# A Validated Stability Indicating HPTLC Method for Determination of Cephalexin in Bulk and Pharmaceutical Formulation

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**Abstract:**   A simple, specific, precise and stability-indicating high performance thin layer chromatographic method of analysis of Cephalexin, both as a bulk drug and in formulation was developed and validated. The method employed TLC (Thin Layer Chromatography) aluminum plates pre-coated with silica gel 60 $F_{254}$ as the stationary phase. The solvent system consisted of Ethyl Acetate : Methanol : Ammonia  (6:4:1, v/v/v). This system was found to give compact spots for Cephalexin ($R_f$, retardation factor, value–0.56). Cephalexin was subjected to hydrolytic, oxidative, dry heat and photo-degradation as per ICH guidelines. The responses for the products of degradation were well separated from the pure drug. Densitometric analysis of Cephalexin was carried out in the absorbance mode at 260 nm. The linear regression analysis data for the calibration spots showed good relationship with (regression) $r^2 = 0.9991$ in the range of 500–1500 ng (nanogram) per spot. The limits of detection and quantitation were 51.03 ng and 154.64 ng, respectively. The drug was observed to not undergo degradation with dry heat and photodegradation, but gets affected in and hydrolytic and oxidative conditions. As the method could effectively resolve the drug from its degradation products, it can be employed as a stability-indicating one.
**Keywords:** Cephalexin, HPTLC, Stability indicating.

## 1. Introduction

Cephalexin 6*R*,7*R*)-7-{[(2*R*)-2-amino-2-phenylacetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene- 2-carboxylic acid  (Figure I) is used to treat urinary tract infections, respiratory tract infections (including sinusitis, otitis media, pharyngitis, tonsillitis, pneumonia, and bronchitis),     and skin and soft tissue infections. In addition to being a rational first-line treatment for cellulitis, it is a useful alternative to penicillin's in patients with penicillin hypersensitivity.[1] Few methods have been reported for the quantification of Cephalexin single drug and in Combination by HPTLC.[2,3,4,5]. The use of RP-HPLC procedures for determination in plasma, serum and urine has been reported.[6,7,8] Few HPLC methods also have been reported for quantification of Cephalexin.[9,10,11,12] Some spectro-photometric and colorimetric methods also have been reported.[13,14] However till now, no stability indicating method for estimation of Cephalexin has been reported. The present work describes the development of a simple, precise and accurate method for the estimation of Cephalexin in presence of degradation products in bulk drugs and marketed formulation. The method validation and forced degradation studies were carried out as per ICH  guidelines.[15,16.]

## 2. Materials and Methods

Cephalexin (purity 99.5 %) was provided as a gift sample by Maxim Pharmaceuticals  Limited, Pune, India. and was used without further purification. All the other reagents used were of analytical grade. Ethyl Acetate (AR grade), Methanol (AR grade), Chloroform (AR grade), Acetone (AR grade), NaOH (AR grade), HCl (AR grade), $H_2O_2$ (AR Grade), Glacial acetic acid  (AR Grade), Ammonia (AR Grade) were purchased from Thomas Baker (chemicals) Pvt Limited, India.

### 2.1 Instrumentation

Chromatographic separation of drug was performed on Merck TLC plate pre-coated with silica gel 60 $F_{254}$ (10 cm ×10 cm with 250 µm layer thickness) from E. Merck, Germany. The samples were applied onto the plates as a

band with 6 mm width using Camag 100 µl sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm). Densitometric scanning was performed using Camag TLC scanner 3 in the range of 500-1500 ng/spot and operated by winCATS software (V 1.4.2, Camag).

## 2.2 Selection of Detection Wavelength
After chromatographic development bands were scanned over the range of 200-400 nm. It was observed that the drug showed considerable absorbance at 260 nm. So, 260 nm was selected as the wavelength for detection (Figure III).

## 2.3. Method validation
### 2.3.1 Linearity
A stock solution of Cephalexin (1000 ng/µL) was prepared in Water. Different volumes of stock solution, 0.5, 0.8, 1, 1.2, 1.5 µL were spotted on TLC plate to obtain concentration of 500, 800, 1000, 1200, 1500 ng per spot of Cephalexin, respectively. The data of peak area v/s drug amount were treated by linear least-square regression analysis.

### 2.3.2. Precision
The intra and inter-day variation for the determination of Cephalexin was carried out at three different concentration levels of 800, 1000 and 1200 ng per spot. The % RSD values were determined for intra-day and inter-day variation.

### 2.3.3. Recovery studies
The analysed samples were spiked with extra 80, 100 and 120 % of the standard Cephalexin and the mixtures were reanalyzed by the proposed method. The experiment was conducted in triplicate. This was done to check for the recovery of the drug by standard addition method.

### 2.3.4. Limit of detection and limit of quantification
The limit of detection (LOD) and limit of quantification (LOQ) were obtained by calculating using the standard formula as per the ICH guidelines, where σ is the standard deviation of response.

