"METHOD DEVELOPMENT AND VALIDATION OF CEFPODOXIME PROXETIL AND OFLOXACIN IN INDIVIDUAL AND COMBINED DOSAGE FOEM "

A Thesis Submitted to

NIRMA UNIVERSITY

in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

BY

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CERTIFICATE

This is to certify that the dissertation work entitled "Method Development and Validation of Cefpodoxime proxetil and Ofloxacin in individual and combined dosage form" submitted by Ms. ARPITA PATEL with Regn. No. (13MPH301) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a bonafide research work carried out by the candidate at the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Method Development and Validation of Cefpodoxime proxetil and Ofloxacin in individual and combined dosage form", is based on the original work carried out by me under the guidance of Mr.Nrupesh R. Patel, Assistant professor, Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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"Don't tell people your dreams, SHOW THEM!"

Date: *ARPITA PATEL*

LIST OF ABBREVIATION

ABBREVIATION	FULL FORM
I.P	Indian Pharmacopoeia
U.S.P	British Pharmacopoeia
B.P	United States Pharmacopoeia
CP	Cefpodoxime Proxetil
OFLO	Ofloxacin
pKa	Partition Coeffcient
min	Minutes
MS	Mass spectrometry
Conc.	Concentration
HPLC	High Performance liquid Chromatography
HPTLC	High Performance thin layer Chromatography
SFC	Super critical Fluid Chromatography
SD	Standard Deviation
RSD	Relative Standard Deviation
°C	Degree Centigrade
cm	centimeter
µm	micrometer
nm	nanometer
mg	miligram
ml	Milliliter
no.	Number
Ref	Reference
Rt	Retention time
Rf	Retention factor
%	Percentage
LOD	Limit of detection
LOQ	Limit of quantification
Sec.	Seconds
CAS	Chemical abstract service
D	Delta
MPa	Megha pascal
UV	Ultra violet spectroscopy
RP	Reverse Phase
λmax	Wavelength
MeOH	Methanol
S.P	Stationary Phase
±	Plus or Minus

INDEX

LIST OF FIGURES

LIST OF TABLES

Abstract

Four simple, accurate, sensitive and reproducible methods are described for the quantitative determination of Cefpodoxime Proxetil(CP) and Ofloxain(OFLO) in their individual and combined dosage form. The first method involves UV Spectrophotometric method for the simultaneous estimation of CP and OFLO in bulk and tablet dosage form. The solvent used was 6 M urea and the absorption maxima for CP and OFLO were found to be 234nm and 286nm respectively.The two drugs follows the Beer- Lambert's law over the concentration range of 3-30 μg/ml. The second method involves the Spectrofluorimetric method includes synchronous mode estimation of ofloxacin in bulk and tablet dosage form using delta value 140 (medium sensitivity mode) that involves measurement of fluorescence intensity at 480 nm synchronous spectra of OFLO drug. Linearity range was observed in the concentration range 0.05-1 μg/ml.The third method is based on separation of drugs by HPTLC followed by densitometric measurements of their spots at 290 nm. The separation was carried out on HPTLC aluminium sheets of silica gel 60 F_{254} using Chloroform: Ethyl acetate:MeOH:TEA (5.0:0.75:0.5:0.3 v/v/v/v) as mobile phase. The linear regression analysis was used for the regression line in the range of 500 -2000 ng/spot for CP and OFLO. This system was found to give compact spots for CP and OFLO, after development. The fourth method is based on SFC separation of CP on the C_{18} column (150 mm length, 4.6 mm i.d, 5 μm particle size) & Agilent ZORBEX SB Phenyl column (150 mm length, 4.6 mm i.d, 5 μm particle size) at ambient temperature using a mobile phase consisting of CO_2 and methanol use as modifier.Condition was optimize by changing flow rate and backpressure.The oven temperature was kept at 35^0C.The detection wavelength was 237 nm.The develop method validated for various parameter and all were found within acceptance criteria.

Key Words:

Cefpodoxime Proxetil,Ofloxacin,UVspectroscopy,Spectrofluorimetry,HPTLC,SFC.

CHAPTER NO 1
INTRODUCTION

1.INTRODUCTION

1.1 Introduction to drugs

Infectious ailment are disorders that are created by life forms, normally infinitesimal in size, for example, microscopic organisms, infections, growths, or parasites that are passed, specifically or in a roundabout way, starting with one individual then onto the next..

These diseases are a leading reason around world, especially in low pay nations, particularly in youthful children.[1]Cefpodoxime is an oral, third-generation cephalosporin antimicrobial. It is dynamic against most gram-positive and gram-negative living beings. Most exceptions incorporate pseudomonas aeruginosa, enterococcus, and bacteroides fragilis.[2]

Gram-positive microscopic organisms:

- Staphylococcus aureus (methicillin-helpless strains, including those creating penicillinases)
- Staphylococcus saprophyticus
- Streptococcus pneumoniae (barring penicillin-safe secludes)
- Streptococcus pyogenes

Gram-negative microscopic organisms:

- Escherichia coli
- Klebsiella pneumoniae
- Proteus mirabilis
- Haemophilus influenzae (counting beta-lactamase creating detaches)
- Moraxella catarrhalis
- Neisseria gonorrhoeae (including penicillinase-producing isolates)[3]

Ofloxacin is a synthetic antimicrobial of the fluoroquinolone medication class thought to be a second-generation fluoroquinolone[4][5]Ofloxacin is additionally accessible for topical utilization,, as eye drops and ear dropsOfloxacin is a racemic mixture, which comprises of

50% levofloxacin(the organically dynamic segment) and 50% of its "reflect picture" or enantiomer dextrofloxacin[6]

Ofloxacin is affirmed for the treatment of bacterial diseases, for example,

- Intense bacterial intensifications of endless bronchitis
- Group gained pneumonia
- Uncomplicated skin and skin structure contaminations
- Nongonococcal urethritis and cervicitis
- Blended Infections of the urethra and cervix
- Intense pelvic incendiary ailment
- Uncomplicated cystitis
- Confounded urinary tract diseases
- Prostatitis
- Intense, uncomplicated urethral and cervical gonorrhea

1.2 INTRODUCTION TO DRUG PROFILE

1.2.1 Drug profile of Cefpodoxime proxetil:-[7-11]

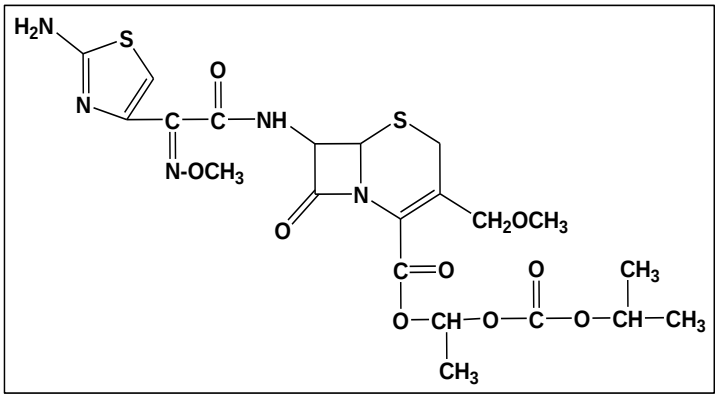


Figure 1.1:-Structure of Cefpodoxime proxetil

Drug class: Lactams

Superclass:- Heterocyclic Compounds

Sub class:- Beta Lactams

Category: Anti-Bacterial Agents

- Cephalosporins

CAS number[7]:- 82619-04-3

Official status[8][9]**:-**It is official in IP 2014 & USP 2012

Chemical name:- (RS)-1(isopropoxycarbonyloxy})ethyl (+)-(6R,7R)-7-[2-(2-amino-4-thiazolyl)-2-{(Z)methoxyimino}acetamido]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0]oct-2-ene- 2-carboxylate.

Molecular formula[8] **:** $C_{21}H_{27}N_5O_9S_2$

Molecular weight[8] :557.61

Physicochemical properties[9]:

Description: Cefpodoxime proxetil is an orally administered, extended spectrum, semi-synthetic antibiotic of the cephalosporin class.

Solubility:Freely soluble in dehydrated ethanol,solution in acetonitrile,methanol,slightly soluble in ether,very slightly soluble in water

Melting point[10]: 111-113°C

Optical rotation: 35.0º and 48.0º

Dissociation constant(pKa):3.20± 0.13

Partition coefficient(log p):((chloroform/water))1.60 at pH1.2

FDA approval: 2/10/2000

Pharmacological action[11]**:** The bactericidal activity of cefpodoxime results from its inhibition of cell wall synthesis. Cefpodoxime proxetil is a prodrug that is absorbed from the gastrointestinal tract and de-esterified to its active metabolite, cefpodoxime.

Mode of action:-

Ofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It acts by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV.

Contraindications:-

Caution in patients with liver disease.

Ofloxacin is also considered to be contraindicated within the pediatric population, pregnan, nursing mothers, patients with psychiatric illnesses and epilepsy or other seizure disorders.

1.2.2 Drug profile of Ofloxacin:-[2-14]

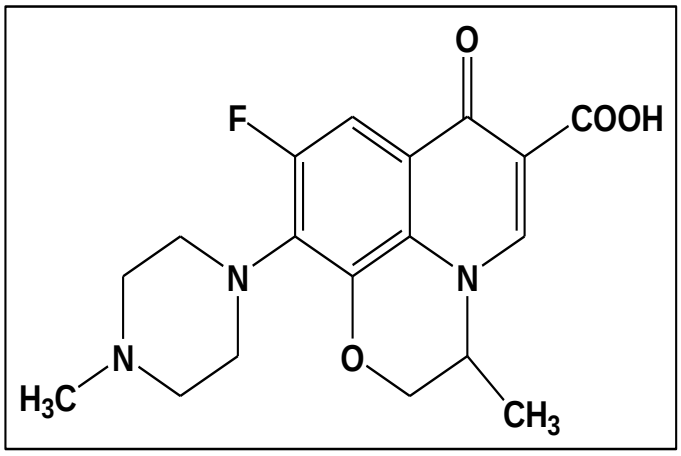


Figure1.2:- Structure of Ofloxacin

Drug class: Quinolines and Derivatives

Superclass:- Heterocyclic Compounds

Sub class:- Quinoline Carboxylic Acids

Category: Anti-Bacterial Agents

- Nucleic Acid Synthesis Inhibitors
- Anti-Infective Agents
- Quinolones

CAS number[13]**:** 82419-36-1

Official status[8]**:-**It is official in IP 2014,E USP 2012,EP 2005 & BP 2010

Chemical name:-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid

Molecular formula [13]**:** $C_{18}H_{20}FN_3O_4$

Molecular weigh [13]: 361.367

Physicochemical properties:[8]

Description: A synthetic fluoroquinolone (fluoroquinolones) antibacterial agent that inhibits the supercoiling activity of bacterial DNA gyrase, halting DNA replication

Solubility: 28.3 mg/ml in water 254 C°, Soluble in Chloroform and DMSO(Dimethyl sulphoxide)

Dissociation constant(pKa):.4.45,6.2

Partition coefficient(log p): -0.39

Melting point[10]**:**-270-275°C

Metabolism[1] **:** Hepatic

Pharmacokinetics[12]**:** After multiple-dose administration of 200 mg and 300 mg doses, peak serum levels of 2.2 μg/mL and 3.6 μg/mL, respectively, are predicted at steady-state. Less than 5% is eliminated by the kidneys as desmethyl or N-oxide metabolites; 4% to 8% by feces.

Absorption & Excreation:- Elimination is mainly by renal excretion Four to eight percent of an ofloxacin dose is excreted in the feces. This indicates a small degree of biliary excretion of ofloxacin.

Bioavability[1]**:** 85% - 95%

Half life[11]**:** 9 hours

Volume of distribution[12]**:** 1.5 l/kg

Indication[14]**:** For the treatment of infections (respiratory tract, kidney, skin, soft tissue, UTI), urethral and cervical gonorrhoea.

Drug interaction[12]**:-**

- Acenocoumarol
- Aluminium
- Anisindione
- Calcium
- Calcium Acetate
- Dicoumarol

1.3 INTRODUCTION TO MULTI COMPONENT ANALYSIS

In present days,market is overflowed with various blends in dosage form and the number is expanding day by day.These multi-component formulations due to high patient acceptability, increased potency,mullti action,fewer reactions and giving easy recovery.Therefore it require this product of standard quality, safety & efficacy.

The aim for quantitative estimation is to ensure that whether a particular drug contains the same strength of medications as specified.

Sometime in addition to main drugs it contain other substance which gives interference in estimation of drug.So it gets to be important to grow new logical strategy for such products for analysis.[15]

The reason for the development of newer methods of drug analysis are:

- The drug or drug combination may not be official in any pharmacopoeia
- A proper analytical methadology for the drug may not be available in the literature due to patent regulations.
- Analytical methods for the assay in combination with other drugs may not be available.
- The existing analytical procedure may have high cost.[15]

Modern instruments use for multi component formulations are UV Visible spetrophotometry,HPTLC,HPLC,Spectrofluorimetry,GC,SFC and Hyphenated technique like LC-MS,LC-NMR,LC-MS/MS,GC-MS,CE-ICP-MS etc. [16][17]

The various UV Visible spetrophotometry techniques used for multi-component analysis are listed below:-

Simultaneous equation method (Vierodt's method)

Concentration of several components present in the same mixture can be determined by solving a set of simultaneous equation even their overlay spectra available.

Two wavelength method

The method is to calculate the concentration of component of interest found in a mixture containing it along some unwanted interfering substance..

The absorption ratio method

The absorbance ratio method is a modification of the simultaneous equation procedure. It depends on the property that for a substance, which obeys Beer's law at all wavelength, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length.

Geometric correction method

The easiest of this methodology is the three-point geometric technique, which may be connected if the unessential assimilation is linear at the three wavelengths chosen. This strategy is just rithmetical estimations of what the standard method in infrared spectrophotometry measurements graphically.

Absorption factor method (Absorption correction method)

It is modification of simultaneous equation method. Quantitative estimation of one drug is carried out by E (1%, 1 cm) value and quantitation of another drug is carried out by subtraction absorption due to interfering drug using absorption factors.

Orthogonal polynomial method In this method absorption spectrum may be represented in terms of orthogonal functions

Difference spectrophotometry

It provides a sensitive method for detection o small changes in the environment of a chromophore or it is use to show ionization of a chromophore leading to identification and quantitation of various substance

Derivative spectrophotometry

Derivative spectrophotometry is for resolving two overlapping spectra and eliminating matrix effect due to an indistinct shoulder on side of an absorption bands.

A**rea under curve method**

In this method, the absorptivity values (ε_1 andε_2) of each of the two drugs were determined at the selected wavelength range. Total area under curve of a mixture at wavelength range is equal to the sum of area under the individual component at wavelength range.

1.4 RATIONAL FOR COMBINATION OF DRUGS:-

Unique Dual Mode Of Action

Ofloxacin-Prevents nucleic acid synthesis

Cefpodoxime-Inhibits cell wall synthesis

Acts synergetically

Gives better patient compliance.[18]

1.5 INTRODUCTION TO METHOD

1.5.1 INTRODUCTION TO UV VISIBLE SPECTROPHOTOMETRY

Principles[19]

Radiation in the wavelength range 200-700 nm is passed through a solution of a substance. The electrons in the bonds within the molecule become excited so that they occupy a higher quantum state and in the process absorb some of the energy passing through the solution.The more loosely held the electrons are within the bonds of the molecule the longer the wavelength (lower the energy) of the radiation absorbed.

Beer-Lambert law[20]

When a beam of light is passed through a transparent cell containing a solution of an absorbing substance,reduction of the intensity of light may occur.

$$\mathbf{A = abc}$$

Where a = absorptivity, b = Pathlength (1 cm)

c = concentration (gm/100 ml),

A = Absorbance

INSTRUMENTATION[19]

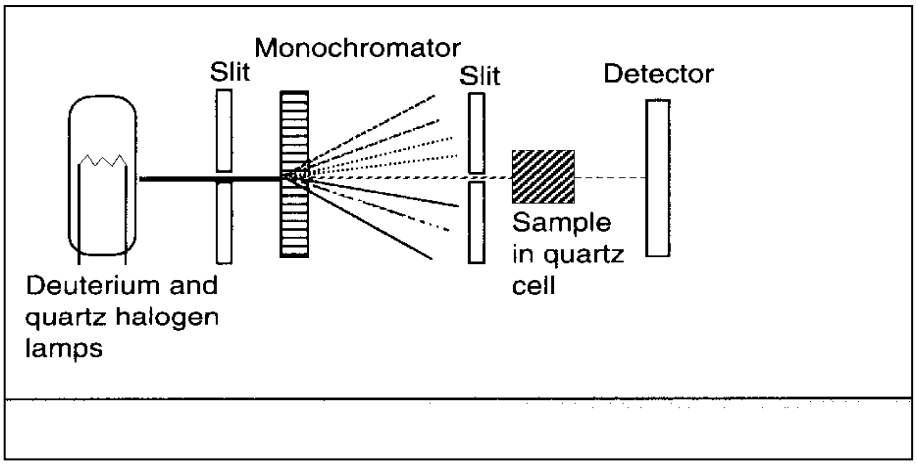


Figure1.3:- Schematic diagram of spectrophotometer

Limitations:-[20] Only moderately selective. The selectivity of the technique relies on upon the chromophore of the individual drugs,e.g medication with a developed chromophore is more particular than a medication with a straightforward benzene ring chromophore.

