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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFIXIME, LINEZOLID AND MOXIFLOXACIN IN FDC TABLET DOSAGE FORMS

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ABSTRACT:

Cefixime, Cefpodoxime, Cefepime are antibiotic drugs under the class of cephalosporin antibiotics. These drugs are used to treat respiratory tract infections, skin infections and urinary tract infections. Brivudine is an antiviral drug (herpes zoster virus). **Conclusion:** The present research work described about a stability-indicating RP-HPLC method development for the simultaneous determination of CEF, LNZ and MOX drug content in CEF+LNZ and CEF+MOX FDC tablets. And the method is validated to show its capability for intended application. Through validation the developed method found to be precise, rugged, accurate, linear, specific and robust. From the forced degradation study it is found that all the degradants formed are well separated from CEF, LNZ and MOX, and it concludes that the method is stability indicating in nature

INTRODUCTION:

Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need by combining the therapeutic effects of two or more drugs in one product. These combination products can present daunting challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that results from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products[1-15].

CHROMATOGRAPHY

Chromatography is unique in the history of analytical methodology and is probably the most powerful and versatile technique available to modern analyst. In a single procedure it can separate a mixture into its individual components and simultaneously determine quantitatively the amount of each component present.

The chromatographic method of separation, in general, involves the following steps:

- Adsorption
- Separation of the adsorbed substances by the mobile phase.
- Recovery of the separated substances by a continuous flow of the mobile phase; the method being called elution.
- Qualitative and quantitative analysis of the eluted substances

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High Performance Liquid chromatography is separation technique where solutes migrate through a column containing a micro-particulate stationary phase at rates dependant on their distribution ratios.

These are functions of the relative affinities of the solute for the mobile and stationary phases, the elution order depending on the chemical nature of the solutes and the overall polarity of the two phases. When a mixture of components dissolved in mobile phases is introduced into the column, the compound with dissolved in mobile phase is introduced into the column, the compound with lesser affinity towards the stationary phase moves faster and hence eluted out of the column first. The one with greater affinity towards the stationary phase moves slower down the column and hence eluted later. Thus the components are separated.

BUFFER SELECTION

Choice of buffer is typically governed by the desired pH. The typical pH range for reversed phase on silica-based packing is pH 2 to 8. It is important that the buffer has a pka close to the desired pH since buffer controls pH best at their pka.

General considerations during buffer selection:

- Phosphate is more soluble in methanol/water than in acetonitrile/water or THF/water.
- Some salt buffers are hygroscopic. This may lead to changes in the chromatography (increased tailing of basic compounds, and possibly selectivity differences).
- Ammonium salts are generally more soluble in organic/water mobile phases·
- TFA can degrade with time, is volatile, absorbs at low UV wavelengths.
- Microbial growth can quickly occur in buffered mobile phases that contain little or no organic modifier. This growth will accumulate on column inlets and can damage chromatographic performance.
- At pH greater than 7, phosphate buffer accelerates the dissolution of silica and severely shortens the lifetime of silica-based HPLC columns. If possible, organic buffers should be used at pH greater than 7.

BUFFER CONCENTRATION:

Generally, a buffer concentration of 10-50 mM is adequate for small molecules. Generally, no more than 50% organic should be used with a buffer. This will depend on the specific buffer as well as its concentration. Phosphoric acid and its sodium or potassium salts are the most common buffer systems for reversed phase HPLC. Phosphate buffers can be replaced with sulfonate buffers when analyzing organophosphate compounds.

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DETECTION:

The detection of the separated components in the elute from the column is based upon the bulk property of the elute (eg. Its refractive index) or the solute property of the individual components (eg. Their ultraviolet absorption, fluorescence or electrochemical activity). Generally, a detector is selected that will respond to a particular property of the substances being separated, and ideally it should be sensitive to at least 10^{-8} g ml^{-1} and give a linear response over a wide concentration range.

SYSTEM SUITABILITY PARAMETERS:

- Resolution
- Retention time
- Tailing factor
- Theoretical plates

Resolution:

Resolution is the parameter describing the separation power of the complete chromatographic system relative to the particular components of the mixture.

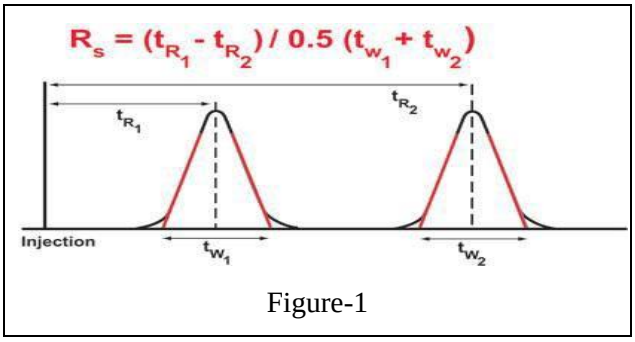

Figure-1

The resolution R_s of two neighbouring peaks is defined as the ratio of the distance between two peak maxima. It is the difference between the retention times of two solutes divided by their average peak width. For baseline separation, the ideal value of R_s is 1.5. it is calculated by using the formula.

Retention time:

The amount of time between the injection of a sample and its elution from the column is known as the retention time; it is given the symbol t_R.

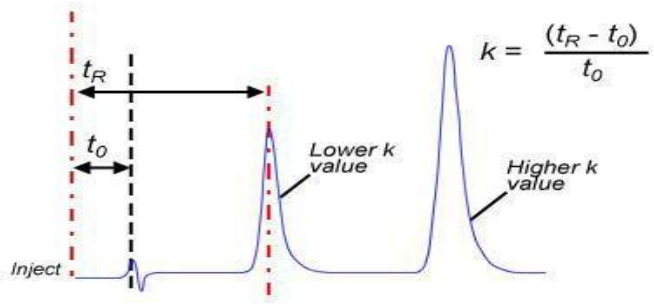

Figure-2

Tailing factor (peak asymmetry factor):

The peak half width, b of a peak at 10% of the peak height, divided by the corresponding front half width, a gives the asymmetry factor.

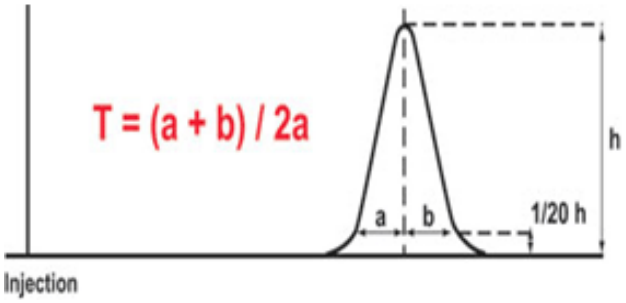

Figure-3

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Theoretical plates (column efficiency):

Efficiency N, of acolumn is measured by the number of theoretical plates per meter. It is a measure of band spreading of a peak. Similar the band spread, higher is the number of theoretical plates, including good column and system performance. Column with N ranging from 5000 to 10000 plates/meter are ideal for a good system.

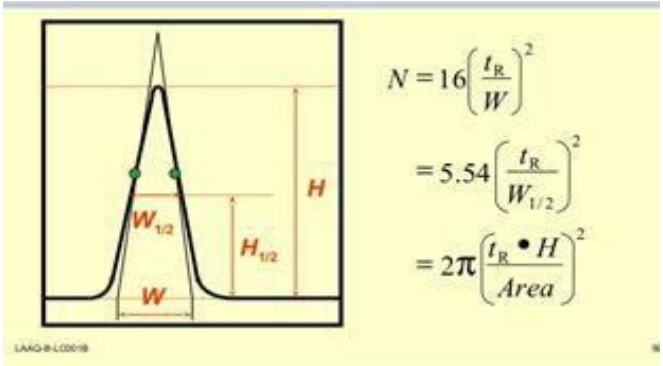

Figure-4

QUANTITATIVE ANALYSIS

Normalisation:

The Area% calculation procedure reports the area of each peak in the chromatogram as a percentage of the total area of all peaks. Area% does not require prior calibration and does not depend upon the amount of sample injected within the limits of the detector. No response factors are used.

If all components respond equally in the detector and are eluted, then Area% provides a suitable approximation of the relative amounts of components.

External Standard Method:

External standard method involves the use of a single standard or up to three solutions. The peak area or the height of the sample and the standard use are compared directly. One can also use the slope of the calibration curve based on standard that contain known concentration of the compound of interest.

Internal standard Method:

A widely used technique of quantitation involves the addition of an internal standard to compensate for various analytical errors. A known compound of a fixed concentration is added to the known amount of samples to give separate peaks in the chromatogram to compensate for the losses of the compounds of interest during sample pretreatment steps. The accuracy of this approach obviously dependence on the structural equivalence of the compound of interest and the internal standard.

$$RF = \frac{\text{standard area or peak height}}{standerd\ concentration}$$

STANDARD ADDITION METHOD:

A known amount of the standard compound is added to the ample solution to be estimated. This method is suitable if sufficient amount of the sample is available and is more realize in the sense that it allow calibration in the presence of excipients or other components.

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An important aspect of the method of standard addition is that the response prior to spiking additional analyte, it should provide a reasonable S/N ratio (> 10), otherwise the result will have poor precision.

ANALYTICAL METHOD DEVELOPMENT

Method development is a continuous process that progresses in parallel with the evolution of the drug product. The notion of phase-appropriate method development is a critical one if time, cost, and efficiency are concerns. The goal and purpose of the method should reflect the phase of drug development. During early drug development, the methods may focus on API behavior. They should be suitable to support pre-clinical safety evaluations, pre-formulation studies, and prototype product stability studies. As drug development progresses, the analytical methods are refined and expanded, based on increased API and drug product knowledge. The methods should be robust and uncomplicated, while still meeting the appropriate regulatory guidelines.

BIOANALYTICAL METHOD

A bio-analytical method is a set of procedures involved in the collection, processing, storage, and analysis of a biological matrix for a chemical compound. The most widely accepted guideline for method validation is the ICH guideline Q2 (R1), which is used both in pharmaceutical and medical science.

Bio-analytical method validation (BMV) is the process used to establish that a quantitative analytical method is suitable for biomedical applications. Characterization of the stability of analytes in biological samples collected during clinical studies together with that of critical assay reagents, including analyte stock solutions, is recognized as an important component of bio-analytical assay validation.

AIM AND OBJECTIVE

Cefixime, Cefpodoxime, Cefepime are antibiotic drugs under the class of cephalosporin antibiotics. These drugs are used to treat respiratory tract infections, skin infections and urinary tract infections.

