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# Development and method validation on stress degradation studies of cefpodoxime proxetil and clavulanic acid in dosage form by hplc method

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**ABSTRACT**

*A simple, precise, accurate and reproducible HPLC method developed and validated on stress degradation studies of Cefpodoxime proxetil and Clavulanic acid in dosage form (tablet). Cefpodoxime proxetil is a prodrug of cefpodoxime acid and supplied as racemic mixture of R- and S- enanatiomers. Clavulanic acid is used as β lactamase inhibitor. Cefpodoxime proxetil and Clavulanic acid were degraded together under different stress conditions by International Conference on Harmonization. The samples so generated were used to develop stability indicating HPLC method for two drugs. The drugs were well separated from degradation products using a reversed phase (C-18) column with a mobile phase composed of 20 mM ammonium acetate-acetonitrile-methanol (70:29:1) pumped at a flow rate of 1.5ml/min. the detection was carried out at 230nm. and the column temperature maintained at 30$^0$C. In this method the S isomer of Cefpodoxime proxetil was not degraded except in thermal condition where as R form of Cefpodoxime proxetil and Clavulanic acid sufficiently degraded in all the conditions. The method was validated for linearity, precision, accuracy, specificity, LOD and LOQ etc. the linearity range of Cefpodoxime proxetil and Clavulanic acid were found to be 150- 750 µg/ml and 200-1000 µg/ml respectively.*

**Keywords**: Cefpodoxime proxetil**,** Clavulanic acid, HPLC.

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## INTRODUCTION

Cefpodoxime proxetil, chemically, [(R, S)-1(isopropoxy carbonyloxy) ethyl (+) - (6R, 7R)-7[2-(2-amino-4-thiazolyl)-2(Z)methoxyiminoacetamido]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0.]Oct-2-ene-2-carboxylate] is an oral third generation abroad spectrum cephalosporin antibiotic. It is active against most gram positive and gram negative bacteria [1]. It is implicated in the treatment of upper respiratory tract and urinary tract infections[2][3]. The drug is official in Indian Pharmacopoeia[4] and United States Pharmacopeia[5]. The recommended dose of cefpodoxime proxetil is 200 to 400 mg per day[6]. The molecular weight of Cefpodoxime Proxetil is 557 [7]. In literature, several analytical methods such as RP-HPLC[7][8][9] and voltammetric [10][11] have been reported for the determination of cefpodoxime proxetil in biological fluids. Few RP–HPLC[12][13] some hyphenated techniques such as LC/MS/MS[14], LC-MS, LC-NMR and LC-IR[15], UVSpectrophotometric[16][17][18] and HPTLC [19][20] methods have been studied for determination of cefpodoxime proxetil in bulk and in pharmaceutical formulations. One RP-HPLC method has been studied for determination of cefpodoxime proxetil in combination with other drugs from pharmaceutical formulation [21].

clavulanic acid (2*R*,5*R*,*Z*)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0] heptane-2-carboxylic acid is a is a ß-lactam, structurally related to the penicillins, which possesses the ability to inactivate a wide range of ß-lactamase enzymes commonly found in microorganisms resistant to penicillins and cephalosporins[22][23]. It has good activity against the clinically important plasmid-mediated ß-lactamases frequently responsible for transferred

drug resistance[24]. it is biosynthetically generated from the amino acid arginine and the sugar glyceraldehyde 3-phosphate combined with penicillin group antibiotics to overcome certain types of antibiotic resistance. It is used to overcome resistance in bacteria that secrete β-lactamase, which otherwise inactivates most penicillins. The name is derived from the *Streptomyces clavuligerus*, which produces clavulanic acid[25][26].

## MATERIALS AND METHODS

*2.1.Chemicals and reagents*

Pure cefpodoxime proxetil was obtained as gift sample from Ranbaxy research laboratories Ltd., Gurgaon; Clavulanic acid was obtained as gift sample from Medreich Ltd, Bangalore. HPLC grade acetonitrile, methanol and ammonium acetate buffer were purchased from Rajesh chemicals. All other chemicals were of analytical reagent grade.

*2.2.Instrumentation*

The HPLC system (Shimadzu Corporation, Japan) comprising of a solvent pump (LC-10AT VP), on-line degasser (DGU-14A), an auto injector (SIL-10AD VP) with temperature control, CTO10AS VP Column oven, UV-visible dual wavelength detector (SPD-10AVP) was utilized for the purpose. Data acquisition, analysis and reporting were done by using Shimadzu LC solution software The separations were achieved on a Thermo scientific ODS hypersil C-18 column (250 mm X 4.6 mm, 5 µm).

*2.3.Preparation and selection of Mobile phases and Chromatographic conditions*

Both drugs cefpodoxime proxetil and clavulanic acid were injected and run in different solvent system. Initially 0.02M ammonium acetate: acetonile: methanol in different ratio (60:39:1, 60:38:2 and 70:29:1) was tried. It was found that 0.02M ammonium acetate: acetonile: methanol (70:29:1) gave acceptable retention time (Rt =3.730 min for Clavulanic acid and Rt = 11.185 min for S form of Cefpodoxime proxetil and Rt = 12.388 min for R form of Cefpodoxime proxetil) at the flow rate of 1.5ml/min and both drugs in combination. Mobile phase consisting of 0.02 M ammonium acetate-acetonitrile-methanol (70:29:1) was selected for validation purpose and stability studies.

Different mobile phases 0.1 M NaH2Po4 : MeOH and 0.02 ammonium acetate-acetonitrile-methanol were tested in order to find the test conditions for separation of its degradation products.

•Composition of mobile phase: 0.02M ammonium acetate: acetonile: methanol (70:29:1) and its PH was adjusted at 4.6 by addition of glacial acetic acid.
•Flow rate (gradient): 1.5ml/min.
•Volume injected: 20 µl.
•Selection of Wavelength By UV analysis of C.P. And C.A. was found that λ max for C.P. in acetonitril solvent determined at 235 nm and λ max for C.A. in acetonitril solvent determined at 270 nm.

