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RESEARCH ARTICLE

Development of Stability-Indicating Liquid Chromatographic Method for Analysis of Cefoxitin Sodium in Sterile Formulation.

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ABSTRACT

Stability-indicating high performance liquid chromatography (HPLC) method was developed and validated for analysis of cefoxitin sodium in its powder for injection dosage form. Chromatographic separation was achieved on a C18 µBondapack (300×3.9, 10µ) column, maintained at 30°C with a mobile phase consisted of water: acetonitrile: glacial acetic acid (800:190:10) and a flow rate of 0.9mL/min. The peak was detected at 254nm & the retention time was obtained at 16.74 min. The peak area plot was linear over the concentration range of 72.16µg/mL to 451.04µg/mL. The different experimental parameters affecting the drug stability were optimized. The method was validated for accuracy, precision, reproducibility, specificity, robustness and ruggedness in accordance with International Conference on Harmonization (ICH) guidelines. The proposed method was successfully applied for the analysis of cefoxitin sodium in drug substance and drug product in the presence of hydrolytic and oxidative degradants.

KEYWORDS: Cefoxitin sodium, Degradation, Stress condition, Stability-indicating.

INTRODUCTION:

The cephalosporins are a class of β-lactam antibiotics originally derived from *Acremonium*, which was previously known as "Cephalosporium". Cefoxitin sodium (Figure 1) is a second generation antibacterial. Cephalosporin compounds were first isolated from cultures of *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu. The 7-aminocephalosporanic acid (7-ACA) proceeds from the hydrolysis of the cephalosporin C biologically active. Chemical structure of cephalosporins derived from the 7-ACA composed of a β-lactam ring fused with a dihydrothiazine ring, but differ in the nature of substituents attached at the 3- and/or 7-positions of the cephem ring. Cephalosporins are bactericidal and have the same mode of action as other beta-lactam antibiotics (such as penicillin's) but are less susceptible to penicillinases. Cefoxitin sodium (1) disrupts the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity. A thorough literature survey has revealed that, colorimetric methods (2-4), first and second derivative UV spectroscopy (5,6), fluorimetric (7), microbiological (8), LC-MS/MS (9), high performance liquid chromatographic (HPLC) method for determination in biological samples (10-13) and HPLC method in dosage forms (14) have been reported for analysis of Cefoxitin Sodium. To best of our knowledge, nothing has been reported for the analysis of cefoxitin sodium in the presence of hydrolytic and oxidative degradants using HPLC method. There is always a necessary for significant stability indicating method for drug analysis to avoid the interaction of degradants during sample preparation. The principle objective of this present study was, therefore, to develop a new, simple and stability-indicating HPLC method for assay of cefoxitin sodium in the powder for injection dosage forms.

EXPERIMENTAL:

CHEMICALS:

Cefoxitin Sodium (99.50 % purity) was kindly supplied from Hospira health care limited, chennai, India as gift sample. HPLC-grade methanol, Monobasic potassium phosphate, Dibasic sodium phosphate; Rankem Fine Chemicals Ltd and high-purity water was prepared using Milli-Q water purification system (Millipore, Milford, USA). Cefoxitin sodium for injection, labeled to contain 1 gram and 2 gram of cefoxitin sodium were manufactured by Hospira Health Care Pvt Limited and were purchased from local market.

EQUIPMENT:

Waters (2695 separation module) with 2996 PDA detector was used for HPLC study along with auto sampler, C18 µBondapack column, (300mm×3.9mm),10 µm packing of octa decyl silane chemically bonded to porous silica particles and Empower 2 software with computer.

CHROMATOGRAPHIC CONDITIONS:

The mobile phase used was water: acetonitrile: glacial acetic acid (800:190:10), at a flow rate of 0.9mL/min. The eluate was monitored at 254 nm. Separation was carried out at temperature of 30°C.

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DILUENT PREPARATION:

Phosphate buffer (pH 7.1) preparation: One gram of mono basic potassium phosphate and 1.8 gram of di basic sodium phosphate were dissolved in 900 ml of Milli-Q water and adjusted the ph up to 7.1 with phosphoric acid and made up to 1000 ml with same.

STANDARD SOLUTIONS:

Cefoxitin sodium standard (1 mg/mL) was prepared by dissolving 100 mg of reference standard in 100 mL of diluent. This stock solution was subsequently used for preparation of working standards in concentration ranges of 25 µg/mL to 500 µg/mL. the stock solution when kept in refrigerator at 5°C, was stable for atleast 2 d.

CONSTRUCTION OF CALIBRATION CURVE:

Aliquots of working solutions equivalent to 75 – 450 µg/mL of cefoxitin sodium (actual concentration 72.168 to 451.049µg/mL) was used for linearity studies. The peak areas of solutions were measured at detection wavelength of 254 nm. The calibration curve representing the relationship between the peak area and the corresponding concentrations of the reference drug was constructed.