1.5.2 INTRODUCTION TO SPECTROFLUORIMETRY

Principles[19]

Certain molecules,particularly those with a chromophore and a rigid structure,can be excited by UV/visible radiation,and will emit the radiation absorbed at a longer wavelength.The radiation emitted is measured.

INSTRUMENTATION[19]

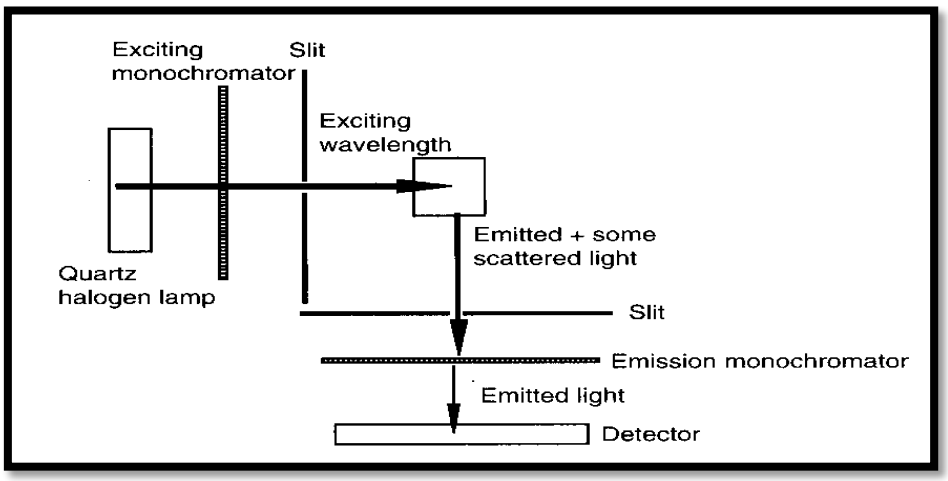


Figure1.4:- Schematic diagram of fluorescences spectrophotometer

Factors affecting fluorescence[21]

- **Molecular** unbending nature
- **Polarity of the solvent**
- Presence of broke up oxygen
- **Changes in pH:-**Neither anion nor cation is fluorescent. Fluorescence is more commonly associated with $\pi-\pi*$ states than with $n-\pi*$ states because $\pi-\pi*$ states possess shorter average lifetime and because deactivation process that compete with fluorescence are much less likely to take place.
- **Quenching**

Advantage of spectrofluorimetry over spectrophotometer[20]:-Selective,High sensitivity

1.5.3 INTRODUCTION TO HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

Principle

Adsorption:-Solute and solvent molecule compete for 'sites' on the adsorbent, to adsorbed, the solute molecule must first uproot a solvent molecule.

Partition:-The capacity of a solute to distribute itself between two stages according to the partition coefficient

Ion-exchange:- If the process involves trading of adversely charged particles, it is known as anion-trade. The correlative methodology is known as cation-trade. .

Size-exclusion:- Retention of solutes on the premise of size or shape. [22]

Steps Involved in TLC/HPTLC Analysis

The expository method for follow natural substances in characteristic lattices includes

- Sampling and sample storage
- Sample preparation and cleaninng
- Selection of TLC/HPTLC plates
- Application of samples
- Selection of solvent
- Separation of the compounds of choice
- Detection and measurement
- Data lessening, record keeping and quality control[22]

Method of Quantification

Scraping and elution: Scraping the isolate analyte, recovery of the compound by elution and analyzed by various instrument.

Visual comparison: Samples and standards are chromatographed one next to the other on the same plate and look at the spot size and power.

Densitometry: It can be completed specifically on the plate in distinctive modes viz., assimilation, transmission and fluorescence. An adjustment bend comprising zones of the norms versus sum analyte spotted is developed and the measure of analyte in the specimen is added from the bend.

Focal points of TLC

TLC has many advantages over other chromatographic methods for separation of simple mixtures-

- Simple, Rapid

• Less costly,Multiple sample application

• Volume of dissolvable utilized every sample is less[22]

1.5.3 INTRODCTION TO SUPER CRITICAL FLUID CHROMATOGRAPHY(SFC)

Principle:-

In supercritical fluid chromatography (SCF) the versatile stage is a supercritical gas or a near critical liquid. Contrasted to gas chromatography, where gas is under ambient pressure, and liquid chromatography, where liquid is used as portable stage, the solvent power of the mobile phase in SFC can be varied by density, e.g., by pressure changes at constant temperature

INSTRUMENTATION

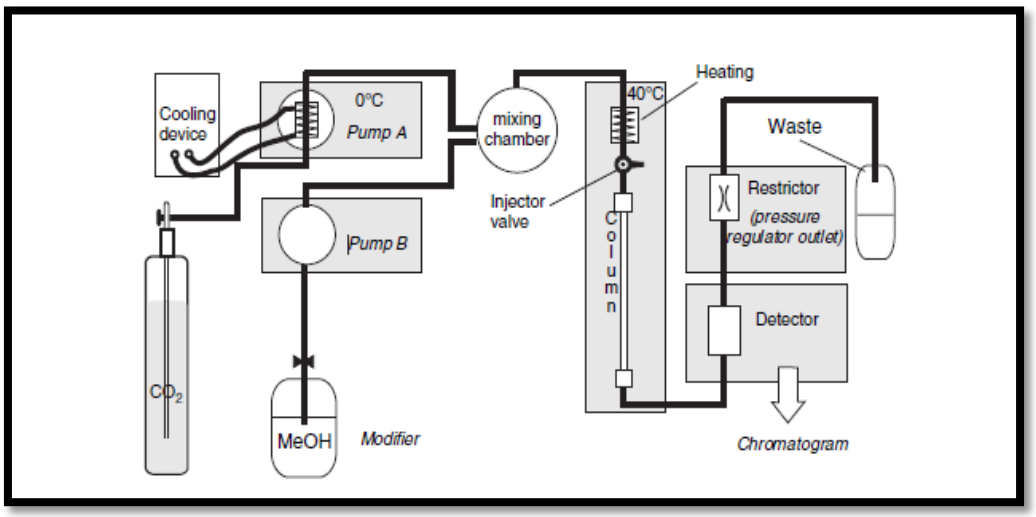


Figure1.5:- Schematic diagram of Super critical fluid chromatography

Advantages

Dissolving power of SCF is controlled by pressure and/or temperature.

SCF is effortlessly recoverable from the concentrate because of its unpredictability

Non-poisonous solvents have no harmful effect

High boiling components are isolated at low temperature

Separation not possible by other processes can sometimes be effected

1.9 INTRODCTION TO METHOD VALIDATION

Linearity/Range

The linearity of an analytical procedure as its ability to obtain test results that are directly proportional to the amount of analyte in the sample.

The range of an analytical procedure between the upper to the lower amounts of analyte in the sample

Linearity is determined by a series of five to six injections of five or more tandards whose concentrations 80–120 percent of specified range.

Acceptance criteria:-Line of best fit, correlation coefficient, Y intercept near to zero[26]

Precision

The precision of an analytical procedure as the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under the specified environment.

Repeatability involve the precision under the same operating conditions over a short interval of time. It is also called intra-assay precision.

Intermediate precision expresses variations within laboratories, different days, different analysts, different Instrument.

Reproducibility (Ruggednnes)expresses expresses the accuracy between research facilities (This parameter use for standardization of methodology).

Acceptance criteria:- RSD < 2%[26]

Accuracy

The accuracy of an analytical method as the closeness of agreement between the ordinary genuine worth or an acknowledged reference quality and the worth found.

Can be evaluated by many approaches:

- By known purity material
- Placebo spiking
- Sample spiking
- **Acceptance criteria:**-Mean Recovery within 90-110%,Individual recoveries within 70-130%[26]

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Acceptance criteria:-The excipient use for formulation of product should not interfere with main drug amount. [26]

Limit of detection & Limit of Quantification

The detection limit of analytical procedure is lowest amount of drug in a sample can be detected but not necessarily quantitated.

The limit of quantitation (LOQ) of analytical procedure is the lowest amount of analyte in a sample which can be quantitatively with given precision and accuracy.

Can be determine by number of way,

- Visual Evaluation
- Signal to noise
- Based on the Standard Deviation of the Response and the Slope
- Based on the Standard Deviation of the blank
- Based on the linearity Curve

Acceptance criteria:-In general Quantitation limit is three times the detection limit[26]

CHAPTER NO 2

Literature Review

2.LITERATURE REVIEW

2.1 Literateure reviews of Cefpodoxime proxetil

Sr.no	Matrix	Method	Method description	Validation parameters	Ref
UV Visible spectrophotometric Methods					
1	Tablet & suspension	UV Visible spectrophotometry	Solvent:-Formation of colored compound of cefpodoxime with 2-hydroxynaphthaldehyde λmax 436nm	Linearity:-02-10 µg/ ml,.	[27]
2	Tablet	UV Visible spectrophotometry	Solvent:-Urea as hydrotropic agent λmax:-231 nm	Linearity:-10-120 µg/ml,Recovery:-99.82 ± 0.106	[28]
3	Bulk and pharmaceutical dosage forms	UV Visible spectrophotometry	Solvent:-Potassium dichromate in acidic condition or medium λmax-570nm	Linearity:- 1-5 µg/ml,	[29]
4	Bulk and pharmaceutical dosage forms	UV-Visible Spectrophotometry	Solvent:-methanol λmax-235nm	Linearity:-5-25 µg/ml	[30]
	Bulk and pharmaceutical dosage forms	UV-Visible Spectrophotometry	Solvent:-ion-pair complex between cefpodoxime Proxetil and bromocresol green in acidic medium and the subsequent extraction of the ion pair in chloroform. λmax-425nm	Linearity:-5-25 µg/ml	[30]
5	Tablet	UV-Visible Spectrophotometry	Solvent:-haematoxylin-chtoramine-T (CAT) and -S:-portion in cefpodoxime proxetil. λmax-555nm	Linearity:- 8-48 µg/ml,LOD-0.0797µg/ml	[31]
6	Bulk powder and in pharmaceutical preparation	Second derivative spectrophotometry	Solvent:-1M NaOH,1M HCl λmax-261nm for acid degradation product	Linearity:- 4.0-40.0 µg/ml	[32]
		Third derivative	1M NaOH,1M HCl λmax-282nm for alkaline degradation	Linearity:- 4.0-40.0 µg/ml	

	ns	spectrophotometry	product		
		First derivative of ratio spectrophotometry	Solvent:-1M NaOH,1M HCl λmax-215 & 255nm for acid degradation roduct, λmax-243nm for lkaline degradation product	Linearity:-4.0-40.0 µg/ml	[32]
		Ratio subtraction spectrophotometry	Solvent:-1M NaOH,1M HCl λmax-555nm for acid or alkaline degradation product	Linearity:-4.0-40.0 µg/ml	
		RP-HPLC (or separtaion of egradation product)	Solvent:-acetonitrile: water: triethylamine (60:40:1, v/v/v) Column:-Zorbax C8,λmax-232nm	-	
7	Bulk &pharmaceutical formulations	UV Visible spectrophotometry	Solvent:-Ninhydrin1 indane-1,2,3- trione hydrate and Ascorbic acid λmax-232nm	Linearity 10-60 µg/ml	[33]
Fluorimetry					
8	CFP in pure form and pharmaceutical dosage form, CFA in human urine	Fluorimetry	Derivatizing reagent in alkaline medium:-1,2-naphthoquinone-4-sulfonate (NQS) λmax:- Emission:440 nm, Excitation:- 330nm	Linearity:-50–2000 ng/ml, 4, LOD:-CFA:-9.17 ng/ml,CFP:-9.48 ng/ml	[34]
TLC & HPTLC Methods					
9	Tablet & capsule	TLC	Solvent:-Water: Ethanol(50:50) λmax:-254 nm	Rf value-0.64	[35]
			Solvent:- Butanol: Ethanol:Water (35:35:30) λmax:-254 nm	Rf value-0.69	
10	Marketed formulation	HPTLC	Solvent:- Chloroform:methanol:toluene(4:2:4 v/v/v) λmax-289nm,S.P.-precoated silica gel	Linearity:-100-700ng/spot,LOD-30ng/ml,LOQ-90ng/spot	[36]

			60F254		
HPLC Methods					
11	Plasma	HPLC (ForCefpodoxime proxetil & cefpodoxime acid	Solvent:-Acetonitrile: ammonium acetate buffer (36:64) (pH 5.0) λmax 235 nm,Flow rate:-1 ml/min,Temperature:-30 ^{0}C		[37]
12	Dry syrup (CP & related substanc)	HPLC	Solvent:-Acetonitrile:0.02mol/L ammonium acetate (40:60, V /V) Column:-Kromasil C ,Flow rate:- 1.0ml/min, λmax:- 235nm		[38]
13	Chinchilla plasma and middle ear fluid (MEF)	HPLC	Solvent:-25 m*M* acetate buffer (pH 4.3)/15 m*M* triethylamine:acetonitrile (92.5:7.5, v/v) Rt:-cefpodxime:-3.5 min,cefuroxime:-.9 min	Recovery in MEF-CP:-90.3 ± 2.9%,cefuroxime:-88.6 %.Recovery in plasma:-CP:-72.1%,cefuroxime:-81.1%	[39]
14	Rabbit plasma	HPLC fluorescence labeling of its active metabolite	Solvent:-10 mM phosphate buffer (pH = 3.5)/CH_3CN (70:30, v/v) Column:-C 8, Excitation λmax:-430 nm	Linearity:-10–1000 ng/ml,correlation coefficient:-0.999,LOD:-3 ng/ml,LOQ:-10 ng/ml	[40]
15	in rat in situ intestinal perfusate samples	RP-HPLC	Solvent:-20 mM ammonium acetate buffer (pH 5.0) and acetonitrile (62:38) C-18 colume,Flow rate-1ml/min,Column temperature - 30 °C,λmax-235nm	-	[41]
16	Related substance	RP-HPLC	Solvent:-water and methanol, Rt:-40 min	-	[42]

17	Bulk and pharmaceutical dosage forms	Stability-Indicating HPLC Method	Solvent:- Acetonitrile and 50 mM ammonium acetate pH 6 (pH was adjusted with o-phosphoric acid) Column:-Phenomenex Luna C18 (250 mm × 4.6 mm i.d., 5 µm particle size)	Linearity of 1–80 µg ml–1,The LOD and LOQ are 0.17 and 0.5 µg ml–1,	[43]
17	Formulations	HPLC(Cefpodoxime proxeti)	Solvent:-acetonitrile : ammonium acetate (38:62)pH 5 λmax-235nm, Flow rate-1ml/min,Temperature-30°C	Linearity of 5–150µg ml–1,LOQ-900ng/ml	[44]
	Bioavability in rat	HPLC(Cefpodoxime acid)	Solvent:-Solvent:-Acetonitrile : phosphate buffer (10:90)pH 3 λmax-269nm,Flow rate-1ml/min	Linearity of 100–5000µg ml–1,LOQ-50ng/ml	
18	Human urine	Dirct injection HPLC	Solvent:-10% methanol in 0.2% phosphoric acid Column:-C18 RP preColumn 3cm	Linearity 1-20 µg/ml	[45]
19	Bulk &pharmaceutical formulations	Stability indicating RP-HPLC	Solvent:-Methanol and phosphate buffer of pH4.0 (65 : 35) λmax-252nm,Column:-Phenomenex (250 × 4.6 mm, 5 μm particle size) ODS,Flow rate of 1ml min−1	Linearity :- 5–100 gm/L,The LOD & LOQ are 53 and 160 ngml−1,	[46]
Miscellanous Methods					
20	Impurities in cefpodoxime proxetil using LC–MS	Formicacid–methanol–water	Column:-C18	-	[47]
21	Solid state,drug formulation & solution	LC-MS,LC-NMR,LC-IR (for degradation product)	LC-MS:-Moleculer weigh t& fragment information,LC-NMR:-structural information,LC-IR:-For	-	[48]

			carboxyl functional group		
22	Bulk powder	Head space Gas chromatography with FID detector	Internal standard:-butanol,Solvent media:-N,N-dimethylformamide Column:-DB-624,carrier gas:-nitrogen,	Linearity:-0.9945-0.9996	[49]
23	Drug-polymer	Differential scanning calorimetry	-	Endothermic fusion peak at 126.8ºC	[50]
23	Drug-polymer	X-Ray Diffraction Studies	Ni filtered,CuKα radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 40/min over a	Linearity of 5ºC to 40ºC	[50]
24	Urine	Stripping differential pulse voltammetry	-	Linearity:- 1 × 10−8 to 1 × 10−7M	[51]