For both the drug the isobestic point was determined at 230 nm so that the wavelength at 230 nm was selected.

*1.3.1.Chromatographic condition*

The mobile phase and samples were filtered using 0.45 um membrane filter. Ultrasonic vibration was used to degas mobile phrase. All determinations were performed at ambient temperature.

*2.3.2.Preparation of mobile phase:*

*2.3.2.1.Preparation of 20 mM ammonium acetate buffer[27]:*

Weighed 1.0974 gm of ammonium acetate and dissolved with HPLC grade of doubled distilled water up to 700 ml and added 290ml of acetonitrile and 10 ml of methanol made the solution acidic by addition of glacial acetic acid at the pH 3.6 ± 0.2, filter the buffer solution in high vacuum pump then keep the ammonium acetate buffer solution in ultrasnicator (Sonica 2200MH) to remove air bubbles for 10 min.

*2.4.PREPARATION OF STANDERD & SAMPLE SOLUTION:*

*2.4.1Preparation of standard stock solution:*

Stock standard Solution was prepared by dissolving 19.23mg of potassium clavulanate in 30 ml of water and filtered, then addition of 30.765mg of cefpodoxime proxetil and diluted with the solution with methanol up to 100ml.

The working standard solutions were prepared by dilution of the stock solution with mobile phrase to reach a concentration range 0.75-1.5 mg/ml for cefpodoxime proxetil and clavulanic acid. Triplicate 20ul injections were

made five times for each concentration and chromatographer under the condition described above (5.3).The peak areas were plotted against the corresponding concentration to obtain the calibration graphs.

*2.4.2. Sample preparation:*
To determine the content of cefpodoxime proxetil and clavulanic acid in conventional tablets label claimed: 325mg (200mg of C.P. and 125 mg of C.A.) [Per tablet: KEFPOD CV200, Glennmark Pharmaceuticals Ltd.] The twenty tablets were weighed, their mean weight determined and they were finely powdered and powder equivalent to 100mg of cefpodoxime proxetil (62 mg) and clavulanic acid (38 mg) were weighed. Then specified quantity of the powder (which contain 100mg of cefpodoxime proxetil + clavulanic acid) was transferred into a 100 ml volumetric flask containing 30 ml of water, the solution was kept for sonication for 10 min, and the volume were diluted up to 100ml by addition of methanol. The resulting solution was solicited for 10min and then filtered using 0.45 micron filter (Millipore, Milford, MA). The above stock solution was further diluted with methanol to get sample solution of different concentrations. A 20 µl of each sample solution was injected into HPLC six times under condition described above. All samples and solvents were filtered through (nylon) 0.45 µm filter before HPLC injections.

*2.5.EXPERIMENTAL METHODS:*
In general degradation studies were carried out at a conc. of 1mg/ml of each drug in the solution. HPLC studies were carried out on a mixture of reaction solution individually and on a mixture of the solution in which decomposition was observed by following different stress testing methods according to ICH [27].

*2.5.1.Acidic stress testing:*
The combined drug showed sufficient degradation behaviors within half an hour refluxation in 5N HCL (43.25ml of conc. HCL dissolved in 100 ml of demineralised CO2 free water[28]) For HPLC study the result and solutions first neutralized by addition of equivalent amount of 5 N NaOH and the solution was diluted by addition of methanol to obtain required solution and 20 µl were injected into the system both show Clavulanic acid and Cefpodoxime proxetil degradation products. 20 µl were injected in to the system and result was observed.

*2.5.2.Alkali stress testing:*
Both the drugs were found to be highly liable to alkaline hydrolysis in 5 N NaOH (20g of NaOH pellets were weighed and dissolved in 100ml of demineralised CO2 free water [29]) most of the drug decomposed within half an hour. After refluxation the drug solution was neutralized by the addition of equivalent amount of 5 N HCL and then diluted by the addition of methanol. Clavulanic acid shows higher degradation as compare to Cefpodoxime proxetil. 20 µl were injected in to the system and results were observed.

*2.5.3.Neutral (water) stress testing:*
Sufficient degradation was observed upon refluxing the combined drug with water for half an hour similar to acid, only Clavulanic acid shows degradation product. 20 µl were injected in to the system and result was observed.

*2.5.4.Oxidative stress testing:*
Both the drugs showed sufficient degradation when the combination was degraded in 30%$H_2O_2$ (Mfg by RFCL Lmt. B.No. K012B07) for half an hour. Then the solution heated in boiling water bath for 10 min to completely remove the excess of Hydrogen peroxide. For HPLC study the resultant solution were diluted by addition of methanol to obtain 1mg /ml of required solution and 20 µl were injected in to the system and result were observed.

*2.5.5.Thermal stress testing:*
A thermal stress study showed that the combined drug was unstable in thermal condition. For HPLC study the drug is dissolved in 30 ml of water and 70 ml of methanol. Enough degradation was observed when the drug in combination was exposed to dry heat at 60˚c for 40hrs and 30 min. 20µl were injected in the system and result were observed.

## RESULS AND DICUSSION

**Cefpodoxime proxetil and Clavulanic acid**
**3.1. Optimization Of Procedure**
**Optimization of HPLC method**
The HPLC procedure was a view to develop a stability indicating assay method. Pure drug along with its degraded products were injected and run in different solvent system. Initially 20 mM ammonium acetate-acetonitrile-methanol in different ratio (60:39:1, 60:38:2 and 70:29:1) was tried. It was found that 20 mM ammonium acetate-acetonitrile-methanol (70:29:1) gave acceptable retention time at (Rt =3.730 min for Clavulanic acid and Rt = 11.185 min for S form of Cefpodoxime proxetil and Rt = 12.388 min for R form of Cefpodoxime proxetil) at the flow rate of

1.5ml/min and both drugs in combination. Mobile phase consisting of 20 mM ammonium acetate-acetonitrile-methanol (70:29:1) was selected for validation purpose and stability studies.

