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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR SIMELTANEOUS ESTIMATION OF CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE IN BULK AND COMBINED DOSAGE FORM

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ABSTRACT

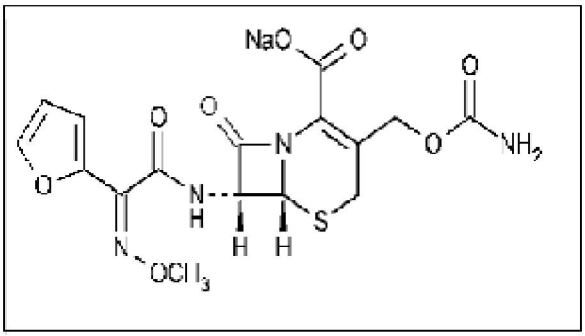
A Stability indicating RP-HPLC was developed and validated for determination of Cefuroxime Sodium (CEF) and Potassium Clavulanate (PCA). The RP-HPLC has shown adequate separation for Cefuroxime Sodium and Potassium Clavulanate from its degradation products. The separation was achieved on Hypersil BDS C_{18} column (250x4.6 mm i.d., 5 particle size) using a mobile phase composition of Acetonitrile: Phosphate buffer Ph4.5 (75: 25) with a flow rate of 1ml/min. Injection volume 20µl and wavelength of detection was kept at 275 nm the retention time of Cefuroxime sodium and Potassium Clavulanate were 3.06 and 7.63min respectively. Linearity was observed over concentration range of 10-40 µg/ ml for Cefuroxime Sodium and 6-20 µg/ml for Potassium Clavulanate. The mean recovery was found to be 100.08±0.68% and 99.95±0.67% for Cefuroxime Sodium and Potassium Clavulanate respectively The limit of detection was 0.34µg/ml and the limit of quantification was 0.112µg/ml for Cefuroxime Sodium and the limit of detection was 0.097µg/ml and the limit of quantification was 0.292µg/ml for Potassium Clavulanate. Cefuroxime Sodium and Potassium Clavulanate does not undergo any degradation so it can be said that Cefuroxime sodium and Potassium Clavulanate is stable to forced degradation conditions. This Method is simple, versatile, and reliable hence it can be used as stability indicating analytical Method for determination of Cefuroxime Sodium and Potassium Clavulanate in bulk and Marketed formulation.

KEYWORDS: Cefuroxime Sodium(CEF), Potassium Clavulanate(PCA), RP-HPLC, **,** stability indicating, Validation.

INTRODUCTION

Cefuroxime Sodium is chemically, is a 5-Thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid,3-[[(aminocarbonyl)oxy]methyl]-7-[[2-furanyl(methoxyimino)acetyl]amino]-8-oxo ,monosodium salt [6*R*-[6a,7b(*Z*)]]-,is a beta-lactam antibiotic., which is used as an Antibacterial Cephalosporins [1,].The Chemical Structure was shown in fig.1.literature survey reveals following methods: chromatography method,U.V.visible spectroscopic, RP-HPLC and liquid chromatography methodsl[2,3,4,5,6,7]. Potassium Clavulanate is chemically 4-0xa-l-

azabicyclo[3.2.0]heptane-2-carboxylicacid,3-(2-hydroxyethylidene)-7-oxo-,monopotassium salt, *2R-(2a,3Z,5a)-* ,which is used as a antibacterial Enzyme inhibitors[8]. Chemical Structure was shown in fig.2. PCA is official in IP 2007,BP 2005,USP 2005[9,10,11]. literature survey reveals following methods: HPLC method,U.V.spectroscopic method and RP-HPLC methods[12,13,14].The combined dosage form of Cefuroxime Sodium and Potassium Clavulanate are available in the market for treatment of patients with mild to moderate infections caused by susceptible strains of the designated microorganisms. . On literature survey, It was found that there is no method available for simultaneous estimation of CEF & PCA by HPLC & Stability study but methods are available for individual drug and in combination with other drug To develop simple, precise and accurate methods for the simultaneous estimation of CEF & PCA in bulk and in combined form using UV Spectrophotometric and HPLC method.



