"FORCED DEGRADATION STUDIES OF CEFUROXIME AXETIL IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD"

A dissertation submitted to

The Tamil Nadu Dr.M.G.R Medical University

Chennai-600032

In partial fulfillment of the requirements

for the award of the degree of

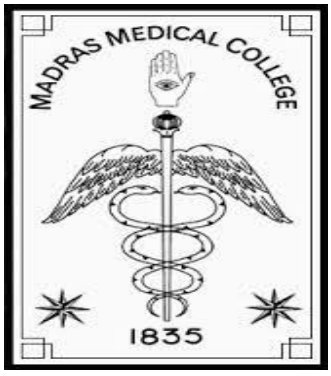
MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

Reg. No.261215703



DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI-600003

APRIL-2014

CERTIFICATE

This is to certify that the dissertation entitled **"FORCED DEGRADATION STUDIES OF CEFUROXIME AXETIL IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD"** is submitted by the candidate bearing the register no **261215703** in partial fulfillment of the requirements for the award of degree in **MASTER OF PHARMACY IN PHARMACEUTICAL CHEMISTRY** by the Tamil Nadu Dr. M.G.R Medical University, Chennai, is a bonafide work done by him during the academic year **2012-2014** at the **Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-03.**

Dr. A. JERAD SURESH, M.Pharm., Ph.D., M.B.A.,

Principal,
Professor& Head,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
Madras Medical College,
Chennai-600003.

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Dr. (Mrs.) V. NIRAIMATHI, M.Pharm, Ph.D.,
Project Adviser,
Department of Pharmaceutical Chemistry,
College of Pharmacy,
Madras Medical College,
Chennai-600003.

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LIST OF ABBREVIATIONS

S.No	ABBREVIATION	EXPANSION
1.	%	Percentage
2.	µg	Microgram
3.	µl	Microliter
4.	Abs	Absorbance
5.	API	Active Pharmaceutical Ingredient
6.	Avg.	Average
7.	Cm	Centimeter
8.	Dil.	Dilution
9.	G	Gram
10.	H_2O_2	Hydrogen Peroxide
11.	HCl	Hydrochloric Acid
12.	HPLC	High Performance Liquid Chromatography
13.	IR	Infrared
14.	KBr	Potassium Bromide
15.	Mg	Milligram
16.	Mins	Minutes
17.	Ml	Milliliter
18.	NaOH	Sodium Hydroxide
19.	Nm	Nanometer
20.	º	Celsius
21.	Rf	Retention Factor
22.	Sam	Sample
23.	Std	Standard
24.	UV	Ultra Violet
25.	Vol	Volume
26.	Wt.	Weight
27.	λ	Lambda

CONTENTS

INTRODUCTION

PHARMACEUTICAL ANALYSIS:

Pharmaceutical Analysis is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air. (**Connors KA (1994)**

Types

There are main two types of pharmaceutical analysis.

1. Qualitative (identification)
2. Quantitative (estimation)

1. Qualitative analysis is performed to establish composition of natural/synthetic substances. These tests are performed to indicate whether the substance or compound is present in the sample or not. It is mainly used in the determination of assay of samples.

2. Quantitative analytical techniques are mainly used to quantify any compound or substance in the sample. It is mainly used in the degradation studies to determine the amount of drug and degraded products present in the sample. (**Sharma BK (2000)**

Various types of Qualitative analysis:

1. Chemical methods
    a) volumetric or titrimetric methods
    b) gravimetric methods
    c) gasometric analysis
2.Electrical methods
3.Instrumental methods
4.Biological and microbiological.

DEGRADATION STUDIES:

The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a lower dose than expected. Therefore it is essential to know the purity profile and behavior of a drug substance under various environmental conditions.

Forced degradation studies are carried out for the following reasons:

- To develop and validate a stability indicating method
- To determine degradation pathways of drug substances and drug products (E.g. during development phase)
- To identify impurities related to drug substances or excipients
- To understand the drug molecule chemistry
- To generate more stable formulations
- To generate a degradation profile that mimics what would be observed in a formal stability study under ICH conditions.
- To solve stability-related problems (e.g., mass balance)

In order to monitor possible changes to a product over time, the applied analytical method (in most cases a chromatographic method) must be stability-indicating; the best case for testing the suitability of a method is using real-time stability samples containing all relevant degradation products that might occur. But due to product development timelines, process characteristics, excipients, and other environmental factors, a forced degradation study (stress test) can serve as an alternative. In a typical study, relevant stress conditions are light, heat, humidity, hydrolysis (acid / base influence) and oxidation or even a combination of described parameters. The formation of degradation products, independent of the strength of stress conditions can vary due to the chemical nature of the drug substance, the kind of drug product, and product specific storage requirements. An individual program has to be set up in order to reach a target degradation of 5 to 20%. A higher level of degradation will be out of the scope of product stability requirements and therefore unrealistic. The scope of the test is to generate degradation products in order to facilitate a method development for determination of the relevant products.
(Sandor Gorog (2006)

IMPURITY PROFILING

The definition of the impurity profile of a new drug material as given in the guidelines of ICH is ‘‘A description of the identified and unidentified impurities, present in a new drug substance’’. As for impurity profiling, it is the common name attributed to analytical activities performed with the aim of detecting, identifying or elucidating the structure and quantitatively determining organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations.

The aim is to minimize the adverse effects of drug materials and the preparations made thereof. After establishing the pharmacological-toxicological profile of a drug substance, pharmacologists, clinicians and drug-registration authorities consider the beneficial and adverse effects to human and, on the basis of the benefit/risk ratio thus obtained, make the decision of introducing the drug substance into therapy. In principle, the adverse effects can originate from two sources:

- It is well known that, in addition to their beneficial effects, all drug materials have adverse (side) effects, which can be considered inherent properties and cannot be influenced by the quality of the drug material and

- If the impurities of a drug material (the difference between 100% and the 98–99% mentioned above) are physiologically highly active (toxic) materials, in principle, they could contribute to the side-effect profile of the drug. Since the impurity profile of a drug material depends on the synthesis route and other factors, this could make the side-effect profile irreproducible, adversely influencing the safety of drug therapy. By estimating the impurity profile of a drug material and setting strict limits for the impurities, this danger can be minimized. **(Saranjit Singh *et al.,* (2000)**

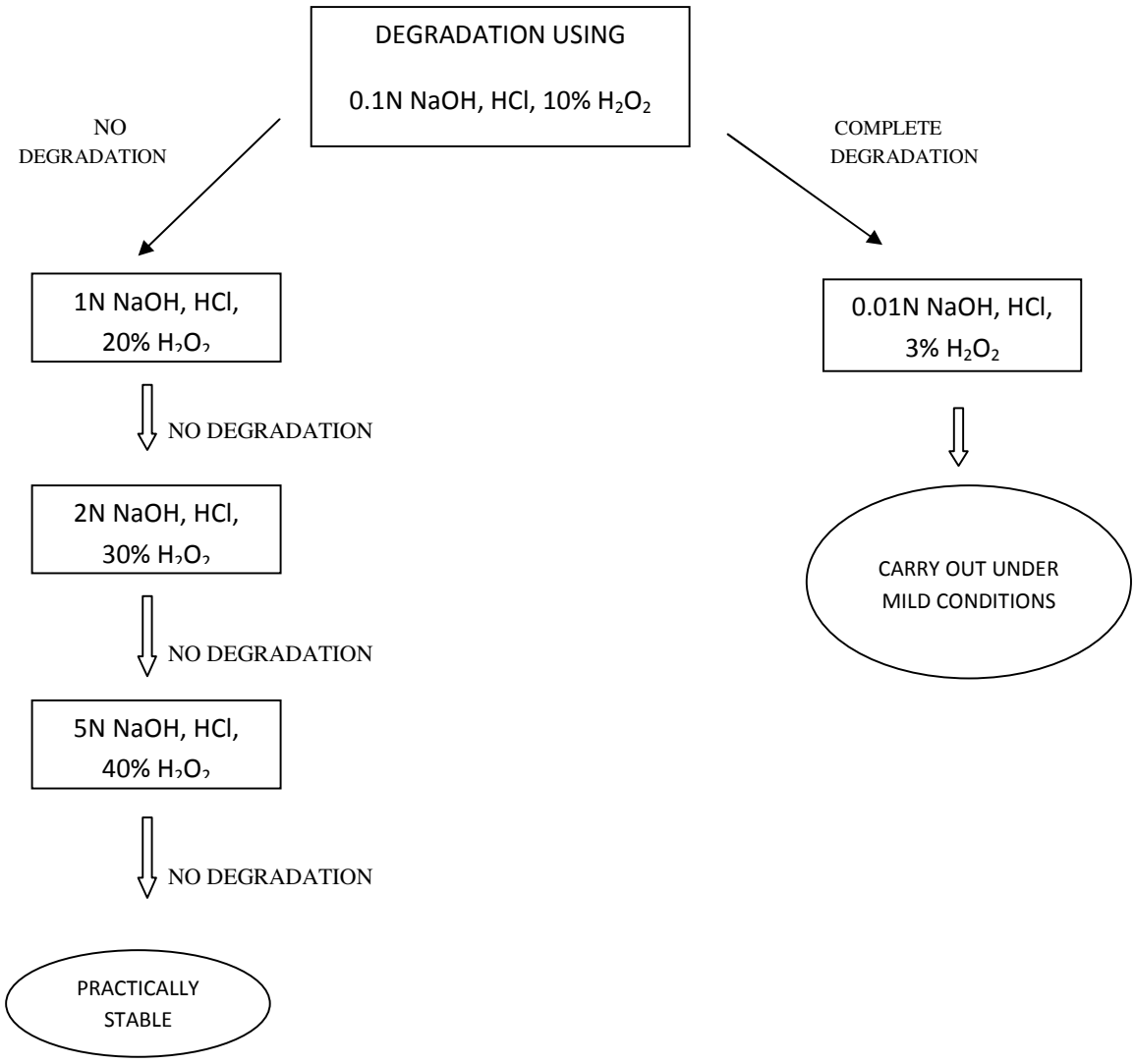
CHARACTERISATION OF DEGRADED PRODUCTS

The characters of the degraded fragments were studied by both Spectrophotometric methods and chromatographic techniques. The degraded products were analyzed by following methods.

- ✓ UV spectroscopy
- ✓ INFRARED spectroscopy
- ✓ TLC
- ✓ HPLC

These methods were used to determine the assay and the amount of sample present in the degraded products and also used in identification of impurities.

FORCED DEGRADATION FLOW CHART



DRUG PROFILE

DRUG NAME : CEFUROXIME AXETIL

BRAND NAME : Ceftin 500mg

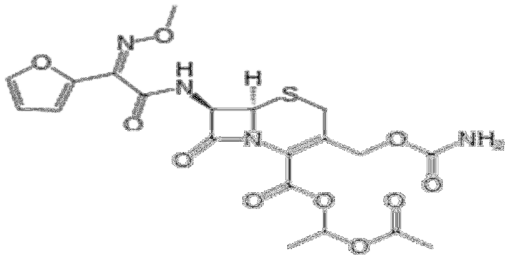
IUPAC NAME :1-Acetoxyethyl(6*R*,7*R*)-3[(carbamoyloxy)methyl]-7-{[(2*Z*)-2-(2-furyl)-2-(methoxyimino)acetyl]amino}-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate.

MOLECULAR FORMULA : $C_{20}H_{22}N_4O_{10}S$

MOLECULAR WEIGHT : 510.475 g/mol

CAS NUMBER : 64544-07-6

CHEMICAL STRUCTURE :



BASIC MOIETY : Beta-Lactam

CATEGORY : Second Generation Cephalosporin Antibiotic

DESCRIPTION : A white or almost white powder

SOLUBILITY : Slightly soluble in water, soluble in acetone, in ethyl acetate and in methanol, slightly soluble in alcohol.

MECHANISM OF ACTION

Cefuroxime axetil is a semi synthetic cephalosporin antibiotic, chemically similar to penicillin. Cephalosporins stop or slow the growth of bacterial cells by preventing bacteria from forming the cell wall that surrounds each cell. The cell wall protects bacteria from the external environment and keeps the contents of the cell together. Without a cell wall, bacteria are not able to survive. Cefuroxime is effective against a wide variety of bacteria, such as *Staphylococcus aureus*, *Streptococcus pneumoniae, Haemophilus influenzae*, *E. coli*, *N. gonorrhea*, and many others. The FDA approved Cefuroxime in December 1987.

INDICATIONS

Cefuroxime is effective against susceptible bacteria causing infections of the middle ear (otitis media), tonsillitis, throat infections, laryngitis, bronchitis, and pneumonia. It also is used for treating urinary tract infections, skin infections, and gonorrhea. Additionally, it is useful in treating acute bacterial bronchitis in patients with chronic obstructive pulmonary disease (COPD).

DRUG INTERACTIONS

Probenecid increases the concentration of Cefuroxime in the blood. Drugs that reduce acidity in the stomach (for example, antacids, H2-blockers, proton pump inhibitors) may reduce absorption of Cefuroxime.

PREGNANCY:

Cephalosporins are usually considered safe for use during pregnancy.

NURSING MOTHERS:

Cefuroxime is excreted in breast milk and may cause adverse effects in the infant. Cefuroxime is approved for pediatric patients 3 months and older.

SIDE EFFECTS:

Cefuroxime is generally well tolerated, and side effects are usually transient. Side effects include diarrhea, nausea, and vomiting, abdominal pain, headache, rash, and hives, vaginitis, and mouth ulcers. Allergic reactions, severe skin reactions, anemia, and seizures also may occur.

DOSING:

Typical adult oral doses are 250 or 500 mg twice daily for 7-20 days depending on the type and severity of the infection. A single 1000 mg dose may used for uncomplicated gonorrhea. The tablets and suspension are not interchangeable. **(www.rxlist.com)**

REVIEW OF LITERATURE

- **Connors KA (1994)** A Textbook of Pharmaceutical Analysis.

- **Saranjit Singh *et al.,* (2000)** have listed out the possible benefits of forced degradation studies to assess the stability of drugs and products.

- **Sharma BK (2000)** Instrumental methods of chemical analysis.

- ✓ **Marianna Zajac *et al.,* (2003)** investigated the effect of temperature and relative atmospheric humidity on the stability of the crystalline form of cefuroxime axetil in solid state. Changes in the concentration of cefuroxime axetil were recorded by means of HPLC with UV detection.

- ✓ **Jain Pritam *et al.,* (2004)** developed a validated UV-Spectrophotometric method for determination of Cefuroxime Axetil in bulk and in Formulation. The λ max of Cefuroxime axetil in 0.1N HCL was found to be 281 nm. The proposed method was applied to pharmaceutical formulation and percentage amount of drug estimated 99.19 % was found in good agreement with the label claim.

- ✓ **Ivanovic Ivana *et al.,* (2006)** determined the assay of cefuroxime axetil after accelerated stability conditions. The study includes the methods to determine the assay of cefuroxime axetil bulk and formulation in various conditions like temperature, humidity and alkali hydrolysis.

- ✓ **Steven W. Baertschi (2006)** has reported the information for discovering and profiling degradation-related impurities. He also reported the technologies, their strengths and limitations, and recommends strategies for successful DRI profiling.

- ✓ **SV Chaudhari *et al.,* (2006)** carried out the Simultaneous UV spectrophotometric method for the estimation of cefuroxime axetil and probenecid from solid dosage forms.

- ✓ **Sandor Gorog (2006)** have reported the importance and the challenges of impurity profiling in modern pharmaceutical analysis.

- ✓ **MV Shinde *et al.,* (2008)** developed a spectrophotometric determination of cefuroxime axetil from bulk and in its tablet dosage form.

- ✓ **Santosh shelke *et al.,* (2009)** developed a Validated UV spectrophotometric method of Cefuroxime axetil in bulk and pharmaceutical formulation which is based on measurement of absorption at maximum wavelength 281nm.

- ✓ **Gandhi Santosh V *et al.,* (2009)** have developed a Simultaneous determination of Cefuroxime axetil and ornidazole in tablet dosage form using reversed-phase high performance liquid chromatography.

- ✓ **Michae Kaza *et al.,* (2010)** developed the validated HPLC method for determination of cefuroxime in human plasma.

- ✓ **M.D.Game *et al.,* (2010)** developed a Validated 1st order derivative spectroscopy for the determination of cefuroxime axetil in bulk drug and tablets.

- ✓ **P Santhosh Kumar *et al.,* (2012)** have reported a Validated High Performance Liquid Chromatography method for the estimation of cefuroxime axetil.

- ✓ **NJ Shah *et al.,* (2012)** have reported a development and validation of a HPTLC method for the estimation of cefuroxime axetil.

- ✓ **K. A. Shaik *et al., (*2011)** have developed a specific stability indicating assay method for the determination of cefuroxime sodium in pharmaceutical formulation by UV-VIS spectrophotometer.

- ✓ **P. N. Patil *et al.,* (2012)** reported a HPLC analysis method of cephalosporins and study of different analytical parameters.

- ✓ **Pramod L. Ingale *et al.,* (2013)** developed the simultaneous determination of cefuroxime axetil and potassium clavulanate in pharmaceutical dosage form by RP-HPLC.

AIM AND OBJECTIVE

The stability of a drug product or a drug substance is a critical parameter which may affect purity, potency and safety. Changes in drug stability can risk patient safety by formation of a toxic degradation product(s) or deliver a lower dose than expected. Therefore it is essential to know the purity profile and behavior of a drug substance under various environmental conditions

The aim of the study is to conduct **"FORCED DEGRADATION STUDIES OF CEFUROXIME AXETIL IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD"** and to evaluate the stability of Cefuroxime axetil in variety of stress conditions. Stability plays an important role in storage of drugs.