Brivudine is an antiviral drug (herpes zoster virus). The FDA approved brivudine June 29, 2011 to treat shingles ((herpes zoster).

Literature reveals very few analytical techniques for the estimation of cefpodoxime and cefepime from tablets. Also there is no HPTLC analytical method reported for the cefixime.

Literatures have proven that there are no analytical method reported for the brivudine in formulation as well as biofluids like plasma.

Hence the major objective of the present research work is,

- Development and validation of HPTLC

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method for determination of cefixime, cefpodoxime, and cefepime in bulk and pharmaceutical dosage form.

- Development and validation of HPTLC and HPLC method for estimation of brivudine in bulk and pharmaceutical dosage form.
- Development and validation of bioanalytical method for the estimation of brivudine in human plasma.

Novel and Stability Indicating HPLC Method for the Assay Determination of Cefixime, Linezolid and Moxifloxacin in FDC Tablet Dosage Forms

Section-1: Brief description of Cefixime, Linezolid and Moxifloxacin

Cefixime (CEF), (6R,7R)-7-[2-(2-Amino-4-thiazolyl) glyoxylamido]-8-oxo-3- vinyl-5-thia-1-azabicyclo[4.2.0]oct-2ene-2-carboxylicacid,7[2]-(Z)-[O-(carboxymethyl)-oxime] trihydrate is a third generation cephalosporin and its molecular weight and molecular formula are, 507.50 g/mol and $C_{16}H_{15}N_5O_7S_2.3H_2O$ respectively. CEF is an antibiotic to treat many bacterial infections, includes strep throat, otitis media, pneumonia, gonorrhea, lyme disease, typhoid fever, sinuses, lower respiratory tract infections such as bronchitis, and urinary tract infections. CEF is highly stable in the presence of beta-lactamase enzymes [1-2]. It is an orally absorbed third generation cephalosporin antibiotic that was approved by the U.S. Food and Drug Administration in 1997 for the treatment of mild to moderate bacterial infections and it is supplied with the brand name of "Suprax" as a single entity product [3]. The structure is presented below in figure-5.

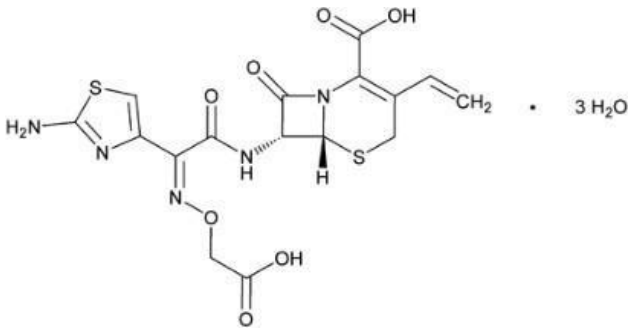

Figure-2: Cefixime

Linezolid (LNZ), (S)- N- [[3- [3- Fluoro- 4-(4-morpholinyl) phenyl] -2-oxo-5-oxazolidinyl] methyl] -acetamide is an antibiotic that used for the treatment of infections caused by gram-positive bacteria which are resistant to other antibiotics and mainly used for the variety of other infections like drug resistant tuberculosis and are infections of the skin and pneumonia, it is an important therapeutic option for the treatment of infections caused by multi resistant Gram-positive bacteria such as vancomycin-resistant Enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA)[4-5]. However, the clinical benefit of LNZ is threatened by the emergence of resistant strains of MRSA and VRE

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reported in North America and Europe. For effective antimicrobial treatment, it is extremely important to have exact knowledge of drug concentrations at the site of action [6], its molecular weight and molecular formula are 337.35 g/mol and $C_{16}H_{20}FN_3O_4$ respectively. And it is supplied with the brand name of "Zyvox" as a single entity product [7]. The structure is presented below in figure-6

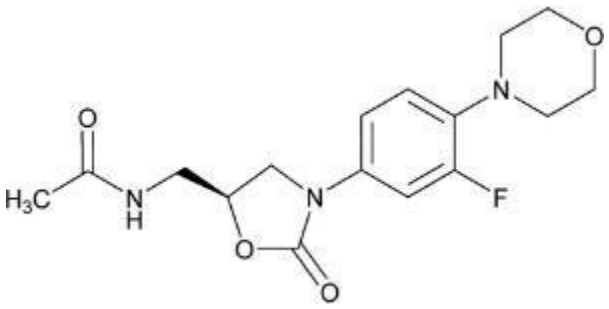

Figure-6: Linezolid

Moxifloxacin (MOX), 1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-[(4a*S*,7a*S*)-octahydro-6*H*-pyrrolo[3,4-*b*]pyridin-6-yl]-4-oxo-3-quinolinecarboxylic acid, is a synthetic antibacterial agent and its molecular weight and molecular formula are, 437.89 g/mol and $C_{21}H_{24}FN_3O_4$.HCl respectively. MOX is an antibiotic used to treat respiratory infections, including acute sinusitis, acute exacerbations of chronic bronchitis, and community-acquired pneumonia, as well as dermatological infections, as a second-line agent in tuberculosis [8]. It is a broad-spectrum antibiotic that functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication [9]. It is used for bacterial conjunctivitis, keratitis, pre & post-operative conditions to control the infections of the eyes and it is supplied with the brand name of "Avelox" as a single entity product [10]. The structure is presented below in figure-7.

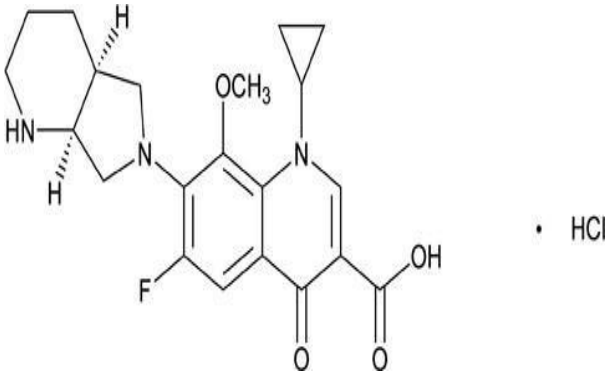

Figure-7: Moxifloxacin

The screening of literature revealed that there was no stability indicating single RP-HPLC method available for the simultaneous determination of CEF+LNZ and CEF+MOX in FDC tablet dosage form. This encouraged the author to develop a, precise, accurate, rugged, robust, simple, fast and stability indicating single RP-HPLC method for the simultaneous determination of CEF+LNZ / CEF+MOX in both the combination drug products.

Section-2: Experimental

This section describes the details of standards, chemicals; instruments used in this

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research work and also briefly described method validation experimental and specificity study.

I. Standards and Chemicals

"Optimus Pharmaceuticals Ltd., Hyderabad" (India) supplied CEF+LNZ+MOX working standard and marketed test drug product "Lincef" (CEF/LNZ: 200/600mg), "Moxinov" (CEF/MOX: 400/400mg) tablets were purchased from pharmacy. Details of the chemicals and solvents are as follows; analytical grade KH_2PO_4 purchased from Sd Fine chemicals, Mumbai and analytical HPLC grade Acetonitrile and Methanol were purchased from Merck Chemicals and highly purified water collected from Millipore (USA) water purification system, milli- Q plus.

II. Equipment's

The equipment's and instruments used in this research work are tabulated below in table-1.

Table-1: List of Equipment / Instrument and manufacturer

S. No	Equipment / Instrument	Manufacturer (Country)
1	Agilent 1100, HPLC system with Ez chrome software to monitor signal.	Agilent (Germany)
2	Cary 60 UV-Spectrophotometer for UV scan	
3	Digital water bath for hydrolysis studies	Equitron (India)
4	Dry air oven and photo stability chamber	Thermo scientific (USA)
5	Centrifuge apparatus	Remi (India)
6	Power sonic 420 ultra sonicator	Labtech (Korea)
7	0.45µm Nylon membrane filters	PALL life sciences (USA)
8	0.45µm Nylon and PVDF Syringe filters	

III. Wavelength determination

Three standard materials were prepared in diluent at about 3 ppm concentration to perform the UV spectral analysis. UV spectrum was scanned from 200 to 400 nm to understand the UV absorbance of the three samples, and based on all the compounds spectra appropriate wavelength for CEF, LNZ and MOX were selected for the determination.

IV. Chromatographic Conditions

The optimized chromatographic conditions, mobile phase and diluent preparations are mentioned below.

Column: Zorbax SB C18, 150 x 4.6 mm; 5µm (Agilent, Germany)

Flow rate: 1.0 mL / min

Column Temperature: 50°C

Detector / Wavelength: UV detector with 270 nm

Injection volume: 20 µL

Mobile phase (MP): MP-A (0.05M KH2PO4: 6.8g in 1L Milli-Q water)

MP-B (Acetonitrile and Methanol: 800:200 v/v).

Diluent: Mixture of MP-A and MP-B (50:50 v/v)

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Gradient Table - 1

Time (min)	MP-A (%)	MP-B (%)
0	100	0
5	92	8
10	75	25
14	75	25
18	50	50
20	100	0
25	100	0

V. Standard solution (STD) preparation (CEF/LNZ/MOX: 40/60/40ppm)

Weighed accurately 80 mg of cefixime, 80 mg of moxifloxacin and 120 mg of linezolid standard materials and transferred into a 100 mL volumetric flask, add 40 mL of diluent and mixed well, further sonicated the solution to dissolve the contents. Allow the resulting solution to reach the room temperature condition, and then diluted the remaining volume with diluent.

Further diluted 5ml of the above stock solution with diluent to 100 ml.

VI. Sample and Placebo solution preparation

For Cefixime and Linezolid Tablets (Lincef: 200 mg / 600mg)

Weigh 20 tablets, and calculated the average weight of one tablet. Selected 20 tablets randomly and crushed in mortar with pestle to get fine powder. Accurately weighed and transferred tablets powder equivalent to 80 mg of cefixime and 120 mg of linezolid in to a 100 mL volumetric flask. 60 mL of diluent was added and mixed well to disperse the contents, then sonicated for 10 min in an ultra sonicator with intermediate shaking at room temperature. Further diluted the solution with diluent to 100 mL and mixed well. Resulting solution was filtered through 0.45µm PVDF or 0.45µm Nylon syringe filters or centrifuged at 3500rpm for 10min and then 5 ml of clear filtered solution was pipetted in to 100 ml volumetric flask and diluted with diluent.

Similarly placebo solution was prepared by taking equivalent placebo present in the test sample preparation.

