International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

VALIDATED SPECTROPHOTOMETRIC & RP-HPLC METHOD FOR THE ESTIMATION OF CEFUROXIME AXETIL & CEFIXIME TRIHYDRATE IN TABLET DOSAGE FORM

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ABSTRACT

HPLC & UV-spectrophotometric method for analysis of Cefuroxime Axetil and Cefixime Trihydrate done a simple, specific, accurate, and precise method has been developed. Separation of drugs was carried out on HPLC & UV system Results were found to be linear in the concentration range of 10-50 µg/ml for Cefuroxime axetil and 5-25 µg/ml for Ceffixime Trihydrate. Intra-day variation, as RSD (%), was 0.124 for Cefuroxime axetil and 0.217 for Cefuroxime Axetil. Interday variation, as RSD (%) was 0.149 for Cefuroxime axetil and 0.254 for Cefixime Trihydrate. The UV-spectrophotometric % assay was found to be 124.5% for Cefuroxime Axetil and 63.07% for Cefixime Trihydrate for HPLC % assay found to be 99.785 for cefuroxime axetil and 101.6 % for Cefixime trihydrate .

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KEYWORDS : *HPLC & UV-spectrophotometric method, Cefuroxime Axetil, Cefixime Trihydrate.*

INTRODUCTION

There are various methods used for the analysis of cephalosporin in the various forms like chromatographic, UV, electrophoresis etc. The applications of HPLC & UV spectrophotometric method of analysis of antibiotics introduce a powerful tool for therapeutic drug monitoring as well as clinical research [(1).] Cefixime trihydrate is an orally active third generation semi synthetic cephalosporin type of β lactamantibiotic. Chemically, Cefiximes is, 5-Thia-1azabicyclo [4,2,0]oct-2-ene-2-carboxylicacid,7-[[(2-amino-4-thiazolyl)[(carboxymethoxy) amino] acetyl] amino]-3-ethenyl-8-oxo-trihydrate. It is soluble in methanol and 0.1M NaOH, insoluble water and 0.1M HCl. It is semi synthetic, broad spectrum cephalosporin antibiotic for oral administration. It is indicated in case of acute bacterial maxillary sinusitis, urinary tract infection,.pharyngitis / tonsillitis, acute bacterial otitis media, chronic bronchitis lyme disease [(2-7)] Cefuroxime axetil (RS)-1 hydroxyethyl(6R,7R)-7-[2-(2-furyl) glyoxyl-amido] -3- (hydroxyl methyl -8-oxo-5- thia-1- azabicyclo[4.2.0]-oct-2-ene-2-carboxylate,7 -(Z)-(O-methyl-oxime),1-acetate3-carbamate) is second generation cephalosporin

used to treat or prevent infections that are proven or stronglysuspected to be caused by bacteria[8]

MATERIAL AND METHODS

Standard solution (10µg/ml) of pure CefiximeTrihydrate and Cefuroxime Axetil was prepared. The pure drug solution was scanned on UV spectrophotometer, which showed maximum absorbance at 237.0 nm and 278 nm respectively. 10 mg of CefiximeTrihydrate and 10 mg of cefuroxime axetil was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the diluents (0.1 NaOH), to give a stock solution of 1000ppm.From stock solutions of Cefixime Trihydrate 1 ml was taken and diluted up to 10 ml. from this solution 0.5,0.1,1.5,2.0, 2.5ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration .similarly From stock solutions of Cefuroxime Axitill 0.1,0.2,0.3,0.4, 0.5 ml gives standard drug solution of 10, 20, 30, 40, 50µg/ ml concentration. The standard drug solutions were taken absorbance 3 times and the mean absorbance of drug was calculated and plotted against the concentration of the drug. A typical calibration curve (fig.2, 4 for UV) and (fig 5 & 8 for HPLC) was obtained. Weight equivalent to 10 mg of sample CefiximeTrihydrate and Cefuroxime axetil and dissolved with 5 ml solvent (0.1 N NaOH) in 10 ml Volumetric Flask and sonicate it for 10 min by ultrasonicator, after that volume was made up to 10 ml with solvent to obtain concentration of 10 µg/ml.Further dilute and make 10 ppm solution and take the absorbance of the sample solutions at 237 nm and 278 nm was measured respectively.