The study includes the following

- To perform the Alkali hydrolysis, Oxidative degradation, and Thermal and Photolytic degradation.
- Quantification of degraded product by UV and HPLC.
- Comparing the results of bulk and sample with standard.
- Identification of Degraded samples by TLC.
- Identification of changes in functional group present in the degraded samples by IR.

MATERIALS AND METHODS

DRUG SAMPLE & STUDY PRODUCTS

Cefuroxime axetil bulk was obtained from Orchid Healthcare, Chennai, and Tamilnadu.

Test product:

Cefuroxime axetil tablets (Ceftin 500mg) were purchased from medical shop.

CHEMICALS AND SOLVENTS USED FOR DEGRADATION:

- Hydrochloric Acid – Merck, AR grade, Mumbai, India.
- Hydrogen Peroxide (30%W/V) – Merck, AR grade, Mumbai, India.
- Sodium Hydroxide – Merck, AR grade , Mumbai, India
- Chloroform – Merck, AR grade , Mumbai, India
- Ethanol – Merck, AR grade , Mumbai, India
- Methanol – Merck, HPLC grade, Mumbai, India
- Acetonitrile – Merck, HPLC grade, Mumbai, India
- Water – Double Distilled

INSTRUMENTS USED:

- Shimadzu 1650 UV Spectrophotometer – Double beam , UV Probe2.31
- ABB-IR ,KBr Press – AB MB 3000
- Agilent HPLC – VWD detector, Chemstation
- Shimadzu electronic balance – AX 200

FORCED DEGRADATION

Forced degradation studies are used to identify reactions which may occur to degrade a processed product. Usually conducted before final formulation, forced degradation uses external stresses to rapidly screen material stabilities. Forced degradation was performed in various conditions to detect the stability of a drug and drug products. Forced degradation plays a role in the storage of drug products. **(Sandor Gorog (2006)**

ANALYTICAL PROCEDURES

Degraded samples were analyzed using Spectrophotometric methods like UV, IR and chromatographic methods like TLC and HPLC. The assays of degraded samples were carried out by UV and HPLC and the changes in the functional group of drug were detected by IR spectroscopy. Degraded products were identified by TLC.

HYDROLYTIC DEGRADATION

Hydrolysis usually means the cleavage of chemical bonds by the addition of water. The main source of impurities in the formulations is from hydrolytic degradation. Water either as a solvent or as moisture in the air comes in contact with pharmaceutical dosage forms is responsible for degradation of most of the drugs. Hydrolytic study under acidic and basic condition cleavage of Beta lactum ring present in the Cefuroxime axetil. NaOH and HCl are employed for generating acidic and basic stress samples respectively.

The hydrolytic degradation of a new drug in acidic and alkaline condition can be studied by treating the drug with 0.1N NaOH /0.1N HCl. If sufficient degradation is not observed, higher strength of reagents are employed to induce degradation (1N NaOH/1NHCl). **(Saranjit Singh *et al.,* (2000)**

OXIDATIVE DEGRADATION

The increase in oxidation state of an atom through a chemical reaction is known as an oxidation. Most of the drugs undergo auto oxidation because of oxygen in air. Therefore it is an important degradation pathway of many drugs. Auto oxidation is a free radical reaction that requires free radical initiator to begin the chain reaction. Hydrogen peroxide, metal ions in a drug substance act as initiators for auto oxidation. Hydrogen peroxide is a common oxidant to produce oxidative degradation products which may arise as minor impurities during long term stability studies. Hydrogen peroxide seems to be much more popular for the purpose than any other oxidizing agent. The strength of H_2O_2 used varies from 1% to 30%. In some drugs extensive degradation is seen when exposed to 3% H_2O_2 for very short time periods at room temperature. In other cases, exposure to high concentrations of H_2O_2 even under extreme conditions does not cause any significant degradation. The behavior is on expected lines, as some drugs are in fact oxidisable, while there are others that are not. The latter are not expected to show any change even in the presence of high dose of oxidizing agents.

THERMAL DEGRADATION

Temperature also plays a role in degradation of drugs. High temperature leads to increase in degradation of drugs. Most of the drugs are sensitive to temperature. Thermal degradation can cause reactions like pyrolysis, hydrolysis, decarboxylation, isomerization, rearrangement and polymerization.

PHOTOLYTIC DEGRADATION

Exposure of Sun light to drug is called photolytic degradation. The rate of degradation is directly proportional to the intensity, quantity of sun light absorbed by the drug. It is carried out by exposing the drug substance and drug product to a sun light. **(ICH Guidelines)**

1. UV SPECTROSCOPY

UV refers to absorption spectroscopy spectroscopy in the ultraviolet spectral region. This means it uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges. The absorption in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. Molecules containing π-electrons or non-bonding electrons (n-electrons) can absorb the energy in the form of ultraviolet or visible light to excite these electrons to higher anti-bonding molecular orbitals. The more easily excited the electrons the longer the wavelength of light it can absorb. In degradation studies UV plays one of the roles in detection of impurities. Assay values of drug products can be calculated using UV spectroscopy. In ultra violet region every molecule will give its own absorption at particular wavelength this is called λmax. Every compound has unique λmax and absorption. Absorption value varies only with the concentration of the compound. Degraded samples spectrum can be compared with standard spectrum to identify the degradation. After degradation the absorption and the λmax of the samples can vary from the standard. This shows the degradation of samples.

Materials and Methods

All absorption spectrums were measured by Shimadzu UV-1650PC spectrophotometer with 1cm matched quartz cells.

Reagents used

0.1N Sodium hydroxide

0.1N Hydrochloric acid

30% Hydrogen peroxide

Ethanol

Distilled water

PREPARATION OF REAGENTS

Preparation of 0.1N Sodium Hydroxide:

4gms of sodium hydroxide pellets were weighed and dissolved in small amount of distilled water then made up the volume to 1000mL.

Preparation of 0.1N Hydrochloric acid

8.33mL of concentrated Hydrochloric acid was measured and diluted with distilled water to 1000mL.

Preparation of 30% Hydrogen peroxide

300 mL of Hydrogen peroxide was diluted with distilled water and the volume made up to 1000mL.

Intraday scheme UV-study of Bulk & Formulation (Table: 1)

DEGRADATION TYPE	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample	Room Temperature	30,60,90mins
Hydrolysis	0.1N NaOH	Room Temperature	30,60,90mins
	0.1N HCl	Room Temperature	30,60,90mins
	Acid Control	Room Temperature	30,60,90mins
	Base Control	Room Temperature	30,60,90mins
Oxidation	30% H_2O_2	Room Temperature	30,60,90mins
	30% H_2O_2(no API)	Room Temperature	30,60,90mins

Interday scheme UV-study of Bulk & Formulation (Table: 2)

DEGRADATION TYPE	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample	Room Temperature	1,3,5 days
Hydrolysis	0.1N NaOH	Room Temperature	1,3,5 days
	0.1N HCl	Room Temperature	1,3,5 days
	Acid Control	Room Temperature	1,3,5 days
	Base Control	Room Temperature	1,3,5 days
Oxidation	30% H_2O_2	Room Temperature	1,3,5 days
	30% H_2O_2(no API)	Room Temperature	1,3,5 days
Thermal	Heating Chamber	50ºC	1,3,5 days
Photolytic		Sunlight	1,3,5 days

INTRADAY STUDY OF HYDROLYTIC DEGRADATION USING 0.1N NaOH

Standard preparation

Cefuroxime axetil was transferred to volumetric flask and dissolved in ethanol to achieve a concentration of 1mg/mL. The solution was kept at room temperature. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm.

Bulk preparation (stress)

50mg of Cefuroxime axetil bulk was weighed and transferred to volumetric flask; dissolved 0.1N NaOH to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask; dissolved 0.1N NaOH to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank preparation

50mL of 0.1N NaOH solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration. This is used as a blank.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

(Jain Pritam et al., (2004)

The percentage content of bulk was determined by following formula

$$\textbf{Percentage content} = \frac{\textbf{Sam Abs.} \times \textbf{Std. Wt} \times \textbf{Dil. factor} \times \textbf{Purity of Std.} \times \textbf{100}}{\textbf{Std. Abs.} \times \textbf{Sam. Wt} \times \textbf{100}}$$

The amount of present was determined by following formula

$$\textbf{Amount present} = \frac{\textbf{Sam Abs.} \times \textbf{Std. Wt} \times \textbf{Dil. factor} \times \textbf{Purity of Std.} \times \textbf{Avg. Wt of tablets}}{\textbf{Std. Abs.} \times \textbf{Sam. Wt} \times \textbf{100}}$$

Percentage content of Cefuroxime axetil was determined by following formula

$$\textbf{Percentage content} = \frac{\textbf{Amount present}}{\textbf{Label claim}}$$

HYDROLYTIC DEGRADATION USING 0.1N HCl

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved in 10 mL of ethanol, and then 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask; dissolved in 10 mL of ethanol. Then 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank preparation

50mL of 0.1N NaOH solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration. This is used as a blank.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were tabulated.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved in 10 mL of ethanol to dissolve the drug substance, and then 30% Hydrogen peroxide was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval**.**

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask; dissolved in 10 mL of ethanol to dissolve the drug product, and then 30% Hydrogen peroxide was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 30mins, the solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 60mins, and 90mins time interval.

Blank preparation

50mL of 30% H_2O_2solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. After 30mins, an aliquot solution was diluted with distilled water to get a final concentration. This is used as a blank.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were tabulated.

INTERDAY FORCED HYDROLYTIC DEGRADATION USING 0.1N NaOH

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved 0.1N NaOH to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask; dissolved 0.1N NaOH to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day (1st day), an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval.

Blank preparation

50mL of 0.1N NaOH solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get a final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

HYDROLYTIC DEGRADATION USING 0.1N HCl

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved in 10 mL of ethanol, and then 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask. It was dissolved in 10 mL of ethanol, and then 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval.

Blank preparation

50mL of 0.1N HCl solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get a final concentration. This procedure is repeated for 3rd and 5th day.

The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to volumetric flask and dissolved in 10 mL of ethanol, and then 30% Hydrogen peroxide was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. Then the next day, an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval. The obtained spectrum is compared with standard spectrum.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask. It was dissolved in 10 mL of ethanol, and then 30% Hydrogen peroxide was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. The next day (1st day), an aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The solution was scanned in the UV region and the maximum absorbance was recorded at 278nm. The same procedure was repeated for 3rd and 5th day time interval.

Blank preparation

50mL of 30% Hydrogen peroxide solution was taken in a 50mL volumetric flask. The solution was kept at room temperature. The next day, an aliquot solution was diluted with distilled water to get a final concentration. This procedure is repeated for 3rd and 5th day. The procedure was repeated thrice. After the stipulated time, the absorption of the resulting solution showed maxima 278nm against reagent blank treated in the same way. Three such determinations were made and the assay value was estimated.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The next day 50mg Cefuroxime axetil bulk was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The same procedure was repeated for 3rd and 5th day.

Sample preparation (stress)

1g of Cefuroxime axetil tablets were crushed weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The next day 50mg equivalent of Cefuroxime axetil tablet powder was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The same procedure was repeated for 3rd and 5th day.

Blank preparation

Distilled water was used as a blank.

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed under a sun light. The next day 50mg Cefuroxime axetil bulk was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The same procedure was repeated for 3rd and 5th day.

Sample preparation (stress)

1g of Cefuroxime axetil tablets were crushed weighed and transferred to a petri dish. This petri dish was placed under a sun light. The next day 50mg equivalent of Cefuroxime axetil was taken from the petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. The same procedure was repeated for 3rd and 5th day.

Blank preparation

Distilled water was used as a blank.

2. INFRARED SPECTROCOPY

Infrared spectroscopy is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, which is light with a longer wavelength and lower frequency than visible light. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far- infrared, named for their relation to the visible spectrum. The IR region is mainly divided into two types

- Finger print region (1500-400cm^{-1})

  This will vary for every compound. In degradation studies this region can show a complete degradation of a sample otherwise no changes in the finger print region.

- Functional group region (4000-1500cm^{-1})

  This will give the peaks based on the functional group present in the compound. In degradation studies it can show the changes in functional groups. Standard can be compared samples to detect the changes after the degradation.

IR spectroscopy is mainly used in thermal and photolytic degradation studies.

Apparatus

All spectral measurements were made on ABB-IR (model no.MB3000) with KBr press (model no. M15).

Interday scheme of IR-study for Bulk & Formulation (Table: 3)

DEGRADATION TYPE	MATERIAL(SOLID)	STORAGE CONDITION	SAMPLING TIME
Normal	Bulk	Room temperature	1,3,5days
	Sample	Room temperature	1,3,5days
Photolysis	Bulk	Sunlight	1,3,5days
	Sample	Sunlight	1,3,5days
Thermal(Heating Chamber)	Bulk	50ºC	1,3,5days
	Sample	50ºC	1,3,5days

2.1 INTERDAY STUDY OF CEFUROXIME AXETIL BY IR

General Procedure

Cefuroxime axetil tablets were weighed and transferred into petri dish. The first one was kept at room temperature, the second one was kept at chamber at 50ºC, and the third one was kept at sunlight. This was referred as 0 day. The bulk drug was weighed and transferred into 3 different Petri dishes. The same procedure was repeated for bulk drug.

Standard preparation (stress)

The first day standard of the Cefuroxime axetil was weighed and reground with dry KBr using agate mortar and pestle. The KBr discs were prepared by using KBr pellet press instrument. Then the percentage transmittance of the standard was measured. The same procedure was repeated for 3rdand 5rdday. The percentage transmittance was recorded in similar way.

Bulk preparation (stress)

The next day, required bulk drug has been taken from petri dish. The required amount of bulk drug was reground with dry KBr using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured. The spectrum obtained from degraded sample was compared with standard spectrum. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

The next day (1st) day, required sample has been taken from petri dish. The required amount of sample was reground with dry KBr using agate mortar and pestle. The discs were prepared by using KBr press instrument. Then the percentage transmittance of the sample was measured. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

THERMAL-50ºC:

Bulk preparation (stress)

The next day, the bulk from chamber was removed and the required quantity has been taken from the petri dish. It was kept in the same place. The required amount bulk drug was reground with dry KBr agate mortar and pestle .The discs were prepared by using KBr press instrument. Then the percentage transmittance of the bulk drug was measured. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

Similar bulk procedure was followed.

SUNLIGHT:

Bulk preparation (stress)

The next day (1st) day, the required quantity has been taken from the petri dish. It was kept in the same place .The required amount of bulk was reground with dry KBr to using agate mortar and pestle. The discs were prepared by using KBr press instrument .Then the percentage transmittance of the bulk was measured .Compared the spectrum with standard spectrum. The same procedure was repeated for 3rd and 5th day. The percentage transmittance was recorded in similar way.

Sample preparation (stress)

Similar bulk procedure was followed.

3. RP-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

High Performance Liquid Chromatography:

High Performance Liquid Chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. This technique is based on the modes of separation like adsorption, partition, including reverse phase partition, ion–exchange and gel permeation. HPLC instrument consists of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector; Compounds are separated by injecting a plug of the sample mixture onto the column. The different compounds in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Intraday scheme HPLC-study of Bulk & Formulation (Table: 4)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	90mins
Hydrolysis	0.1N HCL	Room temperature	90mins
	0.1N NaOH	Room temperature	90mins
	Acid Control (no API)	Room temperature	90mins
	Base Control (no API)	Room temperature	90mins
Oxidative	30%H_2O_2	Room temperature	90mins
	30%H_2O_2(no API)	Room temperature	90mins

Interday scheme HPLC-study of Bulk & Formulation (Table: 5)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	3rd day
Hydrolysis	0.1N HCl	Room temperature	3rd day
	0.1N NaOH	Room temperature	3rd day
	Acid Control (no API)	Room temperature	3rd day
	Base Control (no API)	Room temperature	3rd day
Oxidative	30%H_2O_2	Room temperature	3rd day
	30%H_2O_2(no API)	Room temperature	3rd day
Thermal	Heating chamber	50ºC	3rd day
Photolysis	Powder form	Sun Light	3rd day

REAGENTS AND CHEMICALS USED FOR HPLC:

- Methanol
- Distilled water

Preparation of mobile phase:

Mobile phase was prepared with 60 % of methanol and 40% of distilled water (60:40) respectively. The mobile phase should be prepared freshly, filtered through a 0.45µm membrane filter and sonicated before use.

Standard preparation:

Cefuroxime axetil was transferred to volumetric flask and dissolved in methanol to achieve a concentration of 1mgmL^{-1}. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL.

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed, transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1mg/mL. An aliquot solution was diluted with water to get a final concentration of 10µg/mL.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1mg/mL. An aliquot solution was diluted with water to get a final concentration of 10µg/mL. Before the sample, bulk and standard solutions were filtered through a 0.45µm membrane filter.

Chromatographic conditions:

Column : C 18 (Reversed Phase)

Stationary Phase: Silica

Elution type : Isocratic

Mobile phase : Methanol: Water (6:4)

Detector : PDA detector

Flow rate : 1 mL/min.

Determination of Retention Time:

The mobile phase was injected first to determine the absence of any interference with the base line. The retention time was then determined by injecting 20 µL of the standard in the column and the retention time was determined using 278 nm as the detection wavelength. The retention time was found to be 9.2 and 10.3mins for Cefuroxime axetil.

Analysis of sample solution:

The sample solution was diluted to get the required concentration and used for the estimation of Cefuroxime axetil. 20 µL of the solution was injected into the column, retention time and peak area was determined.