For Cefixime and Moxifloxacin Tablets (Moxinov: 400 mg / 400mg)

Weigh 20 tablets and calculated the average weight of one tablet. Selected 20 tablets randomly and crushed in mortar with pestle to get fine powder. Accurately weighed and transferred tablets powder equivalent to 80 mg of cefixime and 80 mg of moxifloxacin into a 100 mL volumetric flask. 60 mL of diluent was added and mixed well to disperse the contents, then sonicated for 10 min in an ultra sonicator with intermediate shaking at room temperature. Further diluted the solution with diluent to 100 mL and mixed well. Resulting solution was filtered through 0.45µm PVDF or 0.45µm Nylon syringe filters or centrifuged at 3500rpm for 10min and then 5 ml of clear filtered solution was pipetted in

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to 100 ml volumetric flask and diluted with diluent.

Similarly placebo solution was prepared by taking equivalent placebo present in the test sample preparation.

Analysis Procedure:

Inject blank, STD (5 replicates) and test solution into the HPLC system, and calculate % assay of each drug with respect to peak area from standard solution of each component.

System suitability acceptance:

The relative standard deviation for peak areas of CEF, LNZ and MOX from the replicate standard injections should not be more than 2.0%.

The tailing factor of CEF, LNZ and MOX from the replicate standard injections should not be more than 1.5.

% Assay Calculation:

The chromatograms of blank, standard, sample and placebo solutions are presented in figure-8 to 13.

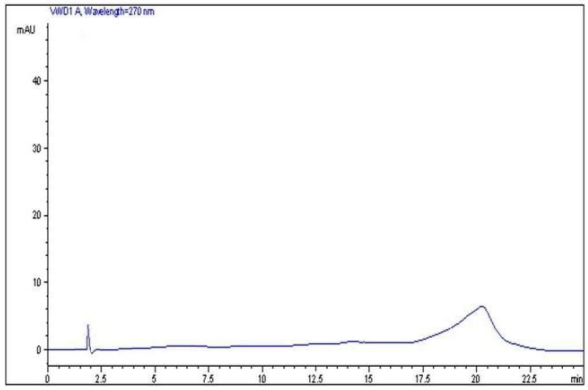

Figure-8: Blank Chromatogram

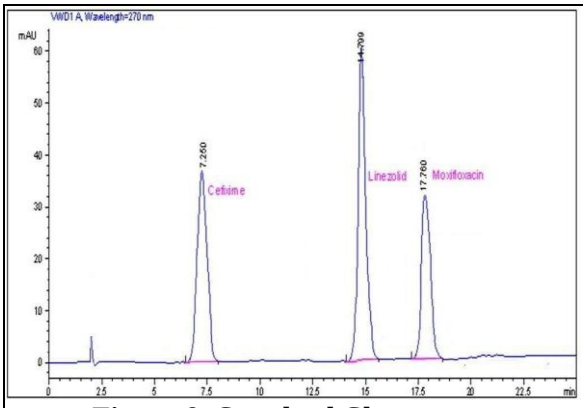

Figure-9: Standard Chromatogram

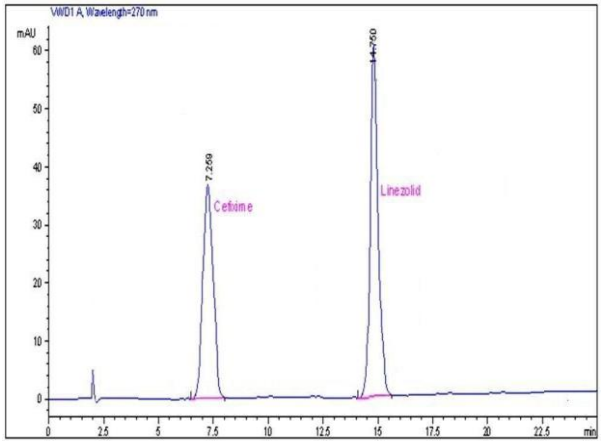

Figure-10: CEF+LNZ sample Chromatogram

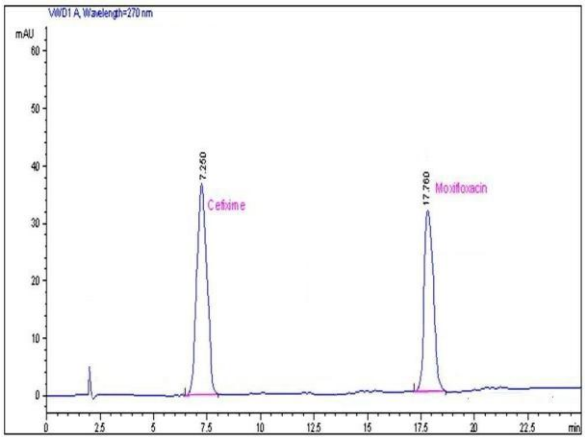

Figure-11: CEF+MOX sample Chromatogram

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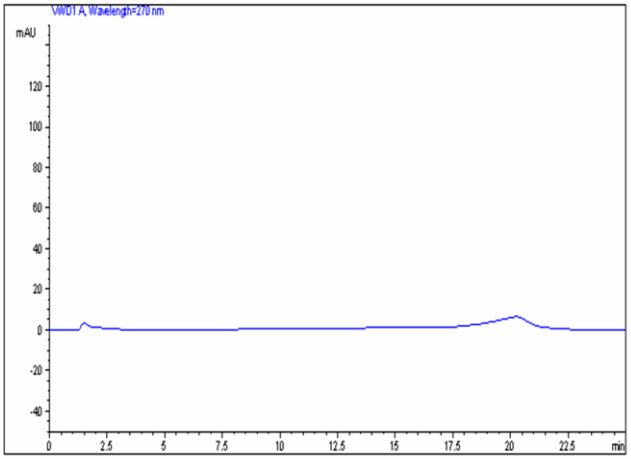

Figure-12: CEF+LNZ Placebo Chromatogram

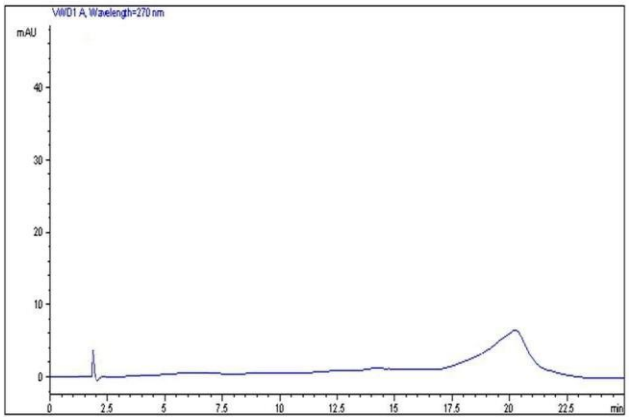

Figure-13: CEF+MOX Placebo Chromatogram

VII: Method Validation

The method validation was performed as per ICH Q2 (R1) guideline recommendations [34], and the validation parameters included are precision, linearity, specificity, recovery, robustness, ruggedness, solution stability and filter interference study. The experimental procedures of each validation parameter are described below.

Forced degradation studies (Specificity)

Forced degradation studies were carried to check the methods stability indicating nature as per the regulatory guidance requirements (ICH Q2A, Q3B and Q1A), and specificity of the method checked along with placebo solutions. These studies were separately carried on CEF+LNZ (200mg+600mg) tablets and CEF+MOX (400mg+400mg) tablets at test concentration and similarly placebo solutions prepared to show that non-interference of placebo peaks at the retention time of CEF, LNZ and MOX. Based on experiments stress conditions optimization was done where total % degradation was not go beyond 20% to make the right assessment of stability indicating nature of the method.

The samples are stressed under heat, humidity, UV-light, visible-light, and under oxidation, acid/base/water hydrolysis. All the stressed test solutions were brought to room temperature after treating to stress conditions and neutralization of acid, base stressed test solutions were done as per requirement. All these stressed solutions were injected into the HPLC system which equipped with PDA detector and also injected placebo, unstressed (control) test

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solutions for comparison. The applied stress conditions are mentioned below.

Stress conditions for Cefixime and Linezolid Tablets:

Heat: at 50°C for 12 hours

Humidity: at 80 % RH (for 1week)

Oxidation (10% H_2O_2): at 25 °C for 10 hours

Photolytic: UV-light (200 watts hr / m^2), Visible-Light (1.2 million lux hours)

Acid Hydrolysis: 1N HCl at 50°C for 10 hours

Base Hydrolysis: 1N NaOH at 50°C for 10 hours

Water Hydrolysis: at 60°C for 12 hours

Precision:

Method precision was evaluated by 6 individual test preparations of test drug products CEF+LNZ (200mg+600mg) tablets and CEF+MOX (400mg+400mg) tablets prepared at test concentration. Injected all six test preparations into the HPLC system and calculated % assay of two drug products respectively, and calculated % RSD for 6 % assay results. System precision was evaluated by injecting standard solution for 5 times and by calculating the % RSD of replicate standard injections.

And intermediate precision (different analyst, day, HPLC column and HPLC system) was evaluated similarly as precision.

Linearity:

Five linearity solutions were prepared at various concentrations ranged from 50% to 150% (50%, 75%, 100%, 125% and 150%) concentration level. Cefixime and moxifloxacin were prepared from 20ppm to 60 ppm, and Linezolid was prepared from 30 ppm to 90 ppm concentration level. Linearity of detector response against concentration for CEF+LNZ+MOX was evaluated by calculating "r" (correlation of co-efficient) value and bias for 100% response.

Recovery study of CEF, LNZ and MOX are performed by placebo spiking. Three recovery level test solutions in triplicate are prepared by mixing placebo with CEF+LNZ+MOX. The concentrations of recovery solutions are ranged between 50% and 150% (50%, 100% and 150%) at the test concentration level, and injected all these recovery solutions into the HPLC system. % recoveries of CEF+LNZ+MOX were calculated with respect to added amount. And also calculated mean % recovery at each spike level and % RSD of triplicate preparations at each level.

Solution stability and mobile phase stability:

Standard and test solutions (from precision) are prepared as per test method and these solutions were kept for 3 days on bench top (room temperature) in volumetric flasks which are closed tightly. All these solutions are tested at day-1 and at day-3. Then CEF, LNZ, MOX

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stability was evaluated in standard and test solution respectively against freshly prepared standard solution at each interval.