2.2 Literature reviews of Cefpodoxime proxetil with other drugs

Sr.no	Drug in combination	Matrix	Method	Method Description	Validation parameters	Ref.
UV Visble spectrophotmetric Methods						
25	Cavulanate potassium(CLA)	Tablet	UV Visble spectrophotmetry	Solvent:-Methanol λmax:-CLA-270nm,CP-235nm	Linearity:-CLA-15-150µg/ml,CP-5-50µg/ml	[52]
26	Ambroxol HCl(AMB)	Tablet	UV Visible spectrophotometry	Solvent:-Methanol λmax:-AMB-248nm,CP-235nm	Linearity:-AMB-8-32µg/ml,CP-9-27µg/ml	[53]
27	Ambroxol HCl(AMB)	Tablet	First Order Derivative Spectrophotometry	Solvent:-0.1 N HCl zero crossing point:-AMB-263nm,CP-307nm	Linearity:-AMB-6-72 µg/ml,CP-10-90 µg/ml	[54]

28	Dicloxacillin	Tablet	First Order Derivative Spectrophotometry	Solvent:-Methanol zero crossing point:-Dicloxacillin-233.8nm,CP-321nm	Linearity:-Dicloxacillin-10-80 µg/ml,CP-40-32 µg/ml	[55]
29	Ambroxol HCl(AMB)	Tablet	UVSpectroscopy,simultaneous equation	Solvent:-Methanol λ max of CEF and AMB are 235nm & 308nm	Linearity of CEF and AMB are 5-30µg/ml and 3-18µg/ml	[56]
30			Derivative Spectrophotometry	Solvent:-Methanol λ max of CEF and AMB are 279nm & 235 nm	Linearity of CEF and AMB is 5-30µg/ml	
31	Ambroxol HCl(AMB)	Tablet	Dual λ max UV Visible Spectrometry	Absorbance difference of AMB & CP are 250.7-279nm & 230-251.8	Linearity of AMB & CP are 6-42 & 10-70 µg/ml	[57]
32	Levofloxacin Hemihydrate(LOFLO)	pharmaceutical formulations	Q-absorbance ratio spectroscopic method	Solvent:-Methanol λ max of LOFLO 300 nm,isoabsorptive point 273nm	Linearity of LOFLO & CP are 2.5-10.5 & 2-10µg/ml	[58]
HPTLC Methods						
33	Ambroxol HCl(AMB)	Human plasma	HPTLC extraction by liquid–liquid extraction	Extraction Solvent:-methanol and acetonitrile,mobile phase:- chloroform: methanol (9:1v/v) λ max:-240nm,Rf values of AMB & CP are 0.49 ± 0.0057 & 0.69 ± 0.005	Linearity of AMB & CP are 1000 to 7000 ng/spot & 500 to 3500 ng/spot & Recovery are 94.50 & 74..40	[59]
34	Levo floxacin Hemihydrate(LOFLO)	synthetic mixture and pharmaceutical dosage form	HPTLC	Solvent:-Butanol: Ethyl Acetate: hexane: triethylamine(2:6:4:0.1) λ max:-237nm, Rf values of LOFLO & CP are 0.66 and 0.33	Linearity of LOFLO & CP are 125-750 ng/spot and 100-600 ng/spot ,Recovery:-LOFLO & CP are 99.89-101.59% & 98.96-102.01%	[60]
35	Ambroxol HCl(AMB)	Tablet	HPTLC	Solvent:-Chloroform: Methanol: Hexane: Glacial acetic acid (6: 2: 4: 0.2v/v/v/v) Rf values 0.22 and 0.64 for AMB & CP,λ	Linearity of CP & AMB are 200-1200 ng/spot & 120-720 ng/spot,LOD & LOQ 21.16 and 64.11 ng/spot per spot for AMB &	[61]

				max:245nm	41.16 and 124.72 ng/spot per spot for CP	
HPLC Methods						
36	clavulanate potassium(CLA)	Tablet	RP-HPLC	Solvent:-methanol:actonitrile:water:tetrahydrofuran(40:20:30:10) λmax:-220nm	Linearity:-CLA-15-200µg/ml,CP-5-50µg/ml	[52]
37	Ambroxol HCl (AMB)	Tablet	RP-HPLC	Solvent:-acetonitrile: methanol: water (30:50:20 v/v/v) pH adjusted to 5.0 with ortho-phosphoric acid Column:-INERTSIL ODS-3V (150 mm x 4.6 mm, 5µm),λmax:-47nm,Rt:-AMB-4.308,CP-3.457,Flow rate-1ml//min	Linearity:-AMB-42-78 mg/ml,CP-70-130 mg/ml	[62]
38	Clavulanate potassium (CLA)	Tablet	RP-HPLC	Solvent:- Phosphate buffer (5.5 pH): acetonitrile (51:49 v/v) Column:-Hypersil-BDS (C-18) (5 µm, 250 mm 4.60 mm),λ max:-233nm,Flow -1ml/min,Rt for CP and CLA are 5.63 min. and 2.49 min	Linearity of CP & CLA are 1-60 µg/ml& 0.5-60 µg/ml ,Recovery:-CP & CLA are 99.71% and 99.51%	[63]
39	Clavulanic acid (CLA-A)	Combined dosage form	HPLC	Solvent: Methanol: water [60:40 (v/v)] Column:-Hypersil-BDS (C-18) (5 µm, 250 mm 4.60 mm),λ max:-225nm,Flow -1.5 ml/min,Rt for CP (S & R Epimer)and CLA-A are 7 &8.2 min. and 1.93 min	Linearity of CP & CLA-A are 50-250 µg/ml &30-150 µg/ml,Recovery:-CP & CLA-A are are 98.14-99.94% & 98.60-99.30%	[64]

40	Dicloxacillin	Extended release tablet dosage form	RP-HPLC	Solvent:-Acetonitrile: Water (70:30 v/v) Column:-Kromasil ODS C18 (5 µm) 250×4.6 mm,λ max:-235nm,Flow rate-1ml/min	Linearity of Dicloxacillin & CP are 12.5-62.5 µg/ml, 5–25 µg/ml	[65]
41	Ambroxol HCl(AMB)	Bulk & tablet	RP-HPLC	Solvent:-acetonitrile: 0.025 M potassium dihydrogen phosphate buffer (70:30 v/v) pH adjusted to 4.0 with orthophosphoric acid Column:-Qualisil RP C-8 (250 mm x 4.6 mm, 5 µm),Flow rate of 1.0 ml/min, λ max-248 nm,Rt of CP & AMB are 3.89, 2.69 min,	Linearity of CP & AMB are 3 - 21 µg/ml & 2 - 12 µg/ml ,LOD and LOQ are 0.18 and 0.55 µg for CP and 0.09 and 0.30 µg for AMB	[66]
42	Ambroxol HCl(AMB)	Bulk and Pharmaceutical dosage form	Stable-HPLC	Solvent:-pH 3.5 phosphate buffer: Acetonitrile (30:70) pH-3.5 was adjusted by using dilute Ortho phosphoric acid	Linearity of CP & AMB are 20- 100 µg/ml & 12-60 µg/ml	[67]
Miscellaneous Methods						
43	Diclofenac sodium	Tablet	Simultaneous Estimation by Ultra fast liquid chromatographic method (UFLC)	Solvent:-1% formic acid in methanol and acetonitrile (80: 20 v/v) Column:Kromasil C18, (250 × 4.6mm, 5µm),λmax:-270nm,Rt:-Diclofenac sodium-3.,CP-2.30min,Flow rate-1ml//min	Linearity:-15 to 50µg/ml	[68]

44	Cefmetazole(CMZ)	Contaminants in pharmaceutical manufacturing environments	LC/MS/MS	A glass plate and a silica fiber filter	LOD:-CMZ & CP are 10 pg/ml & 5 pg/ml,Linearity-0.20 to 3.20 ng/ml	[69]
45	Cefmetazole(CMZ)	Olmesartan medoxomil (OLM) tablets	LC/MS/MS	Solvent:-Water and acetonitrile (11 : 9, v/v)	LOD:-CMZ & CP is 0.002 ppm,Recovery:-CMZ & CP are 96.7 to 102.2% and 88.9 to 94.2%	[70]
46	Clavulanic acid (CLA-A)	Human plasma	LC-MS by solid phase extraction	Solvent:-Methanol:acetonitrilr:2mM ammonium acetate(25:25:50%v/v/v) Column:-SPHER RP C-18 (150 mm x 4.0 mm, 5 µm),Flow rate of 0.8 ml/min, λ max-248 nm,Rt of CP & AMB are 3.89, 2.69 min,	Linearity of CLA-A & CP are 0.1-10 & 0.04-4.4 µg/ml	[71]
47	Cefixime (CEF)	Pharmaceutical formulations and urine	Voltammetry	Solvent:-Britton-Robinson buffer system	Linearity of peak current:-CF and CP are 6.0×10^{-8} to 1.2×10^{-5} mol l^{-1} and 8.8×10^{-8} to 1.1×10^{-5} mol l^{-1}	[72]

2.3 Literateure reviews of Ofloxacin

Sr. no	Matrix	Method	Metod Description	Validation parameters	Ref.
UV Visible spectrophotometric Methods					
48	Tablet	UV Visible spectrophotometry	Solvent:-0.1 N HCL λmax 291.6	Linearity:-1-10 µg/ml% Recovery :-99.08	[73]
49	Injection	UV Visible spectrophotometry	Solvent:-Acetic acid(5%) Linearity:-2-10 µg/ml,	Linearity:-2-10 µg/ml	[74]

			λmax:-294 nm		
50	Bulk & dosage form	Extractive spectrophotometry	Solvent:-phthalate buffer 3.0 & 3.1	Linearity:-0.87-17.35 & 0.58-14.46µg/ml for ofloxacin-bromophenolblue & ofloxacin-bromocresol purple	[75]
51	Pharmaceuticals and urine	Flow-injection UV Visible spectrophotometry	Solvent:-Formation of a yellow complex between ofloxacin and Fe (III), in sulphuric medium λmax-420 nm	Linearity:1.8–289 mg/L,LOD-0.72 mg/L	[76]
52	Tablet	UV-Visible Spectrophotometry method	Solvent:-3 ml of 5% w/v ammonium vanadate and 2 ml of concentrated sulphuric acid λmax:-420 nm	Linearity :- 1.8 – 289 mg/l	[77]
53	Tablet	Colorimetry method	Solvent:-iron (III) nitrate nonahydrate λmax:-370 nm,Stock solution:- 500 mg ml-1	-	[77]
54	Human urine & serum	Solid-phase spectrofluorimetry	λmax-Excitation:-294 nm,Emission:-494 nm	Linearity:-0.5 -16.0 ng ml-1,LOD:-0.14 ng ml^{-1}Recovery:-100 %	[78]
Fluorimetry Methods					
55	Suspension	Spectrofluorimetry	Solvent:-0.1 N H2SO4 λexc:-290 nm	Linearity:-0.3 – 1.4 g/ml	[77]
HPLC Methods					
56	Injection	HPLC	Solvent:-0.5% sodium acetate pH 2.5/acetonitrile,(87:13, v/v) Column:-NovaPak C18 150×3.9 mm id., 4µm particles (Waters Assoc.),Flow rate:- 1ml/min,Ambient temperature (20 1 °C)	Linearity:-20-100 µg/mlRecovery:-coefficient of variation0.72%	[74]
57	Human aqueous humour	RP-HPLC with fluorescence	Solvent:-methanol-acetonitrile-0.4 M citric acid (3:1:10, v/v/v) Column:- NovaPak	Mean Recovery:-103.24 ± 4.45'¼,	[79]

			C18 100×8 mm id., 5µm particles ,Flow rate:- 1ml/min,Rt:-7.32 min		
58	Rat microsome	HPLC(For enantiomer with fluorescence detection)	Solvent:-6mM phenylalamine mixed with 3mM CuSO4 λmax-Excitation:-330 nm,Emission:-505 nm,Column:-C18,Flow rate-1ml/min	LOD:-0.6µM for both enantiomer,LOQ:-5.70±0.45 for S-ofloxacin,5.66±0.45 for R-ofloxacin	[80]
59	Human plasma	HPLC	Solvent:-plasma protein precipitated with acetonitrile Column:-C18, λmax-Excitation:-282nm,Emission:-450 nm	LOQ:-20ng/ml,Linearity-20ng/ml-6900ng/ml	[81]
60	Pharmaceutical formulation	RP-HPLC	Retention time:-not more than 15 min	LOD:-0.03µg/ml	[82]
61	Plasma & lung tissue	HPLC assay with fluorometry detection	Solvent:-Acetonitrile & dichloromethane λmax:-Excitation-280 nm,Emission-500 nm	Linearity plasma:0.1-5 µg/ml,lung:-0.025-2.5 µg/g,LOQ:-5 ng/ml or 5 ng/g	[83]
62	Biological fluid	HPLC with fluorescence	Solvent:-Aqueous phosphate solution:acetonitrile(80:20) λmax:-Excitation-338 nm,Emission-425 nm,Rt:-Ofloxacin-2.66 min,sarafoxacin-4.24 min	Linearity:8-2000 ng/ml,LOD-8 ng/ml,LOQ-15 ng/ml,Recovery-92.9%	[84]
63	Human serum	HPLC-UV with Column switching	Pre Column:-phenyl S.P,Analytical Column:-ODS S.P,λmax-300 nm	Linearity:50-2000 ng/ml,LOD-20 ng/ml,Recovery-88.8-101.7%	[85]
64	Chicken kidney,liver,muscle and fat plus tissue	RP-HPLC with UV detection	Solvent:-Water:acetonitrile:trimethylamine(83:14:0.45) pH 2.3 λmax-295 nm,Flow rate-1ml/min,Rt-5.1 min,for tissue sample-12 min,	Linearity:-kidney-100 µg/kg,liver-1.0 mg/kg,LOQ:-muscle and fat plus skin tissue-50µg/kg,liver and kidney tissues-100µg/kg,LOD:-muscle and fat plus skin tissue-25	[86]