ANALYSIS OF DRUG PRODUCT (1GM CEFOXITIN SODIUM FOR INJECTION):

One vial was reconstituted with 10mL of water for injection or as per labeling to get 95mg/mL of Cefoxitin. Then entire contents was withdrawn from the vial using a suitable calibrated Hamilton syringe & transferred in to 250mL volumetric flask and diluted to volume with Milli Q water and mixed well. Then 2mL of above stock solution was transferred into 25mL volumetric flask and made up to the volume with same and used for analysis.

FORCED DEGRADATION STUDIES:

The drug product was stressed under various conditions to conduct forced degradation studies. Unfortunately, the current guidance documents do not indicate detailed degradation conditions in stress testing. However, the forced degradation conditions, stress agent concentration, and time of stress were selected based on trial and error method. Cefoxitin Sodium was stressed with 0.1N hydrochloric acid in water bath at 70ºC for 15 min (for acid hydrolysis), with 0.1N sodium hydroxide solution for 2 minute on Bench top (for alkaline hydrolysis), with 1% hydrogen peroxide solution for 10 min on Bench top (for oxidation), with dry heat at 105°C for 24 hours, with humidity at 25°C/90 % RH for 312 h , with water at 70°C on water bath for 5 min (water hydrolysis), with visible light radiation for 165 h and with UV light radiation for 34 h (for photo degradation). Then each sample was analysed by above suggested method.

VALIDATION:

The proposed method was validated as per ICH guidelines (15). To determine linearity a calibration graph was obtained by plotting cefoxitin sodium from the concentration range of 75 µg/mL to 450 µg/mL against peak area. Repeatability was carried out using six replicates of same concentration of 1gm/vial and 2gm/vial. The accuracy of the method was assessed by determination of recovery for six concentrations (corresponding to 25, 50, 75, 100, 125 and 150% of test solution concentration) covering the range of the method. For each concentration three sets were prepared and injected. Ruggedness or intermediate precision was assessed by analyzing the test concentration by using two different analysts, columns and instruments. The robustness of the method was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions flow rate (± 0.1 ml/min), the proportions of organic phase in mobile phase (± 10 %, v/v) and changing the column temperature (25°C and 35°C). For each different analytical condition the standard solution was prepared separately and analysed.

RESULTS AND DISCUSSION:

Based on interrelationship between columns, pH, mobile phase composition and its ratio the optimized parameters were set and the peak was obtained at retention time of 16.7 min (Figure 2). The specificity of the method was demonstrated by analysing the sample with different stressed condition. All degradants peaks were well resolved from main peak in the chromatograms which were shown in Figure 3-10. The chromatograms of the stressed samples were evaluated for peak purity using Empower software. For all forced degradation samples, the purity angle for Cefoxitin peak was less than purity threshold and results were tabulated in Table 1. Cefoxitin peak did not have any Flag in purity results table. This indicated that there was no interference from degradants duing quantitating the Cefoxitin using proposed method.

VALIDATION OF DEVELOPED METHOD:

The average percentage content and % relative standard deviation (%RSD) for repeatability study were found to be 102.3 % and 0.3 % for 1 g/vial and 101.4 % and 0.1 % for 2g/vial respectively. The accuracy of the method was determined by performing the recovery experiment at six levels (25%-150%). The % recovery obtained between 99.0– 101.4% proved that the method was accurate.

The method shows good linearity in the range of 75 to 450 µg/mL. The linear regression data for the calibration plot are indicative of a good linear relationship between peak area and concentration over a wide range and the value of correlation coefficient was indicative of high significance. The data for validation parameters were tabulated in Table 2. The results of tailing factor, theoretical plates and % RSD of the peak areas of five replicates were found to be within the limit for ruggedness study. The results were tabulated in Table 3. There was no significant change in the system suitability factors of cefoxitin sodium when the organic composition, flow rate and column temperature were changed. The low values of the %RSD indicated that the method was robust enough and the results were tabulated in Table 4. From the stability study, difference in % assay for drug product with respect to initial was NMT 3.0 %. It was established that the mobile phases, test and standard solutions were stable for 1 day on bench top and stable for 2 days in refrigerator.

Stress Condition	% Degradation	Purity angle	Purity Threshold	Purity flag
Acid hydrolysis	7.86	0.314	1.708	No
Alkaline hydrolysis	18.72	0.059	0.350	No
Oxidation	2.67	0.057	3.890	No
Dry heat	0.69	0.057	1.756	No
Humidity	0.78	0.061	2.161	No
Water hydrolysis	26.30	0.086	1.532	No
Photo degradation with visible light radiation	0.46	0.050	1.458	No
Photo degradation with UV light radiation.	0.48	0.048	0.286	No