Assay:

20 µL of standard and sample solutions were injected separately with the flow rate of 1mL/minute of the mobile phase containing Methanol and Distilled water of (6:4) proportion. The amount of Cefuroxime axetil was calculated from the obtained chromatogram.

$$\textbf{Amount present} = \frac{\textbf{Sam. Area} \times \textbf{Std. Wt} \times \textbf{Dil. factor} \times \textbf{Avg. Wt} \times \textbf{Purity of Std}}{\textbf{Std. Area} \times \textbf{Sam. Wt} \times \textbf{10}}$$

Percentage content of Cefuroxime axetil

$$\textbf{Percentage content} = \frac{\textbf{Amount present}}{\textbf{Label claim}}$$

3.1 INTRADAY STUDY BY HPLC OF BULK AND FORMULATION

Hydrolytic Degradation Using 0.1N NaOH

Standard preparation

50mg of Cefuroxime axetil was weighed and transferred to 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL to achieve a concentration of 1mg/mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL.

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in 0.1N NaOH and the volume made up to 50mL to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in 0.1N NaOH and the volume made up to 50mL to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 0.1N NaOH was taken in a 50mL volumetric flask. After 90mins, as before solution was injected in to the column.

HYDROLYTIC DEGRADATION USING 0.1N HCl

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 0.1N HCl to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using0.1N HCl to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 0.1N HCl was taken in a 50mL volumetric flask. After 90mins, as before solution was injected in to the column.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. After 90mins, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 30% H_2O_2 was taken in a 50mL volumetric flask. After 90mins, as before solution was injected in to the column.

3.2 INTERDAY STUDY BY HPLC OF BULK AND FORMULATION

Hydrolytic Degradation Using 0.1N NaOH

Standard preparation

50mg of Cefuroxime axetil was weighed and transferred to 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL to achieve a concentration of 1mg/mL. An aliquot solution was diluted with distilled water to get a final concentration of 10µg/mL. Standard should be prepared daily.

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in 0.1N NaOH and the volume made up to 50mL to achieve a concentration of 1mg/mL. Then the third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in 0.1N NaOH and the volume made up to 50mL to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 0.1N NaOH was taken in a 50mL volumetric flask. An aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

HYDROLYTIC DEGRADATION USING 0.1N HCl

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 0.1N HCl to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 0.1N HCl to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 0.1N HCl was taken in a 50mL volumetric flask. An aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to a 50mL volumetric flask. It was dissolved in methanol and the volume made up to 50mL using 30% H_2O_2 to achieve a concentration of 1mg/mL. The third day, an aliquot solution was diluted with mobile phase to get a concentration of 10µg/mL. The retention time and peak area were determined by recording the chromatograms.

Blank preparation

50mL of 30% H_2O_2 was taken in a 50mL volumetric flask. The third day, an aliquot solution was diluted with distilled water to get a final concentration. The blank solution was injected in to the column.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, 50mg Cefuroxime axetil bulk was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in mobile phase and the volume made up to 50mL. An aliquot solution was diluted with mobile phase to get a final concentration of 10µg/mL.

Sample preparation (stress)

1g of Cefuroxime axetil tablets were crushed weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, 50mg equivalent of Cefuroxime axetil tablet was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with mobile phase to get a final concentration of 10µg/mL.

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed under a sun light. The third day, 50mg Cefuroxime axetil bulk was weighed from a petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with mobile phase to get a final concentration of 10µg/mL.

Sample preparation (stress)

1g of Cefuroxime axetil tablets were crushed weighed and transferred to a petri dish. This petri dish was placed under a sun light. The third day, 50mg equivalent of Cefuroxime axetil was taken from the petri dish and transferred to 50mL volumetric flask. It was dissolved in ethanol and the volume made up to 50mL. An aliquot solution was diluted with mobile phase to get a final concentration of 10µg/mL.

4. THIN LAYER CHROMATOGRAPHY

TLC is used for identification of degradation whether the drug is degraded or not. Presence of impurities and no of impurities were detected by no of spots were shown after the detection. Various mobile phases given below have been tried for Cefuroxime axetil.

- Hexane: Ethyl acetate (6:4)
- Chloroform: Toluene: Methanol (6:2:2)
- Chloroform: Methanol: Water (6:2:2)
- Chloroform: Methanol (5:5)
- Chloroform: Ethanol (9:1)

Mobile phase containing Chloroform and Ethanol (9:1) were chosen for the study as it gave better resolution. Detection was carried out at by using UV chamber at λ max 366nm.

Rf value was calculated by following formula.

$$\boldsymbol{Rf} = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent}$$

Mobile phase preparation

Mobile phase was prepared by using chloroform and ethanol in the ratio of 9:1. Then the mobile phase was allowed for saturation. Mobile phase should be prepared freshly. Detection was done by UV chamber at λ max 366nm.

Intraday TLC study of Bulk & Formulation (Table: 6)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	90mins
Hydrolysis	0.1N HCL	Room temperature	90mins
	0.1N NaOH	Room temperature	90mins
	Acid Control (no API)	Room temperature	90mins
	Base Control (no API)	Room temperature	90mins
Oxidative	30%H_2O_2	Room temperature	90mins
	30%H_2O_2(no API)	Room temperature	90mins

Interday TLC study of bulk & formulation (Table: 7)

DEGRADATION	EXPERIMENTAL CONDITIONS	STORAGE CONDITION	SAMPLING TIME
	Control Sample (No acid or base)	Room temperature	3rd day
Hydrolysis	0.1N HCL	Room temperature	3rd day
	0.1N NaOH	Room temperature	3rd day
	Acid Control (no API)	Room temperature	3rd day
	Base Control (no API)	Room temperature	3rd day
Oxidative	30%H_2O_2	Room temperature	3rd day
	30%H_2O_2(no API)	Room temperature	3rd day
Thermal	Heating chamber	50ºC	3rd day
Photolysis	Powder form	Sunlight	3rd day

1. INTRADAY HYDROLYTIC DEGRADATION USING 0.1N NaOH

Preparation of standard

10mg of Cefuroxime axetil was dissolved in 10mL of methanol and used as a standard. Standard should be prepared freshly.

Detecting chamber: UV chamber

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved in 0.1N Sodium hydroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the mobile phase containing TLC chamber for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to volumetric flask. It was dissolved in 0.1N sodium hydroxide was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

HYDROLYTIC DEGRADATION USING 0.1N HCl

Bulk preparation (stress)

50mg of Cefuroxime axetil was transferred to volumetric flask and dissolved in 0.1N Hydrochloric acid to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as P. Then standard was applied and denoted as D. Then the TLC was placed in the mobile phase containing TLC chamber for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were weighed and transferred to volumetric flask; dissolved in 0.1N Hydrochloric acid was added to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was noted as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

OXIDATIVE DEGRADATION USING 30% H_2O_2

Bulk preparation (stress)

50mg of Cefuroxime axetil was weighed and transferred to volumetric flask and dissolved in 30% Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After the sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

Sample preparation (stress)

50mg equivalent of Cefuroxime axetil tablets were crushed weighed and transferred to volumetric flask dissolved in 30%Hydrogen peroxide to achieve a concentration of 1mg/mL. The solution was kept at room temperature. After 90mins, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After the sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

4.2.INTERDAY DEGRADATION USING 0.1N NaOH, 0.1N HCl AND 30% H_2O_2

Same procedure was adopted as intraday procedure. After three days exposure samples were collected.

THERMAL DEGRADATION AT 50ºC

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, the sample was spotted on the TLC plate using micropipette. This was noted as D. Then standard was applied and marked as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

Sample preparation (stress)

1g of Cefuroxime axetil tablets were weighed and transferred to a petri dish. This petri dish was placed in a hot air over at the temperature of 50ºC. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

PHOTOLYTIC DEGRADATION USING SUN LIGHT

Bulk preparation (stress)

1g of Cefuroxime axetil bulk was weighed and transferred to a petri dish. This petri dish was placed under a sun light. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection. .

Sample preparation (stress)

1g of Cefuroxime axetil tablets were weighed and transferred to a petri dish. This petri dish was placed under a sun light. The third day, the sample was spotted on the TLC plate using micropipette. This was marked as D. Then standard was applied and denoted as P. Then the TLC was placed in the TLC chamber containing mobile phase for elution. After sufficient elution TLC plate was removed from the TLC chamber and placed in an UV chamber for detection.

RESULTS AND DISCUSSION

1. Hydrolytic Degradation Study Using 0.1N Sodium hydroxide

Hydrolytic degradation study was carried out as per the procedure given in the material and methods. The assay value of active ingredients was calculated using UV spectrophotometry. The respective UV spectrum and the values are given in Fig.1-4 and Table: 8, 9.

Fig.1 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 0.1N NaOH

30mins **90mins**

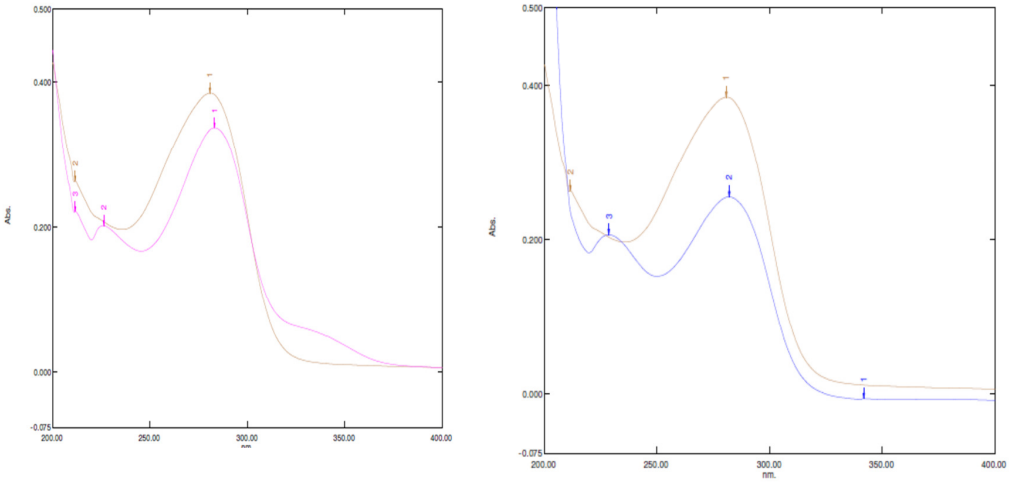


Fig.2 Overlay spectrum of Cefuroxime axetil Sample with Standard in 0.1N NaOH

30mins **60mins**

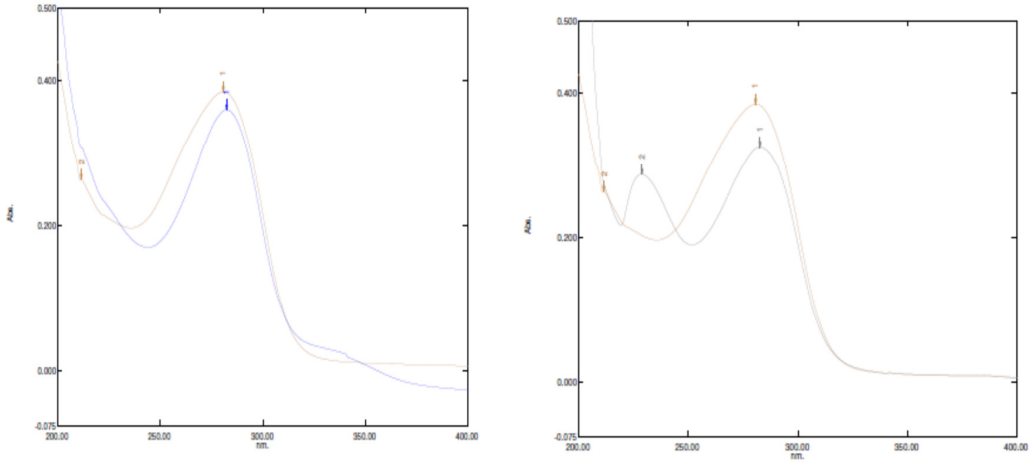


Fig.3 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 0.1N NaOH

1st day **5th day**

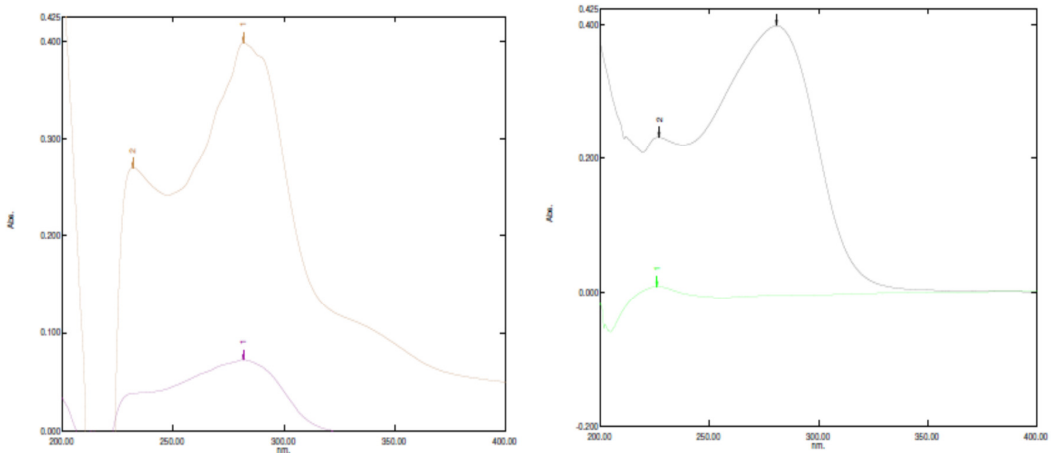


Fig.4 Overlay spectrum of Cefuroxime axetil Sample with Standard in 0.1N NaOH

1st day **5th day**

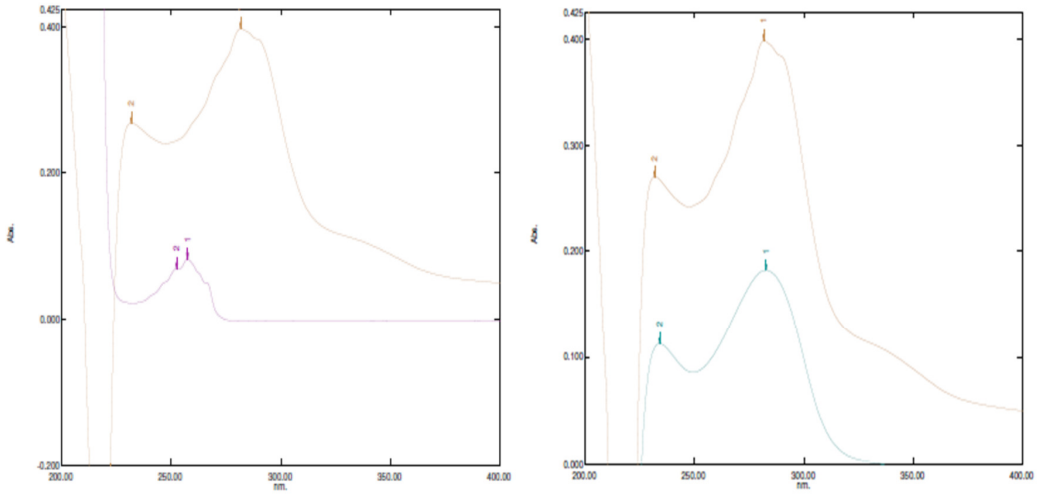


Table: 8 Intraday results of Hydrolytic Degradation Using 0.1 N NaOH

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.331	0.399	30mins
2		0.345		
3		0.338		
1	Sample	0.360		
2		0.354		
3		0.366		
1	Bulk	0.350	0.397	60mins
2		0.345		
3		0.355		
1	Sample	0.332		
2		0.329		
3		0.325		
1	Bulk	0.256	0.398	90mins
2		0.252		
3		0.260		
1	Sample	0.326		
2		0.331		
3		0.321		

Table.8.1: Results Obtained From Hydrolytic Degradation0.1 N NaOH

Stress condition (Alkali Hydrolysis)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1 N Sodium Hydroxide	30mins	84.9	90.5
	60mins	78.1	83.2
	90mins	64.3	81.9

- Each value is the mean of three determinations.