				µg/kg,liver and kidney tissues-100µg/kg	
65	Plasma	RP-HPLC with UV detection	Solvent:-KH_2PO_4(0.03M):methanol(30:70)pH-3.1 adjusted wih formic acid Column:C18,Flow rate-1ml/minLinearity-0.025-2.5 µg/ml	LOD:-10 ng/ml,LOQ:-25 ng/ml	[87]
66	Tablet	Isocratic reversed-phase HPLC	Solvent:-35% (v/v) aqueous acetonitrile together with tetrabutylammonium acetate, sodium dodecyl sulphate and citric acid (pH* 3.4) Column:-ODS C18, λmax-235, 254, 275 and 300 nm.	Linearity:-1.20-4.8mg/100ml,LOD-18µg/100ml,LOQ-36µg/100ml	[88]
	Tablet	RP-HPLC	Solvent:-0.04 M phosphoric acid, tetrabutylammonium as ion-pairingreagent and methanol (pH 2.2) Column with Nucleosil C18 (5 microns)	-	[77]
67	Human serum	HPLC with direct electrogenerated Chemiluminescence detection	Solvent:-$NaNO_3$ solution with dual electrode system	Linearity:-1.0×10-8-4.0×10-6,LOD-4.0×10-9	[89]
68	Human aqueous and vitreous humor	HPLC with fluorescence	Rt:-22 min	Linearity:-10ng/ml-100µg/ml,Recovery-95-104%,LOD:-10ng/ml	[90]
69	Plasma and urine	HPLC with fluorescence	Column:-Reversed phase with C18,λmax for excitation-290 nm,emission-460 nm	Recovery-93%,Linearity:-0.5-10µg/ml	[91]
70	Human urine	Solid phase extraction coupled with ligand exchange	Column:C18,Rt-less than 20 min	Linearity:-0.07-60 µg/ml,correlation coefficient:-0.9996,LOD:-For	[92]

		chrromatography		S(-)& R(+) ofloxacin are 0.03 & 0.044 µg/ml	
Miscellaneous Methods					
71	Bulk	ELISA & immunochromatographic assay	-	Linearity:-0.5-128ng/ml,LOD-0.35ng/ml	[93]
		CGIA(Colloidal gold based immunochromatographic assaay		LOD-0.10ng/ml	
72	Urine	Chemiluminescencesystem for capillary electrophoresis	Solvent:-chiral additive:-Sulfonated β-cyclodextrin (β-CD),runningbuffer:-luminol–diperiodatocuprate (III)(K5[Cu(HIO6)2], DPC)	Linearity:-0.010–100 µM,Recovery:-89.5–110.8%,LOD(S/N=3) :-8.0nM and 7.0nM for levofloxacin anddextrofloxacin	[94]
73	Human urine	capillary electrophoresis with laser induce fluorescence detection	Running buffer:-sulphobutyl β-cyclodextrin λexc:-325 nm	LOQ:-for enantiomer 250 ng/ml,metabolite-100 ng/ml	[95]
74	-	Capillary electrophoresis	Solvent:-methyl-β-cyclodextrin optimization by central composite design	LOQ:-for S-ofloxacin-11.4ng/ml,R-ofloxacin-10.8ng/ml	[96]
75	Tablets & biological fluid	Differential pulse polarography	Solvent:-Britton Robinson buffers pH:-4.1-10.3	current-oncentration relationship:-Rectiliner over Linearity 5×10-5_ 5×10-4, 1×10-5-5×10-4,Minimun detectibility:- 3×10-7	[97]
76	Pharmaceutical	Polarographic and voltametry	Solvent:-Britton Robinson buffers pH 4	Linearity:-8 ×10-4 - 2×10-5 mol/l	[98]

2.4 Literateure reviews of Ofloxacin with other drugs

Sr. no	Drug in combination	Matrix	Method	Method Description	Validation parameters	Ref.
UV Visible Spectrometric Methods						
77	Ornidazole	Tablet & capsule(for dissolution in different media	UV Visible spectrophotometry	Solvent:-0.1 N HCl,phosphate buffer pH 6.8,phosphate buffer pH 7.4 ofloxacin:-λmax-294nm in 0.1 N HCl & 87nmphosphate buffer pH 6.8 & 7.4ornidazole:-λmax-277nm in 0.1 N HCl & 319nmphosphate buffer pH 6.8 & 7.4	Linearity:-ofloxacin-1-8µg/ml,ornidazole-4-26µg/ml	[99]
78	Cefixime	Tablet	First order derivative spectroscopy	λmax:-Cefixime-358.2nm,ofloxacin-312nm	Linearity:-Cefixime&ofloxacin-5-40µg/ml	[100]
79	Ciprofloxacin(CIPRO),norfloxacin (NOR),enrofloxacin (ERF)	Bulk & dosage form	Extractive spectrophotometry	Solvent:-supracene violet 3B9(Method A) & tropaeolin 000(Method B) λmax:-supracene violet 3B-575nm,tropaeolin 000-485,ERF-	LOD:-CIPRO-2.5µg/ml , NOR-5µg/ml & OFLO &ERF-2.5µg/ml	[101]
80	Cefixime	Tablet	Absorbance ratio equation, UV Visible Spectrometry	Solvent:-Methanol λmax:-Cefixime-234nm,ofloxacin-296nm	Linearity:-Cefixime-4-20µg/ml,ofloxacin-2-10µg/ml	[102]
			Simultaneous equations	Solvent:-Methanol λmax:-Cefixime-275nm,ofloxacin-296nm	Linearity:-Cefixime-4-20µg/ml,ofloxacin-2-10µg/ml	
81	Cefixime (CEF)	Oral dosage form	Simulatneous estimation by UV spectrophotometry	Solvent:-0.1N NaOH λmax:-Cefixime-237nm,ofloxacin-288nm	Recovery:-99.15 - 99.52% and 99.43 - 99.85% for CEF and OFL	[103]

82	Ornidazole	Tablet	UV Visibe Spectrophotometry	Solvent:-0.1 N HCl ,phosphate buffer pH 6.8 and phosphate buffer pH 7.4 λmax:-of ofloxacin 294 nm in 0.1 N HCl and at 287 nm in phosphate buffer pH 6.8 and phosphate buffer pH 7.4,ornidazole 277 nm in 0.1 N HCl and at 319 nm in two buffers	Linearity :- 1–8 µg/ ml for ofloxacin and 4–26 µg/ml for ornidazole.	[104]
83	Ornidazole	Bulk & dosage form	Difference absorbance UV Visible spectrophotometry	Solvent:-0.1N NaOH vs 0.1N HCl λ max:-OFLO at 293.4 nm,OZ-277.9 nm	Linearity of 4-24 ug/ml and 5-30 µg/ml for OFLO and,OZ,LOD:-OF and OZ are 0.31 and 0.35 µg/ml,LOQ:-OF and OZ are 0.92 and 1.04 µg/ ml	[105]
84	Cefixime (CEF)	Tablet	Simultaneous equation method	λmax for ofloxacin and Cefixime is 284 nm and 224 nm	-	[106]
85	Cefixime (CEF)	Tablet	Ratio Derivative	Solvent:-methanol 319.11 nm and 347.40 nm λmax of CEF and OFL	Linearity:-5-25 µg/ml,,Recovery:-98.60 – 101.80 % for CEF & 98.75 – 100.2% for OFL	[107]
			Area Under Curve Method	Solvent:-0.1 N HCl λmax Linearitys of 277-279 & 296-298 nm of CEF and OFL	Linearity:-4-20 µg/ml,Recovery:-98.16 – 100.4% for CEF & 98.89-100.21% for OFL	
86	Ornidazole (ORN)	liquid oral dosage form	Simulatneous estimation by UV Visible spectrophotometry	Solvent:-methanol λmax of OFLO & ORN are 295.6 nm and 310.8 nm	Linearity of OFLO & ORN are 2-10 µg/ml & 5-25 µg/ml,Recovery:- OFLO & ORN are 99.58	[108]

					–100.69 % and 99.86 – 101 %,	
87	Diclofenac sodium	Bulk & opthalmic solution	UV-VISIBLE spectrometry	Solvent:-Acidic methanol λmax of Diclofenac sodium & OFLO are 273 & 291nm,isoabsorptive point-263nm	Linearity:-1-20µg/ml,Recovery:- Diclofenac sodium & OFLO are 99.67%,100.35 %	[109]
			Q-value method	Solvent:-Acidic methanol λmax of Diclofenac sodium & OFLO are 273 & 291nm,isoabsorptive point-263nm	Recovery:- Diclofenac sodium & OFLO are 99.99%,100.07 %	
88	Dexamethasone	Dexaflox eye drops	Zero order spectrophotometry methods	λmax-293.4 nm	Linearity-1.5–12 µg/ ml ,mean % Recovery-100.07± 0.66%	[110]
			First derivative of ratio spectra spectrophotometry methods	λmax-266.5 nm	Linearity-2.5–27.5 µg/ ml ,mean % Recovery-100.09± 0.70%, 100.00± 0.72% and 99.92 ± 0.62	
89	Nitazoxanide	Bulk and commercial formulations	UV-spectrophotometry,simultaneous equation method	221.8 nm (kmax of Nitazoxanide) and 244.3 nm (kmax of Ofloxacin)	-	[111]
			First order derivative spectroscopy	263.6 nm for Nitazoxanide and 269.2 nm for Ofloxacin	-	
90	Cefixime (CEF)	Pharmaceutical formulations	Colorimetry methd,ofloxacin	Solvent:-oLinearity colored product in the presence of ferric chloride solution in acidic medium λmax-435nm	Linearity :-15-75 µg/ml	[112]

			Cefixime(CEF)	Solvent:-greenish colored product with Fehling solution λmax-490nm	Linearity :- 5-40 µg/ml	
Fluorimetry Method						
91	Enoxacin (ENO),ciprofloxacin (CIPRO), Norfloxacin (NOR)	Urine & serum	Photoinduced fluorimetry detection & multiemission scanning	Solvent:-Ethanolic-water Emission λmax:-ENO-407,OFLO-490,CIPRO & NOR-444nm	LOD:-ENO-7.3,OFLO-14.5,CIPRO-6.3, NOR-6.0ng/ml	[113]
HPTLC Methods						
92	Nitazoxanide	Bulk and commercial formulations	HPTLC	Solvent:-Toluene:chloroform:carbon tetra chloride:toluene:glacial acetic acid(10:5:3:0.5 v/v/v/v) s.p-aluminum plates pre-coated with silica gel 60 F254,RF values 0.36, 0.57 and 0.63 for Rosiglitazone maleate, Nitazoxanide, and Ofloxacin,λmax-241 nm	Linearity-5–25 µg/ ml	[114]
93	Ketorolac Tromethamine(KETT)	Ophthalmic formulation	HPTLC	Solvent:-Dichloromethane: Methanol: Ammonia 25% (6:3:1) s.p-aluminum plates pre-coated with silica gel 60 F254,RF values of KET & OFLO are 0.79± 0.03 and 0.41± 0.02 ,λmax-241 nm	Linearity of KET & OFLO are 25-75ng/band,15-45ng/band.Recovery:- KET & OFFLO are 99.74-99.95% & 99.78-99.87% w/w	[115]
HPLC Methods						
94	Ciprofloxacin(CIPRO), moxifloxacin (MOXI)	Plasma	HPLC	Solvent:-CH3CN:MeOH:0.025 M TBA·Cl/TFA(eluent A at 75:25:899:1,elent B at 150:50:799:1)at pH 3.5 Emission/Excitaton λmax:,OFLO & MOXI-500/290,CIPRO-	Linearity:-0.02-7.5µg/ml,LOQ:-0.02µg/ml	[116]

				442/279nm		
95	Enoxacin (ENO),ciprofloxacin (CIPRO),norfloxacin (NOR)	Pharmaceutical & blood serum	HPLC	Solvent:-CH3CN:CH3OH:citric acid(7:15:78%,v/v) Column:-Kromasil 100 C8 250×4mm,λmax:-275nm	LOD:-0.02ng/20µl for ENO & 0.01ng/20µl for OFLO,CIPRO, NOR	[117]
96	Levofloxacin	Bulk & dosage form	HPLC -UV detection	Solvent:-Phosphate buffer (pH-3.1) and acetonitrile (70:30 v/v) Column:-WELCHROM C18 (4.6 X 250mm, 5µm),λmax:-293nm,Flow rate-1ml/min,Rt:-3.607min	Linearity of 2-10 µg/ml and LOD and LOQ are 0.1234 and 0.3740 µg/ml	[118]
97	Cefixime (CEF)	Plasma	HPLC-MS	Solvent:-Acetonitrile, methanol and 0.5% formic acid in a ratio of 23:10:67% v/v Zorbax eclipse XBD C18 Column (150 mm × 4.6 mm i.d., 5 µm),□low rate:- 0.6 ml/min	Linearity of 4-500 ng/ml for OFL and 40-6000 ng/ml for CEF,LOQ 4 ng/ml and 40 ng/ml for OFL and CEF	[119]
98	Prednisolone acetate	Bulk & dosage form	HPLC-UV	Solvent:-0.01M phosphate buffer (pH 3.0) and acetonitrile(40:60, v/v) C18 Column (150 mmx4.6 mm i.d, 3µ particle ize),Flow rate of 1ml/min. Rts ofloxacin and prednisolone acetate are 2.7 and 4.6 min,λ max:-240 nm	Linearity of ofloxacin and prednisolone acetate was in the Linearity of 1.5-9 µg/ml and 5-30 µg/ml	[120]
99	Ornidazole(ORN)	liquid oral dosage form	RP-HPLC	Solvent:-0.01M phosphate buffer (pH 4.2 adjusted with ortho phosphoric acid) and acetonitrile(87:13, v/v) Column:-	Linearity of OFLO & ORN are 100 µg/ml& 25-250 µg/ml ,Recovery:-OFLO & ORN	[121]

				thermohypersil phenyl (250 mmx4.6 mm i.d, 5µ particle size),Flow rate of 1ml/min,λmax-294nm	are 00.48% and 99.84%	
100	Tinidazole	Tablet	RP-HPLC	Solvent:-0.5%v/v Triethylamine buffer of pH 3.0 and Acetonitrile(73: 27) Column:-Kromasil C8, 5µ, 15 cm × 4.6 mm id,Flow rate of 1.2ml/min,λmax-303nm,Rts of Ofloxacin & Tinidazole are 2.3, 4.1 min	LOQ of Ofloxacin & Tinidazole are 10 and 30 µg/ml	[122]
101	Tetrahydrozoline Hydrochloride(THC), and Prednisolone Acetate (PAC)	ophthalmic suspension	HPLC	Solvent:-0.05M phosphate buffer–acetonitrile (65:35, v/v), and the pH is adjusted to 2.7 with orthophosphoric acid Column:-Waters Spherisorb, 5 µm ODS 1, 4.6 × 150 mm,λmax-210 nm for OFLO and THC and 254 nm for PAC,Flow rate of 1.2 ml/min,Retention times for OFLO, THC, and PAC are 2.5, 4.5,9.5.	Linearity Linearity and percent recoveries for OFLO, THC, and PAC are 24–120, 4–16, and 16–80 µg/ml and 100.48%, 100.34%, and 100.21%	[123]
102	Malondialdehyde(MDA)	Plasma	HPLC/fluorescence detection system	Solvent:-50mM phosphate buffer (pH-5.8 with KOH) and methanol (45:55 v/v) Column:-RP C18, 4.6 × 250 mm,λmax-excitation & emission are 532 & 553nm,Flow rate of 1.2 ml/min,Retention times OFLO & MDA are 5.9,3.6min	Linearity for OFLO & MDA are 0.06-1.0 mM,0.15-2.43µM	[124]

103	Ornidazole (ORN)	Tablet	RP-HPLC	Solvent:-2mM phosphate buffer and Acetonitrile with pH 3.5 adjusted with orthophosphoric acid (70: 30%v/v)olumn:-Phenomenex C18, (250 mm x 4.6 mm i.d, 5mm, Flow rate of 1 ml / min ,λmax-293 nm,Rt of OFLO & ORN are 2.1,2.5 min	Linearity of OFLO & ORN are 5-50 µg/ml and 12.5-125 µg/ml,	[125]
104	Cefixime (CEF)	Bulk & tablet	RP-HPLC	Solvent:-Ammonium acetate buffer:acetonitrile(40:60v/v%)Column:-Kromasil C18, (250 mm x 4.6 mm i.d, 5µm, Flow rate of 1 ml / min ,λmax-294nm,Rt of OFLO & CEF are 3.24,2.26 min	Linearity of CEF & OFLO are 60-140µg/ml,	[126]
105	Cefixime (CEF)	Tablet	RP-HPLC	Solvent:-Methanol: 0.025 mM potassium dihydrogen phosphate buffer in ratio of (70:30, v/v)λmax-290nm	Linearity :- 1-10 µg/ml	[127]
Miscellaneous Methods						
106	Ciprofloxacin(CIPRO), moxifloxacin(MOXI),gatifloxacin(GOXI)	Pharmaceutical Formulations	Capillary Zone Electrophoresis	Electrolyte:-25 mM-1 of TRIS/ hydrochloride and 15 mmol L-1 of sodium tetraborate buffer mixture (pH 8.87)UV detection at 282 nm	LOD (mg/ml): 2.72 for CIPRO, 1.92 for GOXI, 0.795 for MOXI and 1.05 for OFLX), LOQ (mg L-1: 9.06 for CIPRO, 6.40 for GOXI, 2.65 for MOXI and 3.50 for OFLO	[128]
107	Capreomycin(cp), ,pasiniazide (ipa)	Urine	High-performance capillary electrophoresis method	λmax-280 nm,detection limits (S:N 3) are 0.15, 0.20 and 0.10 mg/ml1 for Cp, oflo and Ipa,	Linearity-0.5 50 mg/ml,Recovery-93.5%	[129]