Table No. 1. Peak purity results for forced degradation studies

Parameters	Results
Linearity range (µg/mL)	75-450
Limit of Detection (µg/mL)	0.172
Limit of Quantification (µg/mL)	0.522
Slope	11318
Standard error of slope	80.727
Confidence limit of slope	11132 to 11504
Intercept	-37329
Standard error of intercept	22814
Confidence limit of intercept	-89939 to 15281
Correlation co-efficient	0.9996

Table No. 2. Results for Linearity Study Data

System Suitability Parameters	Analyst		Instrument		Column		Acceptance Criteria
	1	2	1	2	1	2	
USP tailing factor of Cefoxitin peak in standard preparation	1.5	1.2	1.5	1.2	1.5	1.4	NMT 1.5
Theoretical plates for Cefoxitin peak in standard preparation	3719	6724	3719	7068	3719	4220	NLT 2800
% RSD for peak areas *	0.2	0.1	0.2	0.1	0.2	0.2	NMT 2.0
% RSD for percentage drug content*	0.1	0.2	0.1	1.0	0.1	1.0	NMT 2.0

***(n=5)**

Table No. 3- Data for ruggedness-system suitability and % Assay

Parameters		USP tailing factor	Theoretical plates	% RSD
Flow rate (mL/min)	0.8	1.2	8379	0.5
	0.9	1.3	6935	0.1
	1.0	1.3	6248	0.1
Organic phase composition (%)	90	1.2	6637	0.2
	100	1.3	6935	0.13
	110	1.2	6317	0.2
Column temp. (°C)	25	1.3	6842	0.1
	30	1.3	6935	0.1
	35	1.3	7037	0.1