Table: 9 Interday Result of Hydrolytic Degradation Using 0.1 N NaOH:

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.066	0.395	1st day
2		0.080		
3		0.072		
1	Sample	0.100		
2		0.093		
3		0.097		

Table.9.1: Results Obtained From Hydrolytic Degradation - 0.1 N NaOH

Stress condition (Alkali hydrolysis)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1 N Sodium Hydroxide	1st day	18.0	24.4
	3rd day	0	0
	5st day	0	0

GRAPHICAL REPRESENTATION OF INTRADAY AND INTERDAY STUDY

Fig.5 Assay values of Bulk and Sample at Various Time Intervals

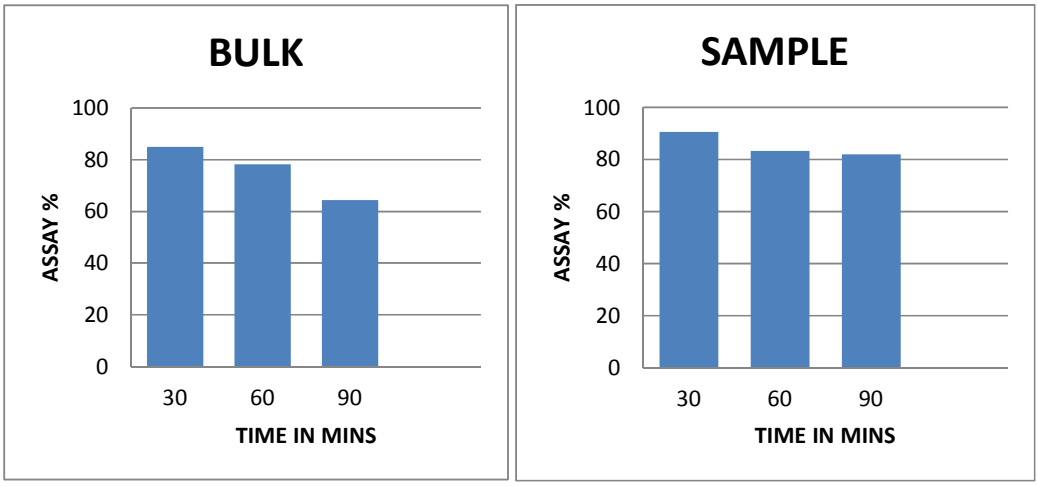
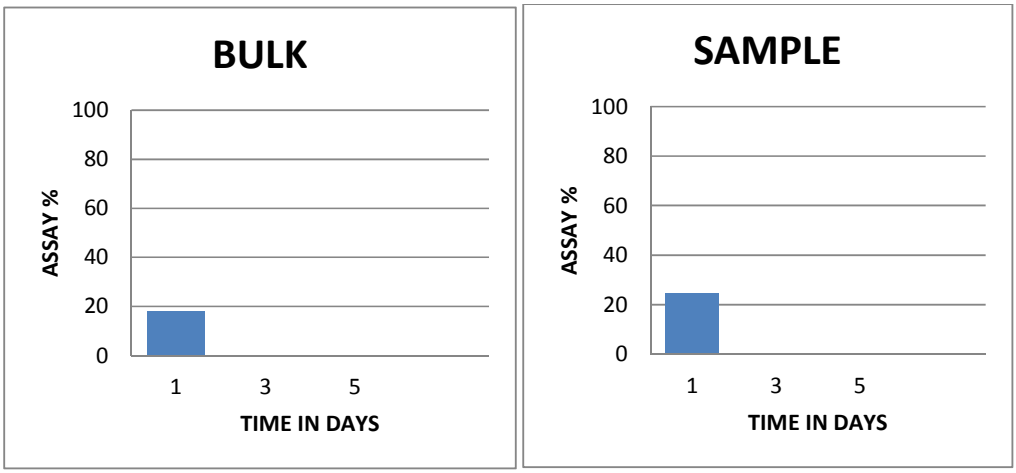


Fig.6 Assay values of Bulk and Sample at Various Time intervals



The study results indicated that Cefuroxime axetil was unstable under alkali hydrolysis condition; the Table: 8 show the results of intraday degradation and remaining assay values of standard and sample. In intraday degradation drug standard and sample showed extensive degradation. Compared to sample, standard had undergone more degradation. The assay values of standard and sample were 64.3% and 81.9% respectively at end of the 90mins degradation. The table: 9 shows that the results of interday degradation. In inter-day degradation study on 1st day standard and sample have undergone maximum degradation. . The assay values of standard and sample were found to be 18.0% and 24.4% respectively. Complete degradation was observed 3rd day onwards.

HYDROLYTIC DEGRADATION USING 0.1N HYDROCHLORIC ACID

Hydrolytic degradation study was carried out as per the procedure given in the material and methods. The assay value of active ingredients was calculated using UV spectrophotometry. The respective UV spectrum and the values are given in Fig.7-10 and Table: 10, 11.

Fig.7 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 0.1N HCl

30mins **90mins**

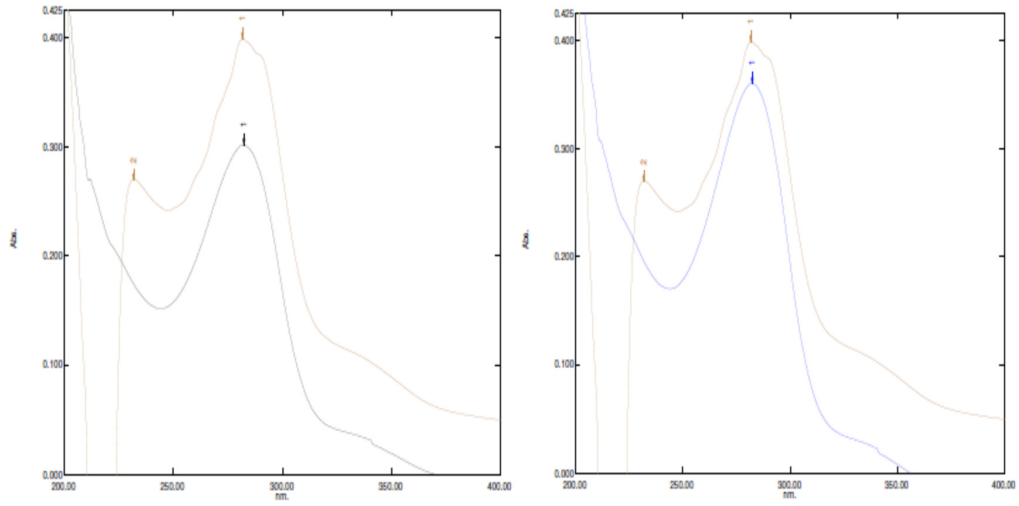


Fig.8 Overlay spectrum of Cefuroxime axetil Sample with Standard in 0.1N HCl

30mins **90mins**

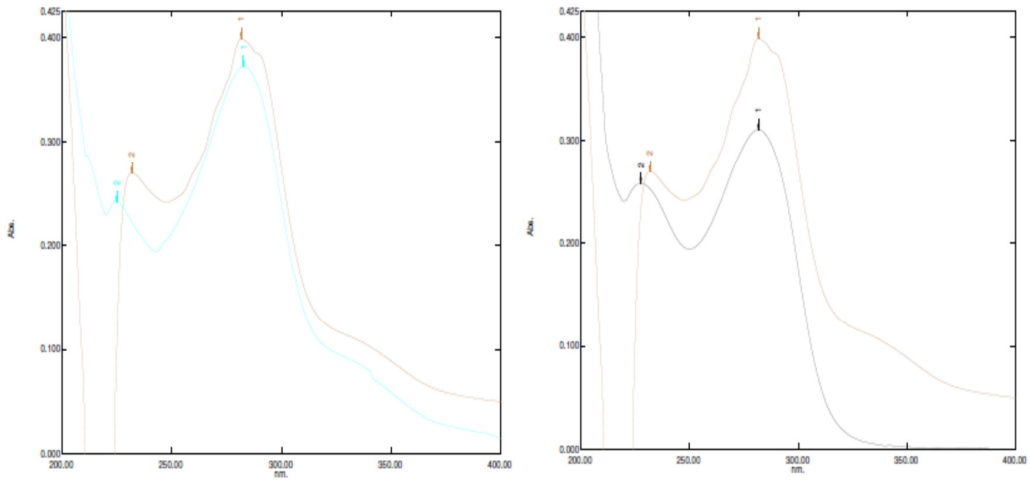


Fig. 9 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 0.1N HCl

1st day **5th day**

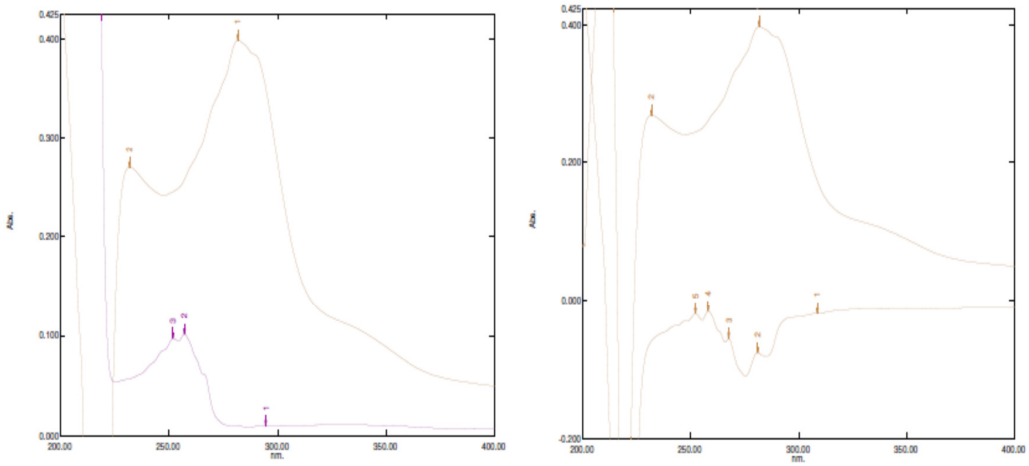


Fig. 10 Overlay spectrum of Cefuroxime axetil Sample with Standard in 0.1N HCl

1st day **5th day**

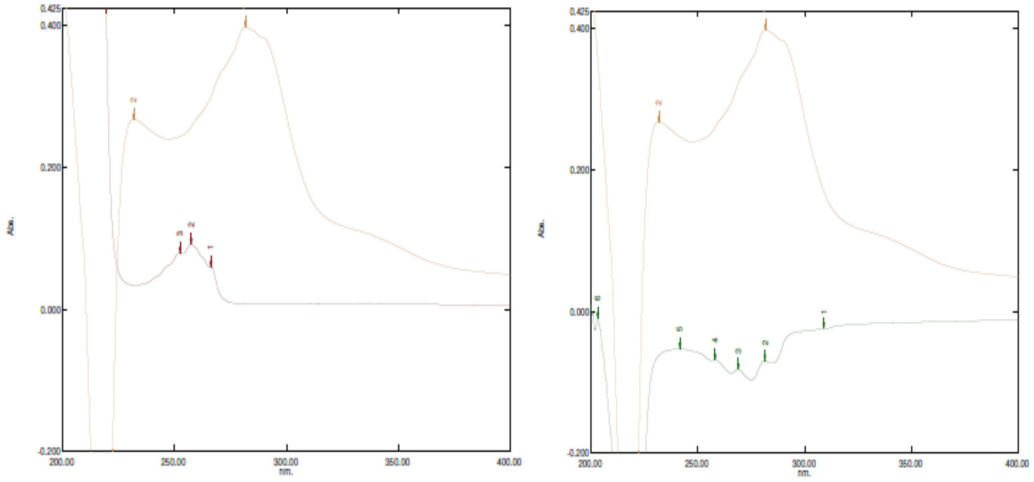


Table 10: Absorbance Values for Hydrolytic Degradation Using 0.1 N HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.355	0.398	30mins
2		0.350		
3		0.360		
1	Sample	0.372		
2		0.378		
3		0.366		
1	Bulk	0.334	0.396	60mins
2		0.339		
3		0.329		
1	Sample	0.334		
2		0.336		
3		0.332		
1	Bulk	0.302	0.397	90mins
2		0.298		
3		0.306		
1	Sample	0.310		
2		0.319		
3		0.301		

Table.10.1: Results Obtained from Hydrolytic Degradation 0.1 N HCl

Stress condition Acid Hydrolysis	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1 N Hydrochloric acid	30mins	89.2	93.4
	60mins	83.9	84.9
	90mins	75.8	78.1

- Each value is the mean of three determinations.

Table: 11 Absorbance Values for Hydrolytic Degradation Using - 0.1 N HCl

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0	0	1st day
2		0	0	
3		0	0	
1	Sample	0	0	
2		0	0	
3		0	0	

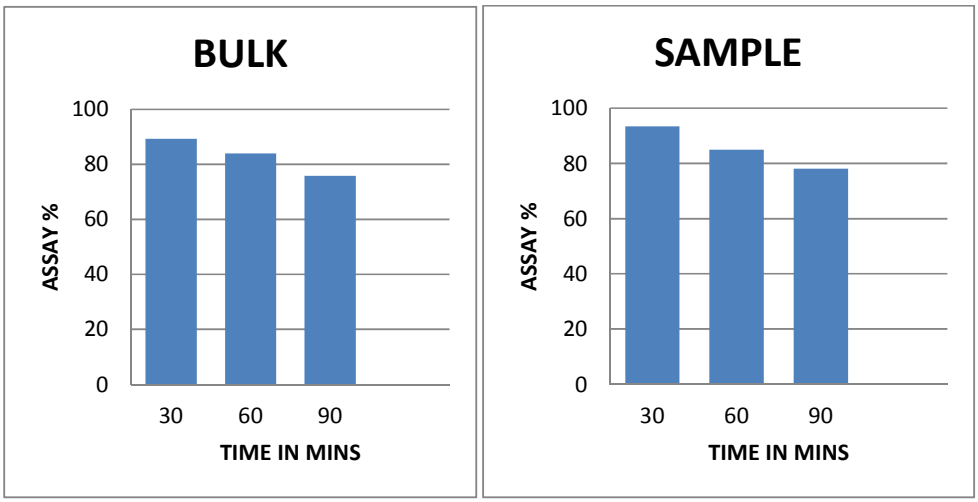
Each value is the mean of three determinations

Table.11.1: Results Obtained From Hydrolytic Degradation - 0.1 N HCl

Stress condition (Alkali hydrolysis)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1 N Hydrochloric acid	1st day	0	0
	3rd day	0	0
	5th day	0	0

GRAPHICAL REPRESENTATION OF INTRADAY AND INTERDAY STUDY

Fig. 11 Assay values of Bulk and Sample at Various Time Intervals



Hydrolytic acid degradation indicated that Cefuroxime axetil was unstable under acid hydrolysis condition. In intraday degradation standard drug and sample showed small amount of degradation. Compared to sample, standard underwent more degradation. The assay values of standard and sample were 75.8% and 78.1% respectively at the end of 90mins degradation. In inter-day degradation study complete degradation was observed on both standard and sample by the end of the first day.

OXIDATIVE DEGRADATION USING 30% HYDROGEN PEROXIDE

Oxidative degradation study was carried out as per the procedure given in the material and methods. The assay value of active ingredients was calculated using UV spectrophotometry. The respective UV spectrum and the values are given in Fig.12-15 and Table: 12, 13.