### 2.3.5. Specificity
The specificity of the method was ascertained by peak purity profiling studies. Purity of the drug peaks was ascertained by analyzing the spectrum at peak start, middle and at peak end. The peak purity was determined on winCATS software using statistical equation.

## 2.4. Analysis of the marketed formulation
To determine the content of Cephalexin in tablets (label claim: 125 mg per tablet). Twenty tablets were powdered and powder equivalent to 25 mg was weighed and transfered to a 25 ml volumetric flask containing about 20 ml water, the solution was filtered through whatman filter paper and finally volume was made upto 25 ml to get the stock solution of (1000 ng/µL) Appropriate

volume of solution was applied on TLC plate followed by development and scanning.

## 2.5. Forced degradation Studies
A stock solution containing 25 mg Cephalexin in 25 ml distilled water was prepared. This solution was used for forced degradation.

### 2.5.1. Degradation under acid catalysed hydrolytic condition
To 2.5 ml stock solution, 2.5 ml of 0.1 N HCl was added. The volume was made upto 25 ml with distilled water. The mixture was refluxed at 80 $^{o}$C for one hour. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

### 2.5.2. Degradation under alkali catalysed hydrolytic condition
To 2.5 ml stock solution, 2.5 ml of 0.01 N NaOH was added. The volume was made upto 25 ml with distilled water. This mixture was kept at room temperature for half hour. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

### 2.5.3. Degradation under neutral hydrolytic condition
To 2.5 ml stock solution, distilled water was added. The volume was made upto 25 ml. The mixture was refluxed for 15 min at 80 $^{0}$C. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

### 2.5.4. Degradation under oxidative condition
To 2.5 ml stock solution, 2.5 ml of 30 % $H_2O_2$ was added. The volume was made upto 25 ml with distilled water. The mixture was kept at room temperature for 3 days. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

### 2.5.5. Degradation under dry heat
Dry heat studies were performed by keeping drug sample in oven (80$^{0}$ C) for a period of 12 hours. Accurately weighed 25 mg of drug was transferred to the 25 ml of volumetric flask and dissolved in water, the volume was made up with distilled water. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

### 2.5.6. Photo-degradation studies
The photochemical stability of the drug was also studied by exposing the drug sample to UV light up to illumination of 200 watt hr/m$^{2}$ followed by fluorescent light up to illumination of 1200 lux-hr. Accurately weighed 25 mg of drug was transfered to the 25 ml of volumetric flask, the volume was made up with distilled water. Appropriate volume of resultant solution (1000 ng per spot) was applied on TLC plate and densitograms were developed.

## 2.6 Stress degradation of Formulation
Cephalexin tablets, each containing 125 mg Cephalexin were purchased from local market, The tablets were

weighed, crushed and sample powder was exposed to stress condition as mentioned under study for bulk drugs. Then the sample was filtered & appropriate volume was spotted on to TLC plate.

# 3. Results and Discussion

## 3.1. Development of the optimum mobile phase
TLC procedure was optimized with a view to develop a stability-indicating assay method. The drug reference standard was spotted on the TLC plate and developed in different solvent systems. Initially, Chloroform: Methanol and Acetone: Methanol with varying proportions were tried, but in both the cases peak shape was not good. Trials were carried out by changing the pH using glacial acetic acid and ammonia. With Ammonia the shape improved, however ammonia was immisible with chloroform. So chloroform was replaced with ethyl Acetate.   Finally, the mobile phase Ethyl Acetate: Methanol: Ammonia (6:4:1, v/v/v) gave sharp and symmetrical peak with $R_f$ 0.56. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature. The representative densitogram is given in Figure II.

## 3.2 Validation of the method
### 3.2.1 Linearity
The response for the drugs was found to be linear in the concentration range 500–1500 ng / spot with correlation co-efficient of 0.9991.
### 3.2.2 Precision
The % RSD value for intra-day and inter-day variation study was found to be not more than 0.7267 % and 1.3623 % respectively, thus confirming precision of the method.
### 3.2.3 Recovery
Excellent recoveries were obtained at each level of added concentration. The results obtained (n = 3 for each 80 %, 100 %, 120 % level) indicated the mean recovery 98.71 %.
### 3.2.4 Limit of Detection and limit of Quantitation
The limit of detection and limit of quantitation as calculated by standard formula as given in ICH guidelines was found to be 51.03 ng/spot and 154.64 ng/spot respectively.
### 3.2.5 Specificity
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be r(s,m) 0.9950 and r(m,e) 0.9938 for Cephalexin, indicating the non interference of any other peak of degradation product, impurity or matrix.
The results are listed in Table I.

## 3.3 Analysis of marketed formulation
A single spot at $R_f$ 0.56 was observed in the chromatogram of the drug samples extracted from tablets. There was no interference from the excipients present in the tablets. The drug content was found to be 102.90 %.