Table No. 4. Data for Robustness studies

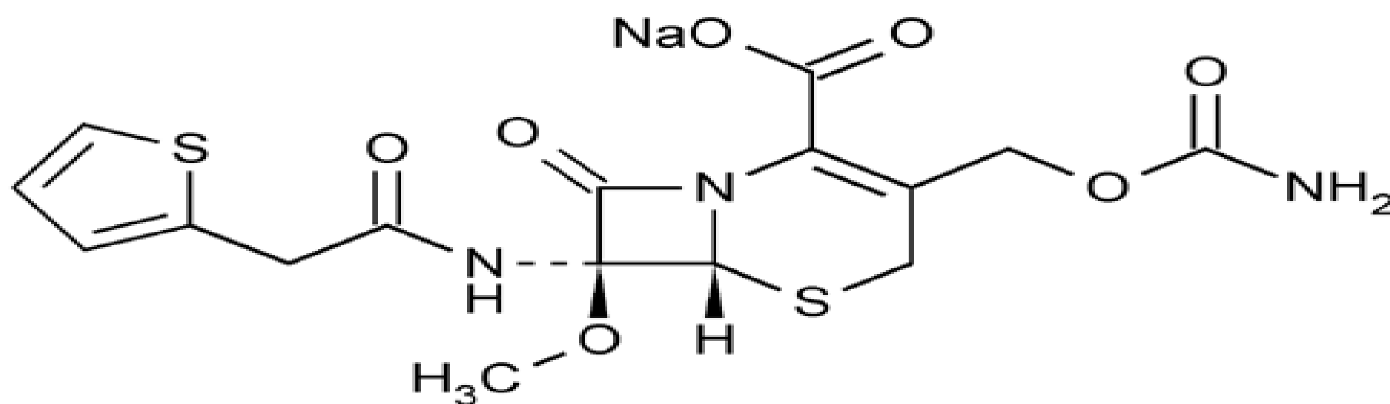

Figure No. 1. Chemical structure of Cefoxitin sodium

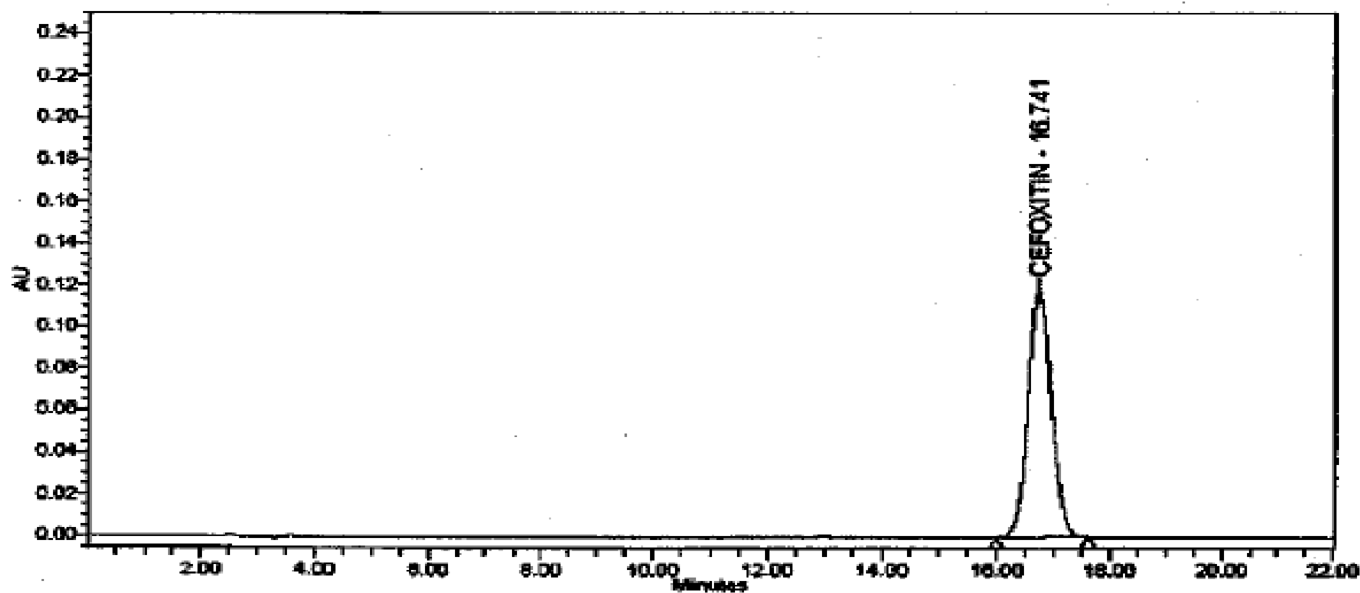

Figure No. 2. Chromatogram of Cefoxitin standard

(The retention time for the cefoxitin sodium was found to be 16.741 minutes.)

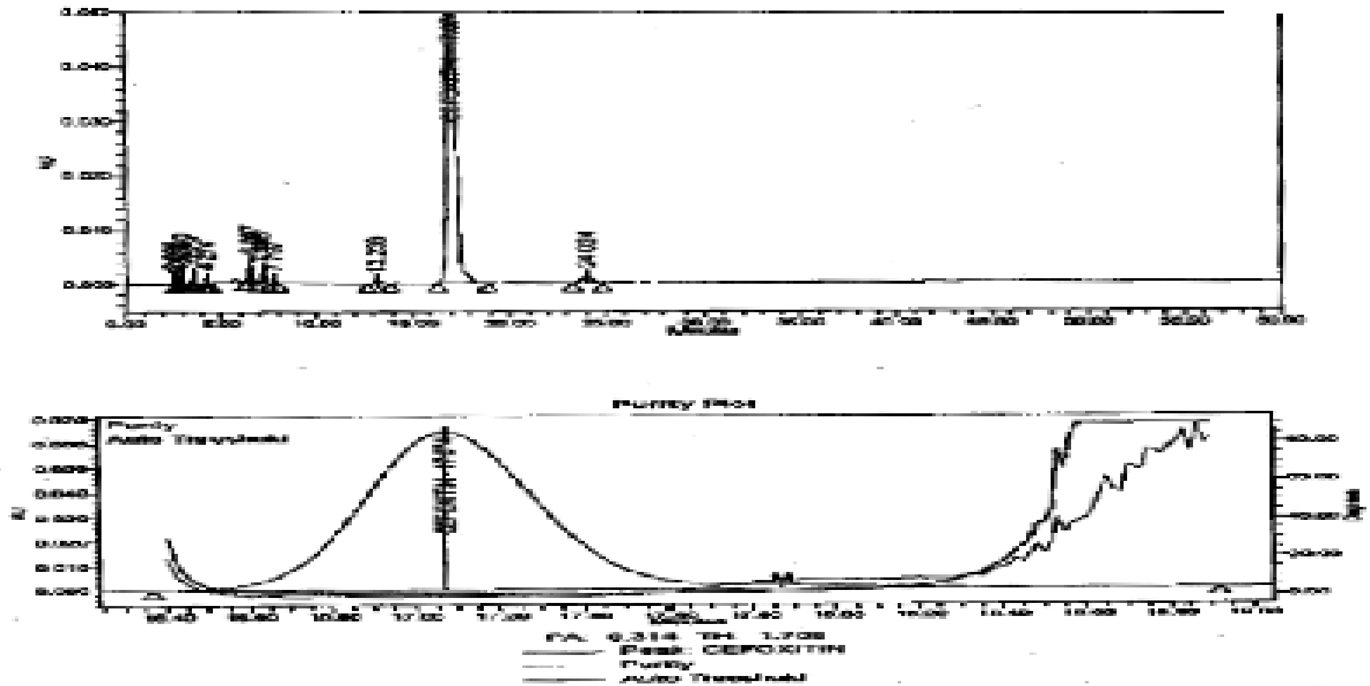

Figure No. 3. Typical chromatogram and purity plot of acid stressed Cefoxitin for injection

(The Cefoxitin sodium eluted at the retention time of 17.074 minute which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 2.0 - 13.233 minutes and also at 24.024 minute**.)**