Fig.12 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 30% H_2O_2

30mins **90mins**

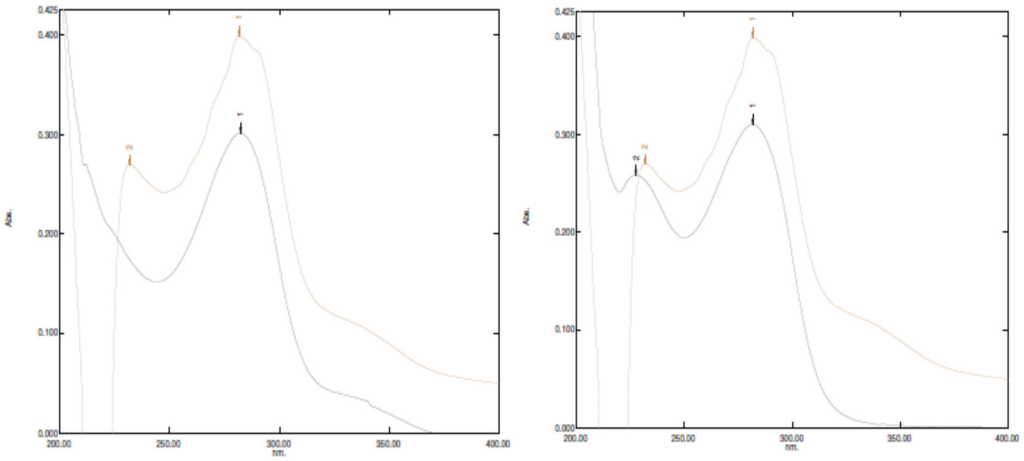


Fig.13 Overlay spectrum of Cefuroxime axetil Sample with Standard in 30% H_2O_2

30mins **90mins**

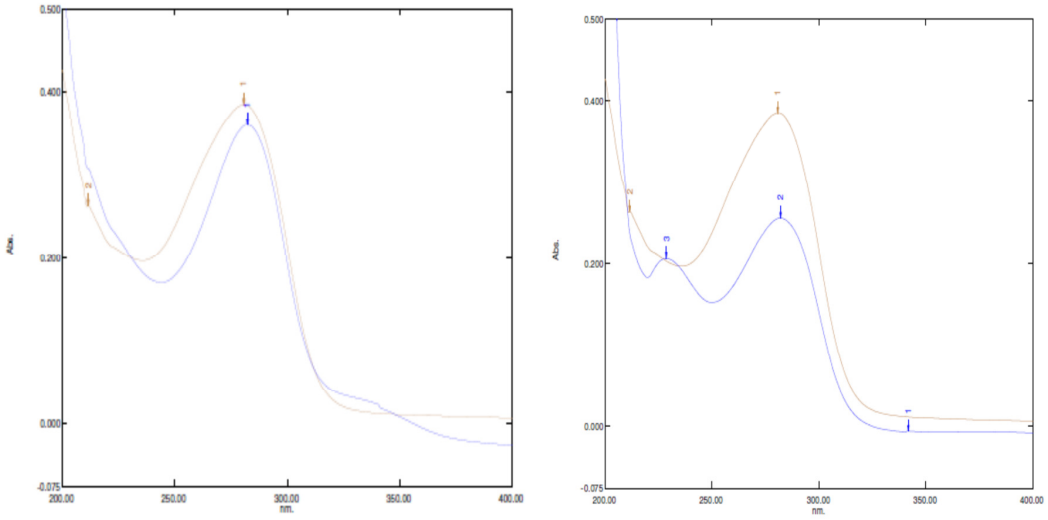


Fig. 14 Overlay spectrum of Cefuroxime axetil Bulk with Standard in 30% H_2O_2

1st day **5th day**

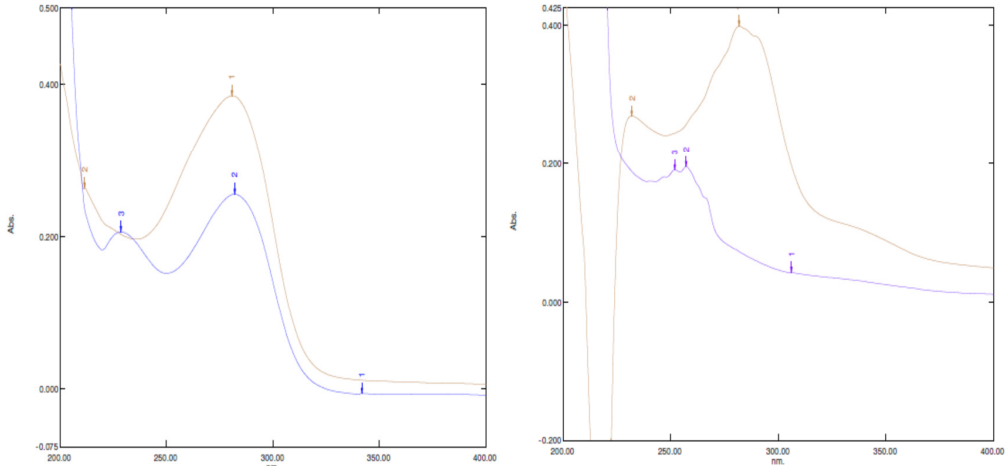


Fig. 15 Overlay spectrum of Cefuroxime axetil Sample with Standard in 30% H_2O_2

1st day **5th day**

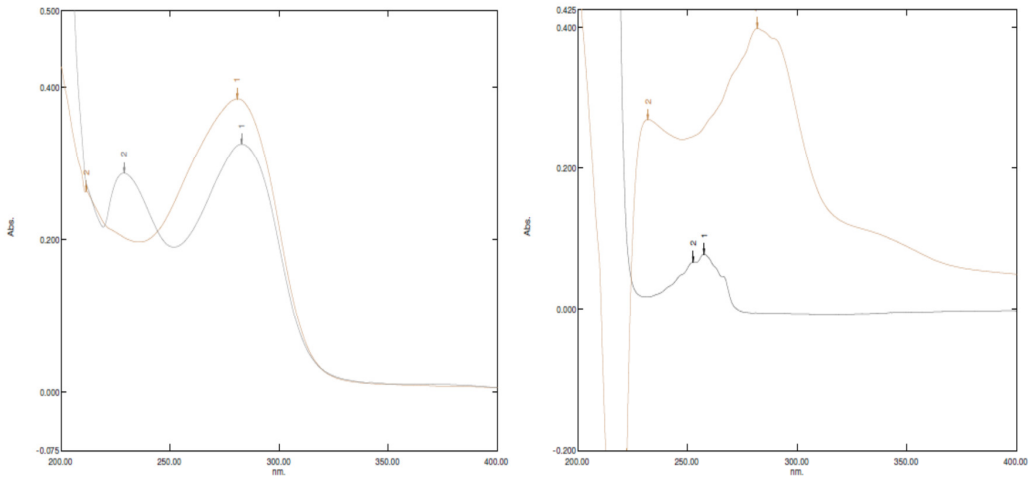


Table: 12 Absorbance Values for Oxidative Degradation Using 30% H_2O_2

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.332	0.398	30mins
2		0.340		
3		0.324		
1	Sample	0.358		
2		0.351		
3		0.365		
1	Bulk	0.305	0.398	60mins
2		0.309		
3		0.301		
1	Sample	0.328		
2		0.333		
3		0.323		
1	Bulk	0.251	0.400	90mins
2		0.250		
3		0.252		
1	Sample	0.306		
2		0.302		
3		0.310		

Table.12.1: Results Obtained from Oxidative Degradation - 30% H_2O_2

Stress condition (Oxidation)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
30% H_2O_2	30mins	83.3	89.9
	60mins	76.6	82.5
	90mins	63.1	76.8

❖ Each value is the mean of three determinations.

Table 13: Absorbance Values For Oxidative Degradation Using - 30% H_2O_2

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.230	0.395	1st day
2		0.216		
3		0.223		
1	Sample	0.250		
2		0.232		
3		0.241		

Table.13.1: Results Obtained From Oxidative Degradation - 30% H_2O_2

Stress condition (Oxidation)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
30% H_2O_2	1st day	56.1	60.6
	3rd day	0	0
	5th day	0	0

GRAPHICAL REPRESENTATION OF OXIDATIVE DEGRADATION STUDY

Fig.16 Assay values of Bulk and Sample at Various Time Intervals

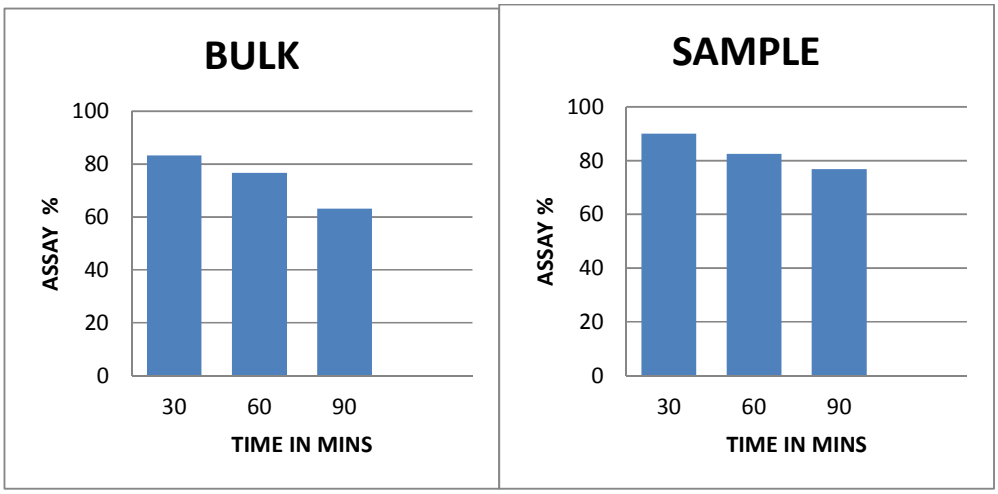
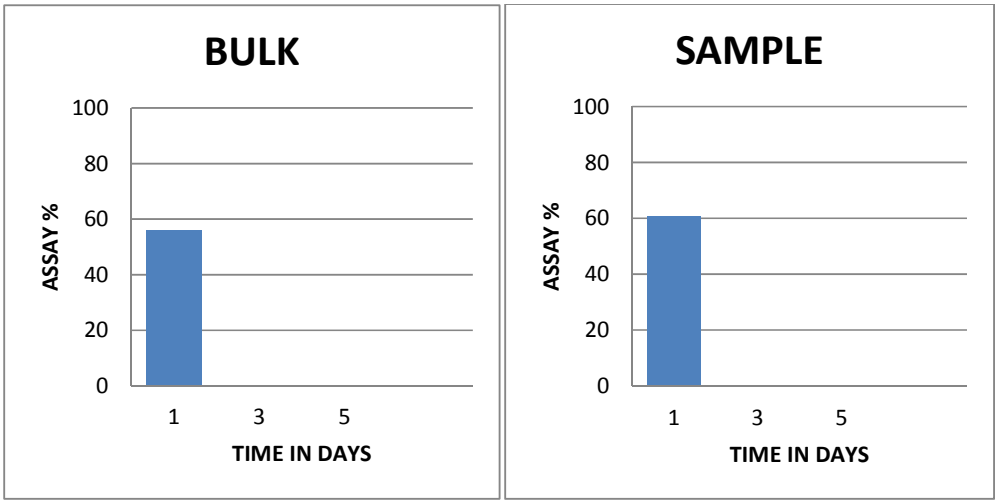


Fig.17 Assay values of Bulk and Sample at Various Time Intervals



Oxidative degradation shows that Cefuroxime axetil was found to be unstable under oxidative condition. Extensive degradation was observed on both standard and sample in intraday and 2nd day onwards. Compared to sample, standard underwent more degradation. The assay values of standard and sample were 63.1% and 73.8% respectively at the end of 90mins degradation. \

In inter-day degradation study, on 1st day standard and sample have undergone maximum degradation. . The assay values of standard and sample were 56.1% and 60.6% respectively. Complete degradation was seen from 3rd day onwards.

THERMAL DEGRADATION

Cefuroxime axetil standard and sample were kept in heating chamber at 50℃. The standard and sample powders were collected at different time intervals and the assay values were calculated by UV spectroscopy. The respective UV spectrum and the values are given in Fig.18, 19 Table-14.

Fig. 18 Overlay spectrum of Cefuroxime axetil Bulk with Standard

1st day **5th day**

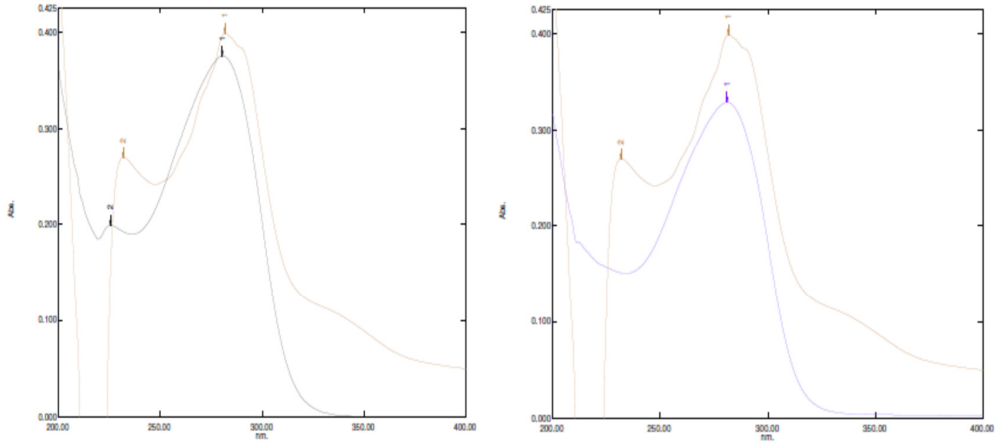


Fig. 19 Overlay spectrum of Cefuroxime axetil Sample with Standard

1st day **5th day**

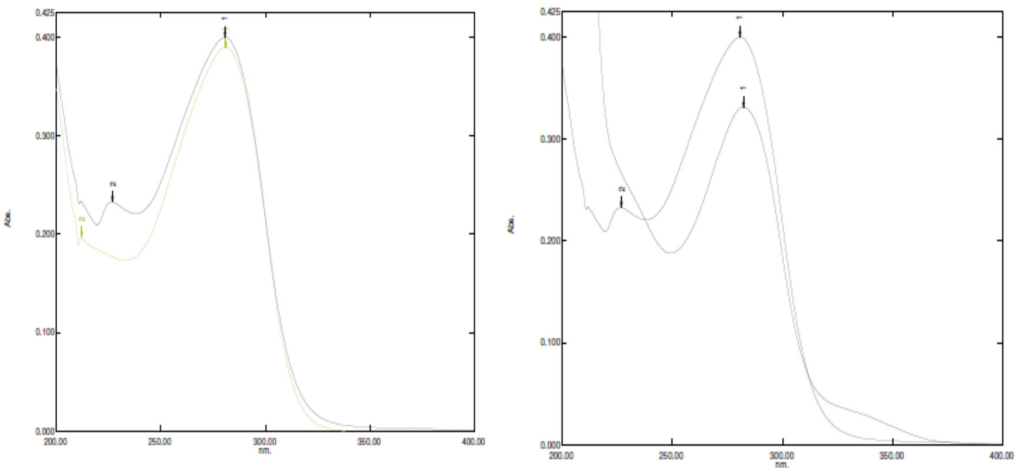


Table 14: Absorbance Values for Thermal at 50ºC

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.382	0.392	1st day
2		0.385		
3		0.379		
1	Sample	0.388		
2		0.391		
3		0.385		
1	Bulk	0.351	0.398	3rd day
2		0.340		
3		0.362		
1	Sample	0.365		
2		0.349		
3		0.333		
1	Bulk	0.330	0.399	5th day
2		0.340		
3		0.335		
1	Sample	0.325		
2		0.332		
3		0.318		

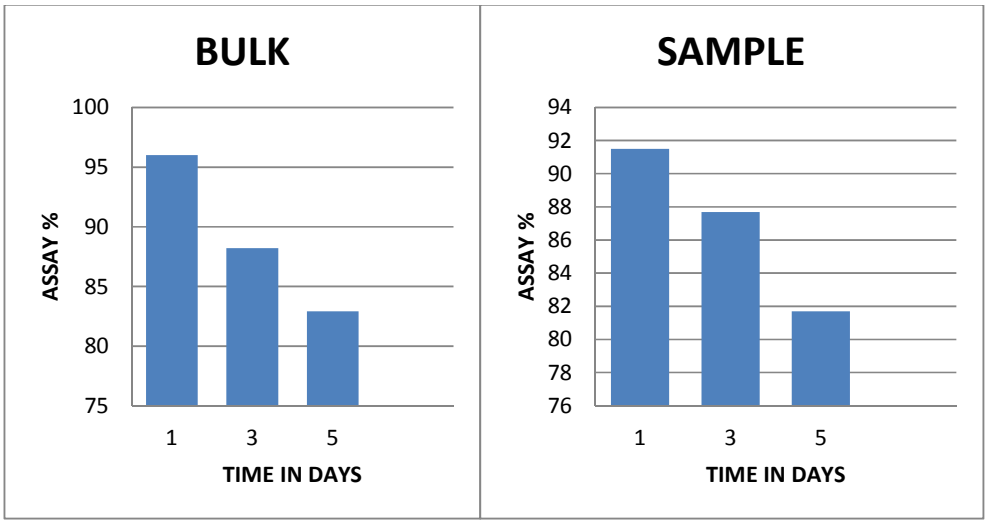
Table.14.1: Results Obtained from Thermal Degradation – 50ºC

Stress condition (Thermal)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
50ºC	1st day	96.0	91.5
	3rd day	88.2	87.7
	5th day	82.9	81.7

- Each value is the mean of three determinations.

GRAPHICAL REPRESENTATION OF THERMAL DEGRADATION STUDY

Fig.20 Assay values of Bulk and Sample at Various Time Intervals



In Thermal degradation only small amount of degradation was observed up to 5th day. It shows that bulk and formulation exhibit stability against temperature. At the end of the 5th day the assay value bulk and sample were found to be 82.9 and 81.7 respectively.

PHOTOLYTIC DEGRADATION USING SUNLIGHT

Cefuroxime axetil standard and sample were kept in heating chamber at 50ºC. The standard and sample powders were collected at different time intervals and the assay values were calculated by UV spectroscopy. The respective UV spectrum and the values are given in Fig.21, 22 Table-15.

Fig. 21 Overlay spectrum of Cefuroxime axetil Bulk with Standard

1st day **5th day**

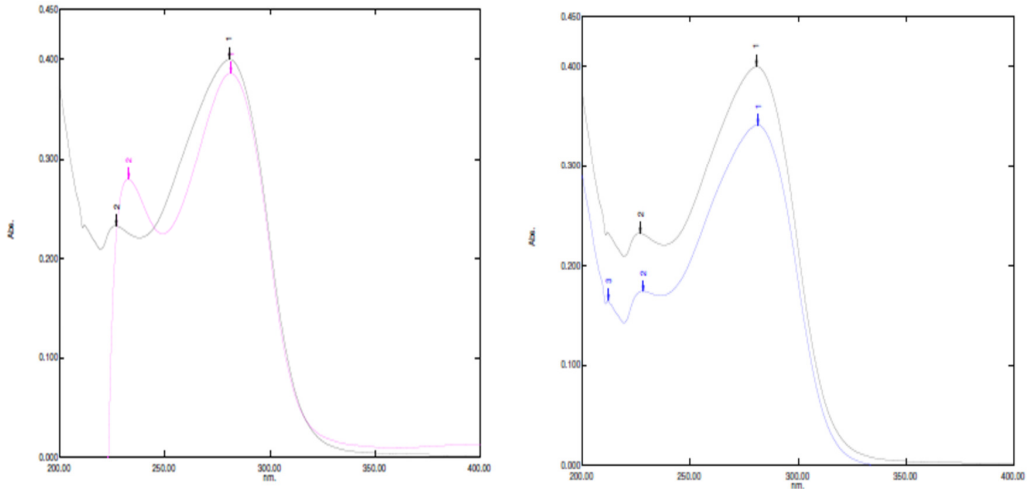


Fig. 22 Overlay spectrum of Cefuroxime axetil Sample with Standard

1st day **5th day**

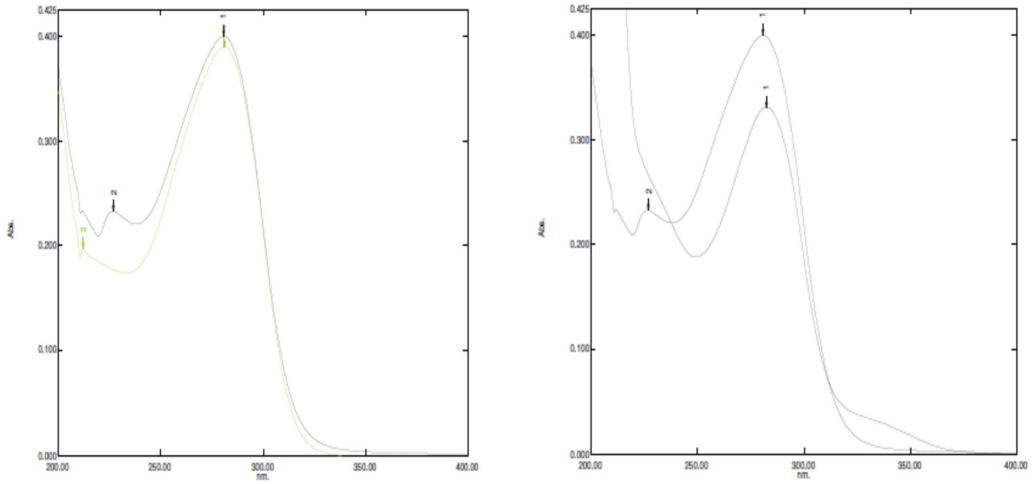


Table 15: Absorbance Values for Photolytic Degradation Using Sunlight

S.No.	Drug	Absorbance	Standard	Time
1	Bulk	0.380	0.399	1st day
2		0.392		
3		0.398		
1	Sample	0.388		
2		0.394		
3		0.382		
1	Bulk	0.363	0.397	3rd day
2		0.371		
3		0.355		
1	Sample	0.340		
2		0.349		
3		0.331		
1	Bulk	0.342	0.399	5th day
2		0.352		
3		0.332		
1	Sample	0.315		
2		0.324		
3		0.306		

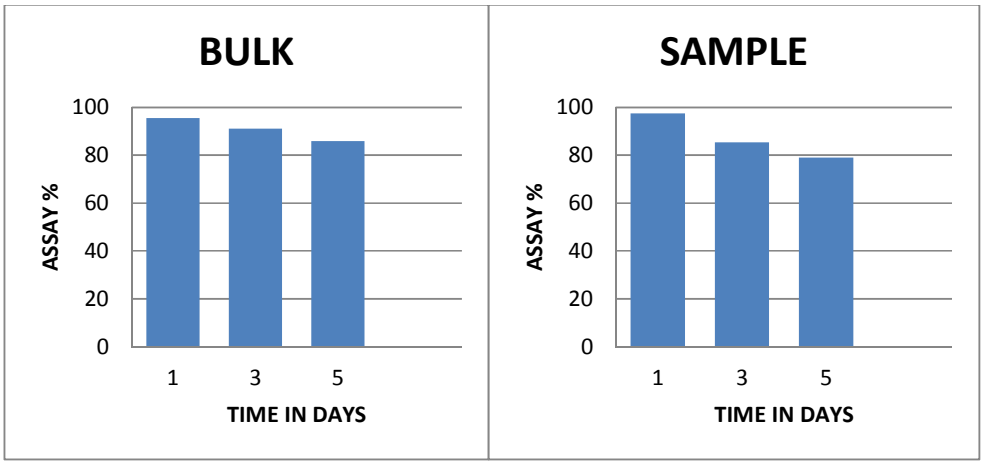
Table.15.1: Results Obtained from Photolytic Degradation – Sunlight

Stress condition (photolytic)	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
Sunlight	1st day	95.5	97.5
	3rd day	91.2	85.4
	5th day	85.9	79.1

- Each value is the mean of three determinations.