The stability of mobile phase is studied by injecting freshly prepared standard solutions. The freshly prepared mobile phase was kept on bench top at room temperature for 3 days and studied at day-1 and day-3 interval by injecting freshly prepared standard solution. The % RSD of replicate standard injections and tailing factor was evaluated.

Robustness:

The method robustness was evaluated by changing the chromatographic conditions deliberately. Injected standard solution at each altered condition and checked % RSD and tailing factor. The altered conditions range are as follows; flow rate (±0.2 mL/min of 1.0 mL/min), and temperature of column compartment (±5°C of 50°C) to evaluate robustness. And also method robustness was evaluated by two different filters, namely 0.45µm PVDF (Millipore) and 0.45µm NY (Millipore) syringe filters against centrifugation (3500rpm / 10min).

VII. a: Wavelength Determination

Detector wavelength was determined by UV spectra (by PDA detector) of CEF, LNZ and MOX which scanned between 200 to 400nm. The UV spectrums illustrates that, CEF showing maximum UV absorbance around 250-300nm, LNZ showing maximum UV absorbance around 250-270nm and MOX showing maximum absorbance around 270-300nm. Hence detector wavelength was determined at 270 nm to get the adequate response, for the quantification of CEF, LNZ and MOX. The individual UV spectrum of each peaks are presented below in figure-14 to 16.

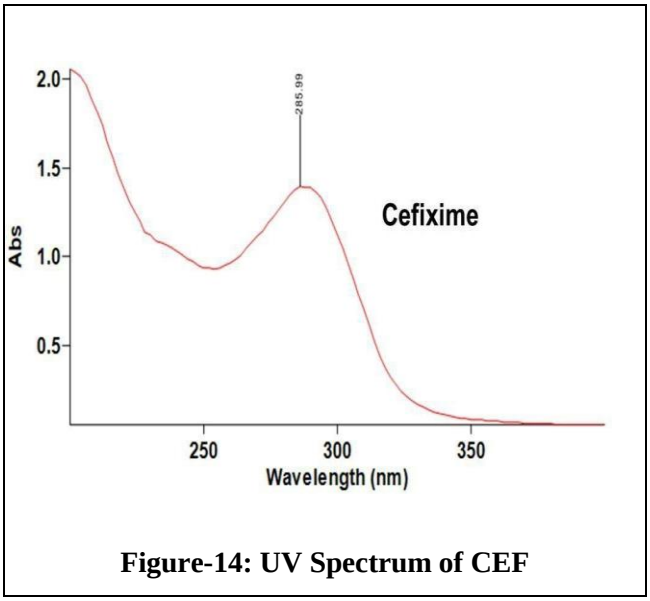

Figure-14: UV Spectrum of CEF

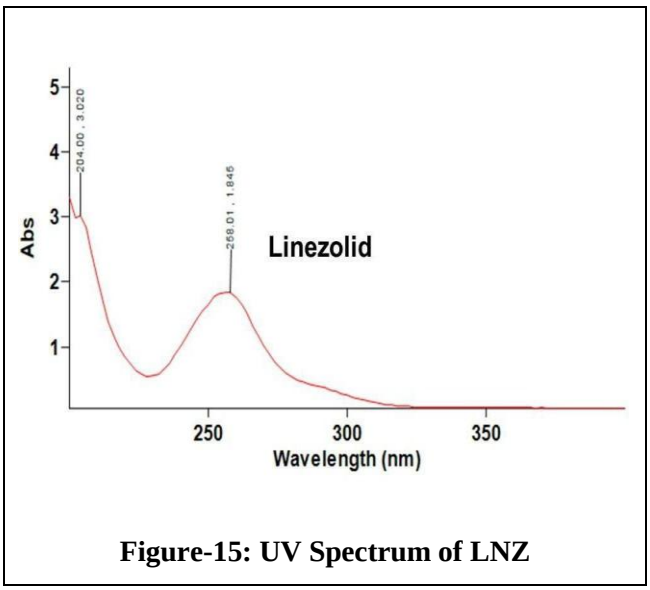

Figure-15: UV Spectrum of LNZ

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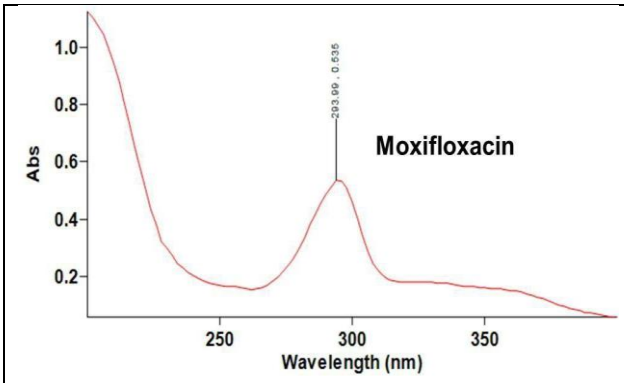

Figure-16: UV Spectrum of MOX

VIII. b: Chromatographic conditions optimization

The main objective of this study was to develop a single RP-HPLC method for the simultaneous determination of CEF+LNZ and CEF+MOX in pharmaceutical drug products, by achieving separation between three components with short run time and by proving stability indicating nature of the method. The development trials were illustrated below.

Trial-1:

Preparation of Buffer:

Accurately weighed and transferred 0.7 g of Ammonium acetate into 500 mL of milli- Q water and mixed well to dissolve. Filtered the solution through 0.45µm membrane filter and degassed.

Mobile Phase A: Buffer

Mobile Phase B: Mixed Acetonitrile and methanol in the ratio of 80:20 v/v respectively.

Chromatographic Parameters:

Column: Zorbax C18, 250 x 4.6 mm, 5µm

Flow rate: 1.0 mL/min

Column Temperature: 30°C

Volume of Injection: 10µL

Wavelength: 270 nm

Run Time: 35 min

Gradient Table - 2

Time (min)	Flow (mL/min)	MP-A (%)	MP-B (%)
0.00	1.00	75	25
10.00	1.00	55	45
18.00	1.00	20	80
25.00	1.00	20	80
28.00	1.00	75	25
35.00	1.00	75	25

Preparation of diluent:

Mixed milli-Q water and Acetonitrile in the ratio of 50:50 v/v respectively and degassed in a sonicator.

Preparation of Standard Solution:

Individual standard solutions were prepared at 250 ppm concentration in diluent solution.

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Procedure:

Blank and individual standard (API) solutions were injected in to the chromatographic system, and checked for elution order.

Observation: Three standard peaks were eluted at different retention time but peak shapes were found not symmetric and LNZ / MOX peaks were closely eluted. Further experiments were carried out to get good peak shape by changing the buffer of mobile phase to KH_2PO_4 and different brand C18 column.

The individual chromatograms of cefixime, linezolid and moxifloxacin are presented below in figure-17, 18 and 19 respectively.

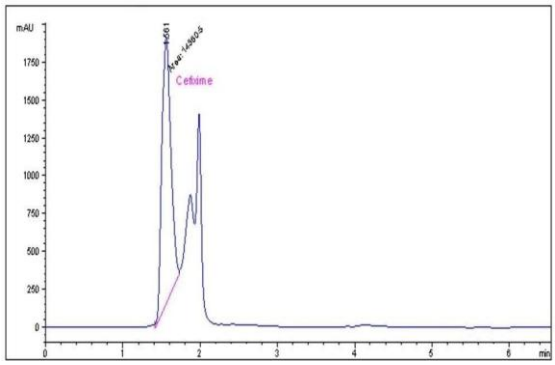

Figure-17: CEF Chromatogram

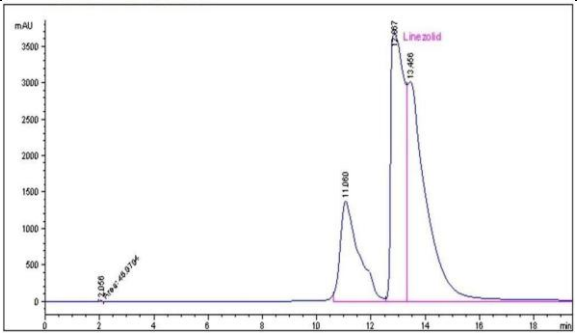

Figure-18: LNZ Chromatogram

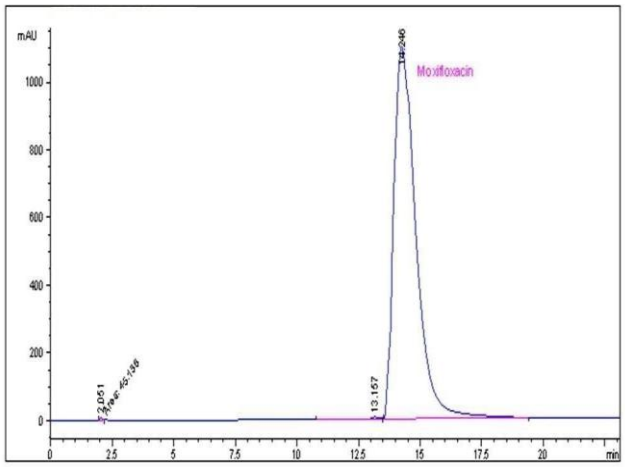

Figure-19: MOX Chromatogram

Trial-2:

Preparation of Buffer:

Accurately weighed and transferred 3.4 g of KH_2PO_4 into 500 mL of milli-Q water and mixed well to dissolve. Filtered the solution through 0.45µm membrane filter and degassed.

Mobile Phase A: Buffer

Mobile Phase B: Mixed Acetonitrile and methanol in the ratio of 80:20 v/v respectively.

Chromatographic Parameters:

Column: Inertsil ODS 250 x 4.6 mm, 5µm

Flow rate: 1.0 mL/min

Column Temperature: 30°C

Volume of Injection: 10µL

Wavelength: 270 nm

Run Time: 35 min

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Gradient Table - 3

Time (min)	Flow (mL/min)	MP-A (%)	MP-B (%)
0.00	1.00	75	25
10.00	1.00	55	45
18.00	1.00	20	80
25.00	1.00	20	80
28.00	1.00	75	25
35.00	1.00	75	25

Preparation of diluent:

Mixed milli-Q water and Acetonitrile in the ratio of 50:50 v/v respectively and degassed.

Preparation of Standard Solution:

Individual standard solutions were prepared at 250 ppm concentration in diluent solution. And mixed standard solution prepared by diluting individual solutions in diluent.

Procedure:

Blank and mixed standard solutions were injected into the HPLC system and evaluated for the selectivity and separation.