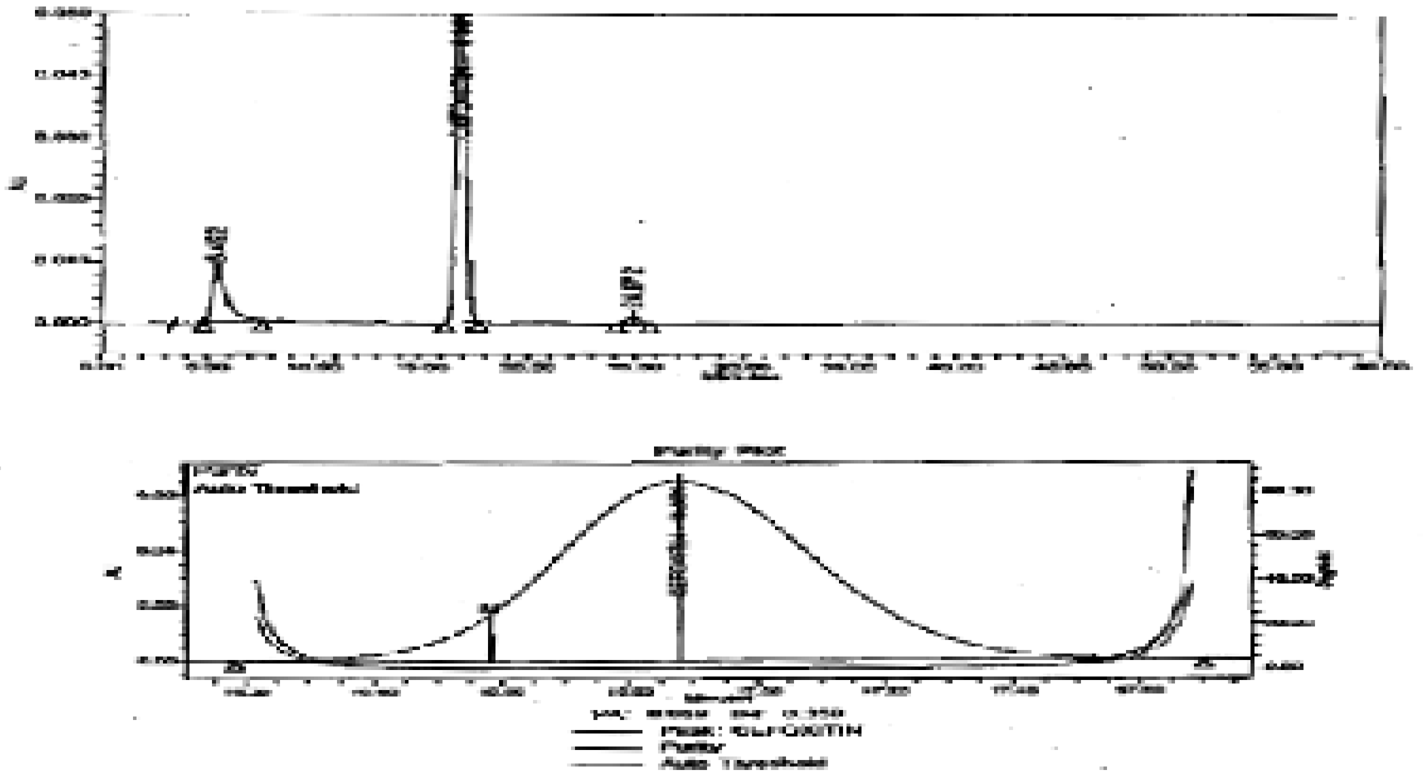

Figure No. 4. Typical chromatogram and purity plot of alkali stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 16.80 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 5.482 minute and also at 24.872 minute.**)**