## 3.4 Stress degradation
### 3.4.1 Hydrolytic Conditions
#### 3.4.1.1 Acidic hydrolytic condition
Cephalexin degrades to considerable extent with acidic hydrolysis. On heating at 80 °C in 0.1 N HCl (1 hour), the height of the drug peak decreased, with corresponding appearance of new degradation peaks. One peak of the degradation product was observed (D1  $R_f$ = 0.76) (Figure- IV).
### 3.4.1.2 Alkaline hydrolytic condition
Cephalexin also degrades rapidly with alkaline hydrolysis. On treatment with 0.01 N NaOH for 1/2 hour, the height of peak was reduced and four new peaks of degradation products were observed (D2 $R_f$ = 0.15,  D3 $R_f$ = 0.62, D4 $R_f$ = 0.78 and D5 $R_f$ = 0.82) (Figure - VI).
### 3.4.1.3 Neutral hydrolytic condition
In neutral condition, considerable degradation of Cephalexin was observed. On heating at 80° C in distilled water for 15 min, the height of the drug peak decreased, with corresponding appearance of one new degradation peak. (D6 $R_f$ = 0.80) (Figure-VIII)
### 3.4.2. Oxidative condition
Cephalexin was found to degrade less rapidly in oxidative conditions. Upon treatment with 30 % hydrogen peroxide at room temperature for three days, peak height was reduced, with the appearance of one new degradation peak. (D7 $R_f$ = 0.73) (Figure - X).
### 3.4.3 Dry heat and Photolytic studies
Under dry heat (Oven, $80^0$ C, 12 hour) and photolytic studies, no additional peaks were observed and drug peak area remained constant. This indicates stability of drugs in dry heat, UV light and fluorescent light for specified period.

# 4. Conclusion
From the above study we can conclude that Cephalexin undergoes degradation to different extent under different, above mentioned, stress conditions. From the peak purity profile studies, it was confirmed that the peak of the degradation product was not interfering with the peak of Cephalexin. It confirms that degradation product of drug can be separated from the drug by this method. Since different formulations of same drug are available in the market so the developed method can also be used for the stability study of marketed formulations also.
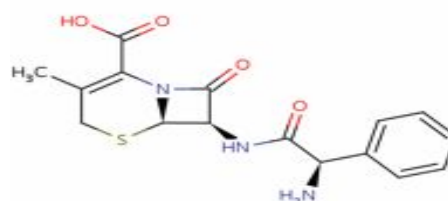
**Fig I: Structure of  Cephalexin.**

**Table I : Validation Parameters.**

| S.N. | Validation Parameter | Cephalexin |
|------|---------------------|------------|
| 1 | Linearity Equation | $y = 3.611x + 146.79$ |
|  | $(r^2)$ | (0.9991) |
|  | Range | 500 – 1500 ng/spot |
| 2 | Precision (% RSD) |  |
|  | Intraday | NMT 0.7267 % |
|  | Interday | NMT 1.3623 % |
| 3 | Accuracy (% mean recovery) | 98.71 % |
| 4 | LOD | 51.03 ng/spot |
| 5 | LOQ | 154.64 ng/spot |
| 6 | Specificity | Specific |
|  | Peak Purity | r(s,m) = 0.9950 |
|  |  | r(m,e) = 0.9938 |

**Table II : : Degradation study results for Cephalexin bulk drug and formulation.**

| S.N. | Conditions | % of Cephalexin degraded | |
|------|-----------|:------------------------:|:---:|
|  |  | Bulk Drug | Formulation |
| 1. | Acid (0.1 N HCl, reflux, 1 hour) | 61.5 % | 69.90 % |
| 2. | Base (0.01 N NaOH, 1/2 hour) | 36.1 % | 27.37 % |
| 3. | Water (reflux, 15 min) | 74.6 % | 69.35 % |
| 4. | Hydrogen peroxide 30% (3 Days) | 20.4 % | 13.62 % |
| 5. | Heat dry (80 $^0$C, 12 hours) | No degradation | No degradation |
| 6. (a) | Photo stability                    UV 200 watt hours/square meter | No degradation | No degradation |
| (b) | Visible 1200 Lux.Hr | No degradation | No degradation |