VALIDATION

Method validation can be defined as (ICH*)* "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre analysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its statistical validation of recovery studies shown in (Table no. 1 & 2).

Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

Analyst to Analyst

The intermediate precision expresses with in laboratories variation: different days, different analysts, different equipment etc. The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods. The statistical analysis method was carried out.

LOD (Limit of Detection)

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve**,** σ = standard deviation of the response.

LOQ (Limit of Quantication)

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula.

$$LOQ = 10 (\sigma / S)$$

TABLE NO.1 VALIDATION RESULTS BY UV-SPECTROPHOTOMETER

S.NO.	Parameter	Cefuroxime Axetil	Cefixime Trihydrate
1	Absorption maxima(nm)	278.0 nm	237.0 nm
2	Linearity Range (mg/ml)	10-50 ug/ml	5-25 ug/ml
3	Standard Regression Equation	Y = 0.013 x +0.0029	Y = 0.055 x +0.002
4	Correlation Coefficient (r2)	0.989	0.999
5	Accuracy (% recovery)	100.61	100.07
6	Precision	99.07(intra day) 98.02(inter day)	99.20((intra day) 97.83(inter day)
7	LOD (mg/ml)	0.15 µg/mL	0.3 µg/ml
8	LOQ (mg/ml)	0.50 µg/mL	1.5 µg/ml
9	SD	0.000816	0.121
10	% RSD	0.147	0.564

TABLE NO.2 VALIDATION RESULTS BY HPLC

S.NO.	Parameter	Cefuroxime Axetil	Cefixime Trihydrate
1	Accuracy (% recovery)	100.30	100.04
2	Precision	99.13(intra day) 97.50(inter day)	100.28(intra day) 98.43(inter day)
3	AUC	5010.445	1401.415
4	Mean response ratio	503.35	141.58
5	SD	0.155	0.007
6	% RSD	0.159	0.001

Table No. 3- Selection of Separation Variable for HPLC

Variable	Condition for cefuroxime	Condition for Cefixime
Column		
Dimension.	250mm x 4.60mm	250mm x 4.60mm
Particle Size	5µ	5µ
Bonded Phase	Octadecylsilane (C_{18})	Octadecylsilane (C_{18})
Mobile Phase- Methanol-ACN		**Mobile Phase-** KH_2PO_4- Methanol
Ratio	50	80
	50	20
Flow rate	1ml/min	1.0 ml/min
Temperature	Room temp.	Ambient
Sample Size	20 µl	20 µl
Detection wavelength	278.0 nm	237.0 nm
Retention time	10.792 $\pm$ 0.3 min	5.470 ± 0.3 min