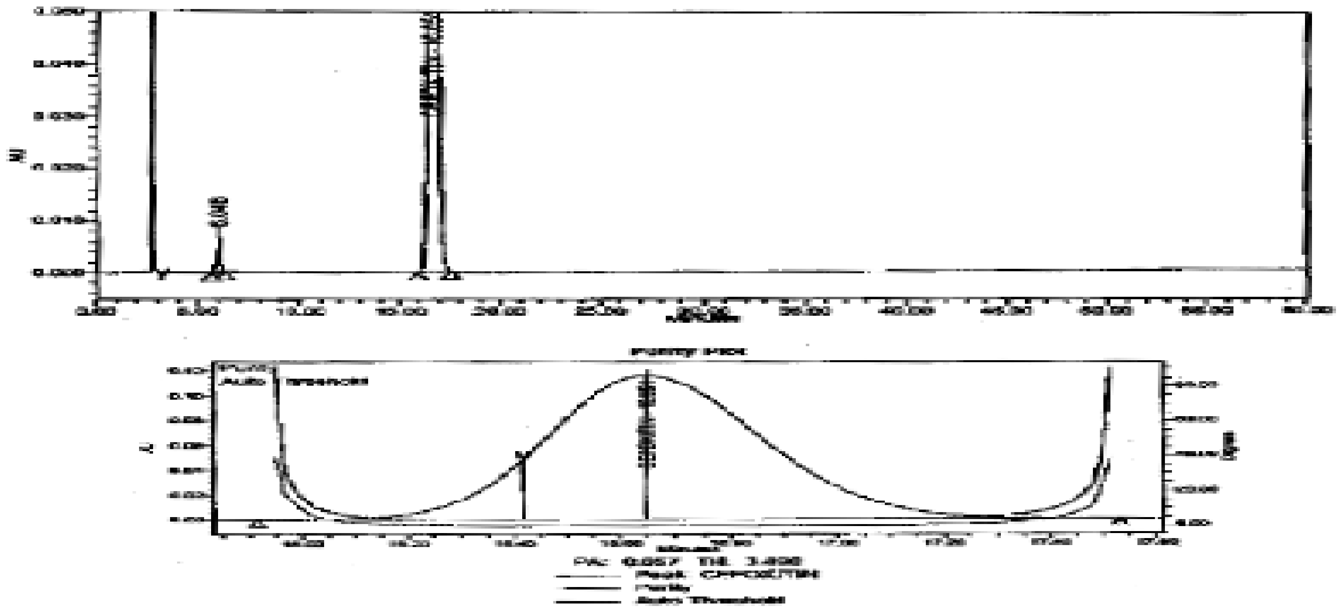

Figure No. 5. Typical chromatogram and purity plot of oxidation stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 16.85 minutes which was well resolved from the degradant peaks. The degradant peak was eluted at the retention time of 6.048 minute.**)**

Purity Plot

PA: 0.057 TH: 1.756
Peak: CEFOXITIN
Purity
Auto Threshold

Figure No. 6. Typical chromatogram and purity plot of dry heat stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 17.10 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 2.570-13.381 minute.**)**

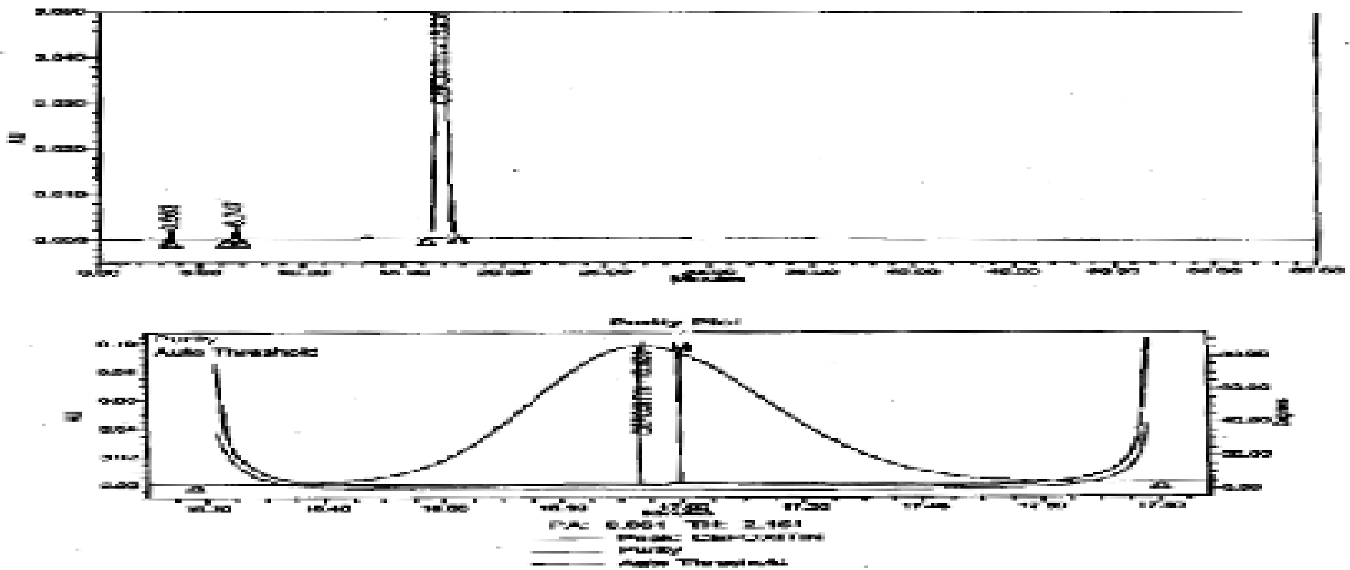

Figure No. 7. Typical chromatogram and purity plot of water stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 16.93 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 3.563 & 6.747 minute.**)**

Figure No. 8. Typical chromatogram and purity plot of dry heat stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 17.0 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 2.685-13.202 minute.**)**