Observation: Three peaks were separated from each other but observed poor peak shape and gradient hump at 20min. Next development trials were carried out with different column and evaluated different gradient program to get satisfactory peak shape and baseline. The mixed standard chromatogram of CEF, LNZ and MOX presented below in figure-20.

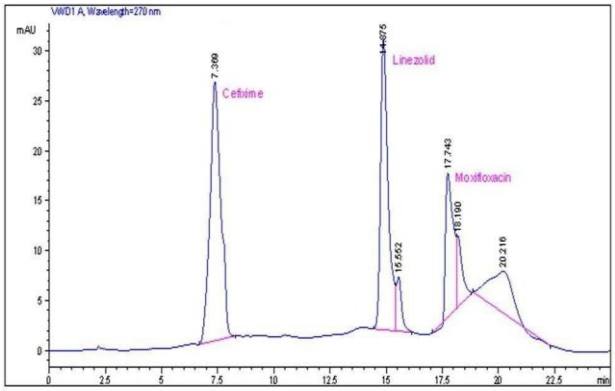

Figure-20: Mixed standard Chromatogram

Trial-3:

Preparation of Buffer:

Accurately weighed and transferred 3.4 g of KH_2PO_4 into 500 mL of milli-Q water and mixed well to dissolve. Filtered the solution through 0.45µm membrane filter and degassed.

Mobile Phase A: Buffer

Mobile Phase B: Mixed Acetonitrile and methanol in the ratio of 80:20 v/v respectively.

Chromatographic Parameters:

Column: Zorbax SB C18 150 x 4.6 mm, 5µm

Flow rate: 1.0 mL/min

Column Temperature: 30°C

Volume of Injection: 10µL

Wavelength: 270 nm

Run Time: 25 min

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Gradient Table - 4

Time (min)	Flow (mL/min)	MP-A (%)	MP-B (%)
0.00	1.00	85	15
10.00	1.00	65	35
14.00	1.00	35	65
16.00	1.00	35	65
20.00	1.00	85	15
25.00	1.00	85	15

Preparation of diluent:

Mixed milli-Q water and Acetonitrile in the ratio of 50:50 v/v respectively and degassed in a sonicator.

Preparation of Solutions:

Individual standard solutions were prepared at 250 ppm concentration in diluent solution and mixed standard solution prepared by diluting individual solutions in diluent. Also prepared placebo solution, to evaluate the non-interference at analyte peaks.

Procedure:

Blank, placebo, individual standard solutions, placebo and mixed standard solutions were injected into the HPLC system.

Observation: From mixed standard solution observed, three peaks were separated from each other but linezolid and moxifloxacin has interference with placebo. Next development trials were taken at 50°C column oven temperature with optimized gradient program.

The mixed standard chromatogram of CEF, LNZ and MOX presented below in figure-21.

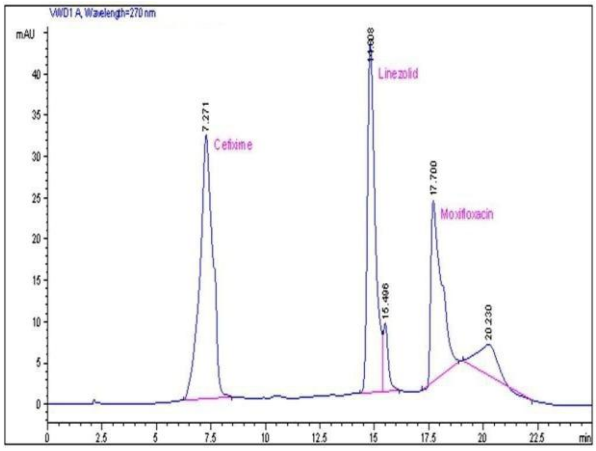

Figure-21: Mixed Standard Chromatogram

Trial-4:

Preparation of Buffer:

Accurately weighed and transferred 3.4 g of H_2PO_4 into 500 mL of water and mixed well to dissolve. Filtered the solution through 0.45µm membrane filter and degassed.

Mobile Phase A: Buffer

Mobile Phase B: Mixed Acetonitrile and methanol in the ratio of 80:20 v/v.

Chromatographic Parameters:

Column: Agilent Zorbax SB C18 150 x 4.6 mm, 5µm

Flow rate: 1.0 mL/min

Column Temperature: 50°C

Volume of Injection: 20µL

Wavelength: 270 nm

Run Time: 25 min

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Gradient Table - 5

Time	Mobile Phase-A (%)	Mobile Phase-B (%)
0.00	100	0
5.00	92	8
10.00	75	25
14.00	75	25
18.00	50	50
20.00	100	0
25.00	100	0

Preparation of diluent:

Mixed mobile phase and Acetonitrile in the ratio of 50:50 v/v and degassed.

Preparation of samples:

Individual standard solutions were prepared with 250 ppm concentration in diluent solution. Individual standard were mixed and used as mixed standard.

Preparation of Placebo Solution:

Placebo samples were prepared with same diluent.

Procedure:

Blank, individual standard solution, Placebo and mixed standard solutions were injected.

Observation: In mixed standard, three peaks were well separated and no interference with placebo. Hence these conditions were considered as final and proceed for method validation.

Figure-22 represents the mixed standard chromatogram.

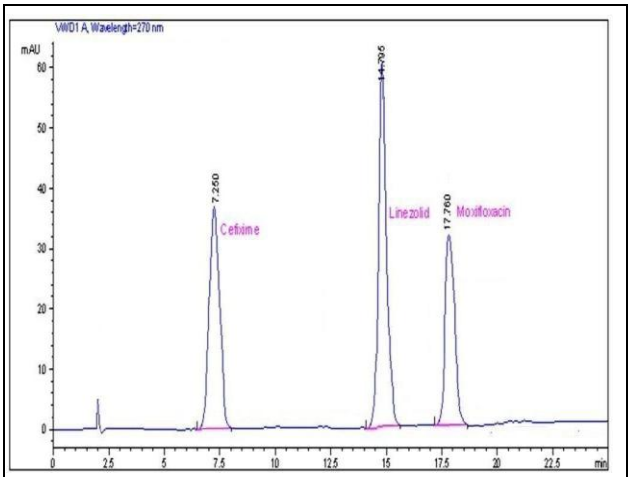

Figure-22: Mixed Standard Chromatogram

Table-2: System suitability results

System Suitability parameter (five replicate injections)	Observations		
	CEF	LNZ	MOX
Retention time (min)	7.3	14.8	17.8
Tailing factor (avg)	1.1	1.0	1.1
Area % RSD (5 replicates)	0.85	1.11	0.69

VIII. c: Method validation

VIII.c.1: Precision

The results of the precision and intermediate precision data were presented in table-3 to 5 respectively. From the results method is found precise and rugged at different conditions (Different analyst, column, HPLC & at different day). % RSD for assay results of

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CEF+LNZ+MOX were found well within the acceptance criteria (not more than 2%) and % assay results found to be between 97.0% - 103.0% at precision and intermediate precision.

Table-3: System Precision

System Suitability parameter (five replicate injections)	Observations		
	CEF	LNZ	MOX
Retention time (min)	7.2	14.7	17.7
Tailing factor (avg)	1.1	1.0	1.2
Area % RSD	0.96	1.04	0.54

Table-4: Precision Results (CEF+LNZ Tablets)

Sample No.	% Assay (97.0% ~ 103.0%)			
	Precision		Intermediate Precision	
	CEF	LNZ	CEF	LNZ
1	101.1	100.1	101.3	101.4
2	99.8	100.2	100.4	99.7
3	100.6	101.3	100.8	101.6
4	100.6	101.1	101.3	100.0
5	101.3	101.1	100.8	101.3
6	101.2	100.6	100.7	101.4
Mean	**100.8**	**100.7**	**100.9**	**100.9**
% RSD (NMT 2.0)	**0.6**	**0.5**	**0.4**	**0.8**

Table-5: Precision Results (CEF+MOX Tablets)

Sample No.	% Assay (97.0% ~ 103.0%)			
	Precision		Intermediate Precision	
	CEF	LNZ	CEF	LNZ
1	101.6	100.1	101.1	101.4
2	100.2	100.2	99.9	100.7
3	101.7	101.3	100.3	101.3
4	100.2	101.1	99.5	100.2
5	100.2	101.2	100.1	100.4
6	100.4	100.7	99.8	101.1
Mean	**100.7**	**100.8**	**100.1**	**100.9**
% RSD (NMT 2.0)	**0.7**	**0.5**	**0.6**	**0.5**

VIII. c.2: Linearity

A linearity graph for CEF, LNZ and MOX were plotted between concentration and peak response (peak area). The concentration range for CEF and MOX are 20 to 60 µg/mL, for LNZ 30 to 90 µg/mL, and the correlation of co-efficient (r) for all the compounds found to be more than 0.999. This indicates that the method proven for good correlation between peak response and concentration. The linearity graphs of CEF, LNZ and MOX, and overlay chromatogram of all linearity levels are presented below in figure-23 to 26. And the correlation co-efficient values and bias at 100 % concentration results (Not more than 2%) are tabulated below in table-6.