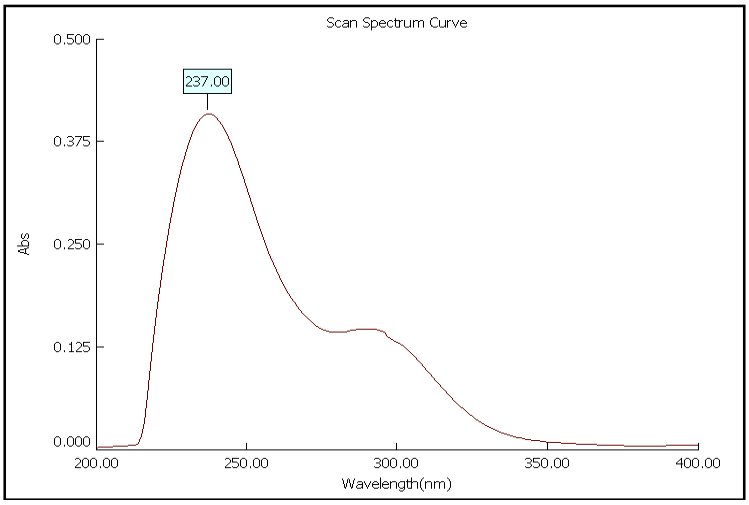

Fig no.1 U.V. Spectra of Marketed Formulation

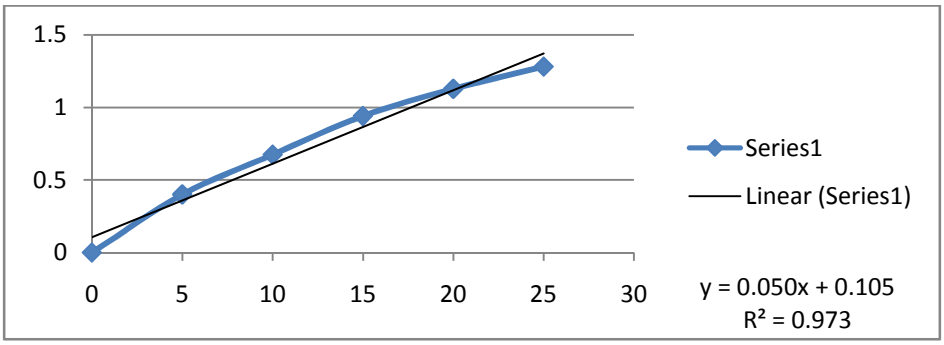

Fig No. 2 Calibration Curve of Standard Cefixime Trihydrate

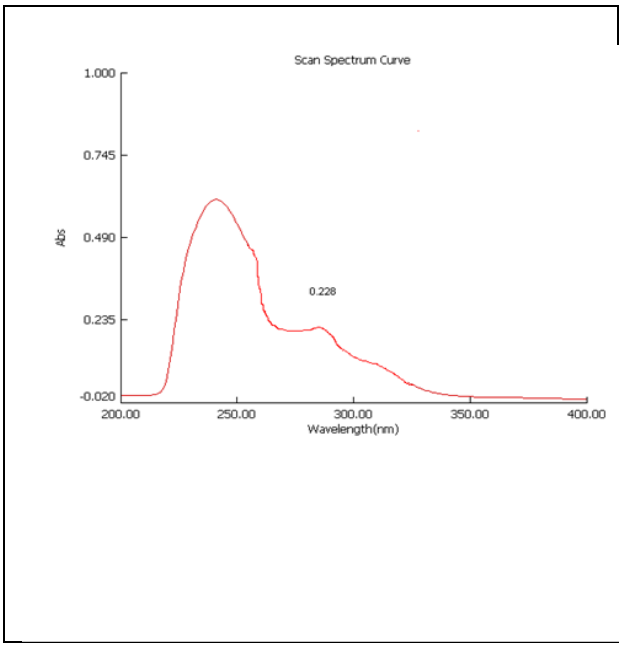

Fig no. 3 U.V. Spectra of Marketed Formulation