2.5 Literateure reviews of Cefpodoxime proxetil and Ofloxacin in combination

Sr. no	Matrix	Method	Method Descripption	Validation parameters	Ref
UV Visible Spectrometric Methods					
108	Tablet	Absorption ratio method	Solvent:-MeOH isoabsorptive point at 272 nm in methanol,λ-max OF Cefpodoxime proxetil is 236	Linearity 5-17 µg/ml	[130]
109	Tablet	Simultaneous spectrophotometry	λmax :-CP:-260 nm,OFLO:-297 nm	Linearity:-cefpodoxime proxetil:-4-40 µg/ml,ofloxacin:-2-12 µg/ml,ofloxacin:-2-12 µg/ml	[131]
		Area under curve method	Linearity:-CP:-240-230 nm,OFLO:-303-293 nm	Linearity:-ofloxacin:-2-24 µg/ml,cefpodoxime proxetil:-4-24 µg/ml	
110	Tablet	Dual λmax Spectrophotometry Method	Solvent:-Methanol Absorbance difference:-CP:-278.2 nm and 320 nm,OFLO:-224 nm and 247.4 nm	Linearity:-CP:-4-24µg/ml ,OFLO-2-12 µg/ml	[132]
111	Tablet	First order derivative Spectrophotometry Method	Solvent:-Methanol λmax :-CP:-236.4 nm(ZCP),OFLO:-208.8 NM(ZCP)	Linearity:-CP:-2-12µg/ml ,OFLO-4-24 µg/ml,Mean Recovery:-CP:-99.80±1.50,OFLO-99.90±0.36	[133]
112	Tablet	Simultaneous spectrophotometry	Solvent:-Methanol λmax :-CP:-236 nm,OFLO:-299 nm	Linearity:-CP:-5-29µg/ml ,OFFLO-1-13 µg/ml	[134]
113	Tablet	Simultaneous equation method	Solvent:-Methanol:Water (70:30) λmax :-CP:-235 nm,OFLO-298 nm	Linearity:-CP:-4-24µg/ml ,OFLO-4-20 µg/ml	[135]
114	Tablet & bulk	Simultaneous equation method	λ_{max} of CP:-234.9 nm,λmax Of OFLO:-298 nmλmax Of OFLO:-298 nm	Linearity:-2–10µg/ml,Recovery:-CP:-100.9±0.16%,OFLO-4-20 µg/ml:-98.22±0.44%	[136]

		Q-absorbance equation	λ_{max} of CP:-271.6 nm,λmax of OFLO:-298 nm	Linearity:-2–10µg/ml,Recovery:-CP:-99.5±0.133%,OFLO-98.90±0.65%	
HPTLC Methods					
115	Tablet	HPTLC	Mobile phase:- Ethanol-Ethyl Acetate-Water-Triethylamine (5:3.2:1.8:0.15 v/v/v/v) Densitometry quantification at 290 nm,Rf of cefpodoxime proxetil:-0.61,Rf of ofloxacin:-0.24	Linearity :-cefpodoxime proxeil:-200-1000 ng/band,ofloxacin:-200-1000 ng/band,correlation co-efficient:-cefpodoxime proxetil:-0.9990,ofloxacin:-0.9997	[137]
HPLC Methods					
116	Tablet	RP-HPLC	Solvent:- 20mM phosphate buffer, Acetonitrile and methanol with pH 3.0 adjusted with ortho,phosphoric acid in the ratio of 60:10%v/v/v.phosphoric acid in the ratio of 30: 60: 10 % v/v/v.ofloxacin Rt 2.65 and 4.17 min	cefpodoxime proxetil-0.17 µg/ml,ofloxacin-0.19 µg/ml,LOQ:-cefpodoxime proxetil-0.51 µg/ml,ofloxacin-0.58 µg/ml	[138]
117	Tablet	RP-HPLC Simultaneous estimation method	Mobile phase:-0.25%v/v triethyl amine buffer of pH 3.5 and acetonitrile (30:70 v/v) Column:-X-terra C8 (4.6 x 250mm, 5µm, Make: ACE),pre packed Column,Flow rate:-1.2ml/min,UV and PDA detection at 227 nm.Rt:-CP:-2.747 min,OFLO:-2.076 min,λmax :CP:-260 nm,OFLO:-297 nm,	Linearity:-CP:-240-230 nm,OFLO:-303-293 nm correlation coefficient:-CP:-0.998,OFLO:-0.999	[139]
118	Tablet	Simultaneous estimation by RP-HPLC	Solvent:-Acetonitrile:phosphate buffer pH 3 (75:25)(pH adjusted with orthophosphoric acid)Column:-Hiber	Linearity:-CP:-5-25 µg/ml,OFFLO:-5-25 µg/ml	[140]

			C18(250 mm×4.6mm, i.d.5 µmFlow rate:-1.0ml/min), w,avelength:-271 nm,Rt:-CP:-3.24 min,OFLO:-2.16 min		
119	Bulk & tablet	Simultaneous estimation by RP-HPLC	Solvent:-0.1M ipotassium Hydrogen Phosphate Buffer: ethanol(90:10%v/v pH: 6.0 Column:- Thermo hypersil C18, 5 µ ,Column,Flow rate:-1,λmax :-265nm,Rt:-CP:-6.9 min,OFLO:-9 min	Linearity:-CP:-200-600µg/ml ,OFLO-0.19 µg/ml:-200–600µg/ml ,Recovery in formulation:-CP:-98% -102% ,OFLO-98% -102%	[141]

CHAPTER NO 3

AIM AND OBJECTIVE OF PROJECT WORK

3.AIM AND OBJECTIVE OF PROJECT WORK

3.1 AIM OF PROJECT WORK

UV visible spectroscopy,Colorimetry,Spectrofluorimetry,HPTLC,HPLC,Hyphenated and miscellaneous methods were reported for estimation of cefpodoxime proxetil and ofloxacin in individual and in combined bulk and pharmaceutical dosage form.Reported methods are also available for cefpodoxime proxxetil and ofloxacin with other drugs combination.

Our main aim is to develop sophisticated method for estimation of cefpodoxime proxetil and ofloxacin in individual and for combined bulk and Pharmaceutical dosage form.

For the cefpodoxime proxetil and ofloxacin in combined dosage form many UV Visible spectrophotometry method are available but it require solvent like methanol which is not eco-friendly to our environment and reagent use for derivatization is having some what high cost.By using urea as hydrotropic agent it is possible to develop organic solvent free method for combined dosage form.

For ofloxacin in literature many UV Visible spectrophotometry,Spectrofluorimetry methods are available.For estimation of ofloxacin in 5% acetic acid solution using UV Visible spectrophotometry for injection dosage form is available.No any spectrofluorimetry method is reported for estimation of ofloxacin in 5 % acetic acid solution for bulk and Tablet dosage form.

As such ofloxacin give fluorescence but by using acetic acid solution it gives a pH control and give ofloxacin in unionize form which having more fluorescence and since ofloxacin is soluble in acetic acid solution.

For the cefpodoxime proxetil and ofloxacin combination only one HPTLC method available and that require water as solvent which require high chamber saturation time so it become time consuming method.

Super critical fluid chromatography is the newer concept in area of chromatography which is having green technology concept .Literature review revels that no any SFC method is reported for cefpodoxime proxetil and ofloxacin in individual and combined dosage form.

3.2 OBJECTIVE OF PROJECT WORK

UV SPECTROPHOTOMETRY:-

A simple,sensitive,accurate,presicise and organic solvent free eco friendly,cost effective Uv spectrophotommetry method development and validation for simultaneous estimation of cefpodoxime proxetil and ofloxacin in combined dosage form in which urea is use as hydrotropic agent for slightly water soluble drug to get solubillize in water.

SPECTROFLUORIMETRY:

A more sensitive than UV Visible spectrophotoetry,accurate,precisie and organic solvent free Synchronous mode spectrofluorimetry method for estimation of ofloxacin in bulk and tablet dosage form using 5 % Acetic acid.

As such ofloxacin give fluorescence but by using acetic acid solution it gives a pH control and give ofloxacin in unionize form which having more fluorescence and since ofloxacin is soluble in acetic acid solution.

HPTLC:-

A simple,sensitive and less time consuming HPTLC method development for estimation of cefpodoxime proxetil and ofloxacin in combined bulk form.

SFC:-

A simple,sensitive,specific and eco-friendly SFC method development using different column in which methanol use as modifier.

CHAPTER NO 4

IDENTIFICATION OF DRUGS

4.IDENTIFICATION OF DRUGS

Identification of drugs

Identification of drugs were carried out by Melting point,UV Visible Spectroscopy,Raman spectroscopy and FT-IR spectroscopy study.

Instrumentation

1. Melting point apparatus(Model T0603160; manufactured by EIE Instruments Pvt Ltd)
2. UV Visible Spectrophotometer(Model UV—2450 PC Series; manufactured by Shimadzu Inc;Japan)
3. Raman Spectrophotometer(Model R-3000 Series; manufactured by Raman systems Inc;USA)
4. FT-IR Spectrophotometer(Model FT-IR 6100; manufactured by Jasco, Inc;Japan)

4.1 MELTING POINT DETERMINATION

Melting point of Cefpodoxime proxetil (CEFPODOXIME PROXETIL) and Ofloxacin(OFLOXACIN) has been determined using melting point apparatus.The melting point of pure drugs was taken by capillary method.

Drug	Reported Melting Point(^{0}C)[10]	Observed Melting Point(^{0}C)
Cefpodoxime proxetil	111-113	100-115
Ofloxacin	270-275	272-277

Table 4.1:- Melting Point of Cefpodoxime proxetil and Ofloxacin

4.2 UV SPECTROMETRIC MEASUREMENT

10 µg/ml solution of standard Cefpodoxime proxetil and Ofloxacin each was prepared in methanol and scanned in UV Visible spectrophotometer in range of 200-400 nm to determine the absorption maxima of both the drugs.

Drug	Reported wavelength(nm)[130]	Observed wavelength(nm)
Cefpodoxime proxetil	237 nm	236 nm
Ofloxacin	299 nm	296 nm

Table 4.2:-UV observed wavelength of Cefpodoxime proxetil and Ofloxacin in 10 µg/ml solution

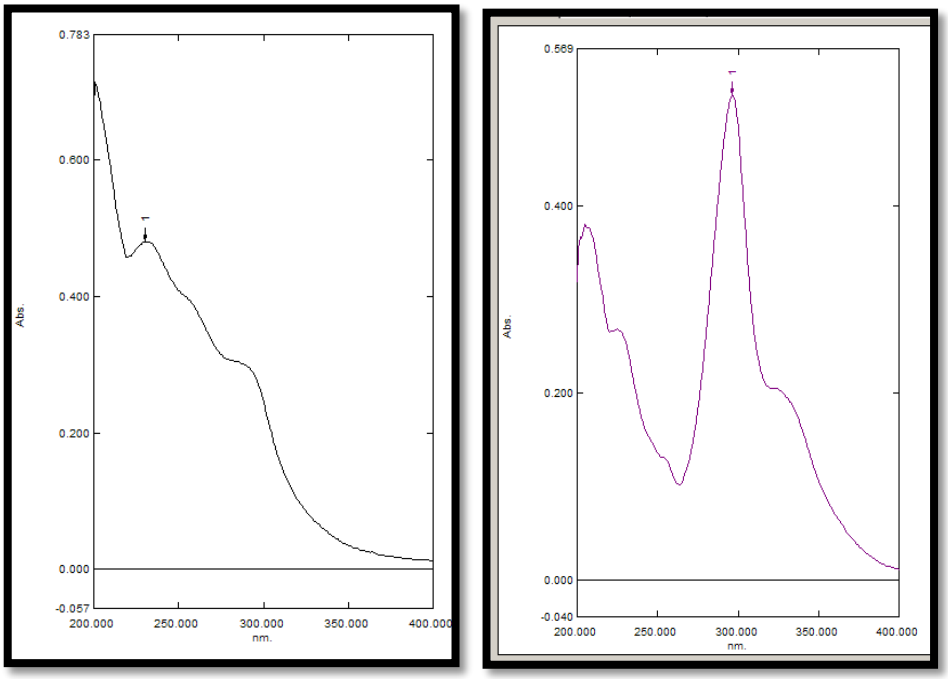


Figure 4.1:- UV spectra of Cefpodoxime proxetil (10 µg/ml) and Ofloxacin (10 µg/ml) in MeOH

4.3 RAMAN SPECTRA MEASUREMENT

Raman spectra of pure drugs of Cefpodoxime proxetil and Ofloxacin was taken by Raman spectrophotometer.

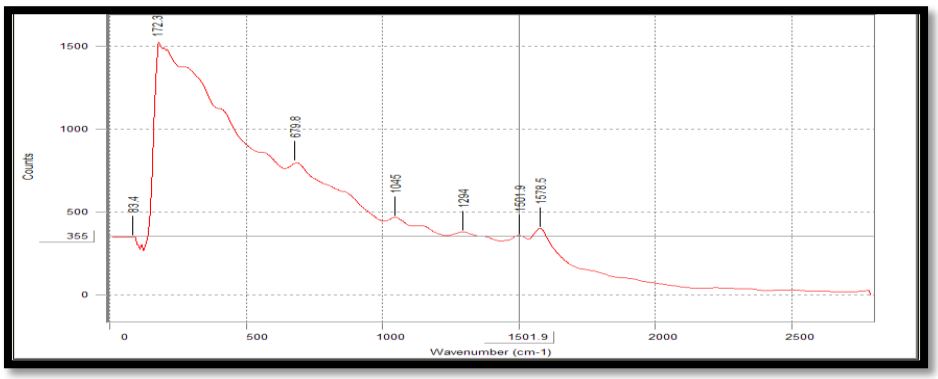


Figure 4.2:- Observed Raman spectra of Cefpodoxime Proxetil

Functional group	Reported wavenumber (cm-1) [142]	Observed wavenumber (cm-1)
-C=N-	1681.98	1578.5
C=O STRETCHING	1053.17	1045
-C-N-(aromatic primary amine)	1377.22	1294

Table 4.3:- Reported and Observed Raman peak of Cefpodoxime Proxetil

Raman spectra of Ofloxacin

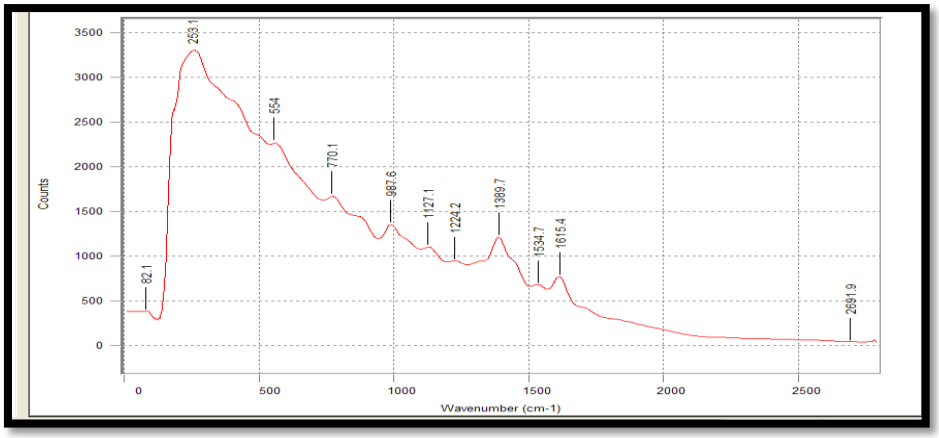


Figure 4.3:-Observed Raman Spectra of Ofloxacin

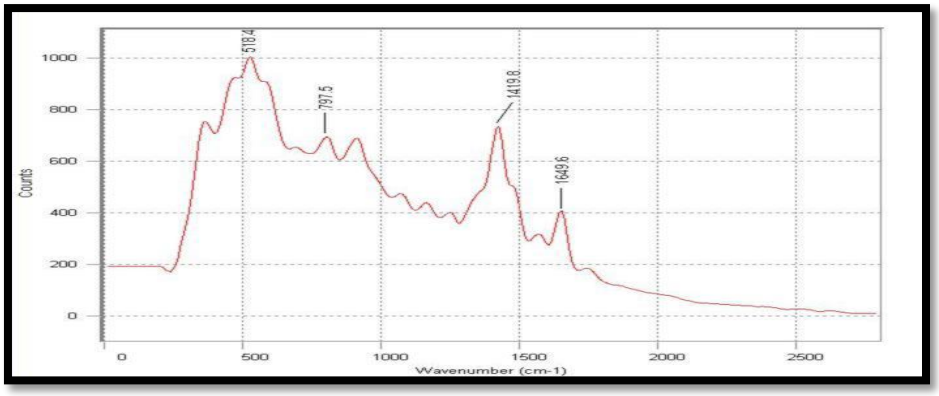


Figure 4.4:-Reported Raman Spectra of Ofloxacin[143]

Functional group	Reported wavenumber (cm-1) [143]	Observed wavenumber (cm-1)
Vibration of aliphatic carbon,C-N stretching,O-H vibration of carboxylic group	518.4	554
Stretching of C-F group	797.5	770.1
Stretching of O-C-O group	1419.8	1389.7
Symmetric stretching of C=F	1649.6	1615.4

Table 4.4:-Reported and Observed Raman Peak of Ofloxacin

4.4 FT-IR SPECTRA MEASUREMENT

IR spectra of Cefpodoxime proxetil and Ofloxacin drugs was taken using FT-IR spectrophotometer.IR spectra obtained was verified with the reported IR spectra.