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Table-6: Linearity data

Linearity Level (%)	CEF		LNZ		MOX	
	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
50	20.06	845	30.50	555	20.20	515
75	30.16	1225	45.51	821	30.31	755
100	40.41	1656	60.25	1083	40.20	1015
125	50.15	2055	75.61	1384	50.21	1245
150	60.17	2487	90.18	1642	60.15	1525
Correlation Coefficient (r)	1.000		1.000		1.000	
Bias at 100% Concentration	0.1		-0.3		-0.01	

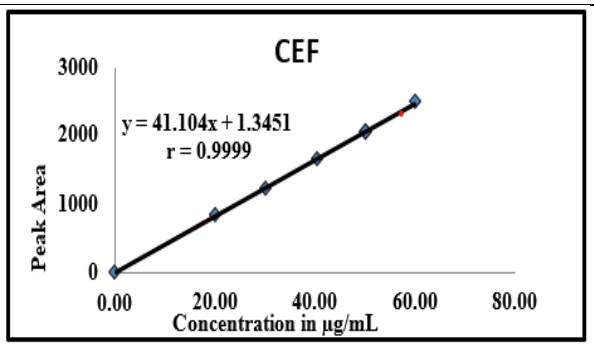

Figure-23: Linearity graph of CEF

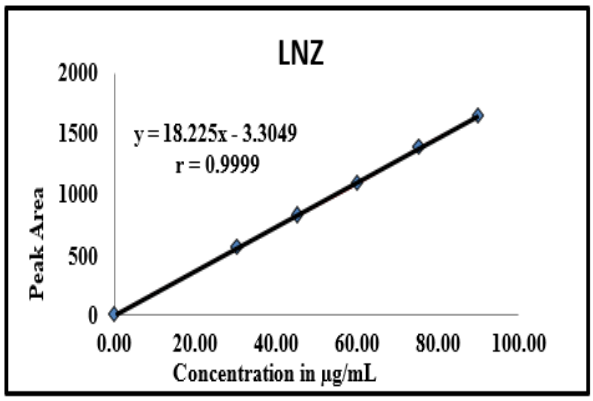

Figure-24: Linearity graph of LNZ

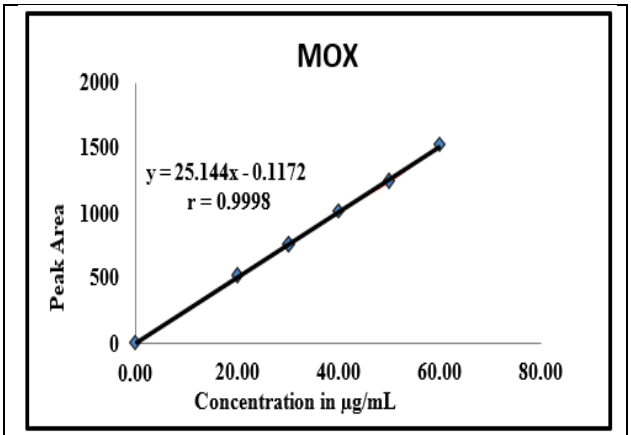

Figure-25: Linearity graph of MOX

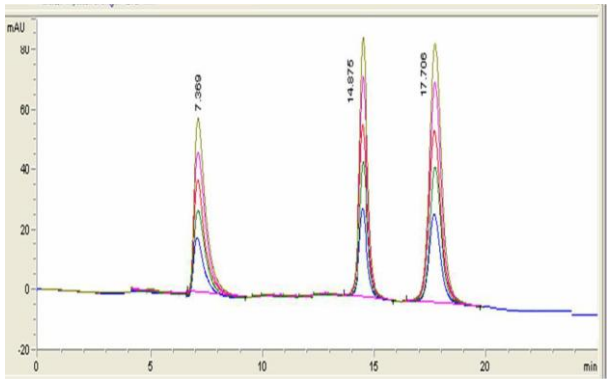

Figure-26: Overlay of Linearity chromatograms

VIII. c. 3: Recovery

The recovery results of CEF+LNZ+MOX are presented below in table-7. The % recovery was ranged from 98.1% to 101.6% for CEF, 99.8% to 101.7% for LNZ and 98.8% to 101.5% for MOX. The individual and mean % recovery results found well within the acceptance criteria (97.0% to 103.0%) and % RSD at each recovery level found within the acceptance criteria (NMT 2.0%). This result indicates that method is accurate within the range selected.

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Table-7: Recovery results

Level	Qty. Added (µg/mL)	Qty. found (µg/mL)	% Recovery	Mean	% RSD
Cefixime					
50%	20.16	19.78	98.1	99.2	1.0
	20.04	19.89	99.3		
	19.99	20.01	100.1		
100%	40.56	40.21	99.1	100.7	1.4
	39.10	39.68	101.5		
	39.52	40.15	101.6		
150%	60.21	60.82	101.0	100.4	0.8
	60.14	60.65	100.8		
	60.18	59.87	99.5		
Linezolid					
50%	30.15	30.17	100.1	100.2	0.2
	30.12	30.20	100.3		
	29.88	29.99	100.4		
100%	60.15	60.25	100.2	100.8	0.8
	60.19	61.20	101.7		
	59.99	60.30	100.5		
150%	90.15	90.01	99.8	100.2	0.4
	90.17	90.25	100.1		
	90.00	90.51	100.6		
Moxifloxacin					
50%	20.19	20.33	100.7	100.5	1.1
	19.79	20.08	101.5		
	20.51	20.35	99.2		
100%	40.61	40.31	99.3	100.0	0.9
	40.25	40.64	101.0		
	40.38	40.31	99.8		
150%	60.21	60.25	100.1	99.7	0.7
	60.28	60.35	100.1		
	60.81	60.10	98.8		

VIII.c.4: Solution stability and mobile phase stability

The stability of standard and test solutions was estimated at day-1 and day-3, at room temperature against freshly prepared standard solution. For test solution the % assay difference at day-1 and day-3 from initial found to be well within the tolerance limit (NMT 1.0%). And for standard, the similarity factor found to be greater than 0.98. From the results it is concluding that the standard and test solution is stable up to 3days at room temperature. The results are tabulated below in table-8 to 9 respectively. For mobile phase stability study results were tabulated below in table-10 and the % RSD of five replicate standard injections results found that less than 2.0% and tailing factor found that less than 1.5. From the results it is concluding that the standard, test solution and mobile phase are stable for 3 days at room temperature.

Table-8: Standard solution stability results

Stage	At room temperature		
	Similarity Factor (0.98 to 1.02)		
	CEF	LNZ	MOX
Day-1	0.99	1.00	0.99
Day-3	1.00	0.99	1.00

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Table-9: Test solution stability results at room temperature

Stage	CEF+LNZ Tablets				CEF+MOX Tablets			
	CEF		LNZ		CEF		MOX	
	T1	T2	T1	T2	T1	T2	T1	T2
Initial	101.1	99.8	100.1	100.2	101.6	100.2	100.1	100.2
Day-1	100.9	99.7	99.8	99.9	101.2	100.1	99.9	99.7
% diff	0.2	0.1	0.3	0.3	0.4	0.1	0.2	0.5
Day-3	100.5	99.2	99.4	99.5	100.8	99.8	99.5	99.5
% diff	0.6	0.6	0.7	0.7	0.8	0.4	0.6	0.7

Table-10: Mobile phase stability results

System Suitability parameter (five replicate injections)		**Observations**		
		CEF	**LNZ**	**MOX**
Tailing factor (avg)	Initial	1.1	1.0	1.2
	Day-1	1.2	1.1	1.1
	Day-3	1.1	1.2	1.1
Area % RSD	Initial	0.96	1.04	0.54
	Day-1	1.01	0.98	0.89
	Day-3	1.06	1.01	0.75

VIII.c.5: Robustness and Filter validation

The method robustness was determined by varying the chromatographic conditions deliberately. All the peaks were well resolved from each other and the elution order was unchanged at each varied condition (Mobile phase Flow rate and Column oven temperature). The % RSD found less than 2.0 and the tailing factor of each peak found less than 1.5. And for filter validation two different filters were evaluated against centrifugation, and the results found that both the filters are suitable for test solution filtration. The robustness and the filter validation results are tabulated below in table-11 and 12.

Table-11: Robustness study results

Parameter	Observed value						
	Variation	Tailing factor (Avg)			% RSD for 5 Standard Injections		
		CEF	LNZ	MOX	CEF	LNZ	MOX
Flow Rate	0.8	1.1	1.1	1.1	0.42	0.32	0.51
	1.0	1.1	1.0	1.2	0.96	1.04	0.54
	1.2	1.2	1.1	1.1	0.66	0.41	0.70
Column Temperature	45	1.1	1.2	1.0	0.11	0.52	0.17
	50	1.1	1.0	1.2	0.96	1.04	0.54
	55	1.0	0.9	1.1	1.49	0.36	1.15

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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFIXIME, LINEZOLID AND MOXIFLOXACIN IN FDC TABLET DOSAGE FORMS

Table-12: Filter validation results

Stage	CEF+LNZ Tablets				CEF+MOX Tablets			
	CEF		LNZ		CEF		MOX	
	T1	T2	T1	T2	T1	T2	T1	T2
Centrifuge	101.1	99.8	100.1	100.2	101.6	100.2	100.1	100.2
0.45µm PVDF filters	100.5	99.8	99.7	99.9	101.1	99.9	99.8	99.9
% diff	0.6	0.0	0.4	0.3	0.5	0.3	0.3	0.3
0.45µm NY filters	100.8	99.5	99.5	99.9	101.3	100.1	100.0	100.1
% diff	0.3	0.3	0.6	0.3	0.3	0.1	0.1	0.1

VIII.c.6: Specificity (Forced degradation study)

Specificity and stability-indicating power of the method was evaluated by forced degradation studies, in which all the unstressed samples, stressed samples, and placebo samples are injected into the HPLC system which connected with PDA detector.

In the placebo chromatograms no peaks were observed at CEF, LNZ and MOX retention time. This indicates that there is no interference from the excipients of CEF+LNZ, and CEF+MOX tablets formulation for quantification of drug content.

All the degradant peaks were well resolved from CEF, LNZ and MOX peaks respectively in all the stressed samples. This concludes that the method is stability indicating. All the forced degradation samples results were tabulated in table-13. And all the stressed samples chromatograms are shown in figure-27 to 42.

Table-13: Specificity results

Stress Condition	% Assay			
	CEF+LNZ		CEF+MOX	
	CEF	LNZ	CEF	MOX
Acid	98.2	99.2	98.4	97.9
Base	98.1	98.5	99.1	98.6
Oxidation	99.2	98.1	98.8	97.5
Water	97.8	99.5	97.8	98.9
UV-Light	99.5	99.8	98.9	99.1
Sun-Light	99.7	99.3	99.2	99.1
Thermal	99.4	100.1	98.3	99.3
Humidity	99.5	97.5	98.8	99.2

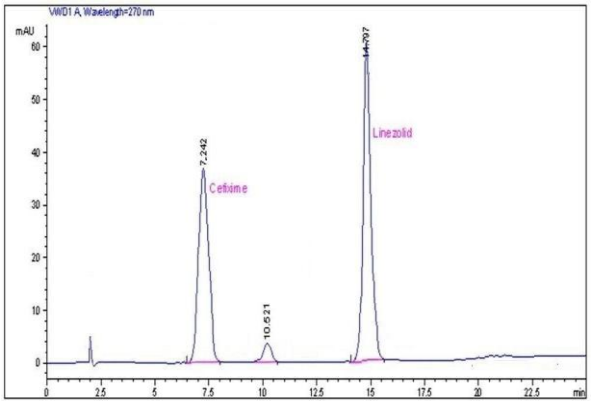

Figure-27: CEF+LNZ acid stressed chromatogram

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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFIXIME, LINEZOLID AND MOXIFLOXACIN IN FDC TABLET DOSAGE FORMS