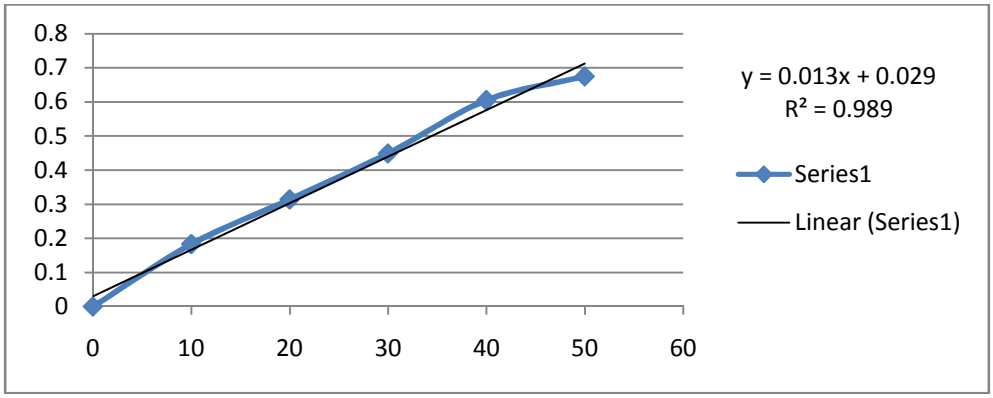

Fig No. 4 Calibration Curve of Standard Cefuroxime axetil

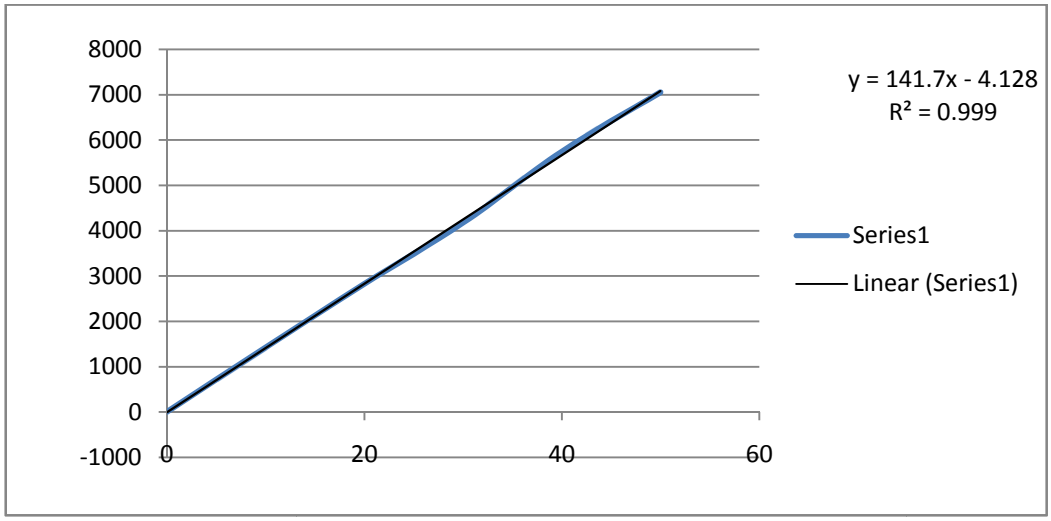

Figure no.5 Calibration Graph of Cefixime trihydrate

Regression Equation

Y= mx +c, Y= AUC, m= slope = 141.7, X = Conc. in µg/ml, c= Intercept = 4.128 r^2= 0.999