GRAPHICAL REPRESENTATION OF PHOTOLYTIC DEGRADATION STUDY

Fig.23 Assay values of Bulk and Sample at Various Time Intervals



In Photolytic degradation studies show that sample was undergone more amount of degradation compared to bulk. The assay values of standard and sample were found to be 85.9% and 79.1% at the end of 5th day degradation.

2) IR STUDY OF BULK&SAMPLE-UNDER STRESS &NORMAL CONDITION:

In interday Thermal and photolytic degradation the samples were kept in heating chamber and sunlight. The next day the samples were taken and ground with KBr. Then the KBr pellets were formed using pellet pressing technique. IR spectrum was taken for pellets. IR spectrum is given in Fig.24-31, Table-16.

THERMAL DEGRADATION

Fig.24 Overlay IR Spectrum of Standard with Bulk in 1st Day

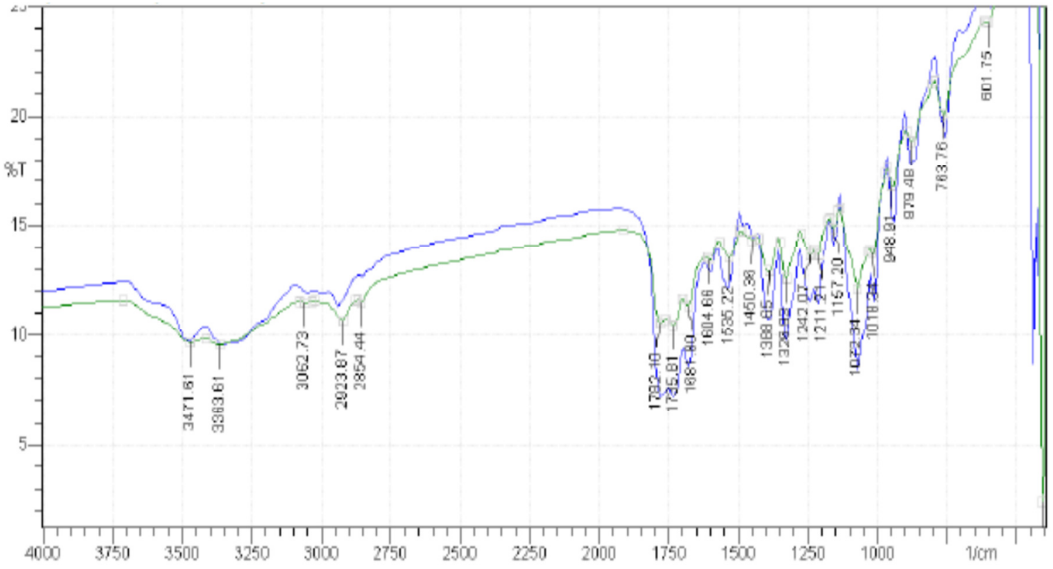


Fig. 25 Overlay IR Spectrum of Standard with Bulk in 5th Day

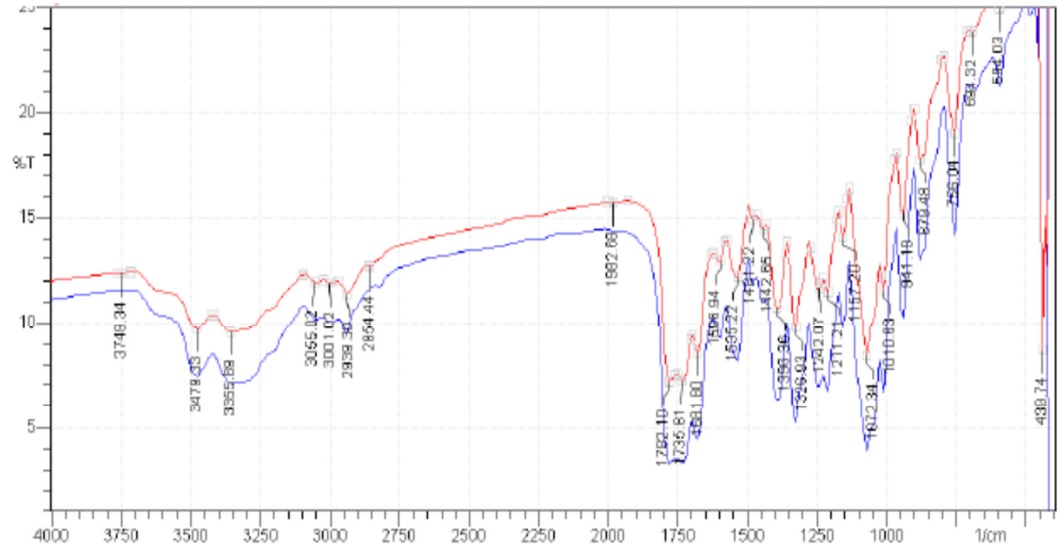


Fig. 26 Overlay IR Spectrum of Standard with Sample in 1st Day

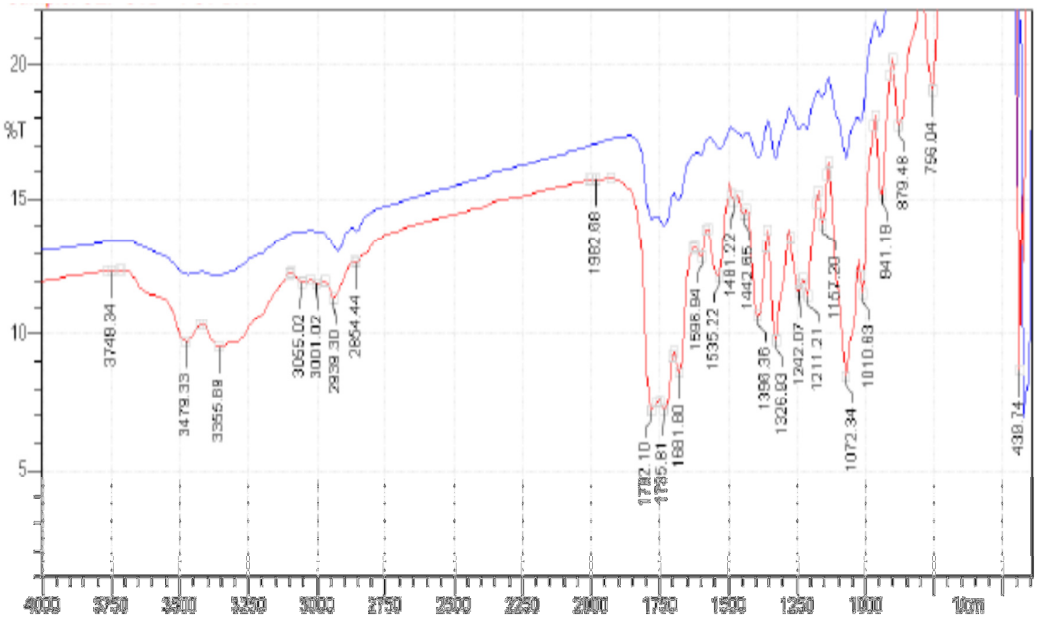
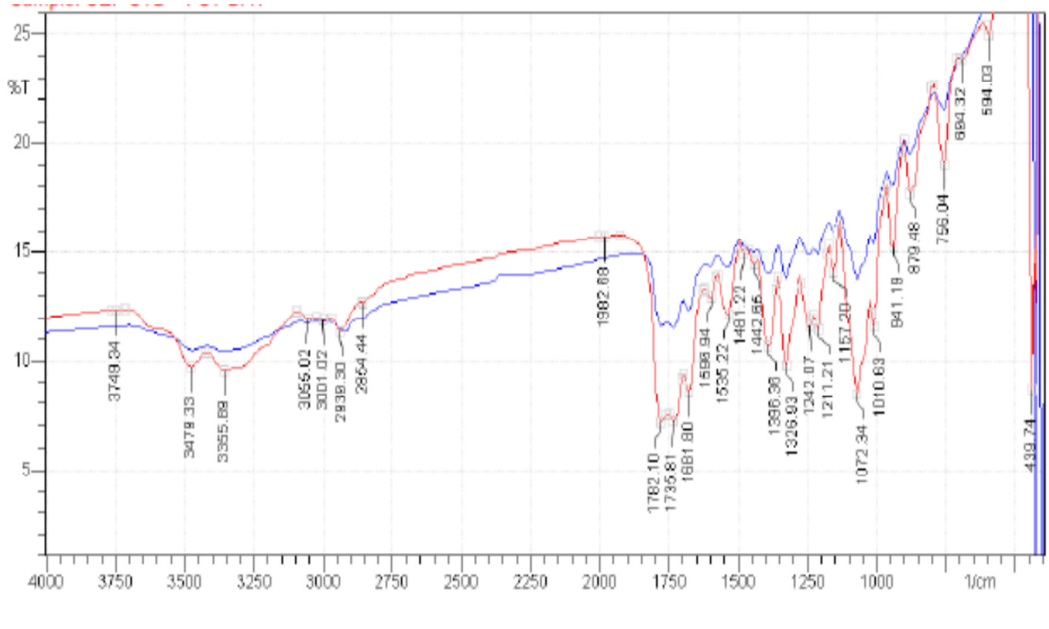