**Fig. II: Representative densitogram of Cephalexin at R$_f$ 0.56.**
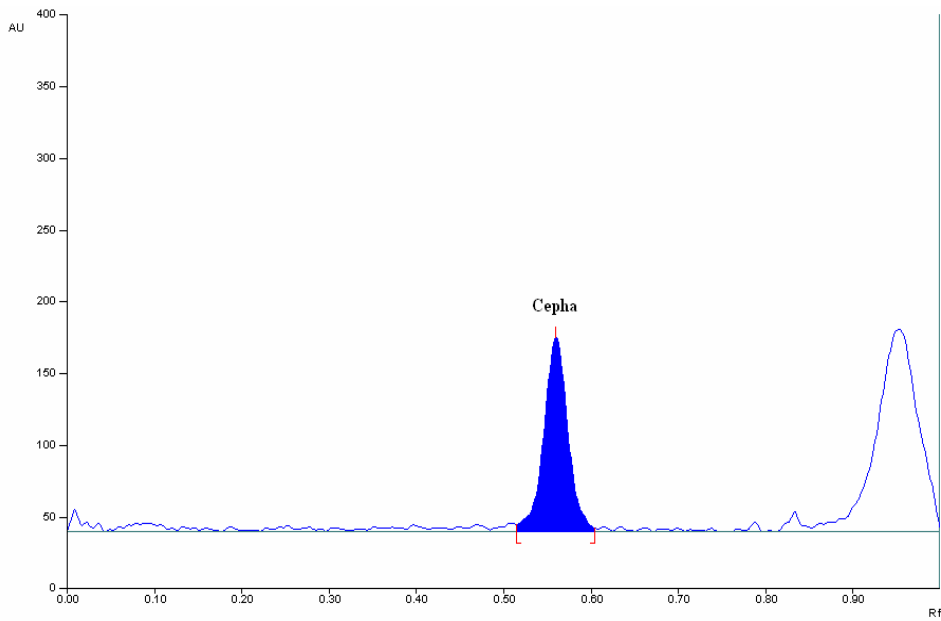


**Fig. III : Spectrum of Cephalexin**
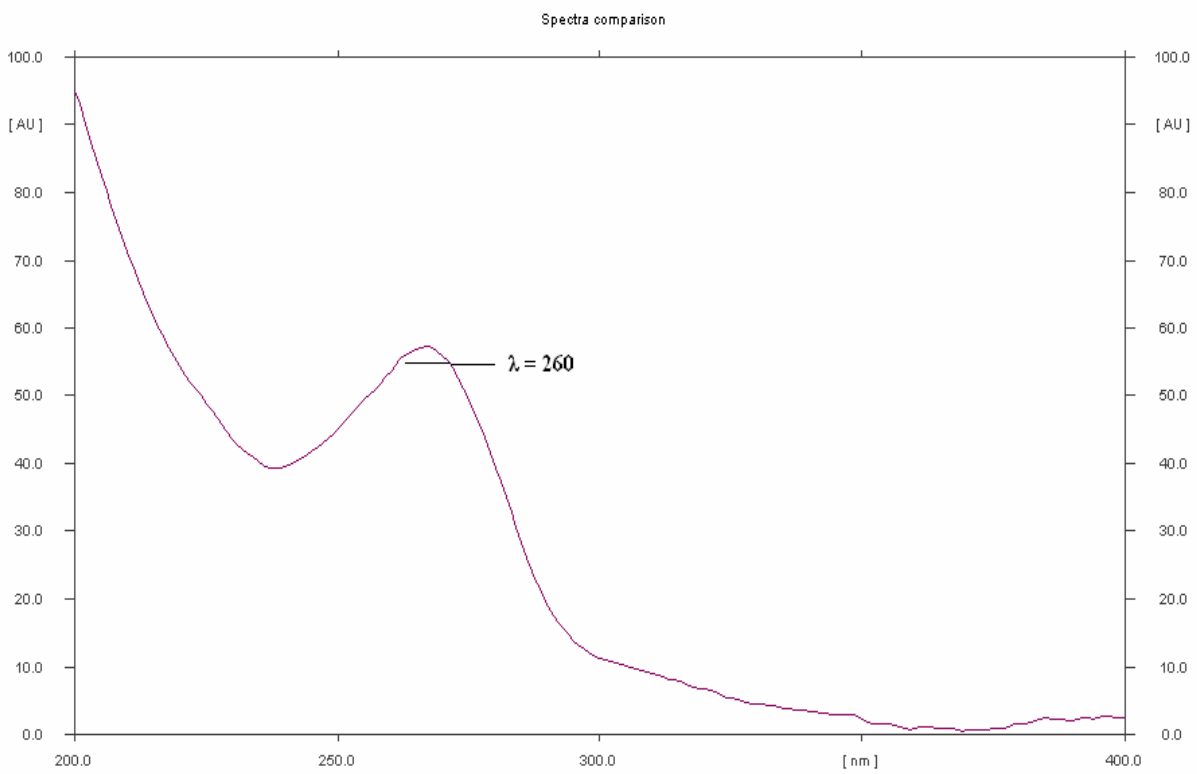
**Fig. IV: Densitogram of Cephalexin and its degradation products after acid catalysed hydrolysis**
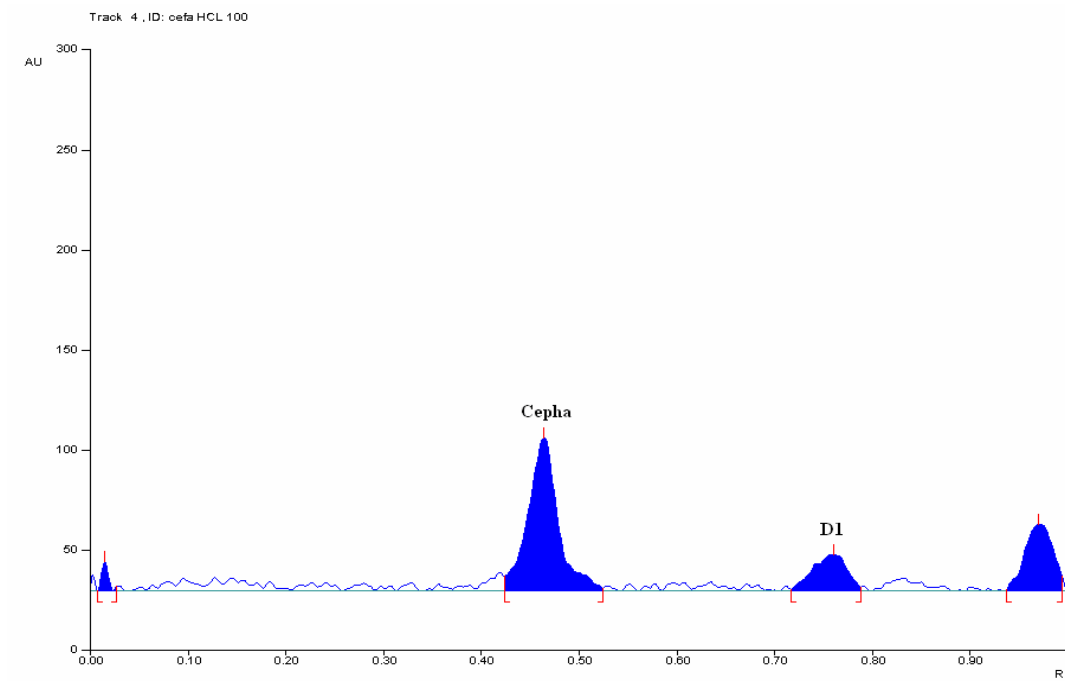