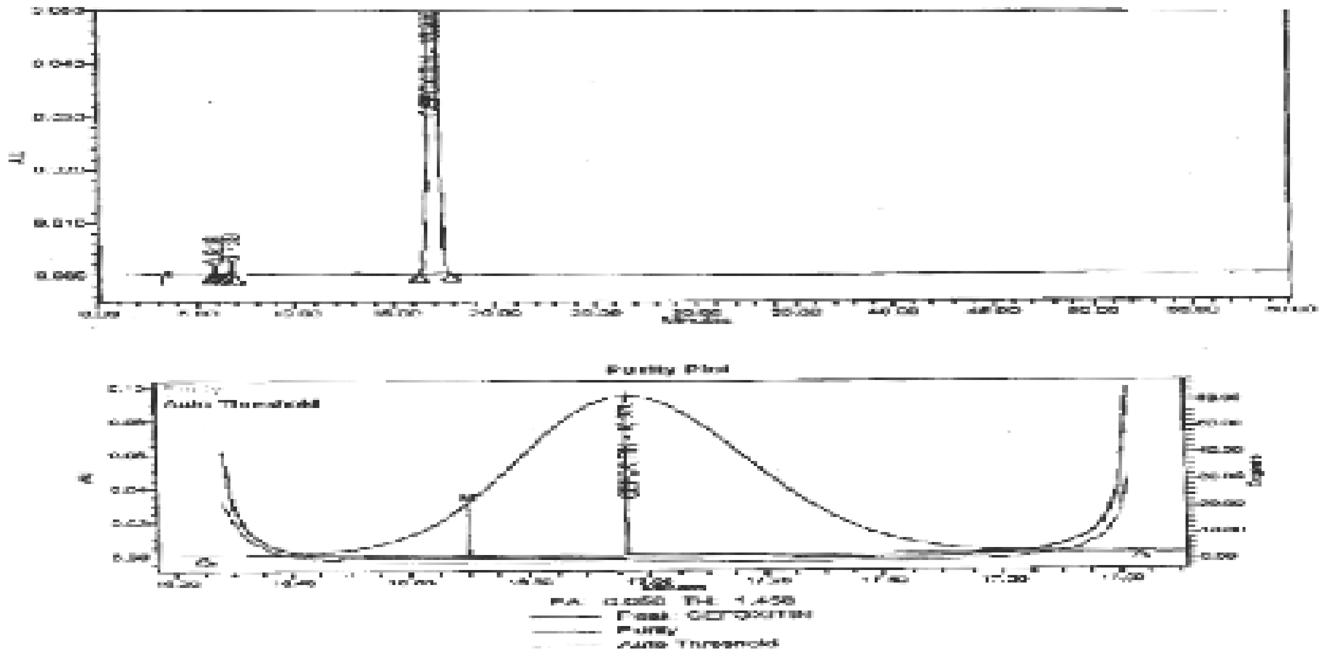

Figure No. 9. Typical chromatogram and purity plot of visible light stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 16.876 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 6.918 & 6.738 minute.**)**