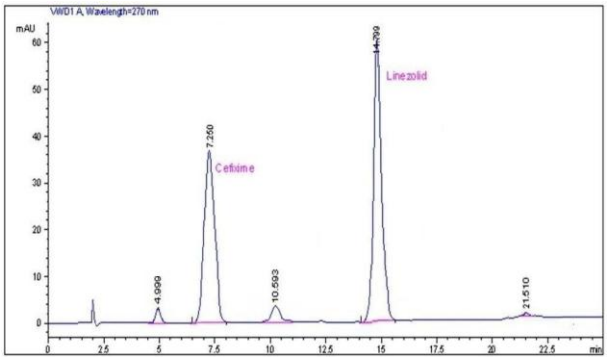

Figure-28: CEF+LNZ base stressed chromatogram

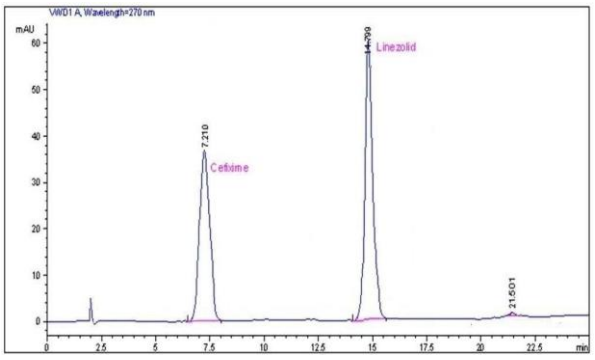

Figure-31: CEF+LNZ UV-Light stressed chromatogram

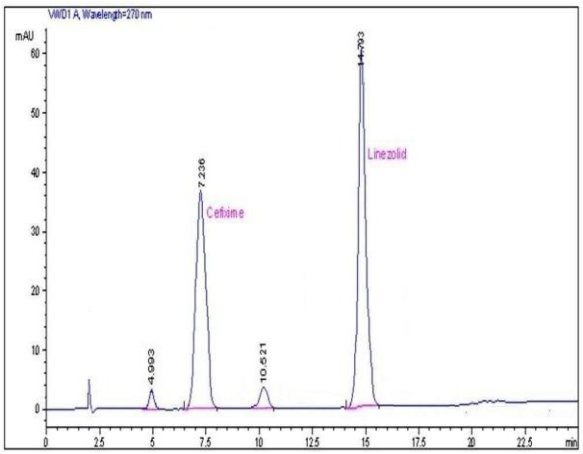

Figure-29: CEF+LNZ peroxide stressed chromatogram

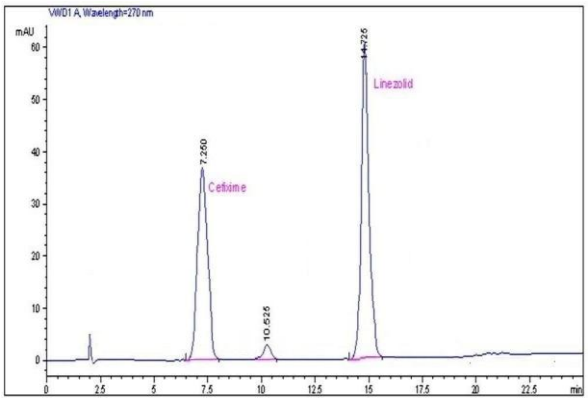

Figure-32: CEF+LNZ Sun-Light stressed chromatogram

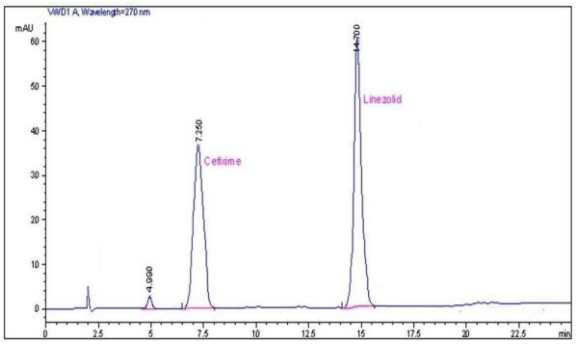

Figure-30: CEF+LNZ water stressed chromatogram

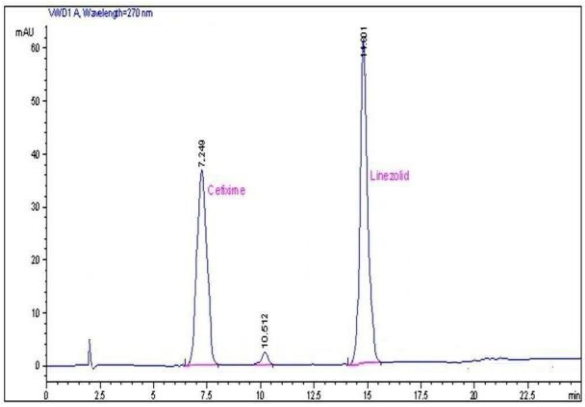

Figure-33: CEF+LNZ heat stressed chromatogram

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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFIXIME, LINEZOLID AND MOXIFLOXACIN IN FDC TABLET DOSAGE FORMS

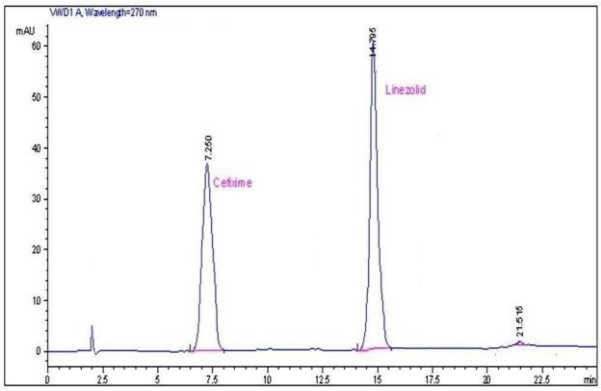

Figure-34: CEF+LNZ humidity stressed chromatogram

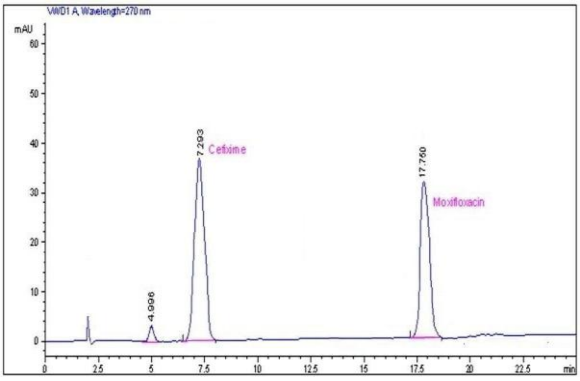

Figure-37: CEF+MOX peroxide stressed chromatogram

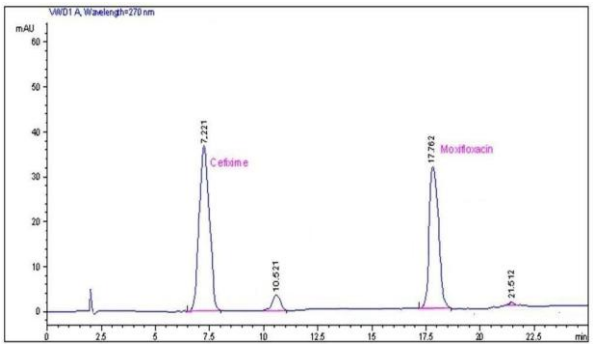

Figure-35: CEF+MOX acid stressed chromatogram

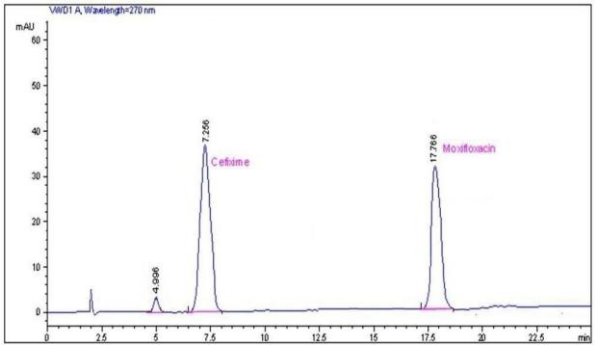

Figure-38: CEF+MOX water stressed chromatogram

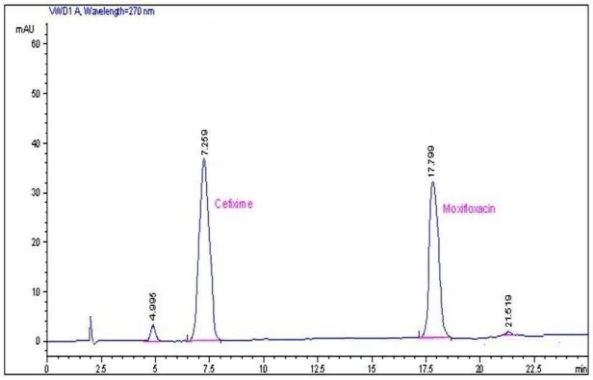

Figure-36: CEF+MOX base stressed chromatogram

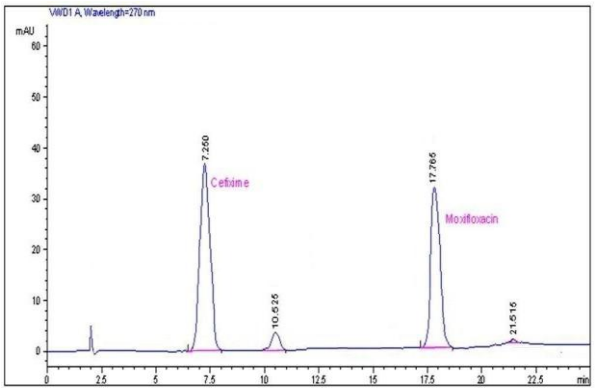

Figure-39: CEF+MOX UV-Light stressed chromatogram

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STABILITY INDICATING HPLC METHOD FOR THE DETERMINATION OF CEFIXIME, LINEZOLID AND MOXIFLOXACIN IN FDC TABLET DOSAGE FORMS