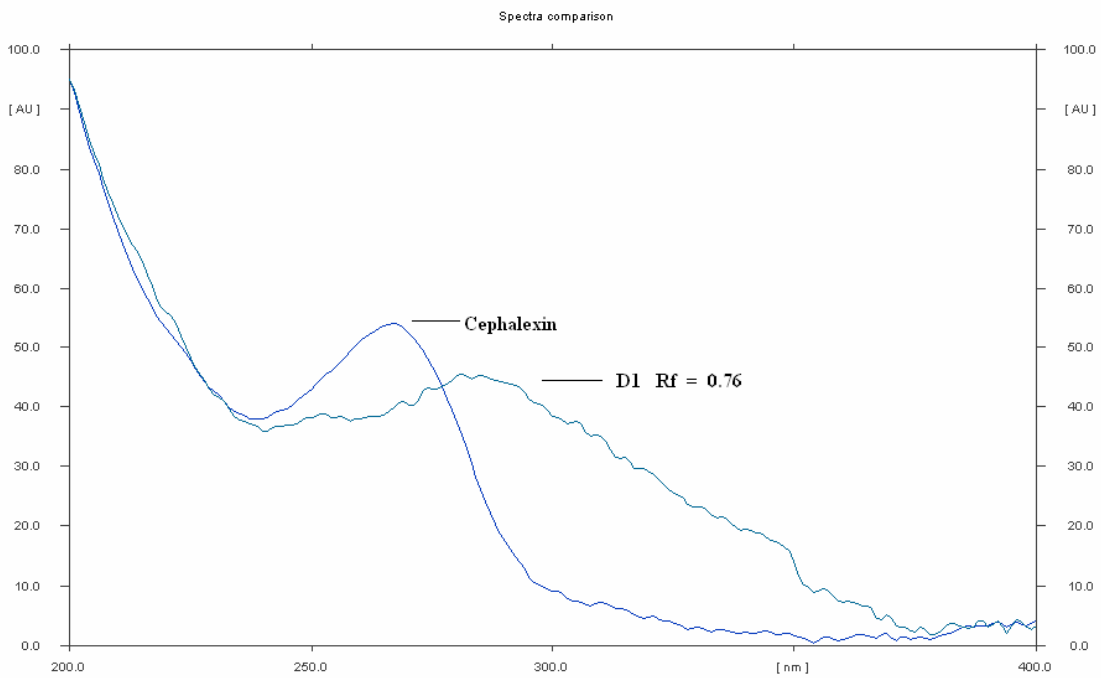**Fig. V: Overlain Spectra of Cephalexin and its degradation products after acid catalysed hydrolysis.**

