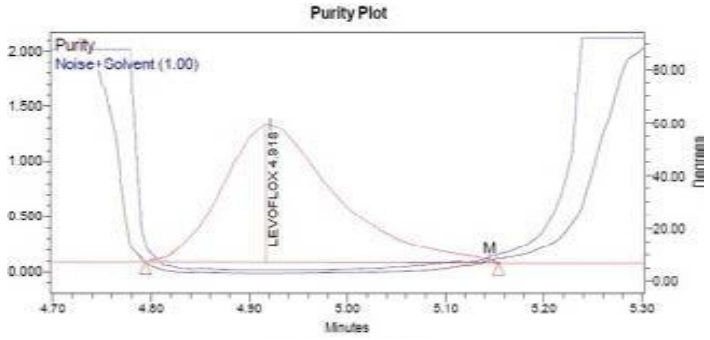


condition

Peak purity curve of Levoflox in alkaline condition



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



Peak purity curve of Levoflox in peroxide condition

Figure 16 (A–H). Peak Purity Plots at Various Stress Conditions

Conclusion

A novel RP-HPLC method was devised to quantitatively determine the levels of Cefpodoxime Proxetil and Levofloxacin in both bulk form and formulations. This approach exhibits characteristics of simplicity, sensitivity, precision, accuracy, robustness, and reproducibility. Notably, it successfully avoids any interference from excipients and degradation products. The method was devised with a mobile phase composed of a buffer solution containing 10 mM potassium dihydrogenphosphate, methanol, and acetonitrile in a ratio of 60:30:10. The pH of the mobile phase was adjusted to 3.2 using orthophosphoric acid. The separation was performed using an isocratic mode on a Hypersil keystone RP C18 column, with a flow rate of 1.2 ml/min. Detection of the analyte was carried out at a wavelength of 230 nm. The Cefpodoxime Proxetil compound exhibited two distinct peaks at 13.21 and 14.20 minutes over a 25-minute run duration. These peaks corresponded to the R and S isomers, respectively, which were present in the racemic mixture. Additionally, Levofloxacin displayed a peak at 4.91 minutes. The tablet formulation known as Glevopod was subjected to analysis using the described method. The results indicated that the average percentage assay for Cefpodoxime Proxetil and Levofloxacin in Glevopod was determined to be 99.82% and 99.28% respectively. The relative standard deviation (RSD) for Cefpodoxime Proxetil was determined to be 0.0836%, while for Levofloxacin it was discovered to be 0.6101%. These values were observed to be within the permitted level. The validation of the devised method was conducted in accordance with the requirements set forth by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). The methodology employed in this study was tailored specifically for the analysis of the medications under investigation, namely Cefpodoxime Proxetil and Levofloxacin. This choice was made due to the absence of any interference observed at the retention time of these two compounds. The precision of the technique was assessed in relation to the system precision, which yielded % RSD values of 0.3114% and 1.372% for Cefpodoxime Proxetil and Levofloxacin, respectively. Additionally, the method precision was determined, resulting in % RSD values of 1.098% and 0.805% for Cefpodoxime Proxetil and Levofloxacin, respectively. The relative standard deviation (RSD) values for the concentrations of Cefpodoxime Proxetil (7.5, 10, 12 µg/ml) and Levofloxacin (10, 12.5, 15µg/ml) were determined to be 0.923%, 0.490%, 0.626%, and 0.744% for the intraday study, and 0.806%, 0.630%, 0.354%, 0.703%, 0.153%, and 0.629% for the interday study. The accuracy investigation revealed that all percentage relative standard deviations (% RSD) were observed to be well within the permitted level, namely not exceeding 2. The accuracy assessment of the assay method that was devised involved the determination of the recovery of the added standards of Cefpodoxime Proxetil and Levofloxacin at three distinct levels: 80%, 100%, and 120% of the labelled claim. This assessment was conducted in triplicate. The average percentage recovery for Cefpodoxime Proxetil and Levofloxacin was determined to be 99.67% and 99.69% at the 80% concentration level, 99.65% and 99.75% at the 100% concentration level, and 99.69% and 99.65% at the 120% concentration level, respectively. The percentage relative standard deviation (% RSD) observed in the recovery investigation was determined to be within the permitted limit, with values not exceeding 2% for both % assay deviation and % RSD. The