### 3.2. Linearity:

The linearity of the method was demonstrated over the concentration range of 150-750 $\mu gml^{-1}$. for both R and S isomers of Cefpodoxime proxetil. It showed good correlation coefficient range $R^2$=0.9902 for S isomer and $R^2$=0.9895 for R isomer for HPLC method the linearity of calibration graphs. (Table: 1, 2 and 3, fig: 1,2 and 3)

**Standard deviation (SD)** = $\sigma = \sqrt{\frac{\Sigma(x - x_1)^2}{n-1}}$

Where, **x** = sample
**$x_i$** = mean value of samples
**n** = number of samples

The **correlation Coefficient** and **Percentage curve fittings** were calculated by using the following formula:

R = $\sum$(X-X) (Y-Y) / (n - 1) $S_x S_y$

Where, **X** = concentration
**Y** = instrumental response
**$S_x$** = Standard deviation of x
**$S_y$** = Standard deviation of y

**Table: 1- Linearity data for Cefpodoxime proxetil (S) isomer: n=3**

| S. NO. | Conc. Of CP (mcg/ml) | Average area (sq. m) | Standard Deviation | % R.S.D |
|---|---|---|---|---|
| 1. | 0 | 0 | 0 | 0 |
| 2. | 150 | 177834 | 5.131 | 0.002 |
| 3. | 300 | 338205.67 | 9.073 | 0.002 |
| 4. | 450 | 462891 | 15.87 | 0.003 |
| 5. | 600 | 590586.67 | 12.055 | 0.002 |
| 6. | 750 | 718272.33 | 9.8488 | 0.001 |

**Table: 2 Linearity data for Cefpodoxime proxetil (R) isomer: n=3**

| S. NO. | Conc. Of CP (mcg/ml) | Average area (sq.m) | Standard Deviation | % R.S.D |
|---|---|---|---|---|
| 1. | 0 | 0 | 0 | 0 |
| 2. | 150 | 177694 | 5.131 | 0.008 |
| 3. | 300 | 321596 | 9.073 | 0.002 |
| 4. | 450 | 460185.7 | 15.87 | 0.001 |
| 5. | 600 | 597472 | 12.005 | 0.001 |
| 6. | 750 | 735071 | 9.8488 | 0.001 |

**Table: 3- Linearity data for Clavulanic acid:**

| S. NO. | Conc. Of CA (mcg/ml) | Average area (sq.m) | Standard Deviation | % R.S.D |
|---|---|---|---|---|
| 1. | 0 | 0 | 0 | 0 |
| 2. | 200 | 24885.33 | 29.280 | 0.117 |
| 3. | 400 | 43875.33 | 20.599 | 0.046 |
| 4. | 600 | 62840.667 | 15.143 | 0.240 |
| 5. | 800 | 81809.33 | 5.686 | 0.006 |
| 6. | 1000 | 100761.33 | 15.144 | 0.015 |