**Fig. VI : Densitogram of Cephalexin and its degradation products after base catalysed hydrolysis.**
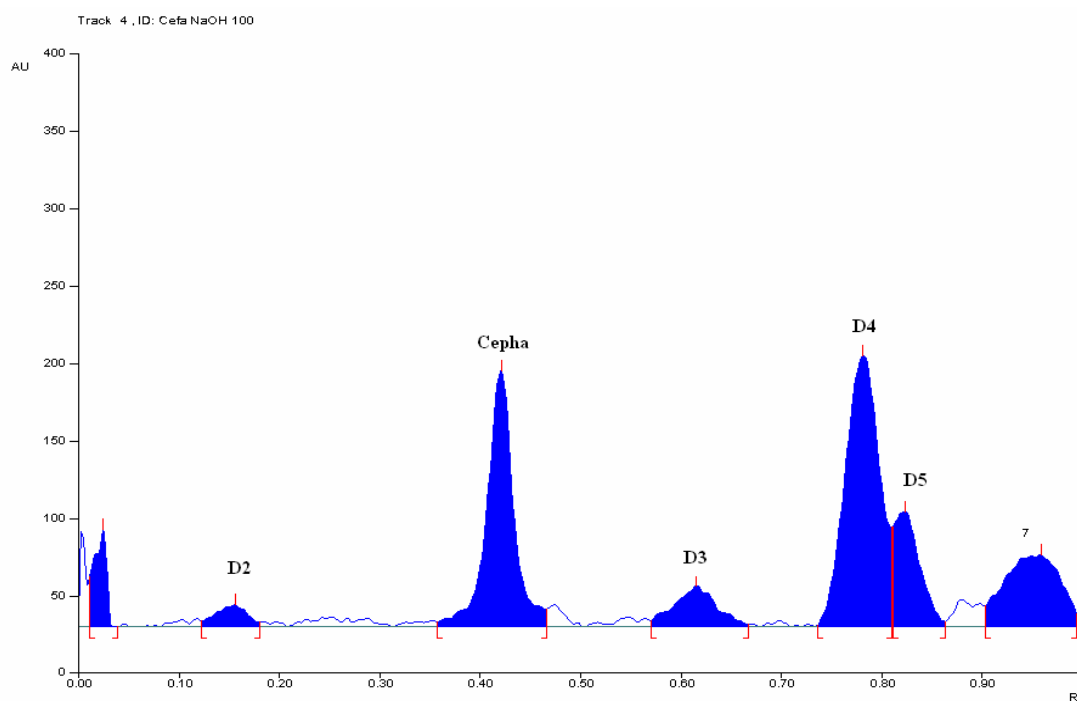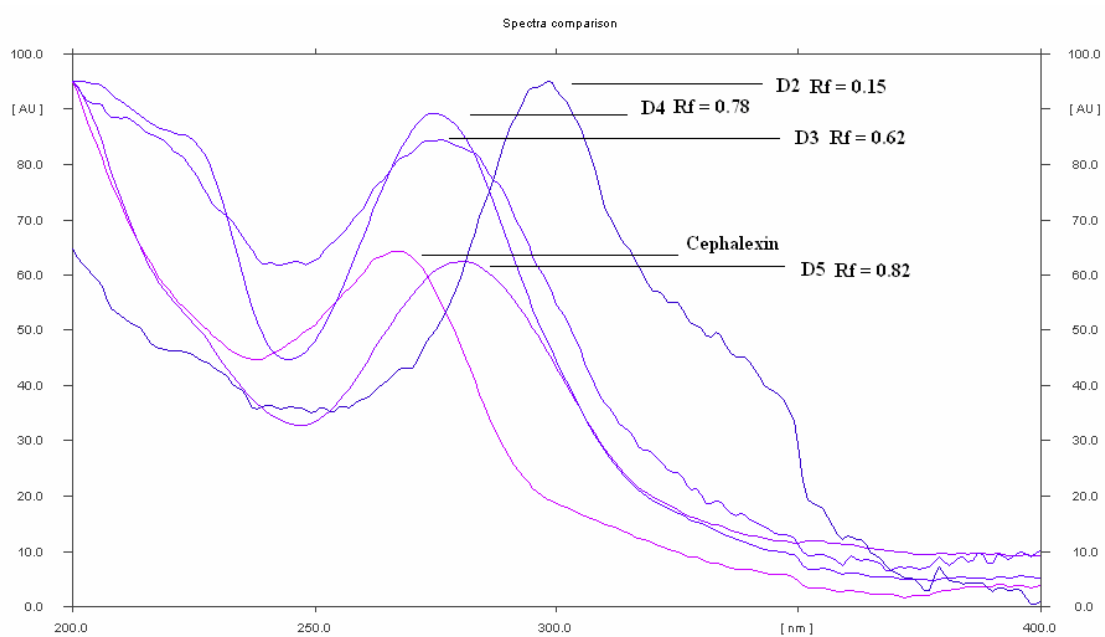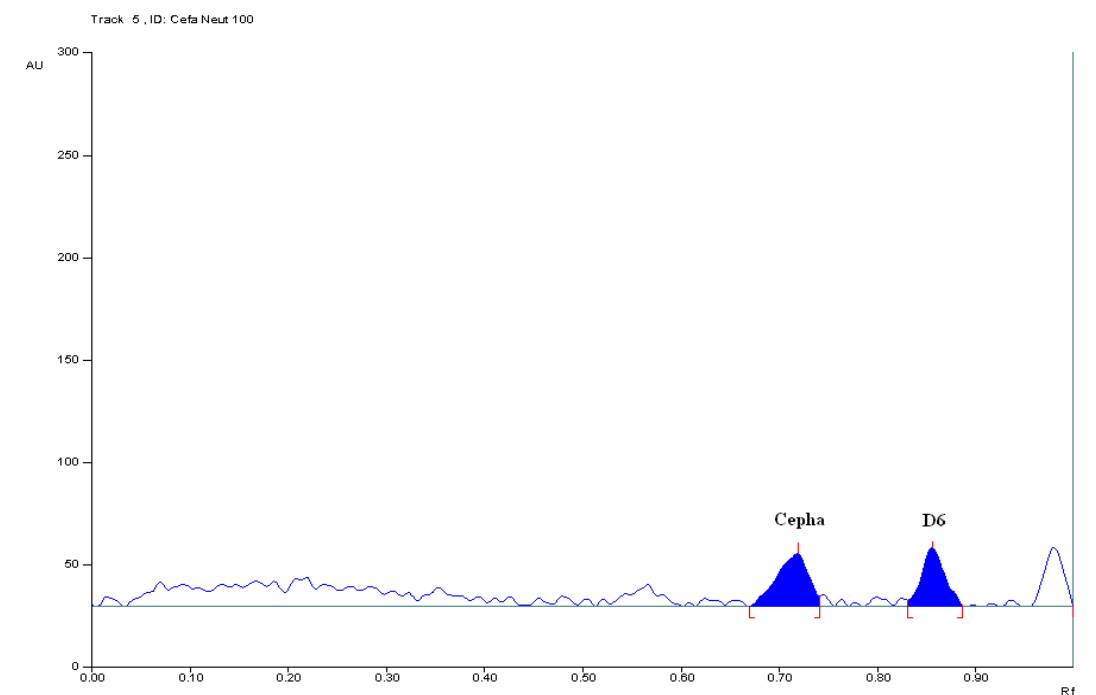