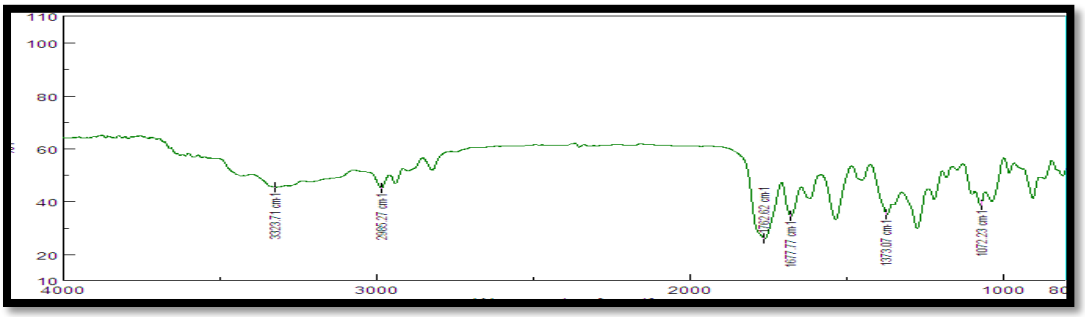


Figure 4.5:-Observed FT-IR Spectra of Cefpodoxime Proxetil

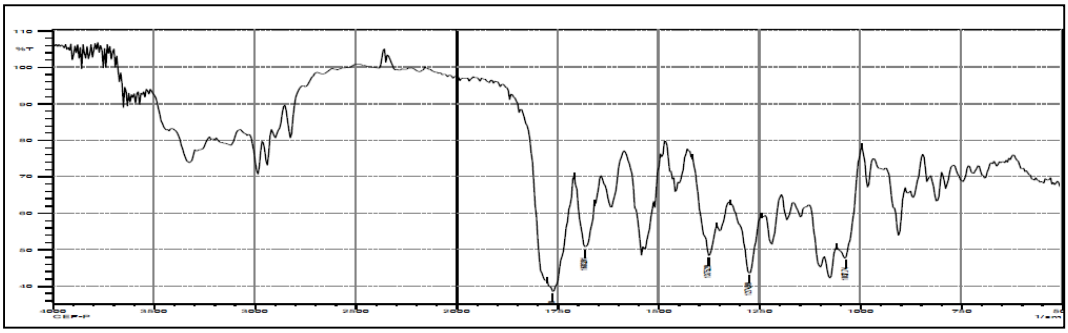


Figure 4.6:-Reported FT-IR Spectra of Cefpodoxime Proxetil[142]

Functional group	Reported wavenumber (cm-1) [142]	Observed wavenumber (cm-1)
-NH2	3317.67	3327.57
S-CH2	2985.91	2939.95
-C=O(lactam)	1763.46	1760.69
-C=N-	1681.98	1616.06
C=O STRETCHING	1053.17	1074.16
-C-N-(aromatic primary amine)	1377.22	1373.07

Table 4.5:-Observed and Reported FT-IR Peaks of Cefpodoxime Proxetil

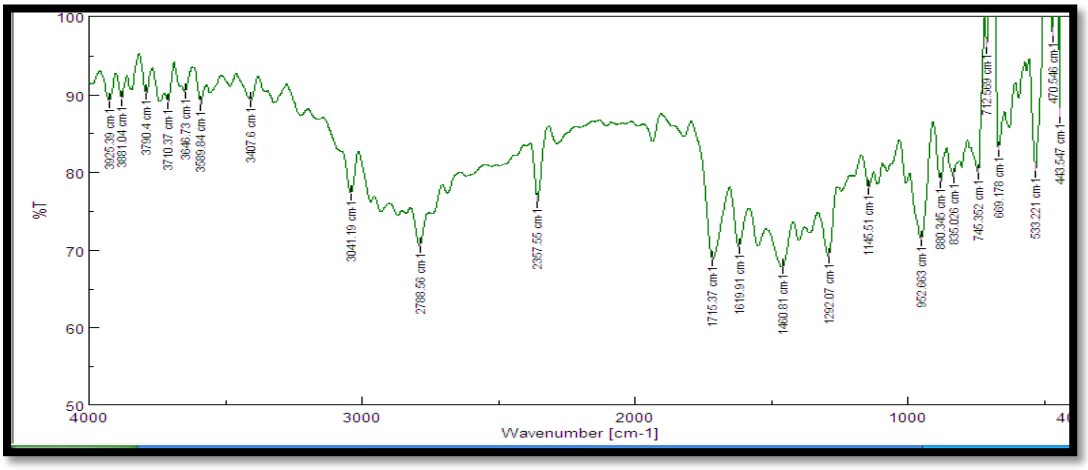


Figure 4.7:-Observed FT-IR Spectrum of Ofloxacin

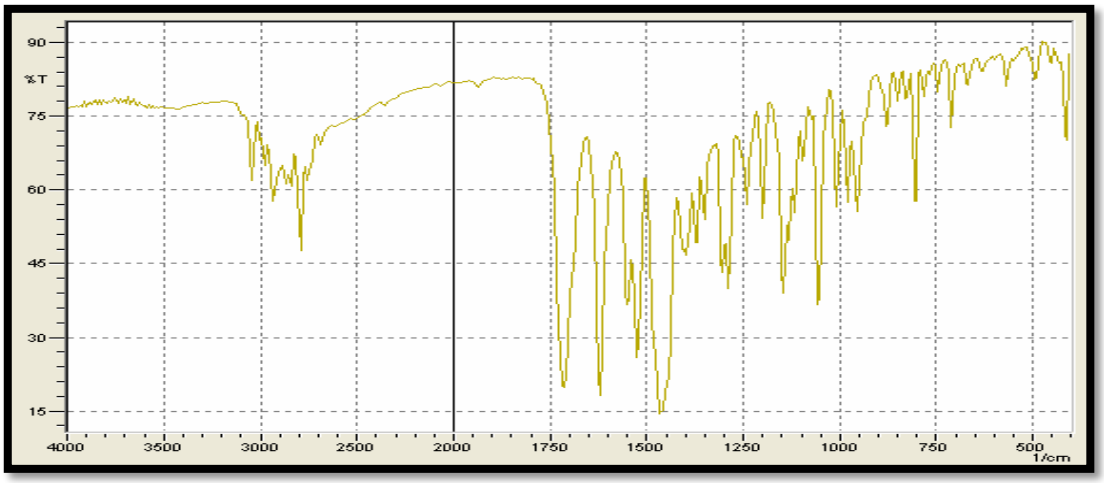


Figure 4.8:-Reported FT-IR Spectrum of Ofloxacin[143]

Functional Group	Reported wavenumber (cm-1)[143]	Observed wavenumber (cm-1)
Streatching of OH group and intramolecular H-bond	30,503,000	3164.61,3041.19
C=O streatching	1750-1700	1713.44
N-H bending vibration	1650-1600	1619.91
Alkyl group	1550-1500	1523.62
C-F group	1050	1075.12

Table 4.6:-Observed and ReportedFT-IR Peaks of Ofloxacin

5.UV VISIBLE SPECTROPHOTOMETERIC METHOD

UV SPECTROPHOTOMETRY METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETIL AND OFLOXACIN IN COMBINED DOSAGE FORM USING UREA AS HYDROTOPIC AGENT.

5.1.1 INSTRUMENTATION AND APPARATUS

- **UV Visible spectrophotometer**
  Model 2450 UV Visible spectrophotometer (Shimadzu Inc,Japan) having UV probe software was used.
- **Analytical balance**
  Model CX 220 analytical balance (CITIZEN,India) having capacity of 10 mg t0 220 mg was used.
- **Sonicator**
  Model Trans-O-sonic.D compact having capacity of 2 liter as used

5.1.2 Reagents and material

- API Cefpodoxime proxetil
- API Ofloxacin
- Urea
- Double distilled water

Market Formulation:-Manufactured by:Relax pharmaceutical Pvt.Ltd.

Brand Name:-GUDCEF®PLUS

Label claim:- Cefpodoximm & Ofloxacin each 200 mg

5.1.3 Preparation of solutions

Preparation of 1 M/6 M Urea solution

1 M UREA:-For 50 ml solution take 3 gm urea in 50 ml volumetric flask and dilute upto 50 ml with distilled water.

6 M UREA:-For 50 ml solution take 18 gm urea in 50 ml volumetric flask and dilute upto 50 ml with distilled water.

Blank solution

From 6M urea take 1 ml and dilue upto 10 ml.From that take 1 ml dilute with distilled water upto 10 ml.

Preparation of Standard Stock solution of Cefpodoxime proxetil (1000µg/ml) and Ofloxacin(1000µg/ml)

Cefpodoxime proxetil (10 mg) and Ofloxacin (10 mg) was weighed accuretly and transferred to individual 10 ml ambert colored volumetric flasks and dissolved in 6 M Urea solution and sonicate for 30 min(1000µg/ml).The flasks were shaken and volume was made up to mark with 6 M Urea solution. The above solution was filtered through Whatmann filter paper(No.41).

Preparation of Standard working solution of Cefpodoxime proxetil (100µg/ml) and Ofloxacin(100µg/ml)

From the above solution (1000µg/ml**)** take 1 ml of each and transferred into 10 ml volumetric flask and volume was made upto 10 ml using distilled water to produce 100µg/ml solution of each drug.

Preparation of stock solution of Marketed formulation

A total of twenty tablets were weighed accuratelty and powdered.An amount of tablet powder equivalent to 10 mg of Cefpodoxime proxetil and Ofloxain was transferred to 10 ml amber colored volumetric flask,volume made upto 10 ml with 6 M Urea solution and sonicated for 30 min to give 1000 µg/ml solution.The above solution was filtered through Whatmann filter paper(No.41).Aliquot 1 ml was pipetted out and transferred to 10 ml amber colored volumetric flask and volume was made up to mark with distilled water to produce 100 µg/ml.

2 METHOD DEVELOPMENT

5.2.1 INSTRUMENTAL PARAMETERS

INSTRUMENT :-UV-Visible Double-Beam spectrophotometer

Matched quartz cell (1cm) **Mode**l: UV-2450

Manufacturer: Shimadzu Inc. Japan,**Wavelength rang**e: 200.00 to 400.00 nm

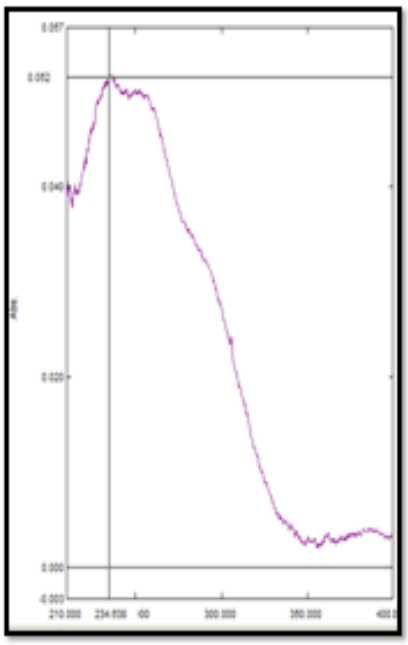
Scanning speed: Fast, **Slit width**: 2 nm

S**oftware**:- Uv probe,**Measurement Mode:-**Spectrum mode,Photometric mode

5.2.2 OPTIMIZATION OF METHOD CONDITION

Step 1:- Selection of urea concentration

10 µg/ml solution of Cefpodoxime proxetil and Ofloxacin individually in 1 M & 6 M Urea solution taken and scan between 200-400 nm range.



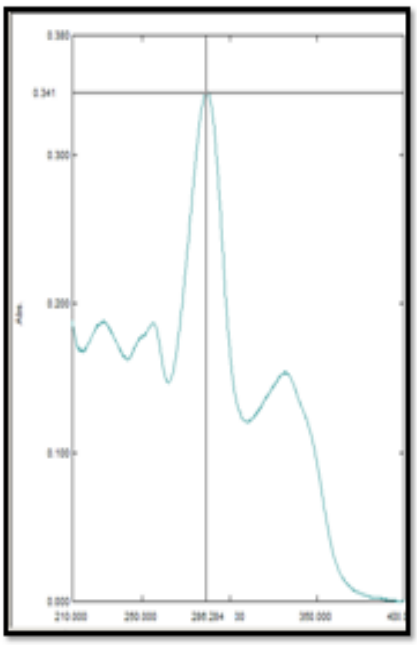


Figure 5.1 UV spectrum of CP (10µg/ml) and OFLO(10µg/ml) in 1 M urea

Figure 5.2 UV spectrum of CP (10µg/ml) and OFLO(10µg/ml) in 1 M urea

Figure 5.3 Iso-bestic point of CEFPODOXIME PROXETIL and OFLOXACIN

Inference:-From the above UV spectra it was conclude that 6M urea it increase absorbance which indicate increase water soliubilty of drug

Step 2:-Selection of wavelength

From the above UV spectra (Figure5.2) it was confirm that wavelength for Cefpodoxime proxetil and Ofloxacinn were 234 nm & 286 nm respectively and isosebastic point was at 267 nm.

Step 3:-Determination of absorptivity

Working standard stock solution:

The stock solution(100µg/ml) of volumes 0.3,0.5,0.7,1,1.2,1.5,2.0 & 3.0 ml was further diluted in separate 10 mL volumetric flasks ith distilled water to get the concentrations of 3,5,7,10,12,15,20,30µg/mL for cefpodoxime proxetil and Ofloxacin respectively.

P**rocedure:**

The absorbances of both the drugs were recorded at 234 and 286 nm and absorptivity (Є) for both the drugs was calculated from the formula:

$$A = abc$$

Where a = absorptivity,**b** = Pathlength (1 cm),

c = concentration (gm/100 ml),**A** = Absorbance

Table 5.1 Absorptivity Data for cefpodoxime proxetil and Ofloxacin

Step 4:- Development of simultaneous equation :

If sample contains two absorbing substance (X and Y) and each of which absorbs at the Wavelength maxima of the other. Then it is possible to determine both the drugs by the technique of simultaneous Equation.

$Cx = (A_2ay_1 – A_1ay_2) / (ax_2ay_1 - ax_1ay_2)$

$Cy = (A_1ax_2 – A_2ax_1) / (ax_2ay_1 - ax1ay_2)$

λ 1: Wavelength maxima for Ofloxacin

λ 2: Wavelength maxima for cefpodoxim eproxetil

ax_1 and ax_2: Absorptivity of Ofloxacin at 286 nm and 234 nm

ay1 and ay2: Absorptivity of cefpodoxim proxetil at 286 nm and 234 nm

A1: Absorbance of Ofloxacin at 286 nm

A2: Absorbance of cefpodoxime proxetil at 234 nm

Developed simultaneous Equation using absorptivity value

At 286 nm, A1=468.055Cx + 134.223Cy

At 234 nm, A1=251.491Cx + 229.490Cy

5.3 METHOD VALIDATION

(1) Linearity:-Linearity was evaluated through a linear regression analysis.

Table 5.2 Linearity data of cefpodoxime proxetil(n*=6)

Figure 5.4 Linearity Overlay spectra of CP and OFLO(3-30 µg/ml) in 6 M Urea

Table 5.3 Linearity data of Ofloxacin(n*=6)

Figure 5.5 Linearity Graph of CP **Figure 5.5 Linearity Graph of OFLO**

(2) Accuracy:-

By standard addition method

Sample concentration was taken 6 µg/ml for Cefpodoxime proxetil and Ofloxacin. After that accuracy of the method was determined by standard addition method at three different levels (80%, 100% and 120%).