**Fig. 1 Linearity for Cefpodoxime proxetil (S) isomer:**

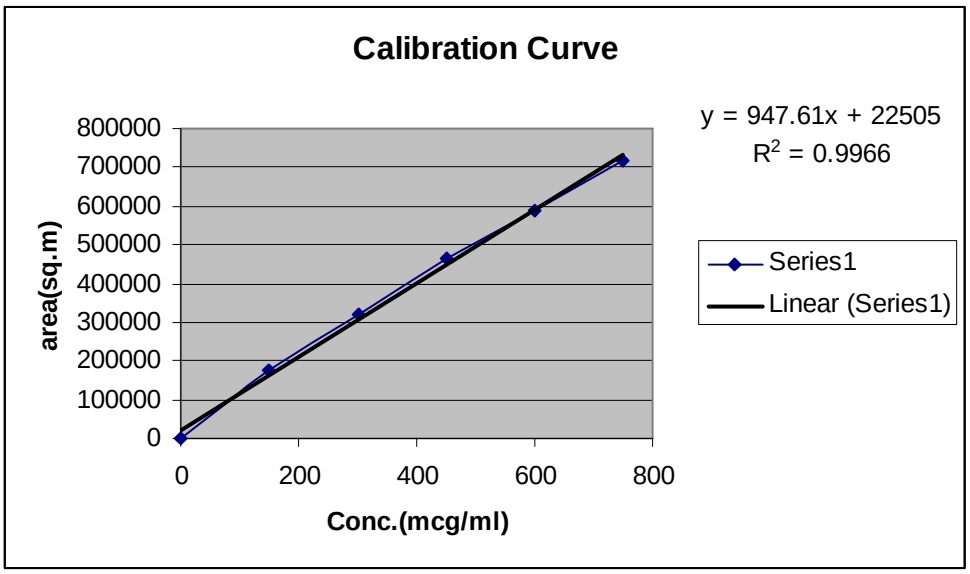



**Fig. 2: Linearity for Cefpodoxime proxetil (R) isomer:**

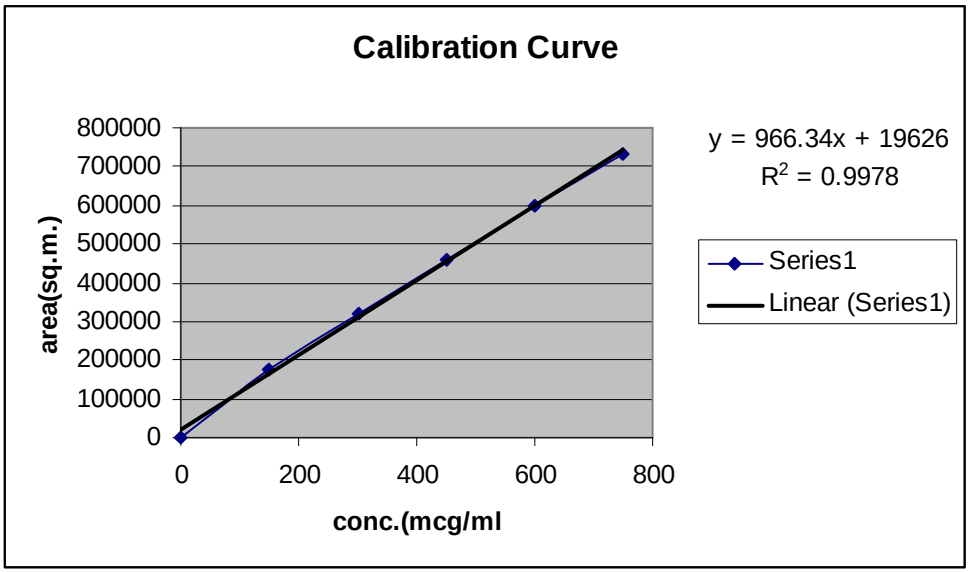
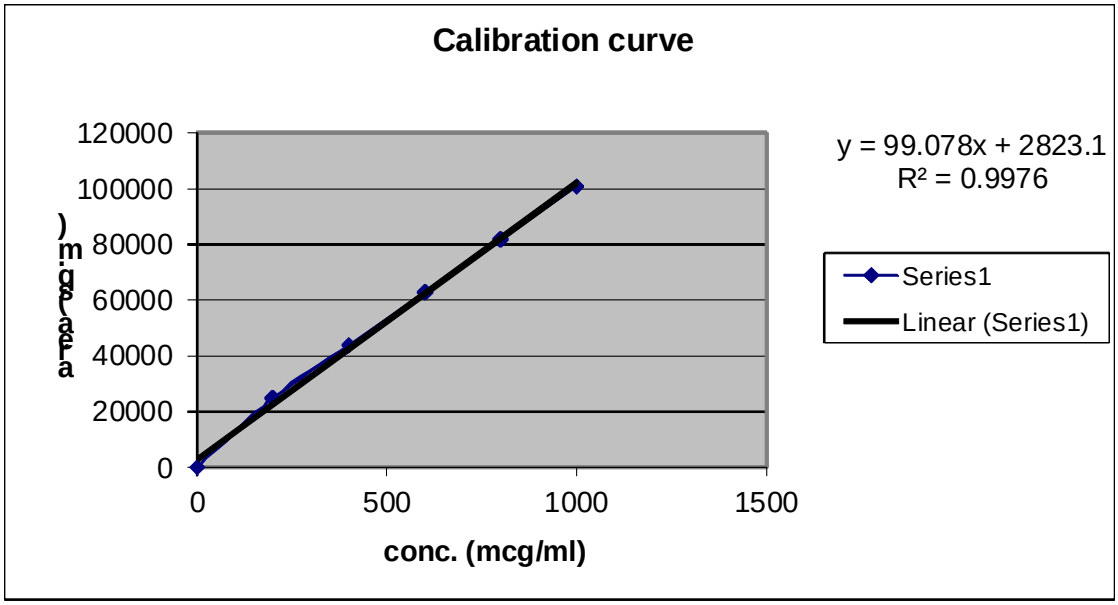



**Fig. 3 Linearity for Clavulanic acid:**



### 3.3. Precision:

The interday and intraday precision of the proposed HPLC method were determined by assaying the tablets three times per day for consecutive six days and expressed as % RSD. The intra-day and inter-day precision has been depicted in the precision of the analytical method was studied by analysis of multiple sampling of homogeneous sample. The precision expressed as standard deviation or relative standard deviation.

### 3.3.1.Procedure

5 Different concentrations of working standard solution were prepared with mobile phase these concentrations were in linearity range. 20 µL of the standard solutions was injected and chromatograms were recorded. The concentration of Cefpodoxime proxetil and Clavulanic acid were calculated for each trial. The experiment was repeated three times in a day (Intra day precision started at 8:00 am to 9:30 am, 9:30 am to 11:00 am and 11:00 am to 12:30 pm ) and the average % RSD values of the results were calculated. Similarly the experiment was repeated on three different days (Inter day precision) the average % RSD values for the determination of Cefpodoxime proxetil and Clavulanic acid. (Table: 4 and 5)

**Table: 4 Interday Precision: n = 3**

| Drug | Std. conc. (µg/ml) | Amount found (µg/ml) | ± S.D. | %RSD |
|---|---|---|---|---|
| **Cefpodoxime proxetil** | 150 | 152.13 | 0.471 | 0.30 |
| | 300 | 304.44 | 2.380 | 0.78 |
| | 450 | 447.94 | 1.296 | 0.28 |
| | 600 | 603.53 | 0.727 | 0.119 |
| | 750 | 757.43 | 2.38 | 0.31 |
| **Clavulanic acid** | 200 | 201.86 | 0.88 | 0.43 |
| | 400 | 396.85 | 2.18 | 0.54 |
| | 600 | 605.43 | 2.24 | 0.36 |
| | 800 | 805.99 | 4.51 | 0.56 |
| | 1000 | 1004.18 | 3.71 | 0.36 |

**Table: 5 Intraday Precision: n=3**

| Drug | Std. conc. (µg/ml) | Amount found (µg/ml) | ± S.D. | %RSD |
|---|---|---|---|---|
| **Cefpodoxime proxetil** | 150 | 152.63 | 2.29 | 1.5 |
| | 300 | 303.18 | 1.84 | 0.59 |
| | 450 | 449.40 | 2.11 | 0.47 |
| | 600 | 599.36 | 1.65 | 0.27 |
| | 750 | 751.69 | 1.71 | 0.22 |
| **Clavulanic acid** | 200 | 202.18 | 1.77 | 0.875 |
| | 400 | 397.80 | 4.88 | 1.2 |
| | 600 | 603 | 4.34 | 0.71 |
| | 800 | 801.6 | 1.77 | 0.22 |
| | 1000 | 1004.27 | 4.15 | 0.41 |