Fig. 27 Overlay IR Spectrum of Standard with Sample in 5th Day



PHOTOLYTIC DEGRADATION USING SUNLIGHT

Fig. 28 Overlay IR Spectrum of Standard with Bulk in 1st Day

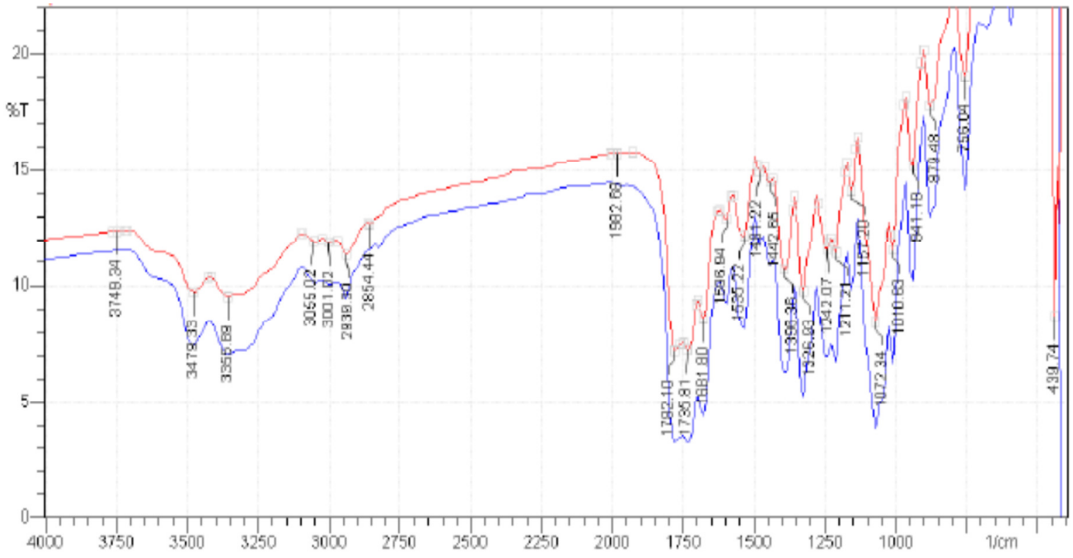


Fig. 29 Overlay IR Spectrum of Standard with Bulk in 5th Day

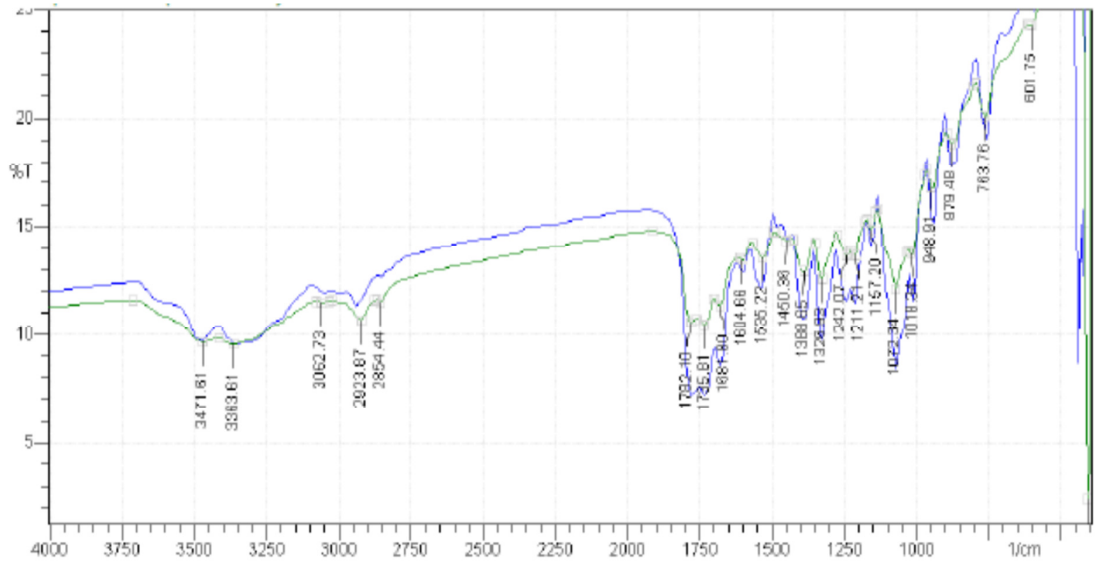


Fig. 30 Overlay IR Spectrum of Standard with Sample in 1st Day

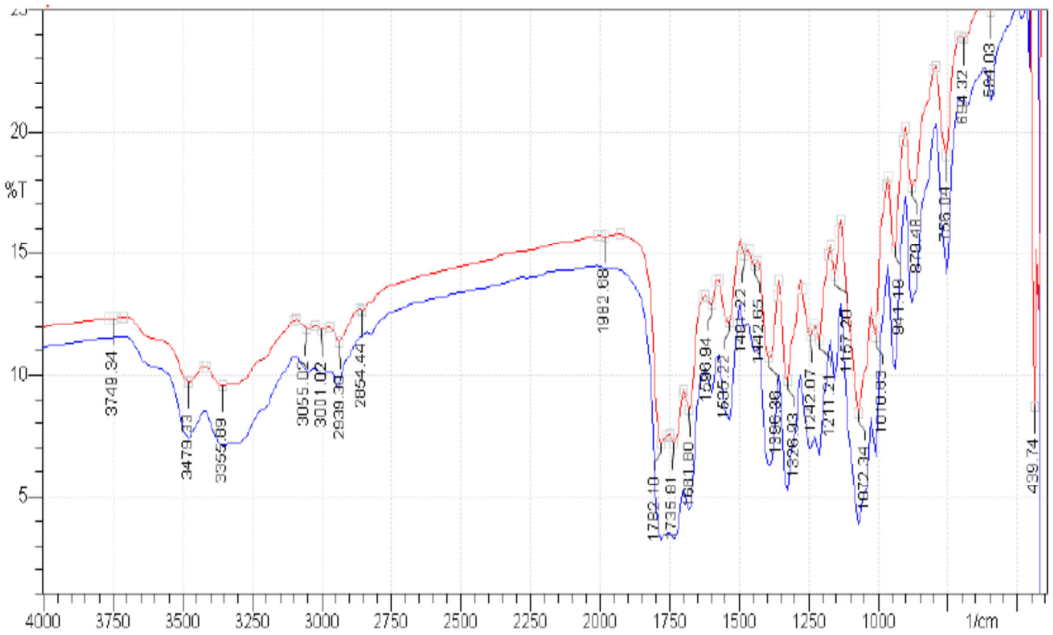


Fig. 31 Overlay IR Spectrum of Standard with Sample in 1st Day

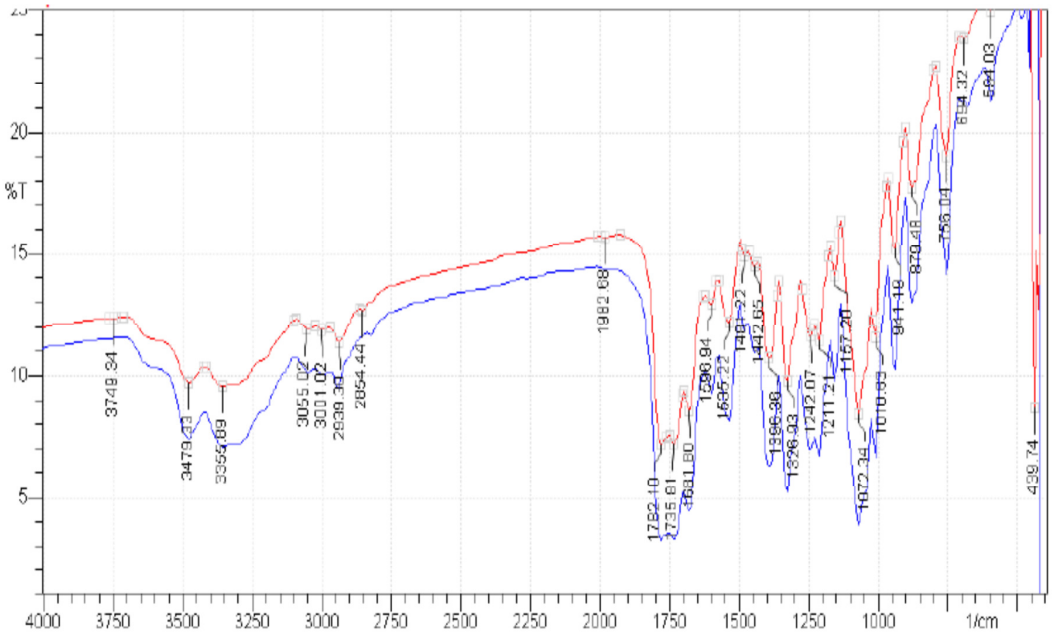


Table: 16 IR STUDY OF THERMAL & PHTOLYTIC DEGRADATION

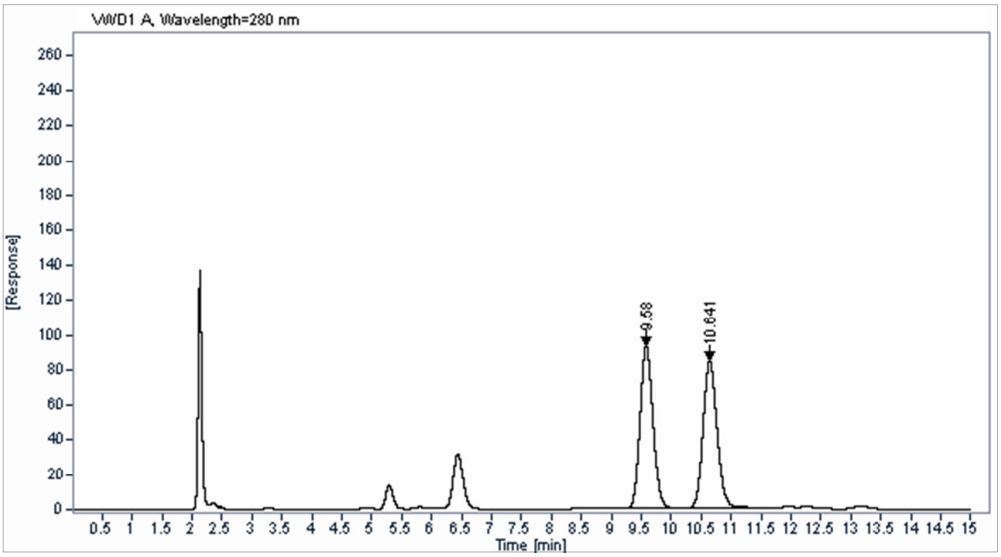
Band Frequency (cm^{-1})	Bulk	Sample	Result
3478	N-H Stretching	N-H Stretching	No changes observed
3001	C-H Stretching	C-H Stretching	No changes observed
1735	C=O Stretching	C=O Stretching	No changes observed
1681	C=N Stretching	C=N Stretching	No changes observed
1596	C=C Stretching	C=C Stretching	No changes observed

It was observed that the standard and sample were less susceptible to Photolytic degradation during the interday study. The assay value was found to be around 80% at the end of 5^{th} day. The degraded samples were subjected to IR study where no structural changes were observed in Thermal and Photolytic degradation.

3) RP-HPLC STUDY OF BULK&SAMPLE-UNDER STRESS CONDITIONS

In RP-HPLC C-18 silica column was found to be suitable for Cefuroxime axetil. The mobile phase used for assay was methanol and water in the ratio of 6:4. The flow rate maintained was 1mL/min. The PDA detector was used in the wavelength of 280nm. The retention time was found to be 9.5 and 10.6. The standard chromatogram is given in Fig.32.

Fig.32 STANDARD CHROMATOGRAM



INTRADAY RP-HPLC STUDY

The chromatograms for intraday study are given in Fig.33-38 and Table-17.

Fig.33 Chromatogram for Cefuroxime axetil Bulk in 0.1N NaOH (90mins)

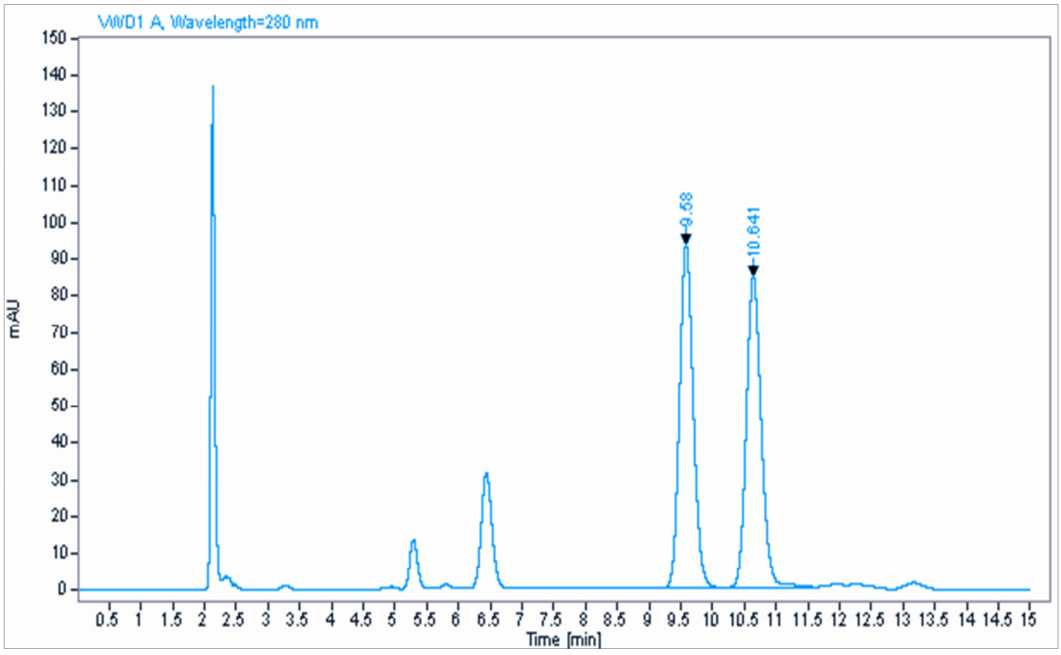


Fig.34 Chromatogram for Cefuroxime axetil Sample in 0.1N NaOH (90mins)

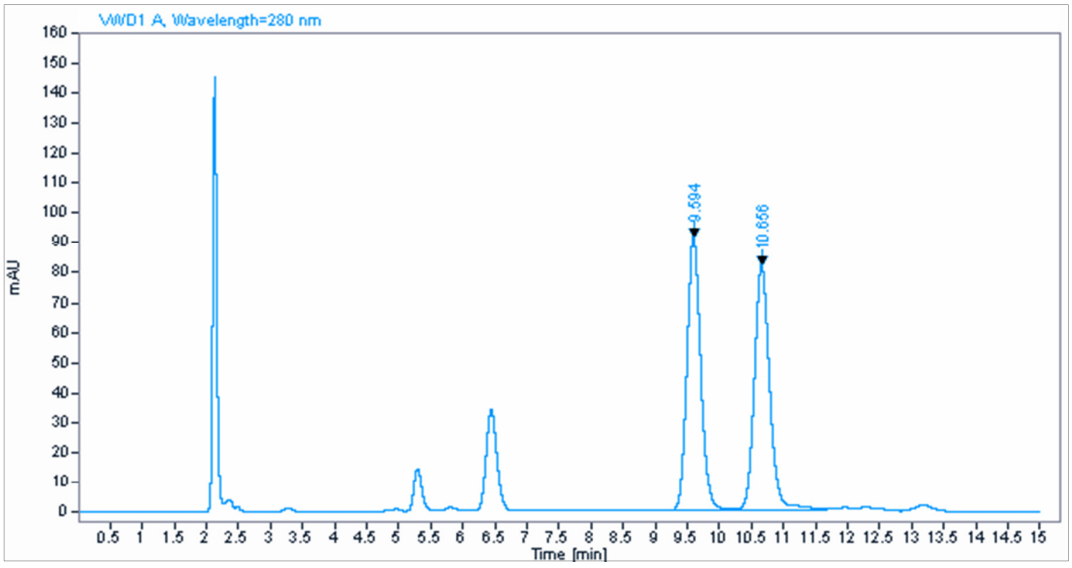


Fig.35 Chromatogram for Cefuroxime axetil Bulk in 0.1N HCl (90mins)

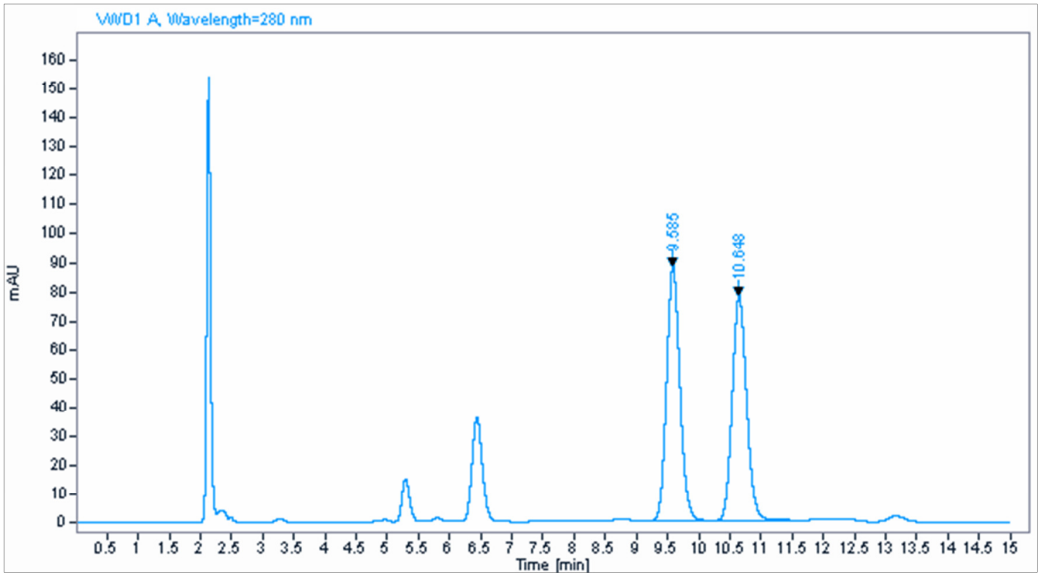


Fig.36 Chromatogram for Cefuroxime axetil Sample in 0.1N HCl (90mins)

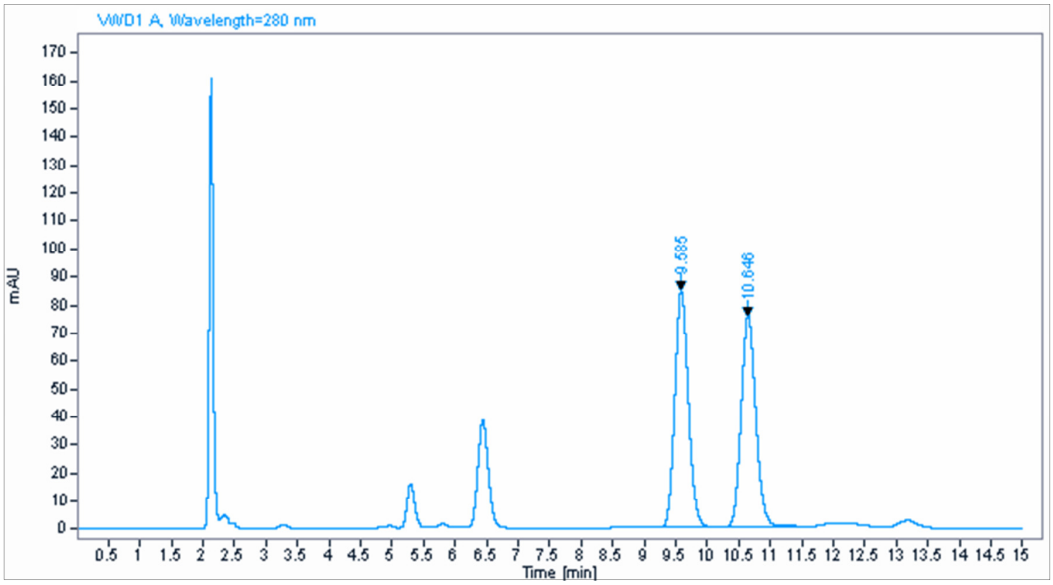


Fig.37 Chromatogram for Cefuroxime axetil Bulk in 30% H_2O_2 (90mins)

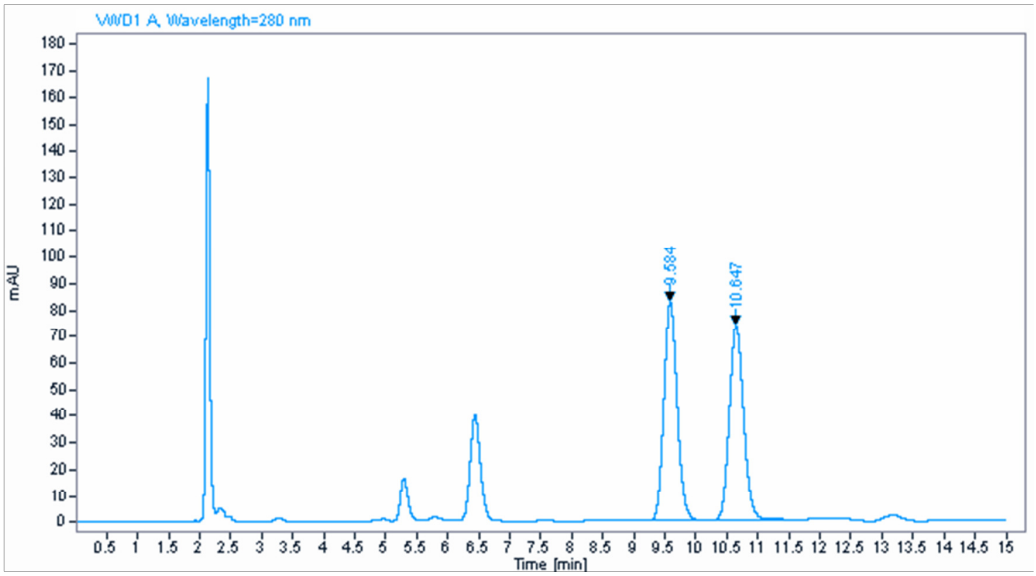


Fig.38 Chromatogram for Cefuroxime axetil Sample in 30% H_2O_2 (90mins)

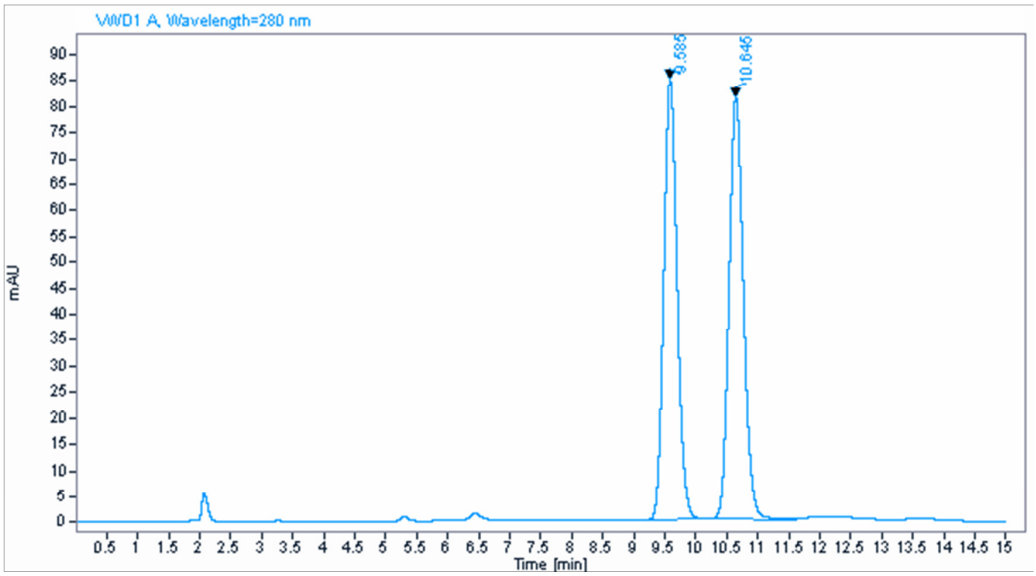


Table: 17 Intraday Study for Degradation

Stress condition	Time	Bulk Peak Area	Sample Peak Area
0.1N NaOH(Alkali)	90mins	942.5	1080.0
0.1N HCl(Acid)	90mins	1050.2	1080.4
30% H_2O_2(Oxidation)	90mins	944.0	1005.5

Stress condition	Time	Bulk Percentage Content (%)	Sample percentage Content (%)
0.1N NaOH(Alkali)	90mins	68.7	78.7
0.1N HCl(Acid)	90mins	76.6	78.8
30% H_2O_2(Oxidation)	90mins	68.8	73.3

Intraday RP-HPLC study indicates that Cefuroxime axetil was undergone moderate degradation in alkali hydrolysis and oxidative degradation compared to acid hydrolysis. It was observed that bulk was undergone more amount of degradation compared to sample.