Table 5.4 Accuracy data for Cefpodoxime proxetil

Table 5.5 Accuracy data for Ofloxacin

(3) Precision

For Precision concentration selected for both drugs Cefpodoxime proxetil and Ofloxacin was 5,10,15 µg/ml.For both drugs intraday precision was carried out by taking 3 different concentration (5,10,15 µg/ml) for 3 times in a day & for interday precision it was carried out on three different days.

(4) Repetability

For repetabilty stydy of both drugs Cefpodoxime proxetil and Ofloxacin,10 µg/ml solution absorbance was measured six times at fixed wavelength.

Table 5.10 Repetability data of Cefpodoxime proxetil(n*=6)

Table 5.10 Repetability data of Ofloxacin(n*=6)

(5) Ruggedness

For ruggedness study of both drugs Cefpodoxime proxetil and Ofloxacin 3,5,7,10,12,15,20,30 µg/ml solution was made by different analyst and their absorbance was measured.

Table 5.11 Ruggednes data of Cefpodoxime proxetil

Table 5.12 Ruggednes data of Ofloxacin

(6) Specificity

For specificity of both drug Cefpodoxime proxetil and Ofloxacin same concentration of sample(tablet) and standard solution taken and scan between 200-400 nm.

Figure 5.6 Uv spectra of standard and sample solution

From the spectra of standard and sample solution it shows no interference of excipient.

(7)Limit of detection & Limit of Quantification

The calibration curve was repeated six times and the standard deviation of response(absorbance) was calculated.LOD was calculate using following equation

$$LOD=\sigma/s*3.3$$

Where,σ= standard deviation of response

s=slope

Here standard deviation of response is Absorbance

Limit of Quantification

The calibration curve was repeated six times and the standard deviation of response(absorbance) was calculated.LOQ was calculate using following equation

LOQ=σ/s*10

Where,σ= standard deviation of response

s=slope

Analysis of Marketed formulation

From the marketed formulation solution **(**100 µg/ml) pippet out 1.2 ml and diluted upto 10 ml with 6 M Urea solution in 10 ml ambert colored volumetric flask.

5.4 RESULTS AND CONCLUSION

Parameters	Cefpodoxime proxetil	Ofloxacin
λmax (nm)	234 nm	286 nm
Beer lambert's law limits (µg/ml)	3-30	3-30
Absorptivity	229.49	468.05
Regression equation	y = 0.026x - 0.029	y = 0.055x - 0.079
Slope (m)	0.026	0.055
Intercept (c)	0.029	0.079
Correlation coefficient (r)	0.998	0.998
Accuracy(% Recovery)	98.4-100.9	98-100.7
Interday Precision % RSD	< 2%	< 2%
Intraday Precision % RSD	< 2%	< 2%
Repetabilty % RSD	< 2%	< 2%
Specificity	No inteference	No inteference
Ruggedness % RSD	< 2%	< 2%
LOD (µg/ml)	0.1	0.32
LOQ (µg/ml)	0.32	0.99
% Assay	102.6	101.13

CONCLUSION:-

From the results it conclude that using Urea increase solubility of drugs in water .The validation parameters are under the specified range.The developed method is accurate,precisie,rugged and specific.The developed method is useful for routine analysis of Pharmaceutical formulation.

6.SPECTROFLUORIMETRIC METHOD

SYNCHRONOUS MODE SPECTROFLUORIMETRY METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF OFLOXACIN IN BULK AND TABLET DOSAGE FORM.

6.1.1 INSTRUMENTATION AND APPARATUS

- **Spectrofluorimeter Model :**
  FP- 6500 PC series, Matched quartz cell (1cm),Manufacturer JASCO Japan, Wavelength range: 220.00 to 750.00 nm
- **Analytical Balance Model :**
  CX -220, Citizen, Bombay, India
- **Sonicator Model :**
  TRANS-O-SONIC; D-compect., Capacity: 2 Lit., Ahmedabad,India
- **Hot air oven Model :**
  Hot air oven with digital temperature controller, EIE Instrument Pvt. Ltd., Ahmedabad, India

6.1.2 Reagents and material

- API Ofloxacin
- Glacial acetic acid, (AR Grade), S.D.Fine Chemicals Ltd., Bombay, India..
- Doubled Distilled water
- Formulation:-Ofloxacin Tablet,
- Manufactured by:- Cipla LTD, Label claim:-200 mg

6.1.3 Preparation of solutions

5 % Acetic acid solution:-For preparation of 100 ml 5% acetic acid solution take 5 ml conncentrated actic acid in 100 ml volumetric flask dilute upto 100 ml with distilled water.

Blank solution :- 5 % Acetic acid solution

Preparation of standard stock solution of Ofloxacin

Ofloxacin 10mg accurately weighed and transferred to 10 mL amber colored volumetric flasks. Dissolved in 5 % acetic acid,sonicated for 20 min and volumes were made up to mark with aceticacid to obtain standard stock solutions having concentration 1000 µg/mL.

Preparation of woring stock solution of Ofloxacin

From the above solution pipette out 1 ml solution in 10 ml volumetric flask and volume made upto 10 ml with acetic acid solution to produce 100 µg/mL.

Preparation of stock solution of Marketed formulation

Quantity of tablet powder equivalent to 10 mg of Ofloxacin was weighed and transferred to a 100 mL amber colored volumetric flask volume made upto mark with acetic acid solution sonicated for 20 min. The solution was shaken and filtered through Whatmann filter paper.

Preparation of working solution of Marketed formulation

From the filtered solution(1000µg/ml) pipette out 1 ml solution in 10 ml volumetric flask and volume made upto 10 ml with acetic acid solution to produce 100 µg/mL.

6.2 METHOD DEVELOPMENT

6.2.1 INSTRUMENTAL PARAMETER:-

INSTRUMENT MODE:-Spectrum measurment

MEASUREMENT MODE:-Synchronous

BAND WIDTH:-5 nm,**DELTA VALUE**:-10-160

DATA PITCH:--5 nm,**SCANNING SPEED**:- 500 nm/min

SENSITIVITY:-Medium

6.2.2 Optimization of condition for method

Step 1:-Scanning of solution in Uv spectrophotometer to confirm excitation wavelength.

100 µg/ml Ofloxacin solution in 5% acetic acid solution taken and scan between 200-400 nm in UV Visible spectrometer.

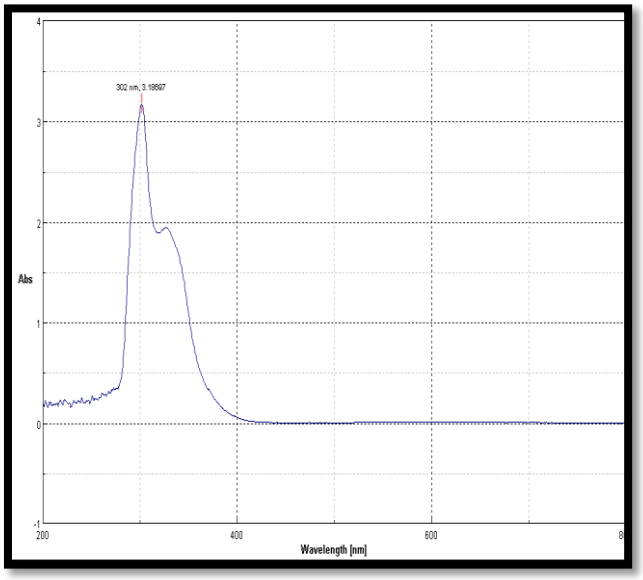


Figure 6.1 **Uv spectrum of Ofloxacin(100µg/ml) in 5%Acetic acid solution**

Step 2:-Selection of Delta (D) value:- For better sensitivity and resolution

Table 6.1 Trials for optimization of delta value in spectrofluorometer

Optimized Delta value:-From the number of trial it indicate that at **140 D** value it give sharp peak at 480 nm with high sensitivity

Figure 6.2 Ofloxacin 1 µg/ml solution synchronous mode spectra

Step 3:-Scanning of Ofloxacin 1 µg/ml solution in emission and excitation mode

Figure 6.3 Emission spectra at 295 nm excitation wavelength

Figure 6.4 Excitation spectra at 480 nm emission wavelength

6.3 METHOD VALIDATION

(1) Linearity:- Linearity was evaluated through a linear regression analysis.

From the Standard working solution take 1,0.1 ml & dilute with 10 ml with 5% Acetic acid to produce 10 µg/ml & 1 µg/ml solution in 10 ml volumetric flask. From 1 µg/ml solution take 0.5,1,3,5,8 ml dilute with 10 ml 5% Acetic acid to produce 0.05,0.1,0.3,0.5,0.8 µg/ml solution .Solutions were scanned in spectrofluorometer.

Figure 6.5 Linearity overlay spectrum of OFLOXACIN(0.05-1µg/ml)

Table 6.2 Linearity data of Ofloxacin (n*=6)

Figure 6.6 Linearity graph of Ofloxacin

(2) Accuracy

By standard addition method

Sample concentration was taken 0.3 µg/ml for Ofloxacin. After that accuracy of the method was determined by standard addition method at three different levels (80%, 100% and 120%) by spiking 0.24,0.3,0.36µg/ml in 0.3µg/ml.

Table 6.3 Accuracy data of Ofloxacin(n=3)

(3) Precision

For Precision concentration selected for Ofloxacin was 0.05,0.5,1 µg/ml.For Ofloxacin intraday precision was carried out by taking 3 different concentration (0.05,0.5,1 µg/ml) for 3 times in a day & for interday precision it was carried out on three different days.

Intraday precision

Table 6.4 Intraday precision data of Ofloxacin(n*=3)

Interday precision

Table 6.5 Interday precision data of Ofloxacin(n*=3)

Repetability

For repetabilty stydy of Ofloxacin,0.05,0.5,1 µg/ml solution intensity was measured.

Table 6.6 Repetability data of Ofloxacin(n*=6)

(4) Ruggedness

For ruggedness study of Ofloxacin 0.05,0.1,0.3,0.5,0.8 µg/ml solution was made by different analyst and their intensity was measured.

Table 6.7 Ruggedness data of Ofloxacin(n=2)

Specificity

For specificity of Ofloxacin same concentration of sample(tablet) and standard solution scan in synchronous mode.

Figure 6.7 Spectra of standard and sample solution

Limit of detection and Limit of Quantification

The calibration curve was repeated six times and the standard deviation of response(absorbance) was calculated.LOD was calculate using following equation

LOD=σ/s*3.3

Where,σ= standard deviation of response

s=slope

Limit of Quantification

The calibration curve was repeated six times and the standard deviation of response(absorbance) was calculated.LOQ was calculate using following equation

LOQ=σ/s*10

Where,σ= standard deviation of response

s=slope

Table 6.8 LOD & LOQ data of Ofloxacin(n*=6)

Analysis of Marketed Formulation

Table 6.9 Assay results of Markted formulation (n=3)

6.4 RESULTS AND CONCLUSION

Parameters	Observation
Synchronous mode λmax (nm)	480 nm
Linearity rang (µg/ml)	0.05-1
Excitation λmax (nm)	295 nm
Emission λmax (nm)	480 nm
Delta value	140 D
Regression equation	y = 385.3x - 28.44
Slope (m)	385.3
Intercept (c)	28.44
Correlation coefficient (r)	0.995
Accuracy(% Recovery)	99.3-100.7
Interday Precision % RSD	< 2%
Intraday Precision % RSD	< 2%
Repetabilty % RSD	< 2%
Specificity	No inteference
Ruggedness % RSD	< 2%
LOD (µg/ml)	0.01
LOQ (µg/ml)	0.03
% Assay	105.43

CONCLUSION

From the results it conclude that determination of ofloxacin using 5% acetic acid solution enhance the sensitivity in terms of results.The develop method is accurate,precisie,rugged and specific.The method validation parameters are as per specified range. The developed method is useful for routine analysis of Pharmaceutical formulation

7. HPTLC(HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY) METHOD

A SIMPLE,SENSITIVE AND LESS TIME CONSUMING HPTLC METHOD DEVELOPMENT FOR ESTIMATION OF CEFPODOXIME PROXETIL AND OFLOXACIN IN COMBINED BULK FORM.

7.1 INSTRUMENTATION AND APPARATUS

- Pre-coated silica gel aluminum Plate 60F–254 (20 × 20 cm with 250 μm thickness) (E. Merck)
- Camag – 100 μl Applicator syringe (Hamilton, Bonaduz, Schweiz)
- Camag Applicator-Linomat V
- Camag – Twin trough chamber (10× 20) with stainless steel Lid
- Camag TLC scanner3
- UV cabinet with dual wavelength UV lamp (254 nm and 366 nm)
- Balances Model:Citizen Cx-220, Citizen Pvt. Ltd.
- Ultra Sonicator, Trans-o-sonic, India
- Hot air oven, EIE Instruments Pvt. LTD.
- WinCATS software

7.1.1 REAGENTS AND MATERIAL

- API Cefpodoxime proxetil & Ofloxacin
- Methanol (AR grade, S.D. Fine chemicals Ltd., Mumbai, India)
- Chloroform (AR grade, S.D. Fine Chemicals, Mumbai)
- Ethyl Acetate (AR Grade, CDH Pvt LTD, New Delhi)
- Toluene (AR grade, S.D. Fine Chemicals, Mumbai)
- Triethylamine(AR grade, S.D. fine chemicals, Mumbai)

7.1.2 Preparation of solution

Preparation of Standard stock solutions of Cefpodoxime proxetil and Ofloxacin

Cefpodoxime proxetil (10 mg) and Ofloxacin (10 mg) was weighed accuretly and transferred to individual 10 ml ambert colored volumetric flasks and dissolved in metanol and sonicate for 5 min. The flasks were shaken and volume was made up to mark with methanol. (1000µg/ml)

Preparation of working standard stock solution

From the above solutions of Cefpodoxime proxetil and Ofloxacin 1 ml aliquot was taken and transferred to 10 ml ambert colored volumetric flask. The flasks were shaken and volume was made up to mark with methanol. (100µg/ml)

7.2 METHOD DEVELOPMENT AND VALIDATION

7.2.1 Optimization of method condition

Step 1:- Optimization of mobile phase on TLC(Thin layer chromatography)

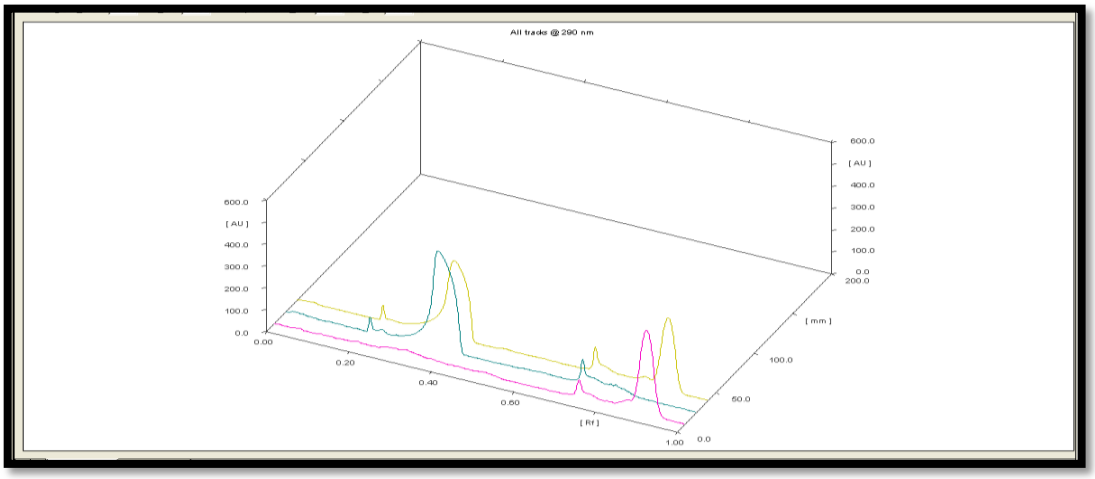
Figure 7.1 Trials on TLC (Thin layer chromatography) Visulization in UV Chamber

Table 7.1 Trials of mobile phase optimization on TLC(Thin layer chromatography)