### 3.4. Accuracy: n=3

Subsequent estimation of Cefpodoxime proxetil and Clavulanic acid from pharmaceutical dosage form after spiking with additional drug afforded recovery of 98 – 102 % and mean recovery for Cefpodoxime proxetil and Clavulanic acid. Standard addition technique for determination of Cefpodoxime proxetil and Clavulanic acid by HPLC (n=3). Accuracy was found out by recovery study from prepared mixture at three levels of standard addition, from 75%, 100% and 125% of the label claim. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy is calculated as the percentage of the analyte response after sample workup compared to that of a solution containing the analyte at a concentration corresponding to 100% recovery. (Table:6)

**Table:6 -Percentage recovery of tablet formulation of C.P. and C.A n = 3**

| Drug | Level of std. Drug adds. (mcg/ml) | Conc. of sample (µg/ml) | Recovery of the sample | % recovery | Mean% recovery |
|---|---|---|---|---|---|
| **Cefpodoxime proxetil** | 200 | 150 | 350.56 | 100.16 | 100.15 ± 0.54 |
| | 300 | 300 | 601.28 | 100.21 | |
| | 400 | 500 | 900.91 | 100.10 | |
| **Clavulanic acid** | 200 | 150 | 349.97 | 99.99 | 100.083 ± 0.34 |
| | 300 | 300 | 602.81 | 100.33 | |
| | 400 | 500 | 900.26 | 100.02 | |

**3.4.1.Formula**

$$\% \text{ Recovery} = \frac{\text{Recovery of the sample}}{\text{Level of std. Drug adds. (mcg/ml)+ Conc. of sample (µg/ml)}} \times 100$$

**3.5. LOD and LOQ for Cefpodoxime proxetil and Clavulanic acid:**

**Limit of detection (LOD):**

Limit of detection is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. Signal to noise ratio shall be 3:1.

**Limit of quantitation (LOQ):**

Limit of quantitation is the lowest concentration of analyte that can be quantified by measuring the magnitude of analytical background response by analyzing a number of blank samples and calculation standard deviation of the response. The std. deviation multiplied by a factor 10 provides an estimate of the blank. (Table no. 7)

**Table: 7-LOD and LOQ for Cefpodoxime proxetil and Clavulanic acid n = 3**

| S. no | Drug | LOD | LOQ |
|---|---|---|---|
| 1. | C.P. | 25 mcg/ml | 84.6mcg/ml |
| 2. | C.A. | 100 mcg/ml | 334.2 mcg/ml |

**Formula:**

$$\text{LOD} = Cs \frac{\mathbf{3}}{S/N}$$

$$\text{LOQ} = Cs \frac{\mathbf{10}}{S/N}$$

Where S/N = hs / 2hn

**Cs** = amount of conc. of injected analyte.
**hn** = Largest deviation (Positive or negative) of the detector signal from the average base line level measured over a span of at least 10 peak width

**hs** = peak height of the analyte measured from the average baseline level of the top of the peak measured in the same unit as hn.

**3.6. Analysis Of Marketed Formulation:** Cefpodoxime proxetil and Clavulanic acid were degraded together under different stress conditions prescribed by ICH. The samples so generated were used to develop stability indicating HPLC method for two drugs. The drugs were well separated from degradation product using a reversed phase (C-18) column with a mobile phase composed of 20 mM ammonium acetate-acetonitrile-methanol (70:29:1) pumped at a flow rate of 1.5ml/min. the detection was carried out at 230nm. and the column temperature maintained at $30^0$C. The major degradation products of Clavulanic acid was found at retention times (Rt) 3.285 min., for Cefpodoxime proxetil only R isomer was showing degradation product at retention time (Rt) 9.765 min. In neutral (water) degradation studies, upon refluxing the combined drug for half an hour. Only Clavulanic acid shows degradation product appeared at retention times (Rt) 3.285, 3.513 min. In alkali degradation studies, both drugs are highly liable to hydrolysis in 5N NaoH. The major degradation products were found at retention times (Rt) 3.285, 3.568, 10.586 and 11.735 min. In oxidative degradation studies drugs showed sufficient degradation in 30%$H_2O_2$ for half an hour and the three major degradation products appeared at retention times(Rt) 3.405,10.586 and 13.416 min. In solid state or thermal degradation studies showed that the combination was unstable. Enough degradation was observed when the combination was exposed to dry heat at $60^O$c for 40hr and 30 min. The major degradation products resolved at 3.285, 3.576, 10.389, 10.476, 10.724, 11.735 and 13.416 min. The method was validated for linearity, precision, accuracy, specificity etc. the linearity range of Cefpodoxime proxetil and Clavulanic acid were found to be 150- 750 µg/ml and 200-1000 µg/ml respectively. The Limit of detection was found to be 25 $\mu gml^{-1}$ for Cefpodoxime proxetil and 100 $\mu gml^{-1}$ .For Clavulanic acid.LOQ was found to be 84.8 $\mu gml^{-1}$ for Cefpodoxime proxetil and 334.2$\mu gml^{-1}$ for Clavulanic acid This indicates that the proposed single method allowed selective analysis of both Cefpodoxime proxetil and Clavulanic acid in the presence of there degradation products form under a variety of stress conditions. The developed method was also applicable to the determination instability of the drug in commercial product. Analysis of marketed formulation is shown in Table : 8- Table- 15 & Fig 4-Fig 10.