**Fig. VII : Overlain Spectra of Cephalexin and its degradation products after base catalysed hydrolysis.**

**Fig. VIII : Densitogram of Cephalexin and its degradation products after neutral hydrolysis.**



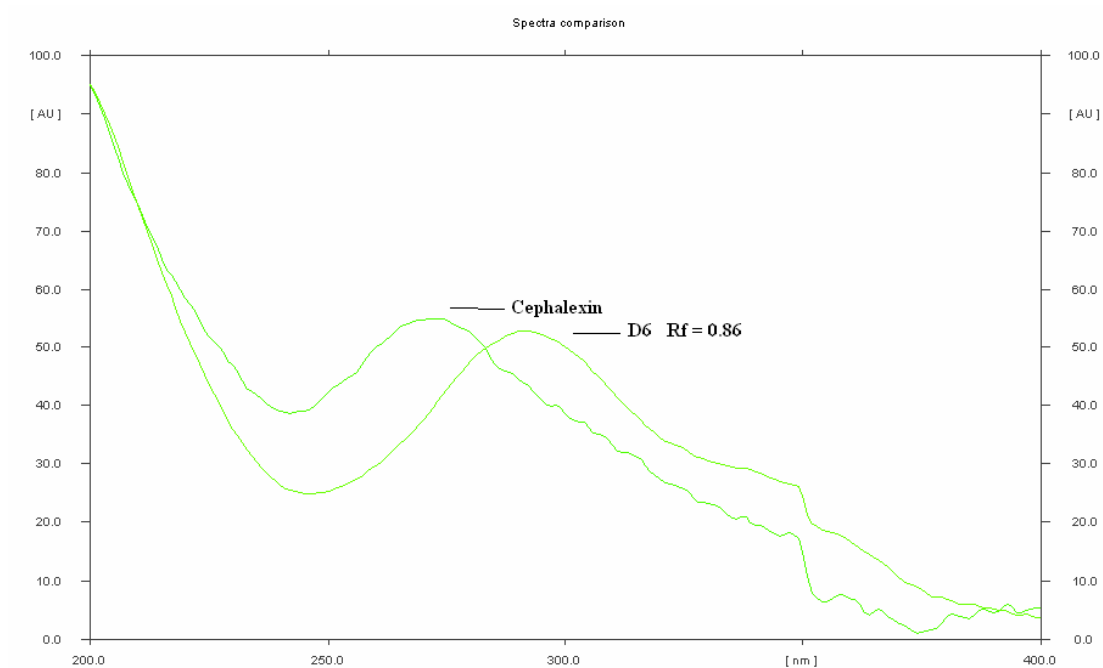**Fig. IX : Overlain Spectra of Cephalexin and its degradation products after neutral hydrolysis.**