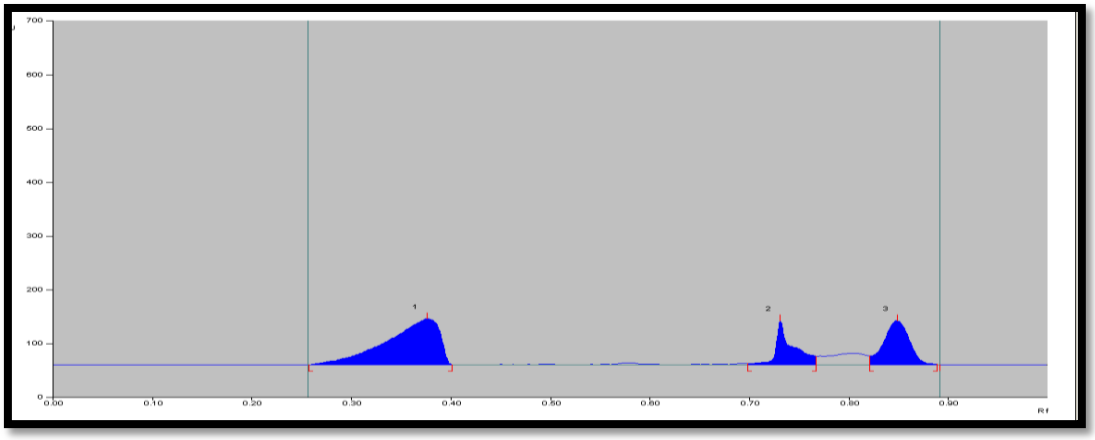
Step 2 Optimization of mobile phase on HPTLC system

Figure 7.2 Trials on HPTLC Visulization in UV Chamber

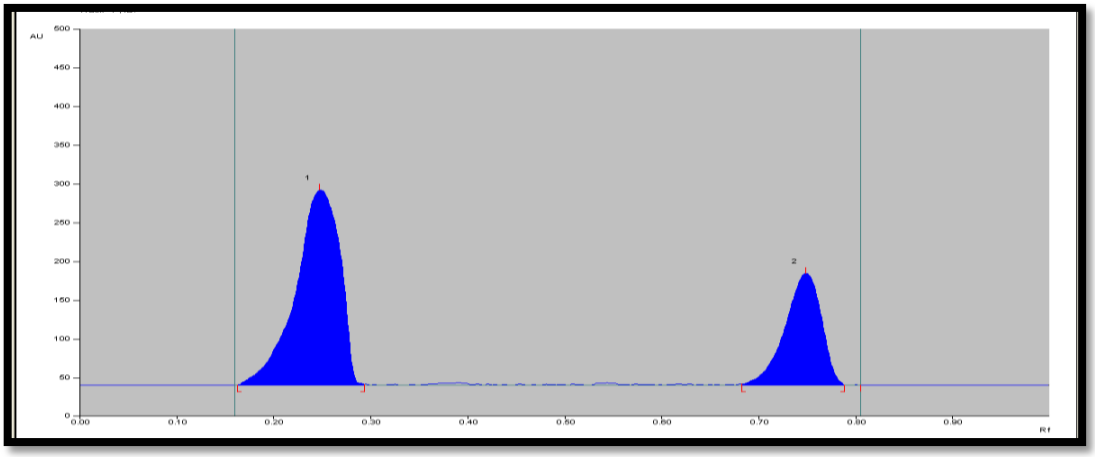
Trial No 1



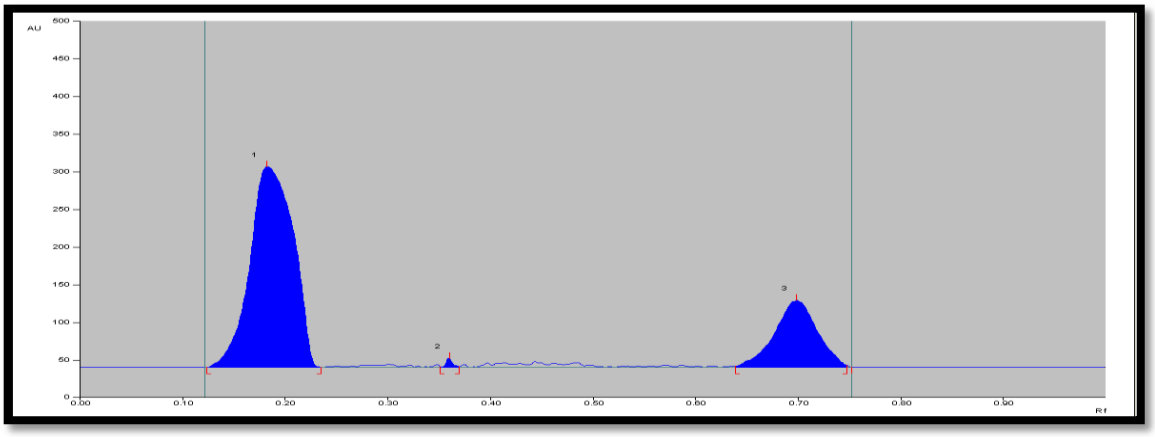
Trial No 2



Trial No 3



Trial no 4



able 7.2 Trials of mobile phase optimization on HPTLC

Step 3:-Selection of(optimize) wavelength for scanning on HPTLC-UV system

1) By observing area at different wavelength

Selection of wavelength from scanning of tracks 200-450 nm wavelength

(2) By peak purity

Figure 7.3 Peak purity spectra of Cefpodoxime proxetil & Ofloxacin

From the above observation it can be conclude that 290 nm wavelength is optimum for scanning of Cefpodoxime proxetil and Ofloxacin

OPTIMIZED HPTLC METHOD PARAMETERS

- **Mobile Phase** :- Chloroform: Ethyl acetate:MeOH:TEA (5.0:0.75:0.5:0.3 v/v/v/v)
- **Scanning Wavelength** :- 290 nm
- **Chamber Saturation Time: -** 15min.
- **Band Width** :- 5mm
- **Distance Run** :- 80mm**Slit Dimension** :- 5mm x 0.33mm
- **Scanning Speed** :- 20mm/s

METHOD VALIDATION

(1) Linearity:- Linearity was evaluated through a linear regression analysis.

From the working standard mixture(100 µg/ml), aliquots were spotted on the TLC plate under nitrogen stream using Linomat V to obtain final concentration 500,700,1200,1500,2000 ng/spot.

Figure 7.4 Linearity HPTLC graph scanning at 290 nm wavelength

Table 7.3 Linearity data of Cefpodoxime proxetil (n*=6)

Table 7.4 Linearity data of Ofloxacin (n*=6)

Figure 7.5 Linearity graph of Cefpodoxime proxetil and Ofloxacin

(2) Specificity(Peak Purity)

Figure 7.6: Peak purity HPTLC chromatogram of Cefpodoxime proxetil and Ofloxacin

Table 7.5 Specificity data of Cefpodoxime proxetil and Ofloxacin

From the HPTLC-UV Peak purity chromatogram it shows that each bands separated was pure drug Cefpodoxime proxetil and Ofloxacin.

(3) Limit of detection & Limit of Quantification

The calibration curve was repeated six times and the standard deviation of response(Area) was calculated.LOD & LOQ was calculate using equations given in section (5.3)

Table 7.6 LOD & LOQ data of Cefpodoxime proxetil and Ofloxacin(n*=6)

7.3 RESULTS AND CONCLUSION

Parameters	Cefpodoxime proxetil	Ofloxacin
Scanning wavelength	290 nm	290 nm
Linearity rang (ng/spot)	500-2000	500-2000
R_f	0.68	0.28
Regression equation	y = 4.321x + 3716	y = 6.652x +4996
Slope (m)	4.321	6.652
Intercept (c)	3716	4996
Correlation coefficient (r^2)	0.999	0.999
Specificity(Peak purity)	PASS	
LOD (µg/ml)	0.73	2.21
LOQ (µg/ml)	5.8	17.7
Saturation time	15 min	

CONCLUSION

From the results it conclude that method optimize for the method validation parameters are as acceptance criteria.Chamber saturation time is 15 min.Rf value for both drugs are within limit.It is having less time consuming than reported method in which water use in composition of mobile phase.

8.SFC(SUPER CRITICAL FLUID CHROMATOGRAPHY) METHOD

A SIMPLE,SENSITIVE,SPECIFIC AND ECO-FRIENDLY SFC METHOD DEVELOPMENT FOR ESTIMATION OF CEFPODOXIME PROXETIL USING DIFFERENT COLUMN IN WHICH METHANOL USE AS MODIFIER.

8.1 INSTRUMENTATION AND APPARATUS

- **Super critical fluid chromatography** with model no.JASCO 900 series,manufactured by Jasco Inc.JAPAN with pump (Jasco PU-950),Column oven (JASCO-CO-965),Injector (Rheodyne model 7125 with 20 µL,fixed loop),Back pressure (JASCO-880-81),Detector(JASCO UV-975),Software (BORWIN Jasco) and column

- **Analytical balance**
  Model CX 220 analytical balance (CITIZEN,India) having capacity of 10 mg t0 220 mg was used.
- **Sonicator**
  Model Trans-O-sonic.D compact having capacity of 2 liter as used

5.1.2 Reagents and material

API Cefpodoxime proxetil

Carbon dioxide used was 99.9 % pure,obtained from BOC Pvt.Ltd.India

Methanol HPLC grade was obtained from S D Fine Chemicals,Mumbai

5.1.3 Prepartation of solutions

Preparation of (1000 µg/ml)standard stock solution

Standard Cefpodoxime proxetil was accurately weighed and transferred to 10 ml ambert colored volumetric flask. It was dissolved properly and diluted up to mark with methanol to obtain final concentration. (1000 µg/ml).

Preparation of(100 µg/ml) standard working solution

From the standard stock solution take 1ml and transferred to 10 ml ambert colored volumetric flask.Volume was made upto mark with methanol to get final concentration. (100 µg/ml).

Preparation of solution for acid or base degradation:-

For degradation take 10 mg Cefpodoxime proXetil in 10 ml volumetric flask add 5 ml methanolic HCl/NaOH(1 M) and kept it overnight in dark place.Then neutralize with 1M NaOH/HCl.

Preparation of solution for oxidative degradation:-

For degradation study take 10 mg Cefpodoxime proxetil in 10 ml volumetric flask add 2 ml 30% Hyderogen Peroxide.Add 2 ml methanol and kept it overnight in dark place.Then dilute upto mark with methanol.

Preparation of solution for Thermal and Photo degradation:-

For thermal 10 mg Cefpodoxime proxetil taken in petri plate and kept it at 80^0C for 48 hrs.

For thermal 10 mg Cefpodoxime proxetil taken in petri plate and kept it in sun light for 48 hrs.

5.2 METHOD DEVELOPMENT

Optimization of Method condition

[1] Method optimization using C_{18} column:-

Constant parameter

Column:- C18, 150*4.6 mm, 5µm particle size

Wavelength:-237 nm

Temperature:-35^0C

Back pressure:-150 MPa

Table no:8.1 Trials of Cefpodoxime proxetil on SFC using C_{18} column for method optimization

[2] Method optimization using Phenyl column:-

Constant parameter

Column:- ZORBEX SB phenyl, 150*4.6 mm, 5µm particle size

Wavelength:-237 nm

Temperature:-35^{0}C

Table no:8.2 Trials of Cefpodoxime Proxetil on SFC using phenyl column for method optimization

8.3 RESULTS

Trial no	Conc(µg/ml)	Flow rate(ml/min)		Back pressure Mpa	Retention time (min)	Assymetry
		CO2	MeOH			
C_{18} column						
1	25	1.7	0.3	150	1.067	2.078
2	25	1.8	0.2	150	1.158	1.794
3	1000	0.9	0.1	150	2.342	1.909
Phenyl Column						
4	1000	0.9	0.1	150	11.76	1.797
5	1000	1	0.25	150	3.217	2.308
6	1000	1	0.15	170	5.042	2.51
7	1000	1	0.15	100	5.975	1.978
8	1000	1.8	0.15	150	6.958	1.483
9	1000	1.8	0.15	200	5.158	3.111
10	25	1.8	0.2	150	4.917	1.728
11	1000	1.8	0.2	150	4.625	2.855
DEGRADTION STUDY						
12	1000	1.8	0.2	150	3.717	3.435
13	1000	1.8	0.2	150	2.992	1.931
					12.792	0.96
14	1000	1.8	0.2	150	4.242	2.708
15	1000	1.8	0.2	150	4.125	2.432
16	1000	1.8	0.2	150	4.117	2.557

[Trial no 1-3 using C18 column, Trial no 4-11 using Phenyl column,

Trial no 12-16 for degradation study]

CONCLUSION

From the results it conclude that it is possible to develop method for determination of Cefpodoxime proxetil using phenyl column on Super critical fluid chromatography.

As the composition of methanol increase the retention time of drug is decrease.By changing back pressure at constant flow rate it affect the retention time .As back pressure increase retention time get decrease.

9.SUMMARY & FUTURE SCOPE

9.1 SUMMARY

UV Spectrophotometric method includes simultaneous equation method that involves measurement of absorbances at two wavelengths i.e. at 234 nm (λmax of CEFPODOXIME PROXETIL) and 286 nm (λmax of OFLOXACIN) in methanol. Linearity range was observed in the concentration range 3-30 µg/mL with recovery range 98.4-100.9 for CEFPODOXIME PROXETILand 3-30 µg/mL with recovery range of 98-100.7 for OFLOXACIN respectively. The correlation coefficients for CEFPODOXIME PROXETILand OFLOXACIN was found to be 0.998 and 0.998, respectively. LOD and LOQ were found to be 0.1 and 0.32 µg/mL for CEFPODOXIME PROXETIL0.32 and 0.99 µg/mL for OFLOXACIN, respectively. The R.S.D. values for precision studies were found to be less than 2 for both the drugs.

Spectrofluorimetric method includes synchronous mode estimation of OFLOXACIN using delta value 140 (medium sensitivity mode) that involves measurement of fluorescence intensity at 480 nm wavelengths in synchronous spectra of drug in 5 % acetic acid solution.Linearity range was observed in the concentration range 0.05-1 µg/mL with recovery range of 99.3-100.7. The correlation coefficients for Ofloxacinxacin was found to be 0.995.LOD and LOQ was found to be 0.01 and 0.03 µg/ml. The RSD values for precision studies were found to be less than 2 for both the drugs.

In HPTLC method quantification of CEFPODOXIME PROXETILand OFLOXACIN was done by peak area.The separation of drugs was carried out using Chloroform: Ethyl acetate:MeOH:TEA (5.0:0.75:0.5:0.3 v/v/v/v) as mobile phase on TLC silica gel G60 F254.The distance run was 80 mm.The densitometric detection was done at 290 nm wavelength. Linearity range was observed in the concentration range 500-2000 ng/spot The correlation coefficients for CEFPODOXIME PROXETILand OFLOXACIN were found to be 0.9989 and 0.9984, respectively.LOD and LOQ were found to be 0.999 ng/spot for both drugsThe Rf value for CEFPODOXIME PROXETILand OOfloxacin were found to be 0.68 & 0.28.Peak purity spectra shows that the method is specific for both drugs determination.

Super critical fluid chromatography includes optimization of various parameter to improve selectivity,sensitivity of method.The parameters optimize are flow rate of carbon dioxide & methanol,temperature of oven,back pressure,The wavelength selected for the estimation CEFPODOXIME PROXETILis 237 nm.Different flow rate was tried for sensitivity of drug.Degradation profile of CEFPODOXIME PROXETILwas done in 1 M HCl,1 M NaOH,30 % Hydrogen peroxide,Thermal,Photo.CEFPODOXIME PROXETILshows degradation in 1 M NaOH.From the result table it shows that by changing back pressure fluid property change which affect retention time of compound.

9.2 FUTURE SCOPE

A Literature survey revels that no single super critical chromatography method is reported for estimation of CEFPODOXIME PROXETILand OFLOXACIN in individual or in combined dosage form.So by using develop method of CEFPODOXIME PROXETILit is possible to do validation of the method for various parameter.

It is also possible to develop method for determination of degradation study.Develop method for determination of drugs in combined dosage form.

Literature surve revels that no any raman spetcrophotometric method for quantification of both drug in individual or combined dosage form.

For spectrofluorimetry only one method for determination of CEFPODOXIME PROXETILis available through derivatization using reagent,so it is possible to develop spectrofluorimetry method for determination of CEFPODOXIME PROXETILand OFLOXACIN in combined dosage form.

By using develop HPTLC method of CEFPODOXIME PROXETILand OFLOXACIN in combined bulk form it is possible to use for determination of CEFPODOXIME PROXETILand OFLOXACIN in combined dosage form.

By using urea as hydrotropic agent it is possible to develop method for dissolution profile of drugs in combination.

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