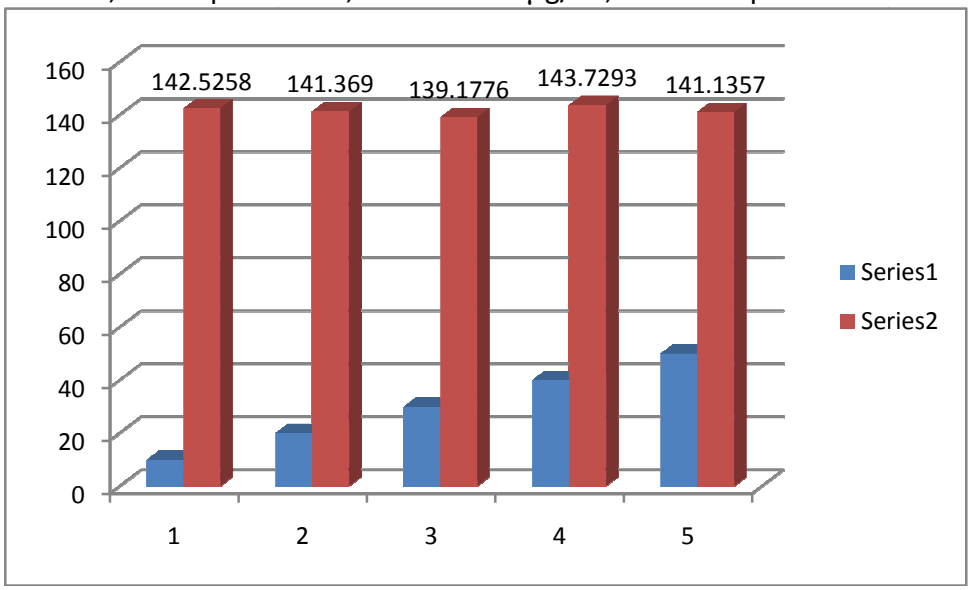

Figure no.6-Response Ratio Curve of Cefiximetrihydrate

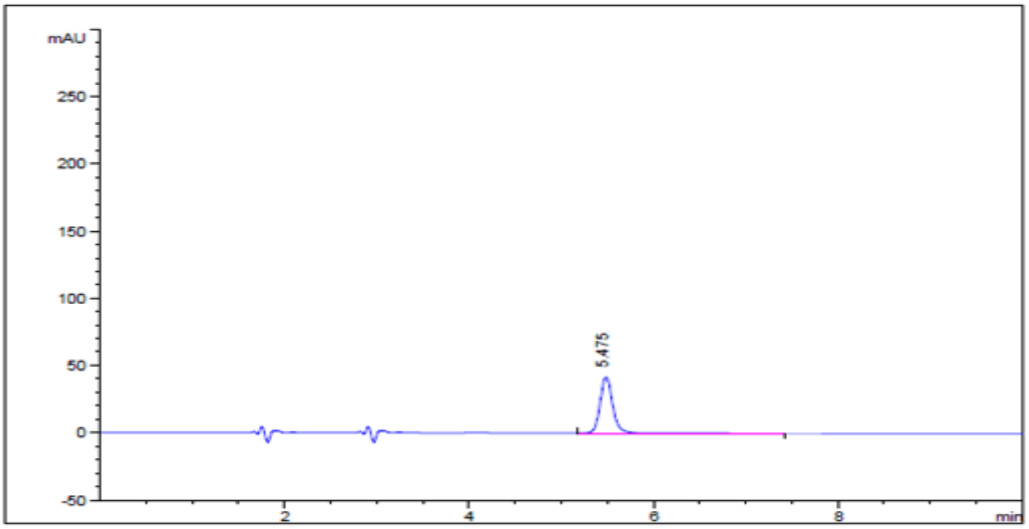

Figure no.7 Chromatogram of marketed formulation Cefiximetrihydrate

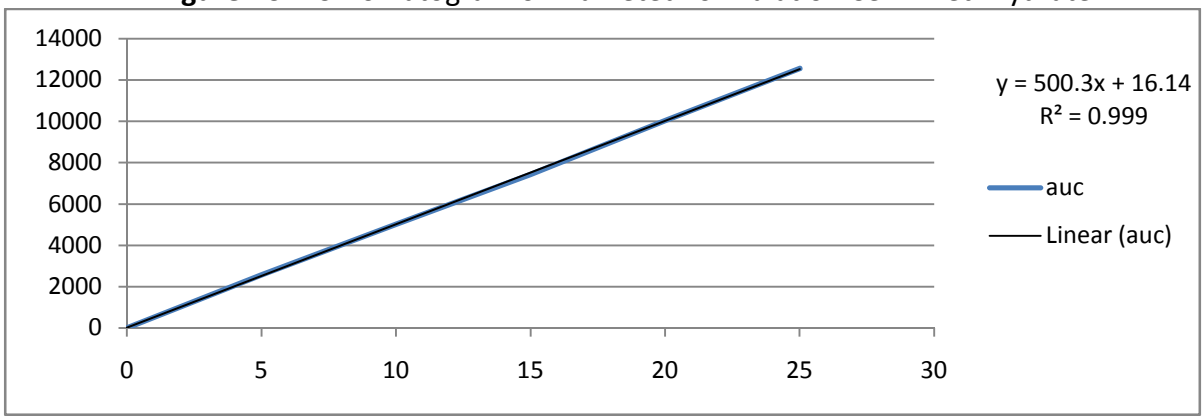

Figure no-8 Calibration Graph of Cefuroxime Axetil

Regression Equation

Y= mx +c, Y= AUC, m= slope = 500.3, X= Conc. in µg/ml, c= Intercept = 16.14, r^2= 0.999