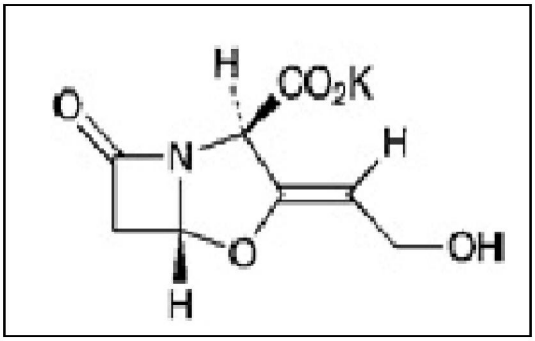


Fig.1. Cefuroxime Sodium **Fig.2. Potassium Clavulanate**

MATERIALS AND METHODS

Chemiacals and Reagents:

The raw materials of CEF and PCA are manufactured by Macleods Pharma Ltd, Mumbai respectively and received as gift sample. Acetonitrile,water,methanol,Disodium hydrogen phosphate (AR grade),0.05M potassium di hydrogen phosphate (75:25 v/v) were used as solvent.

Selection of Detection Wavelength:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study 10 µg/ml of CEF and 15µg/ml of were, therefore, prepared in methanol. These drug solutions were than scanned in the UV region of 200-400 nm and the overlain spectrums were recorded.

Chromatographic Condition:

The high performance liquid chromatograph (shimadzu) was composed of an LC-20AT. Prominence solvent delivery module, a manual rheodyne injector with a 20-µl fixed loop and a SPD-20A. Prominence ultraviolet- visible detector. Separation was performed on an enable C18 G column (particle size 5 µm; 250mm × 4.6mm) preceded by an ODS guard column (10µm, 10mm×5mm) at ambient temperature. Data were acquired on a spinchrome Chromatographic station® CFR Version 2.4.0.195.

Preparation of Mobile Phase:

A mixture of 75 ml ACN and 25 ml 0.05 M Phosphate Buffer (pH 4.5) of HPLC grade water filtered through 0.45 µm filter paper, Sonicate for 10 minutes to degas the mixture and used as mobile phase.

Preparation of Standard Stock Solution:

A 25 mg of standard Cefuroxime Sodium and 12.5 mg of standard Potassium Clavulanate were accurately weighed and transferred to a 100 ml volumetric flasks separately and dissolved in 25 ml mobile phase separately. The flasks were shaken and volume was made up to the mark with mobile phase to give a solution containing 250 µg/ml Cefuroxime Sodium and 125 µg/ml Potassium Clavulanate respectively.

Preparation of calibration curve:

Appropriate volume of aliquots from standard Cefuroxime Sodium and Potassium Clavulanate stock solutions were transferred to same volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with mobile phase give a solution containing 12.5, 18.75, 25,31.25,and37.5 µg/ml Cefuroxime Sodium and6.25,9.37,12.5,15.62, 18.7 µg/mL Potassium Clavulanate. The mixed standard solution was chromatographed for 10 minutes using mobile phase at a flow rate of 1.0 ml/min. The Calibration Curve were plotted for peak area vs. concentration for both the drugs.(Table no.1)

VALIDATION OF THE DEVELOPED RP-HPLC METHOD:

The proposed method has been validated for the simultaneous determination of Cefuroxime Sodium and Potassium Clavulanate in bulk and tablet dosage form. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80, 100 and 120%) taking into consideration percentage purity of added bulk drug samples(Table: 5). It was determined by calculating the recovery of Cefuroxime Sodium and Potassium Clavulanate by standard addition method. The method was found to be precise based on the results obtained in the intraday and interday precision evaluation study. Standard solutions containing 12.5, 25

and 37.5µg/ml CEF and 6.25, 12.5 and 18.75 µg/ml PCA were analyzed 3 times on the same day for intraday precision and on three different days for interday precision(Table:2). Chromatogram of each sample was taken. SD and RSD were calculated. Calibration Curve was repeated for 3 times and the SD of the Intercept was calculated then Limit of detection(LOD) was calculated. LOD values for Cefuroxime Sodium and Potassium Clavunalate were found to be 0.112and 0.0966 µg/ml, respectively, and limit of quantification (LOQ) values for Cefuroxime Sodium and Potassium Clavulanate were found to be 0.32 and 0.292 µg/ml respectively(Table:1). These data show that the proposed method is sensitive for the determination of Cefuroxime Sodium and Potassium Clavulanate.