INTERDAY RP-HPLC STUDY

Chromatograms for interday study are given in Fig.39-44, Table-18.

Fig.39 Chromatogram for Cefuroxime axetil Bulk in 0.1N NaOH (3rd day)

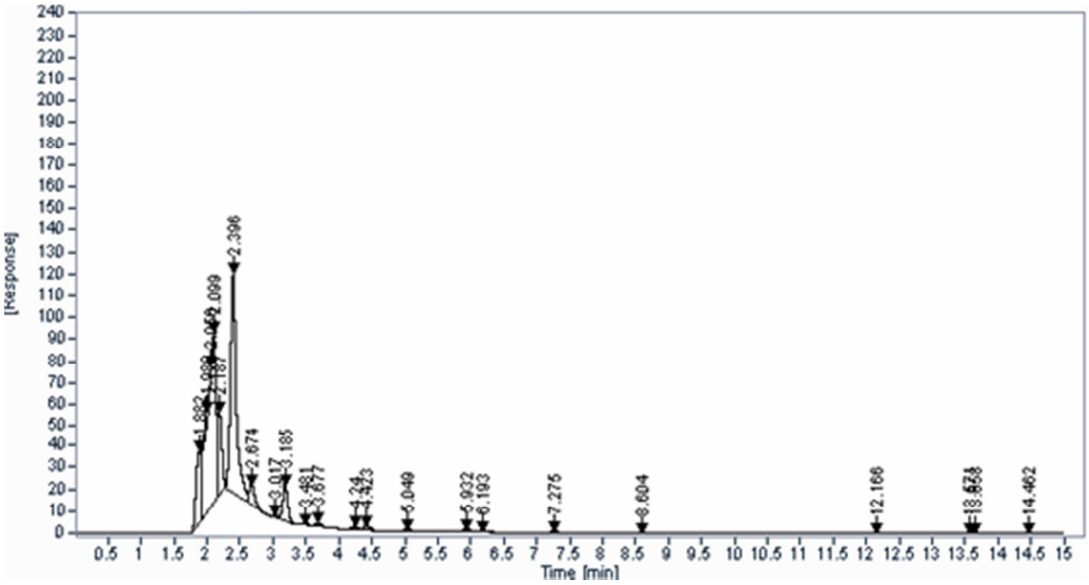


Fig.40 Chromatogram for Cefuroxime axetil Sample in 0.1N NaOH (3rd day)

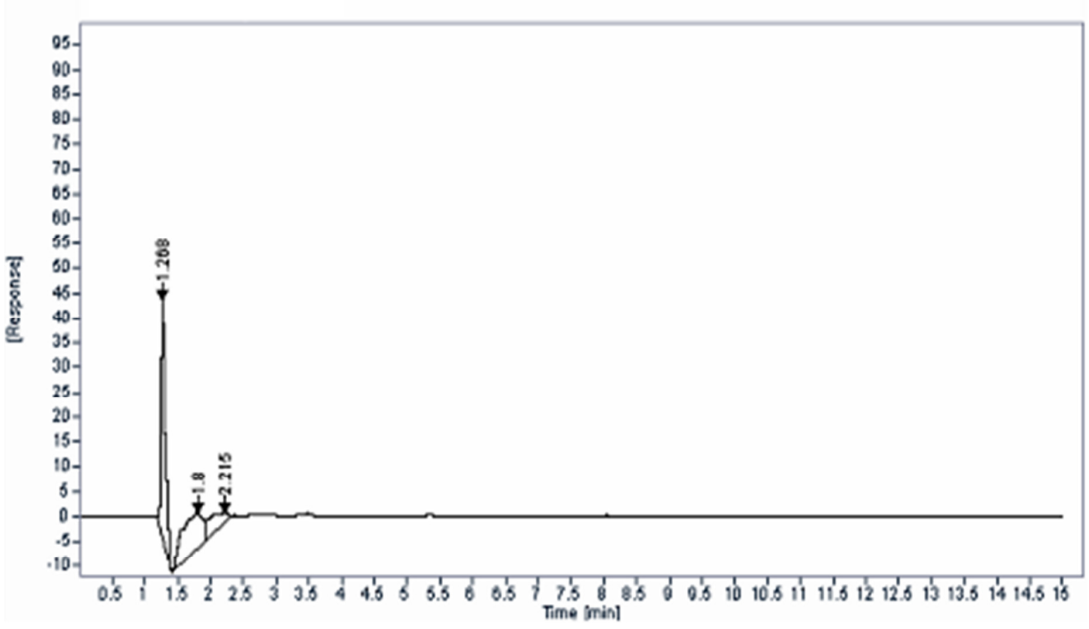


Fig.41 Chromatogram for Cefuroxime axetil Bulk in 0.1N HCl (3rd day)

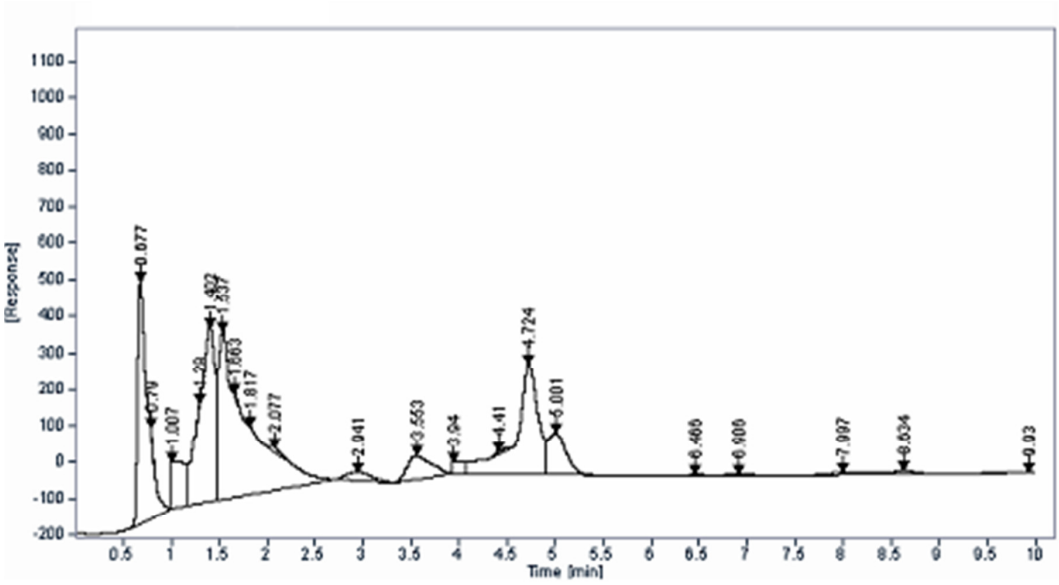


Fig.42 Chromatogram for Cefuroxime axetil Sample in 0.1N HCl (3rd day)

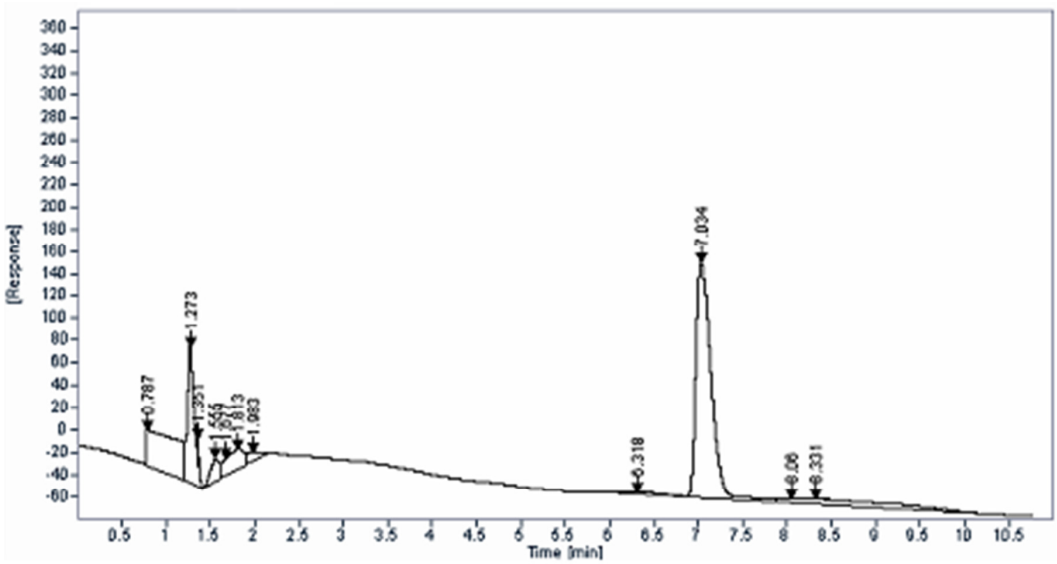


Fig.43 Chromatogram for Cefuroxime axetil Bulk in 30% H_2O_2 (3rd day)

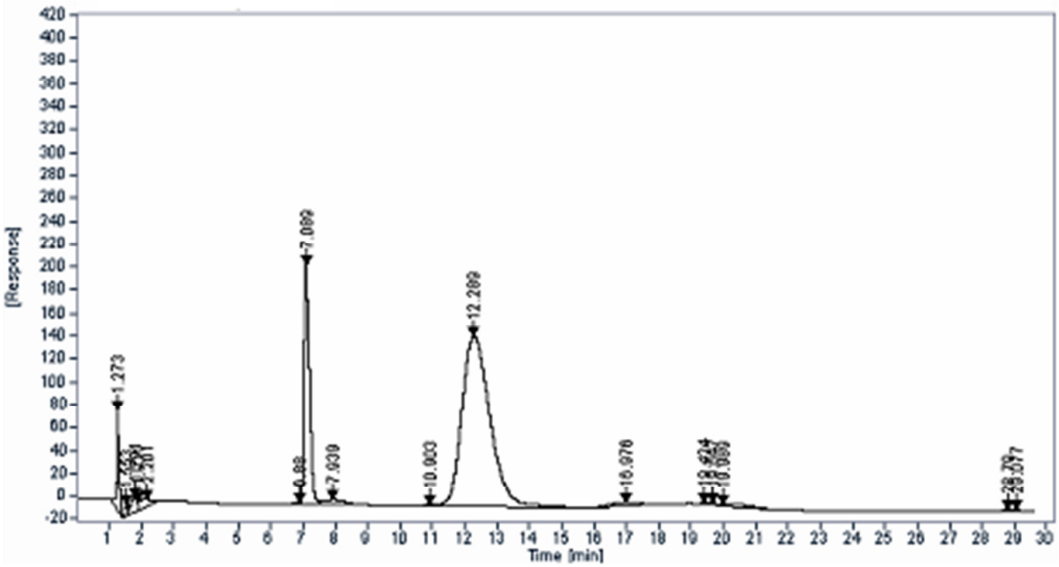


Fig.44 Chromatogram for Cefuroxime axetil Sample in 30% H_2O_2 (3rd day)

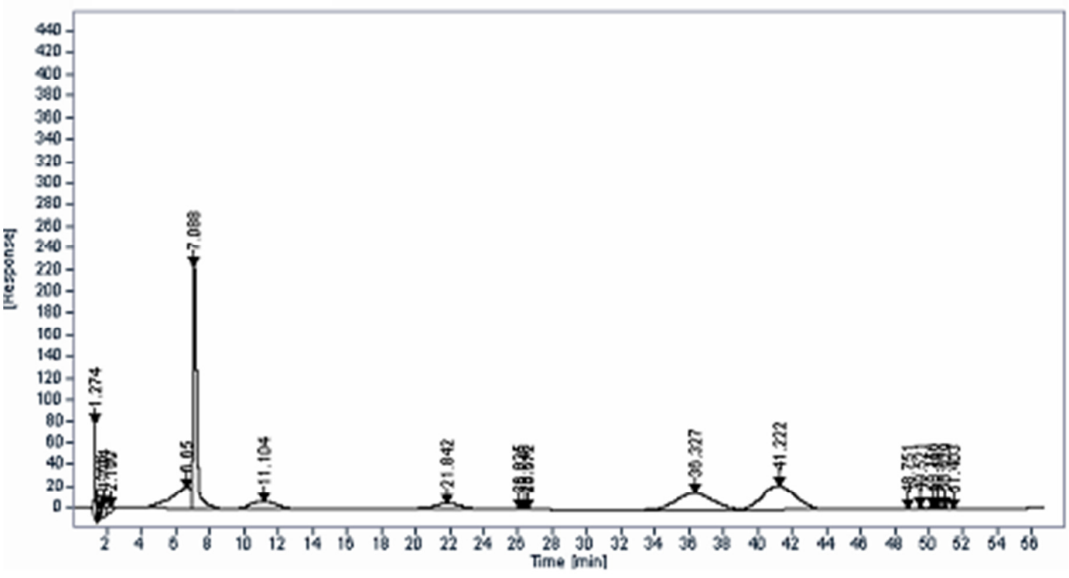


Table: 18 Interday Results for Degradation

Stress condition	Time	Bulk Peak Area	Sample Peak Area
0.1N NaOH(Alkali)	3rd Day	-	-
0.1N HCl(Acid)	3rd Day	-	-
30% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50ºC)	3rd Day	1198.6	1165.0
Sunlight (Photolysis)	3rd Day	1222.2	1128.2

Stress condition	Time	Bulk Percentage Content (%)	Bulk Percentage Content (%)
0.1N NaOH(Alkali)	3rd Day	-	-
0.1N HCl(Acid)	3rd Day	-	-
30% H_2O_2(Oxidation)	3rd Day	-	-
Thermal (50ºC)	3rd Day	87.4	84.9
Sun Light (Photolysis)	3rd Day	89.1	82.2

Complete degradation was observed at the end of 3rd day hydrolytic and oxidative degradation. This indicates that Cefuroxime axetil is vulnerable to hydrolytic and oxidative degradation. Photolytic and Thermal degradation show only small amount of degradation compared to other degradation methods.

4. THIN LAYER CHROMATOGRAPHY

TLC is used for identification of degraded products. The mobile phase used for degradation of Cefuroxime axetil was found to be Chloroform and Ethanol in the ratio of 9:1. Various mobile phases were tried for Cefuroxime axetil. The results of intraday and interday are given Table19 and 20 respectively.

Table: 19 Intraday study

STRESS CONDITION	SAMPLE TYPE	NO OF SPOTS	SECONDARY SPOTS	Rf VALUE
Alkali Hydrolysis 0.1N NaOH	Bulk	1	0	0.51
	Sample	1	0	0.50
Acid Hydrolysis 0.1N HCl	Bulk	1	0	0.60
	Sample	1	0	0.59
Oxidative Degradation 30% H_2O_2	Bulk	1	0	0.55
	Sample	1	0	0.53

It was observed that there was no major degradation in intraday degradation. No secondary spots were observed. The Rf value of standard and sample were found to be around 0.55.

Table: 20 Interday Degradation Study

STRESS CONDITION	SAMPLE TYPE	NO OF SPOTS	SECONDARY SPOTS	Rf VALUE
Alkali Hydrolysis 0.1N NaOH	Bulk	1	0	0.26
	Sample	1	0	0.15
Acid Hydrolysis 0.1N HCl	Bulk	0	0	0
	Sample	0	0	0
Oxidative Degradation 30% H_2O_2	Bulk	1	0	0.98
	Sample	0	0	0
Thermal Degradation	Bulk	1	0	0.55
	Sample	1	0	0.52
Photolytic Degradation	Bulk	1	0	0.55
	Sample	1	0	0.53

Changes in Rf values were observed in hydrolytic and oxidative degradation. But there was no major variation in thermal and photolytic degradation. This shows no major degradation in thermal and photolytic degradation. There was no spot observed in bulk, sample in acid hydrolysis and sample in oxidative degradation.

SUMMARY AND CONCLUSION

The present work entitled as "**FORCED DEGRADATION STUDIES OF CEFUROXIME AXETIL IN BULK AND FORMULATION BY UV, IR SPECTROPHOTOMETRY, TLC, AND RP-HPLC METHOD".** This study is to evaluate the stability of Cefuroxime axetil in bulk and formulation.

The study includes the following

- The Alkali hydrolysis, Oxidative degradation, and Thermal and Photolytic degradation were performed.
- Degraded samples were quantified by UV spectroscopy and HPLC.
- The results of bulk and sample compared with standard.
- Degraded samples were identified by TLC.
- Functional group changes in degraded samples were identified by IR.

Quantification of Cefuroxime axetil in bulk and formulation was done by UV spectroscopy and HPLC method.

Interday studies of Photolytic and Thermal degradation was evaluated by IR spectroscopy to detect changes in functional group.

Cefuroxime axetil bulk and formulation were subjected to Hydrolytic degradation, Oxidative degradation, Thermal and Photolytic degradation.

The main findings of the study are

Compared to sample, standard has undergone more amount of degradation in intraday alkali degradation.

Complete degradation was observed in interday alkali hydrolytic degradation on 3rd day.

It indicates that Cefuroxime axetil is vulnerable to alkali hydrolysis.

Complete degradation was observed in interday acid hydrolytic degradation. It shows Cefuroxime axetil is sensitive to acid.

Oxidative degradative study indicates Cefuroxime axetil is vulnerable to oxidation.

The further scope of study includes

- To develop and validate a stability indicating method
- To determine degradation pathways of drug substances and drug products (E.g. during development phase)
- To identify impurities related to drug substances or excipients
- To understand the drug molecule chemistry
- To generate more stable formulations.

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