**Table: 8 ANALYSIS OF MARKETED FORMULATION: n=3**

| Formulations | Labeled amount | | Amount found | |
|---|---|---|---|---|
| | C.A. (mg) | C.P. (mg) | C.A. (mg) | C.P. (mg) |
| 1. KEFPOD CV $^{200}$ (Glennmark) | 125 | 200 | 124.86 | 201.21 |
| 2.CEPODEM XP $^{325}$ (Ranbaxy) | 100 | 200 | 100.67 | 199.19 |

**Table:9 Table of standard Cefpodoxime proxetil and Clavulanic acid:**

| Peak # | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. C.A. | 3.730 | 43205 | 3.458 |
| 2. C.P. (S) | 11.185 | 599583 | 47.996 |
| 3. C.P. (R) | 12.388 | 606470 | 48.546 |
| | | 1249258 | 100 % |

**Table : 10 Tablet formulation of Cefpodoxime proxetil and Clavulanic acid:**

| Peak # | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1.C.A. | 3.703 | 43186 | 3.451 |
| C.P. (S) | 11.276 | 601346 | 48.062 |
| C.P. (R) | 12.508 | 606673 | 48.487 |
| | | 1251183 | 100 % |

**Table: 11 Degradation of Cefpodoxime proxetil and Clavulanic acid in 5 N HCl:**

| Peak | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. | 3.285 | 5216 | 0.417 % |
| 2. | 3.703 | 37996 | 3.040 % |
| 3. | 9.765 | 78138 | 6.253 % |
| 4. | 11.185 | 599589 | 47.983 % |
| 5. | 12.388 | 528656 | 42.307 % |
| | | 1249564 | 100 % |

**Table: 12 Degradation of Cefpodoxime proxetil and Clavulanic acid in 5N NaOH:**

| Peak | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. | 3.285 | 5374 | 0.429 % |
| 2. | 3.568 | 1260 | 0.100 % |
| 3. | 3.713 | 36538 | 2.920 % |
| 4. | 10.586 | 16380 | 10.309 % |
| 5. | 11.185 | 601254 | 48.056 % |
| 6. | 11.735 | 51698 | 4.1320 % |
| 7. | 12.388 | 538698 | 43.056 % |
| | | 1251133 | 100 % |

**Table : 13 Degradation of Cefpodoxime proxetil and Clavulanic acid in Neutral condition:**

| Peak | Ret. Time(min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. | 3.285 | 5459 | 0.436 % |
| 2. | 3.513 | 1986 | 0.158 % |
| 3. | 3.713 | 36796 | 2.865 % |
| 4. | 11.185 | 599596 | 47.991 % |
| 5. | 12.388 | 606585 | 48.550 % |
| | | 1249381 | 100 % |

**Table : 14 Degradation of Cefpodoxime proxetil and Clavulanic acid in 30 % $H_2O_2$:**

| Peak | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. | 3.405 | 6248 | 0.500 % |
| 2. | 3.719 | 36970 | 2.959 % |
| 3. | 10.586 | 16356 | 1.3092 % |
| 4. | 11.276 | 599570 | 47.992 % |
| 5. | 12.496 | 65268 | 5.224 % |
| 6. | 13.416 | 52487 | 42.013 % |
| | | 1249308 | 100 % |

**Table : 15 Degradation of Cefpodoxime proxetil and Clavulanic acid in thermal condition:**

| Peak | Ret. Time (min) | Area (sq. m) | % Area |
|---|---|---|---|
| 1. | 3.285 | 5461 | 0.4 |
| 2. | 3.576 | 385 | 0.03 |
| 3. | 3.730 | 36482 | 2.9 |
| 4. | 10.398 | 241 | 0.01 |
| 5. | 10.476 | 298 | 0.02 |
| 6. | 10.724 | 1972 | 0.15 |
| 7. | 11.276 | 597078 | 47.8 |
| 8. | 11.735 | 51705 | 4.25 |
| 9. | 12.496 | 502481 | 40.24 |
| 10. | 13.416 | 52489 | 4.20 |
| | | 1248592 | 100 % |