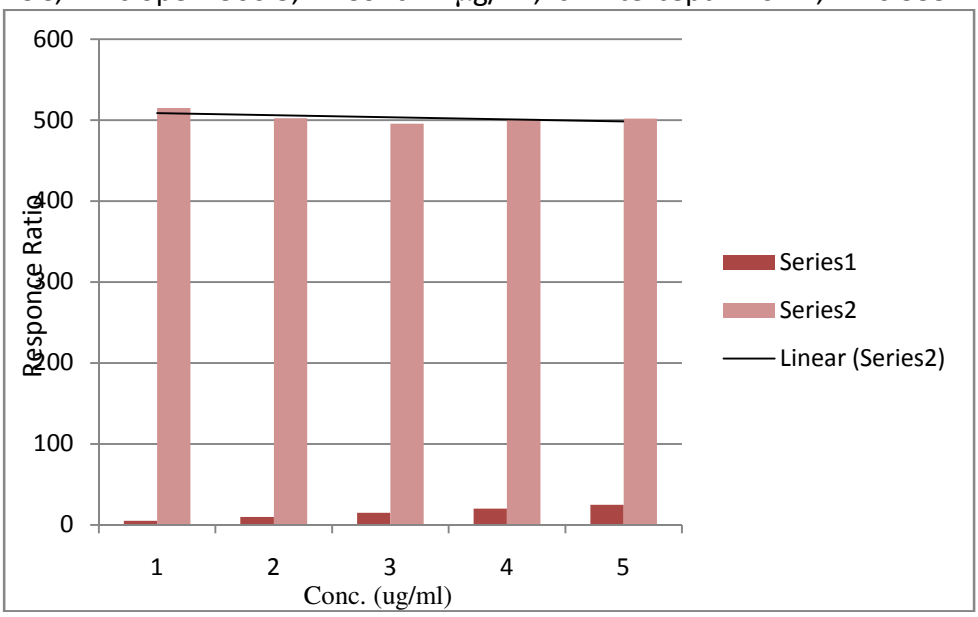

Figure no.9-Response Ratio Curve of Cefuroxime Axetil

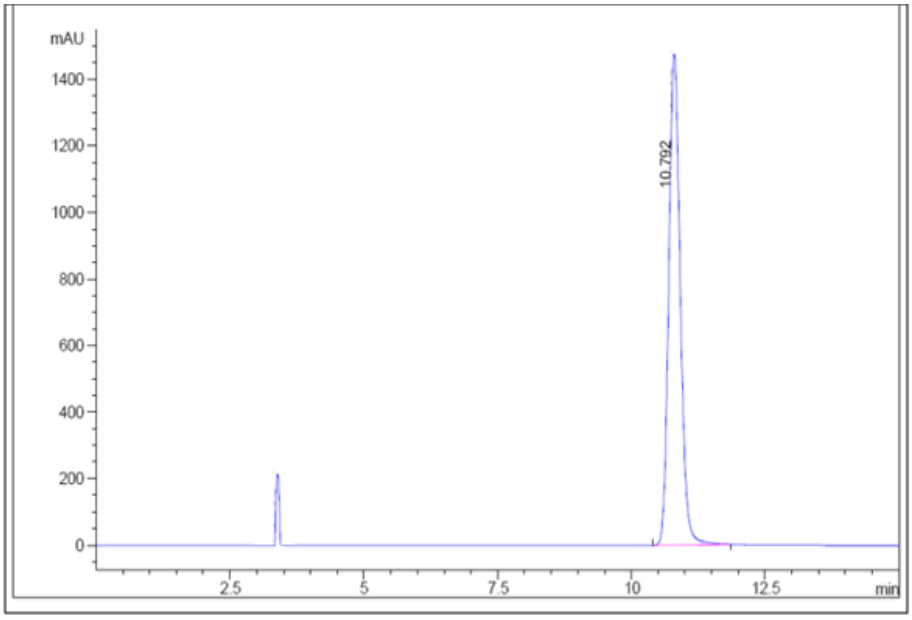

Figure no-10-Chromatogram of marketed formulationCefuroxime Axetil

RESULTS AND DISCUSSION

In this work, we analysed two cephalosporin drug cefuroxime axetil and cefixime trihydrate. We can done a simple ,precision and accurate validation method by ICH(international conference of harmonization) guideline For HPLC method different mobile phases were tried and the mobile phase containing Methanol-ACN in ratio 50:50(% *v/v*)for cefuroxime and KH_2PO_4- Methanol in ratio 80:20(% *v/v*) for cefixime .System suitability parameters for HPLC method are listed in Table 3. The LOD and the LOQ for the Cefuroxime axetil were found to be 0.15 µg/mL and 0.50 µg/mL respectively, and for cefixime trihydrate were found to be 0.3 µg/ml and1.5 µg/ml, respectively. The % recovery was found to be 99.50 % for Cefuroxime Axetil and 99.98 % for Cefixime Trihydrate. Results of recovery studies are represented in (Table1 & 2). The % RSD values were satisfactorily low indicating reproducibility of the method. The UV-spectrophotometric % assay was found to be 124.5% for Cefuroxime axetil and 63.07% for Cefixime trihydrate and for HPLC % assay found to be 99.785 for cefuroxime axetil and 101.6 % for cefixime trihydrate .

CONCLUSIONS

The developed method was found to be simple, sensitive, accurate, precise, reproducible, and can be used for routine quality of drug of Cefuroxime axetil and Cefixime trihydrate in bulk and pharmaceutical formulation. % RSD values were satisfactorily low indicating reproducibility of the method. The proposed HPLC method gives good resolution between Cefixime and Cefuroxime with short analysis time. The method is simple, accurate rapid and no complicated sample preparation is needed.

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