**Fig X : Densitogram of Cephalexin and its degradation products after oxidation treatment.** .
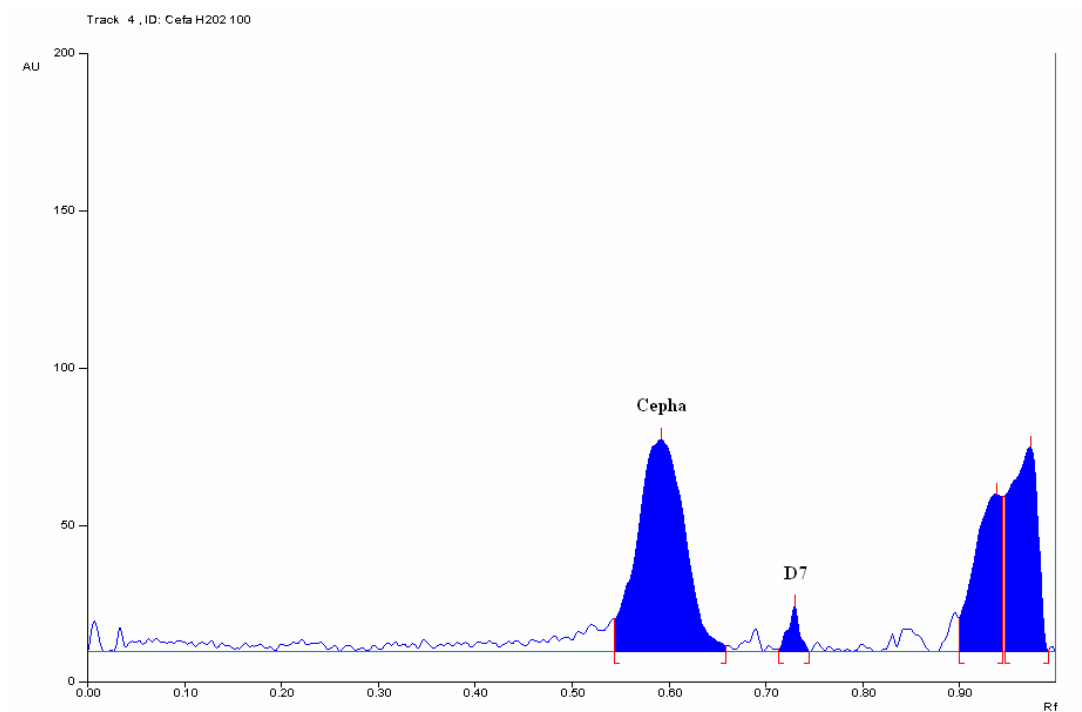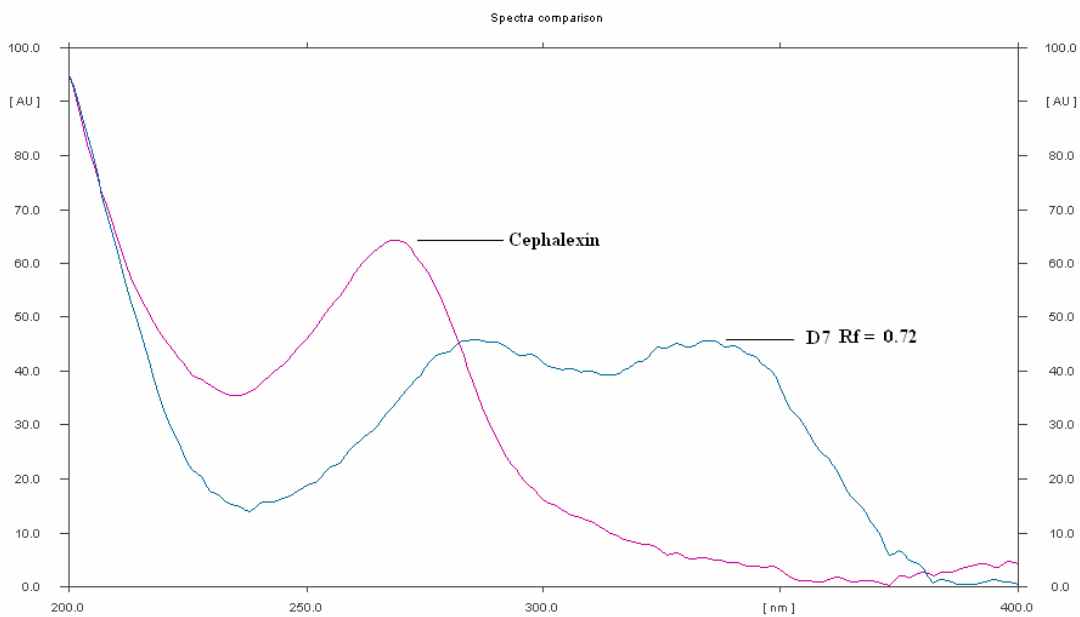


**Fig. XI: Overlain Spectra of Cephalexin and its degradation products after oxidation treatment.**

## 5. Acknowledgement

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