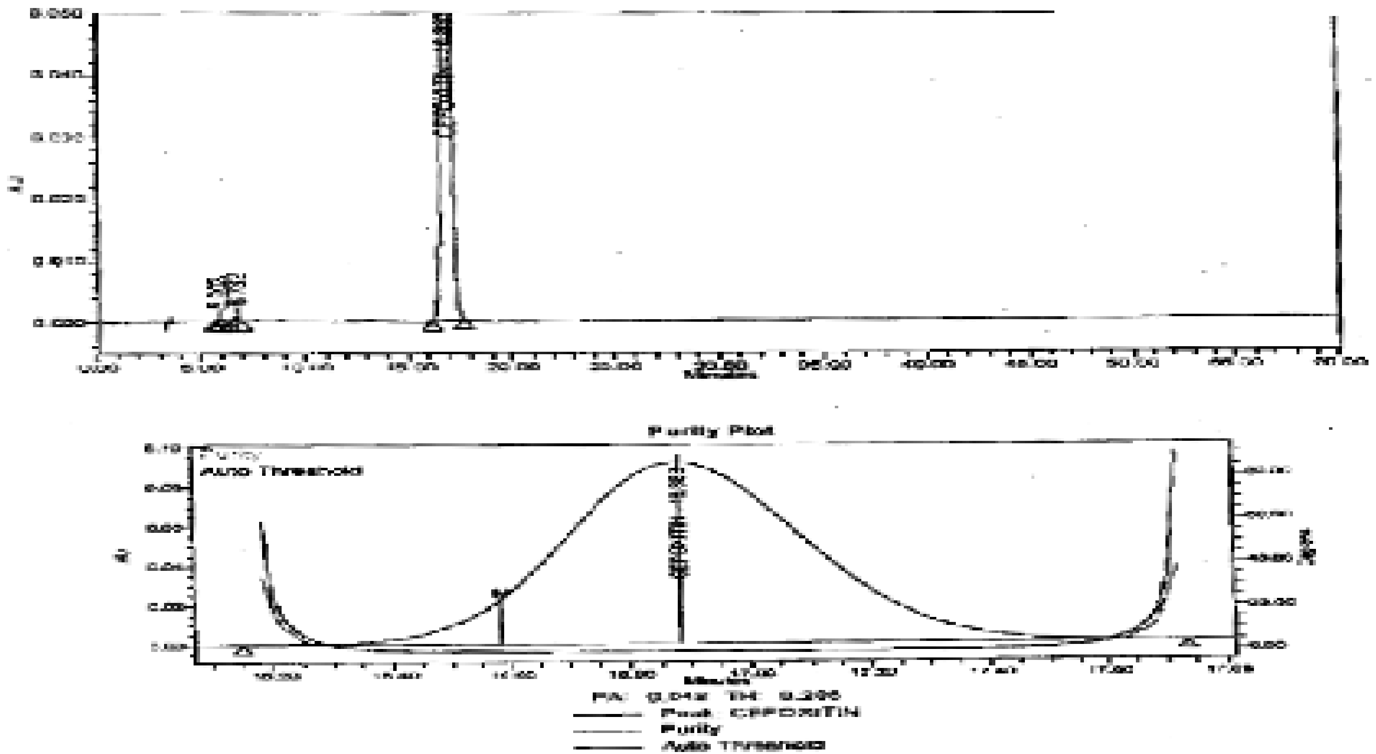

Figure No. 10. Typical chromatogram and purity plot of UV light stressed Cefoxitin for injection

(The cefoxitin sodium eluted at the retention time of approximately 16.880 minutes which was well resolved from the degradant peaks. The degradant peaks were eluted at the retention time of 5.903 & 6.732 minute.**)**

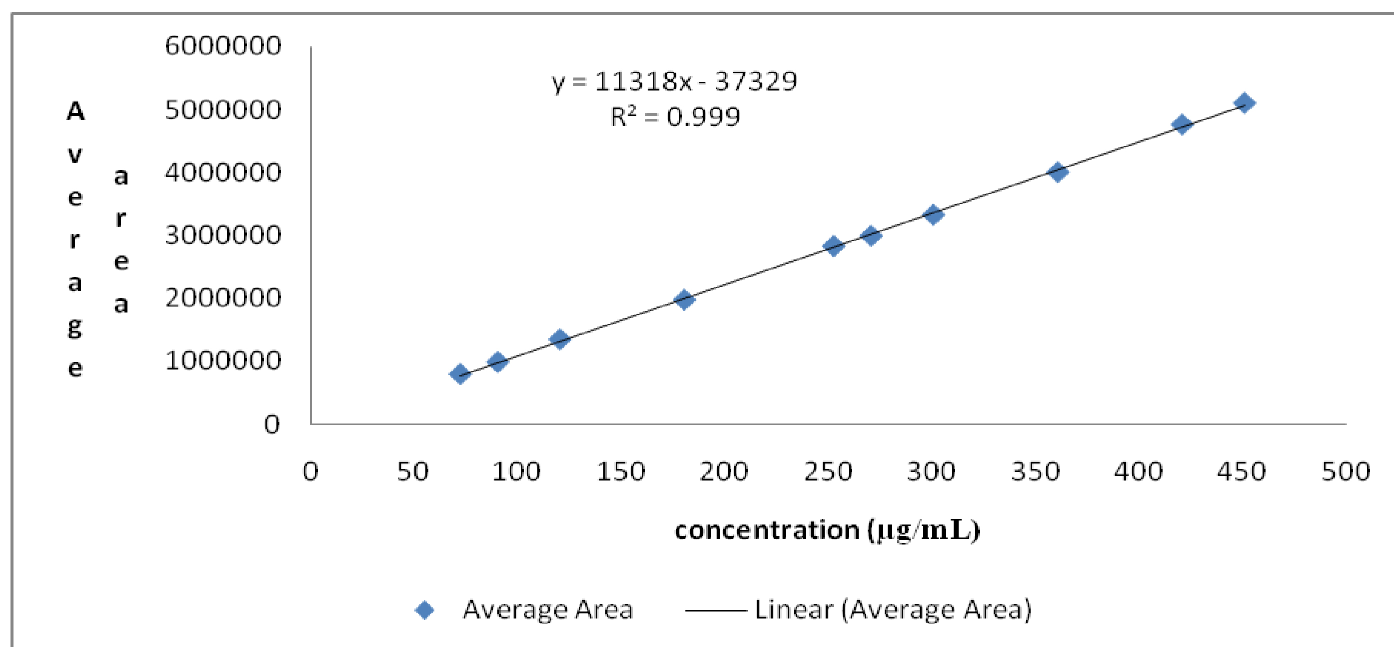

Figure No. 11. Linearity of Detector Response

(Regression equation y=Mx+C (y=11318x+37329) and Correlation coefficient R^2=0.999.**)**

CONCLUSION

From the results of analysis, it was concluded that the proposed method showed high accuracy, repeatability and specificity and can be used as stability indicating method. Moreover, this method is simple and specific and can be applied to routine analysis of drug in sterile preparation.

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