RESULTS AND DISCUSSION:

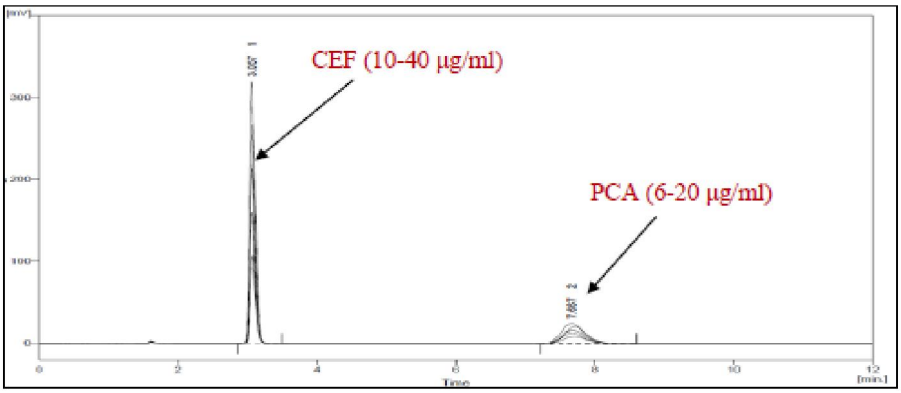


Fig 3: Chromatograms of Cefuroxime Sodium and Potassium Clavulanate for Linearity

TABLE 1: CALIBRATION CURVE OF CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE

Cefuroxime Sodium		Potassium Clavulanate	
Concentration(µg/ml)	Peak area*(mV*s)	Concentration(µg/ml)	Peak area*(mV*s)
12.50	0580.14	6.25	192.26
18.75	0869.96	9.37	288.01
25.00	1157.47	12.50	384.73
31.25	1410.94	15.62	457.19
37.50	1730.28	18.75	556.05
Slop=90.921		Slop=28.697	
Intercept=13.424		Intercept=16.998	
r^2=0.9991		r^2=0.9979	
LOD=0.34		LOD=0.097	
LOQ=0.112		LOQ=0.292	

(*n=5)