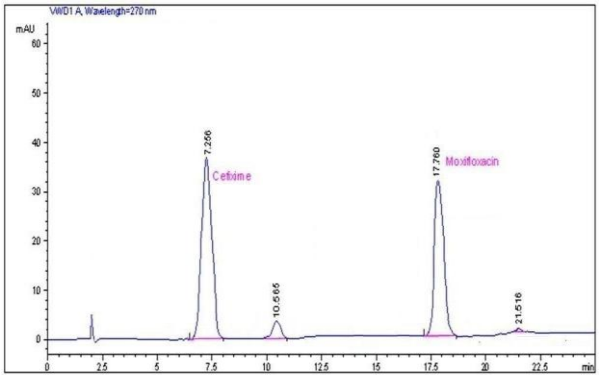

Figure-40: CEF+MOX Sun-Light stressed chromatogram

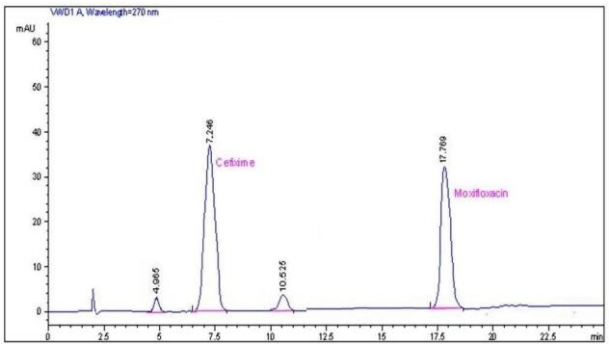

Figure-41: CEF+MOX heat stressed chromatogram

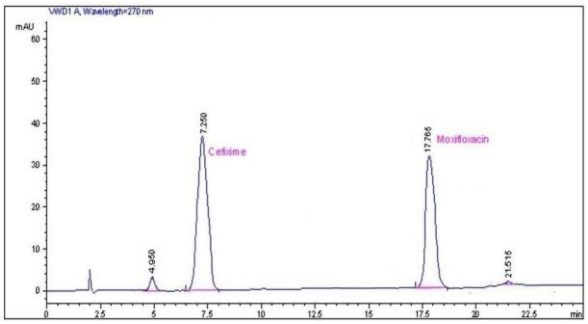

Figure-42: CEF+MOX humidity stressed chromatogram

CONCLUSION:

The present research work described about a stability-indicating RP-HPLC method development for the simultaneous determination of CEF, LNZ and MOX drug content in CEF+LNZ and CEF+MOX FDC tablets. And the method is validated to show its capability for intended application. Through validation the developed method found to be precise, rugged, accurate, linear, specific and robust. From the forced degradation study it is found that all the degradants formed are well separated from CEF, LNZ and MOX, and it concludes that the method is stability indicating in nature. Hence the described method is useful for checking quality of production samples and also useful in stability studies [35] to screen the quality of CEF + LNZ and CEF + MOX tablets during storage.

REFERENCE:

1. Yonatan HGrad et. al., Genomic epidemiology of *Neisseria gonorrhoeae* with reduced susceptibility to cefixime in the USA: a retrospective observational study, The Lancet Infectious Diseases, 2014, 14 (3) 220-226.
2. Muhammad Imran, Muhammad Raza Shah, Farhat Ullah, Shafi Ullah, Abdelbary M.A.Elhissi, Waqas Nawaz, FaridAhmad, Abdul Sadiq, ImdadAli, Glycoside-based niosomal nanocarrier for enhanced in-vivo performance of Cefixime, International Journal of Pharmaceutics, 2016, 505 (1–2), 122-132.

DOI: 10.63712/bpsrj-v1i2p001

3. Cefixime Tablets: https://www.rxlist.com/suprax-drug.htm
4. Myungsun Lee, Taeksun Song, Youngran Kim, Ina Jeong R.N., Sang Nae Cho, Clifton E. Barry, Linezolid for XDR-TB Final Study Outcomes, N Engl J Med 2015; 373: 290-291.
5. Shorr AF, Lodise TP, Corey GR, De Anda C, Fang E, Das AF, Analysis of the phase 3 ESTABLISH trials of tedizolid versus linezolid in acute bacterial skin and skin structure infections. Antimicrob Agents Chemother 2015, 59:864–871.
6. Gregory J Moran et. al., Tedizolid for 6 days versus linezolid for 10 days for acute bacterial skin and skin-structure infections (ESTABLISH-2): a randomised, double-blind, phase 3, non-inferiority trial, The Lancet Infectious disease, 2014, 14 (8) 696-705.
7. Linezolid Tablets: https://www.rxlist.com/zyvox-drug.htm
8. Rodney Dawson et.al., Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis, Lancet, 2015, 385 (9979), 1738-1747.
9. Zilu Li, Daniel L. Clemens, Bai-Yu Lee, Barbara Jane Dillon, Marcus A. Horwitz, and Jeffrey I, Zink Mesoporous Silica Nanoparticles with pH-Sensitive Nanovalves for Delivery of Moxifloxacin Provide Improved Treatment of Lethal Pneumonic Tularemia, *ACS Nano*, 2015, *9* (11), 10778–10789.
10. Moxifloxacin Tablets: https://www.rxlist.com/avelox-drug.htm
11. Cefixime API Monograph: USP-40 NF35 S2.
12. Cefixime Tablets Monograph: USP-40 NF35 S2.
13. Linezolid API Monograph: USP-40 NF35 S2
14. Cefixime API Monograph: BP 2018 Vol-I.
15. Moxifloxacin HCl API Monograph: USP-40 NF35 S2.
16. Moxifloxacin HCl Tablets Monograph: USP-40 NF35 S2.
17. Moxifloxacin HCl API Monograph: BP 2018 Vol-II.
18. Jérôme Toutain, Emmanuel Boselli, Sarah Djabarouti, BernardAllaouchiche, Fabien Xuereb, Jean-Marc Bernadou, BoubakarBa, Marie-Claude Saux, Dominique Breilh, Determination of linezolid in plasma and bronchoalveolar lavage by high-performance liquid chromatography with ultraviolet detection using a fully automated extraction method, Journal of Chromatography B, 2004, 813, 1–2, 145-150.
19. Madan LalMaheshwari, Ayaz AliMemon, ShahabuddinMemon, Fakhar-un- NisaMemon, Ubed Ur RahmanMughal, AbdullahDayo, NaheedMemon, Mohammed AliGhoto, M.Khan Leghari, Optimization of HPLC method for determination of cefixime using 2-thiophenecarboxaldehyde as derivatizing reagent: A new approach, Saudi Pharmaceutical Journal 2015: 23 (4), 444-452.
20. Rachel Taylor, Bruce Sunderland, Giuseppe Luna, and Petra Czarniak, Evaluation of the stability of linezolid in aqueous solution and commonly used intravenous fluids, Drug Des Devel Ther, 2017: 11, 2087–2097.
21. Prashanth S.N., Nagappa L.Teradal, Seetharamappa J., Ashis K.Satpati, Reddy A.V.R., Fabrification of

DOI: 10.63712/bpsrj-v1i2p001

electroreduced graphene oxide–bentonite sodium composite modified electrode and its sensing application for linezolid, Electrochimica Acta, 2014: 133; 49-56;

22. Satish A. Patel and Jinalben V Patel, RP-HPLC method for simultaneous estimation of cefixime trihydrate and linezolid in tablet dosage form, IJPCBS 2013, 3(2), 372-379.
23. Naga M., Rubesh Kumar S., Uma Maheswara Rao V., RP-HPLC Method Development and Validation for Simultaneous Estimation of Linezolid and Cefixime in API and Pharmaceutical Dosage Form, Int J Pharma Res Health Sci., 2017, 5 (4): 1731-35.
24. Alka Verma, Rathor D. S., VidyaSagar G., Verma, Bhupesh K, Development and Validation of RP-HPLC method for Simultaneous Estimation of Cefixime and Linezolid, International Journal of Pharmacy & Life Sciences, 2016,7 (8),5130-5134.
25. Nidhi S. Patel, Falguni B. Tandel, Yogita D. Patel, and Kartavya B. Thakkar, Development and Validation of Stability-indicating HPLC Method for Simultaneous Estimation of Cefixime and Linezolid, Indian J Pharm Sci. 2014 76(6): 535–540.
26. Joshi HV., Patel JK., Shah UA., Patel K,; Simultaneous Estimation of Linezolid and Cefixime in their Combined Dosage Form; Pharma Tutor; 2015; 3(2); 48-52
27. Mohammed Imran Anees, Mirza Shahed Baig and Ashwini Tathe, Determination Of Cefixime And Moxifloxacin In Pharmaceutical Dosage Form By Simultaneous Equation and area Under Curve UV spectrophotometric Method, World Journal of Pharmacy and Pharmaceutical Sciences, 2015 :4 (030), 1172-1179.
28. Mirza Shahed Baig and Dehghan M. H. G., Effect Of Accelerated Aging Conditions On In-Vitro Dissolution Studies Of Moxifloxacin Hydrochloride And Cefixime Trihydrate From Its Combined Tablet Dosage Form By HPLC Assay Methods, World journal of pharmacy and pharmaceutical sciences, 2015: 4 (08), 1949-1964.
29. Fahim Ullah, Khan Fazli Nasir, Zafar Iqbal, smail Khan Naila, Shahbaz Muhammad Hassan, Farhad Ullah, Simultaneous determination of moxifloxacin and ofloxacin in physiological fluids using high performance liquid chromatography with ultraviolet detection, Journal of Chromatography B, 2016: 1017–1018, 120-128.
30. Desai S. P., Kalaveni K. J., Malairajan P., Development and Validation for the Simultaneous Estimation of Moxifloxacin Hydrochloride and Bromfenac Sodium by RP- HPLC, Journal of Pharmacy Research, 2015: 9 (2), 129-133.
31. Hassouna MEM., Abdelrahman MM., Mohamed MA., 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, J Forensic Sci & Criminal Inves. 2017; 2(4) 1-9.
32. Rimakumari N. Chavda, Hitendra N. Yadav, Madhuri A. Hinge, Shuchi P. Desai, Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Cefuroxime Axetil and Linezolid in Tablet Dosage Form, J Pharm Sci Bioscientific Res. 2016: 6(3), 365-370.
33. Bharat G. Chaudhari , Heena J. Patel, Development and Validation of Dual Wavelength UV Spectrophotometric Method for Simultaneous Estimation of Cefixime and Dicloxacillin Sodium

DOI: 10.63712/bpsrj-v1i2p001

in Pharmaceutical Dosage Form, International Journal of Pharmaceutics and Drug analysis, 2014: 2 (3), 148- 154.

34. ICH guideline Q2 (R1): Validation of Analytical Procedures-Text and Methodology, November 2005.
35. ICH guideline Q1A (R2): Stability Testing of New Drug Substances and Products February 2003.

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