linear relationship between concentration and response was observed within the concentration range of 2 – 24 µg/ml for Cefpodoxime Proxetil (r 2 = 0.999) and 2.5–30 µg/ml for Levofloxacin (r 2 = 0.999). There was no statistically significant variation observed in the percentage assay of both medications when comparing their values before and after being stored for a duration of 24 hours under refrigeration and at room temperature. This observation validates the stability of the medications when dissolved in solutions. The limits of detection (LOD) and limits of quantification (LOQ) for Cefpodoxime Proxetil were determined to be 0.0064 µg/ml and 0.00211 µg/ml, respectively. For Levofloxacin, the LOD and LOQ were found to be 0.0011 µg/ml and 0.0003 µg/ml, respectively. The method's robustness was assessed through purposeful modifications to the pH of the mobile phase, temperature of the column, and flow rate, while keeping the alterations minimal. The system suitability parameters exhibited % RSD values ranging from 0.0013 to 1.90%, all of which fall comfortably within the specified acceptability requirements of not exceeding 2% for % RSD. This finding demonstrates the strength and effectiveness of the method that was devised. The percentage assay for both Cefpodoxime Proxetil and Levofloxacin was determined at the same level in duplicate by multiple analysts in order to demonstrate the robustness of the devised method. The mean assay percentage for Cefpodoxime Proxetil was determined to be 99.81%, while for Levofloxacin it was discovered to be 99.39%. The relative standard deviation (RSD) for Cefpodoxime Proxetil was 0.282%, while for Levofloxacin it was 0.329%. The relative standard deviation (RSD) was determined to be within an acceptable range.

References

1. Akhgari, F., Samadi, N., & Farhadi, K. (2017). Fluorescent carbon dot as nanosensor for sensitive and selective detection of cefixime based on inner filter effect. *Journal of fluorescence*, *27*, 921-927.
2. Khan, F. U., Nasir, F., Iqbal, Z., Khan, I., Shahbaz, N., Hassan, M., & Ullah, F. (2016). Simultaneous determination of moxifloxacin and ofloxacin in physiological fluids using high performance liquid chromatography with ultraviolet detection. *Journal of Chromatography B*, *1017*, 120-128.
3. Maheshwari, M. L., Memon, A. A., Memon, S., Mughal, U. U. R., Dayo, A., Memon, N. ... & Leghari, M. K. (2015). Optimization of HPLC method for determination of cefixime using 2-thiophenecarboxaldehyde as derivatizing reagent: A new approach. *Saudi Pharmaceutical Journal*, *23*(4), 444-452.
4. Ramadan, A. A., Mandil, H. A. S. N. A., & Dahhan, M. A. R. W. A. (2013). UV-VIS Spectrophotometric study for determination of cefixime in pure form and in pharmaceuticals through complexation with Cu (II) using acetate-NaOH buffer in water: methanol. *International J. of Pharmacy and Phar. Sci*, *5*(1), 428-433.
5. Hassouna, M. E. M., Abdelrahman, M. M., & Mohamed, M. A. (2017). Validation of a novel and sensitive RP-HPLC method for simultaneous determination of cefixime trihydrate and sodium benzoate in powder for oral suspension dosage form. *Journal of Forensic Sciences & Criminal Investigation*, *2*(5).
6. Sireesha, K. R., & Prakash, K. (2012). HPLC-UV method for simultaneous determination of ofloxacin and dexamethasone sodium phosphate. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*, 415-418.
7. Vichare, V., Tambe, V., Joshi, S. V., & Dhole, S. N. (2013). New Simultaneous UV-Visible Spectrophotometric Methods for Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form. *Asian journal of pharmaceutical analysis*, *3*(2), 53-57.
8. Saravanakumar, K., Adaikala, R., & Umaiyambigai, D. (2016). GC-MS and FTIR Profiling of leaves methanol extract from the Pleiospermium alatum (Wall. ex Wt. & Arn) Swingle Rutaceae family. *J Phytopharmacol*, *5*, 201-204.
9. Shekhar, S., Kumar, A., Rana, V., Kumar, R., Mittal, C., & Tariyal, K. (2023). Recent Approaches of Matrix Release Tablet in NDDS System. *Journal for Research in Applied Sciences and Biotechnology*, *2*(3), 64-71.
10. Umama, Y., Venkatajah, G., Shourabh, R., Kumar, R., Verma, A., Kumar, A., & Gayoor, M. K. (2019). Topic-The scenario of pharmaceuticals and development of microwave as; sisted