**Fig. 4 Standard Cefpodoxime proxetil and Clavulanic acid:**

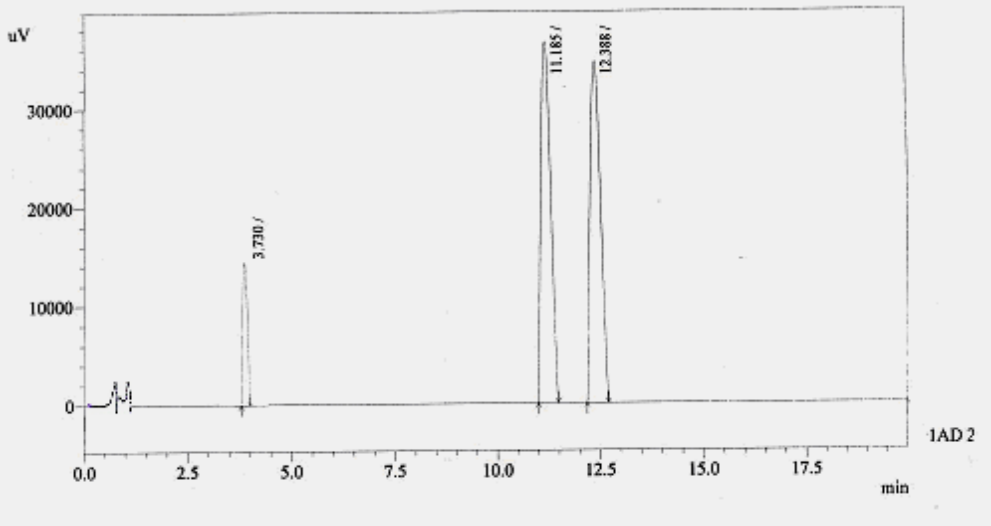



**Fig. 5 Tablet formulation of Cefpodoxime proxetil and Clavulanic acid:**

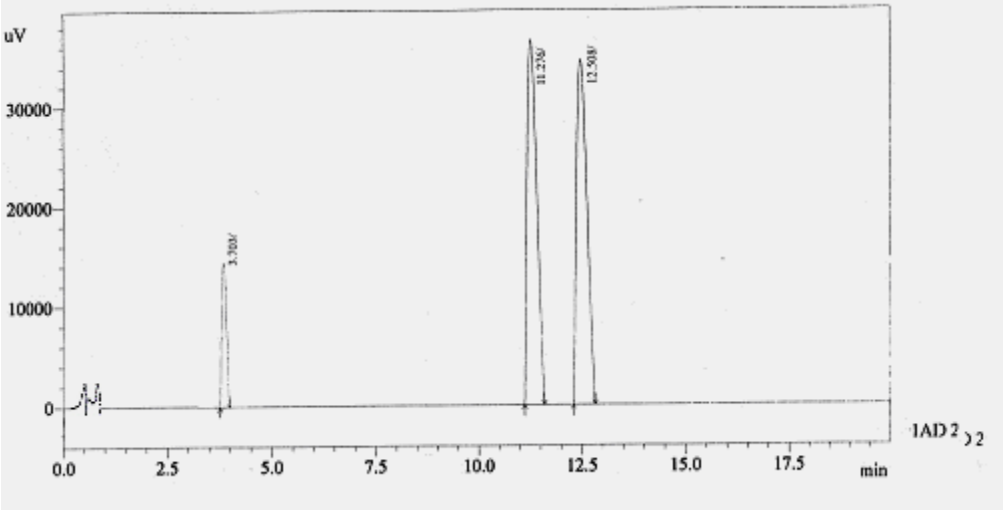


**Fig. 6. Degradation of Cefpodoxime proxetil and Clavulanic acid in 5 N HCl:**

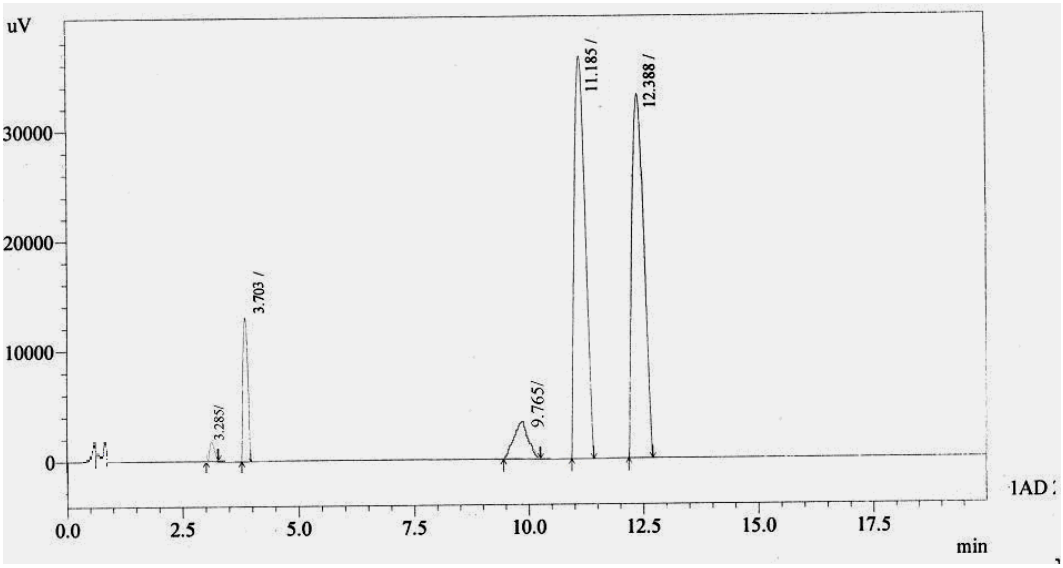



**Fig. 7 Degradation of Cefpodoxime proxetil and Clavulanic acid in 5N NaOH:**

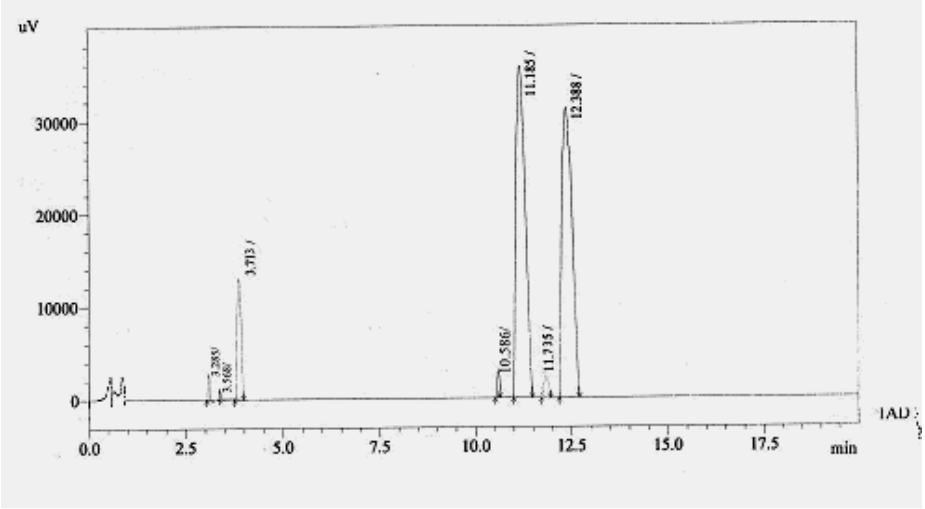



**Fig. 8 Degradation of Cefpodoxime proxetil and Clavulanic acid in neutral (water) medium:**

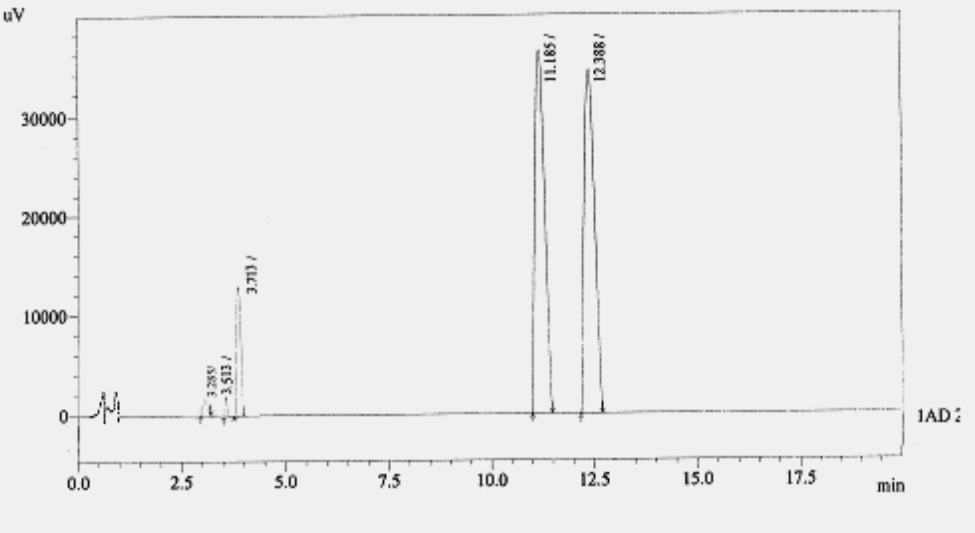


**Fig. 9 Degradation of Cefpodoxime proxetil and Clavulanic acid in 30 % $H_2O_2$:**

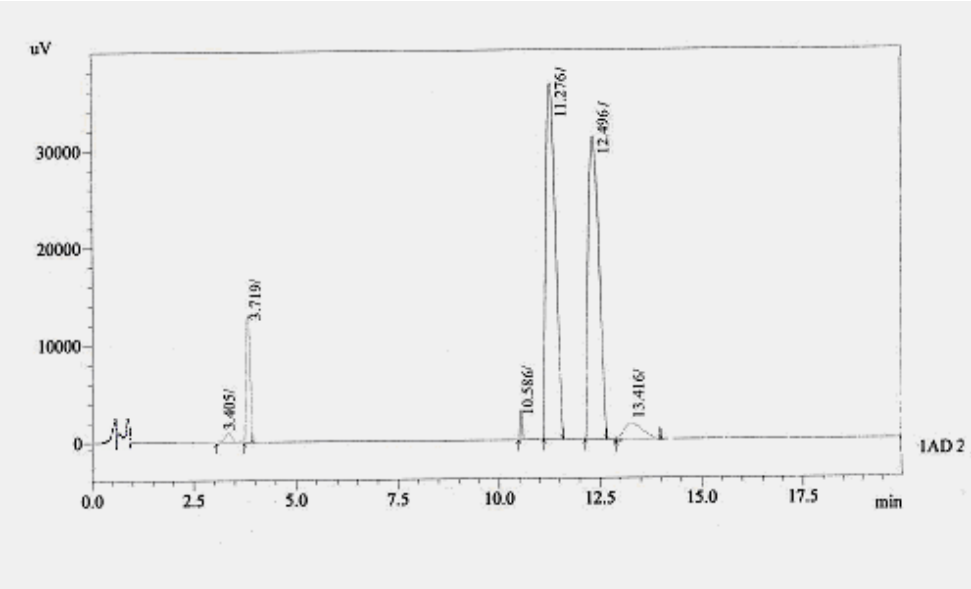
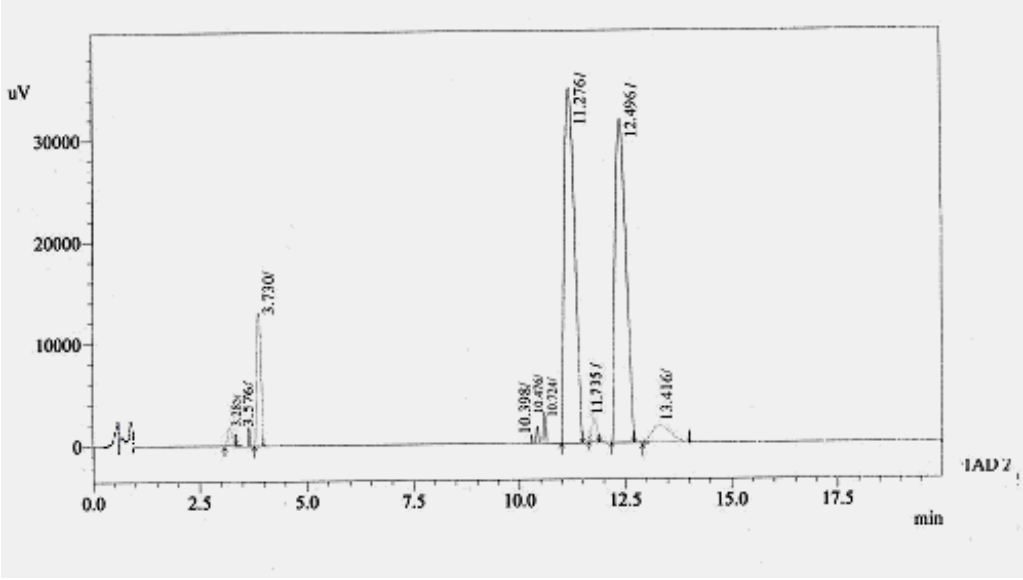



**Fig. 10 Degradation of Cefpodoxime proxetil and Clavulanic acid in thermal condition :**




## CONCLUSION

The present study is a simple and validated indicating HPLC method simultaneous estimation of Clavulanic acid and Cefpodoxime proxetil in the presence of degradation products.

Clavulanic acid and R- form of Cefpodoxime proxetil show sufficient degradation in acidic, basic, oxidative and thermal condition.

S- form of Cefpodoxime proxetil show degradation products in thermal condition (dry heat at $60^0$C for 40.5 hrs) only.

In neutral medium only Clavulanic acid is degraded.

So the highly unstable drug is Clavulanic acid compare to an R form of Cefpodoxime proxetil whereas the S- form of Cefpodoxime proxetil is highly stable.

The method yielded results similar to those determined by application of compendia procedures on individual drugs.

The method could be applied with success even to the analysis of marketed formulation/products as no interference was observed due to excipients or other components present.

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