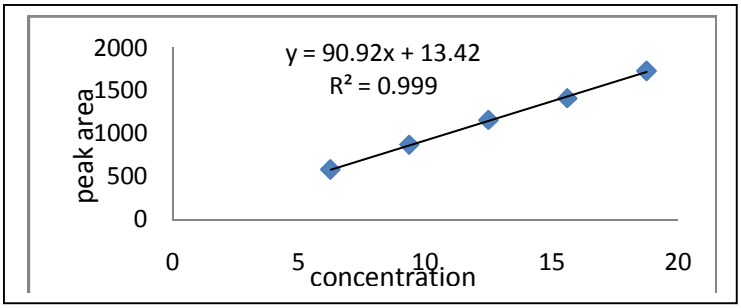


Fig 4: Calibration Curve of Cefuroxime Sodium

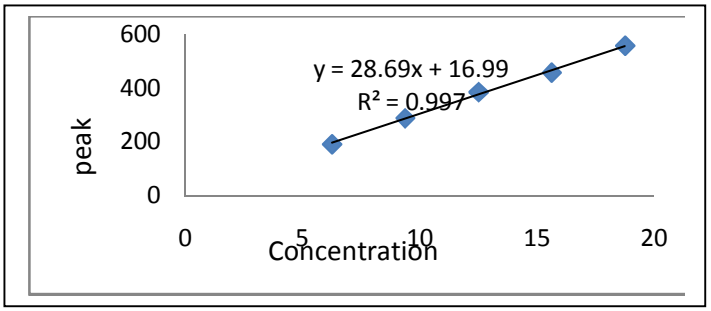


Fig 5: Calibration Curve of Potassium Clavulanate

TABLE 2: INTRADAY AND INTERDAY PRECISION OF CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE

Target concentration(µg/ml)	Intraday Avg±SD	%RSD	Interday Avg±SD	%RSD
Cefuroxime Sodium				
12.5	12.51	1.18	12.53	0.80
25	25.16	0.90	24.58	0.79
37.5	37.78	1.02	37.84	0.85
Potassium Clavulanate				
6.25	6.25	1.20	6.24	1.02
12.5	12.64	0.75	12.61	1.16
18.75	19.02	1.07	18.99	0.42

(*n=3)

TABLE 3: ROBUSTNESS DATA FOR CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE

Parameters (n=3)	Validation	Average Peak Area (mV*s)		%RSD	
		CEF	PCA	CEF	PCA
Flow rat (±0.02 of 1ml/min)	1.02	1102.92	366.62	0.65	0.65
	0.98	1218.64	403.37	0.79	1.14
Mobile phase ACN:0.05Mbuffer (75:25)(±2ml)	73.27	1187.34	376.32	1.16	1.05
	77.23	1134.47	398.96	1.23	1.11
PH(4.5)(±2%)	4.7	1154.97	381.50	0.90	1.48
	4.3	1161.14	385.21	1.11	1.22

TABLE 4: % ASSAY OF CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE

Drug	Label claim mg/tablet	Concentration taken for % assay (µg/ml)	Concentration estimated (µg/ml)		%Assay ±SD
Cefuroxime Sodium	250	25	25.63		100.24±1.33
Potassium Clavulanate	125	12.5	13.53		103.89±0.48

TABLE 5: % RECOVERY OF CEFUROXIME SODIUM AND POTASSIUM CLAVULANATE

%of spike	Amt of spiked std.CEF (µg/ml)	Total Amt. CEF (µg/ml)	Avg ± SD	RSD	Mean % recovery
Cefuroxime Sodium					
80%	20	45	20.31±0.42	2.06	100.07
100%	25	50	25.06±0.82	3.27	100.28
120%	30	55	29.96±0.80	2.67	99.89
Potassium Clavulanate					
80%	10	22.5	9.99±0.42	4.20	99.89
100%	12.5	25	12.51±0.82	6.55	100.18
120%	15	27.5	14.96±0.79	5.28	99.79

TABLE 6: SYSTEM SUITABILITY PARAMETERS

Name	Retention TIme	Theoretical Plate	Tailing factor	Resolution
CEF	3.060	7470	1.400	12.366
PCA	7.6832	2571	1.303	

CONLUSION

The RP-HPLC method was developed and validated using Acetonitrile and Phosphate buffer as mobile phase. The linearity range was found between 10-40 µg/ml for CEF and 6-20 µg/ml for PCA with correlation coefficient 0.999 for CEF and 0.998 for PCA respectively. The proposed method was successfully applied for the simultaneous estimation of both drugs in dosage form. The method was found to be precise as indicated by the intraday, inter-day and repeatability

analysis; showing % RSD was less than 2. Assay value of CEF was found to be 100.24 % of labeled claim and that of PCA was found to be 103.98 % of labeled claim. Mean recovery were found to be 100.08% and 99.95% for CEF and PCA respectively. LOD and LOQ were found to be 0.34 µg/ml and 0.112 µg/ml for CEF and 0.097µg/ml and 0.292µg/ml for PCA respectively. The developed and validated method is used for simultaneous estimation of both the drugs in bulk and dosage form. A validated stability-indicating HPLC analytical method has been developed for the determination of CEF & PCA in bulk and in dosage forms. CEF and PCA does not undergo any degradation so it can be said that CEF and PCA is stable to forced degradation conditions.

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