extraction technique. *World J Pharm Pharm Sci*, *8*(7), 1260-1271.

11. Kumar, R., Saha, P., Sarkar, S., Rawat, N., & Prakash, A. (2021). A Review on Novel Drug Delivery System. *IJRAR-International Journal of Research and Analytical Reviews (IJRAR)*, *8*(1), 183-199.
12. Saravanakumar, K., Adaikala, R., & Umaiyambigai, D. (2016). GC-MS and FTIR Profiling of leaves methanol extract from the Pleiospermium alatum (Wall. ex Wt. & Arn) Swingle Rutaceae family. *J Phytopharmacol*, *5*, 201-204.
13. Gadhiya, D. T., & Bagada, H. L. (2013). Simultaneous equation method for the estimation of cefixime trihydarte and linezolid in their combined tablet dosage form by UV-visible spectrophotometry. *Asian J Biomed Pharm*, *3*(5), 29-38.
14. Muchtaridi, M. U. C. H. T. A. R. I. D. I., Yuliani, E. S. T. E. R., & Sopyan, I. Y. A. N. (2015). Application off-line SPE-HPLC/UV methods in analysis of ofloxacin in human urine (In Vitro). *Int J Pharm Pharm Sci*, *8*(1), 255-261.
15. Hussein, L. A., Hussien, E. M., Magdy, N., & Mohamed, H. S. (2017). Simultaneous estimation of Ofloxacin and Cefixime in tablet form in presence of the inactive Ofloxacin USP Related compound A. *Bulletin of Faculty of Pharmacy, Cairo University*, *55*(1), 171-176.
16. Babu, G. R., Rao, A. L., & Rao, J. V. (2016). Bioanalytical Method Development and Validation for Simultaneous Estimation of Paracetamol and Cefixime by using RP-HPLC in Rabbit Plasma. *Oriental Journal of Chemistry*, *32*(1), 701-707.
17. Elgizawy, S. M., Hassan, N. Y., Lotfy, H. M., & Saleh, S. S. (2014). Comparative study of RP-HPLC versus TLC-spectrodensitometric methods applied for binary mixtures of fluoroquinolones and corticosteroids. *Acta Chromatographica*, *26*(3), 439-456.
18. Ibrahim, F., Magdy, G., & Wahba, M. E. (2020). Utility of micellar liquid chromatography as an analytical tool for the estimation of three binary antibiotic mixtures. Application to biological fluids. *Journal of Taibah University for Science*, *14*(1), 931-942.
19. Kaushal, P., Kushawaha, S. K., Majumder, M., & Ashawat, M. S. (2023). Validated Analytical Method for Multicomponent Analysis of Famotidine and Ofloxacin in Bulk drug and Tablet Formulation by using UV-Visible Spectrophotometer and RP-HPLC. *Asian Journal of Pharmaceutical Analysis*, *13*(3), 3.
20. Mohammmed, M. A., & Mezaal, E. N. (2016). Simultaneous determination of cephalexin and cefixime by first and second derivative ultraviolet spectrophotometry. *Journal of the college of basic education*, *22*(93), 89-100.