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Simultaneous estimation of cefuroxime axetil and potassium clavulanate-analytical method development and validation

Pramod L. Ingale[1*], Sampada D. Dalvi[1], Deepali D. Jadav[2], Sachin V. Gudi[2], Laxmileena D. Patil[2] and Yogita A. Kadam[2]

[1]Department of Pharmaceutical Chemistry, Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, India
[2]Department of Quality Assurance Techniques, Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune, Maharashtra, India

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ABSTRACT

A simple, rapid and sensitive spectrophotometric method has been developed for simultaneous estimation of cefuroxime axetil and potassium clavulanate in combined dosage form. Maximum absorbance of cefuroxime axetil and potassium clavulanate was measured in methanol at 284 nm and 271nm. The calibration curve of both the drug obey's the Beer's Law in the concentration range 5-50µg/ml for cefuroxime axetil and 1-30µg/ml for potassium clavulanate with correlation coefficient value 0.999 and 0.998 at 284nm and 271nm respectively. The present method was validated as per ICH guidelines. The result obtained of this method were in good agreement recommended for routine analysis where time, cost effectiveness and high specificity of analytical techniques are of great importance.

Keywords: Cefuroxime Axetil, Potassium clavulanate, Simultaneous estimation, Method development, Validation.

INTRODUCTION

Chemically Cefuroxime axetil is, (1RS)-1-(acetyloxy) ethyl (6R, 7R)-3-[(carbamoyloxy)methyl]-7-[[(z)-2-(furan-2-yl)-2-(methoxyimino)acetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0] oct-2-ene-2 carboxylate. It is used as an antibiotic for the treatment of many type of bacterial infections such as bronchitis, sinusitis, tonsillitis, ear-infections, skin-infections, urinary tract infections. Cefuroxime axetil (CA) is a second-generation cephalosporin that contains the classic β-lactam ring structure. (Fig.no.1)

Potassium clavulanate (PC) is chemically, (2R, 5R)-3-[(1Z)-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo [3.2.0] heptanes-2-carboxylate. It is a powerful inhibitor of β-lactamase enzyme and is most often formulated in combination with antibiotics for treatment of infection caused by lactamase producing bacteria. Both are official in IP, BP and USP. (fig.no.2)

Fig. No.1: Cefuroxime axetil

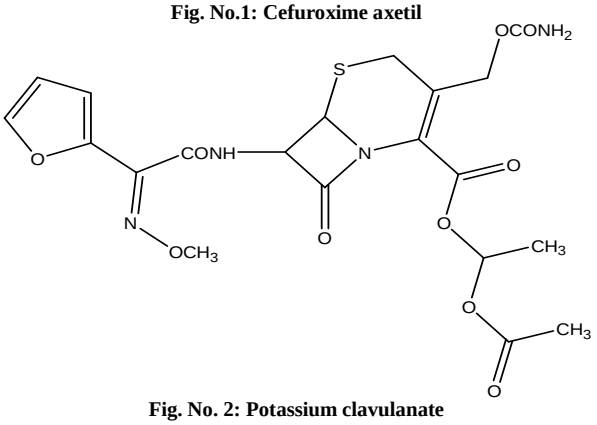
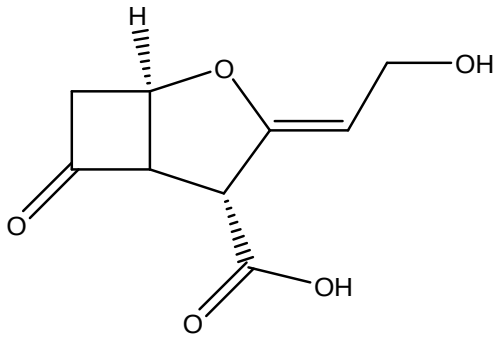


Fig. No. 2: Potassium clavulanate



Literature survey reveals; spectrophotometric, mercurimetric, DSC, HPLC and HPTLC methods for CA determination in single or in combination with other drugs are reported. RP-HPLC determination of PC with other drugs and bioanalytical methods for determination of PC as single drug are reported. No reports were found for simultaneous determination of CA & PC by UV- spectrophotometric method.

Hence attempts were made to develop simultaneous estimation of cefuroxime axetil and potassium clavulanate in combined dosage form by UV- spectroscopy.

MATERIALS AND METHODS

Cefuroxime axetil and Potassium clavulanate was gift sample from Lupin Pharmaceuticals. Methanol used was of analytical grade. The UV spectra of standard and sample solutions were recorded in 1 cm quartz cells using a Shimadzu UV/Vis-1800 double beam UV/Vis spectrophotometer (Japan). Shimadzu AUX 220 balance was used for weighing the samples. Oratil CV 500 tablets (Cefuroxime axetil 500mg, Potassium clavulanate 125mg, (MACLEODS PHARMACEUTICALS LTD.) were procured from local market.

Preparation of sample solution

A stock solution of Cefuroxime axetil and Potassium clavulanate (100µg/ml) was prepared by dissolving 10 mg of both the drug in 100 ml of methanol taken in a clean 100 ml volumetric flask. Aliquots of 100µg/ml solution were suitably diluted with methanol to give the final concentration in the range of 5 - 50 µg/ml for cefuroxime axetil and 1-30µg/ml for potassium clavulanate.

Calibration curve procedure

The following procedure has been adopted for obtaining the standard curve. An aliquot each from 5-50µg/ml for cefuroxime axetil and 1-30µg/ml for potassium clavulanate was prepared. The solution was scanned in the range of 200 to 400 nm against methanol as blank, the excitation wavelengths were found to be 284nm for Cefuroxime axetil and 271nm for Potassium clavulanate. The calibration curve was obtained by plotting absorbance values against

amount of standard drug in µg/ml was shown in Fig No. 3 and 4. The calibration data and statistical parameters are summarized in Table No. 1 and 2.

Fig. No. 3: Standard Calibration curve of CA at 284nm

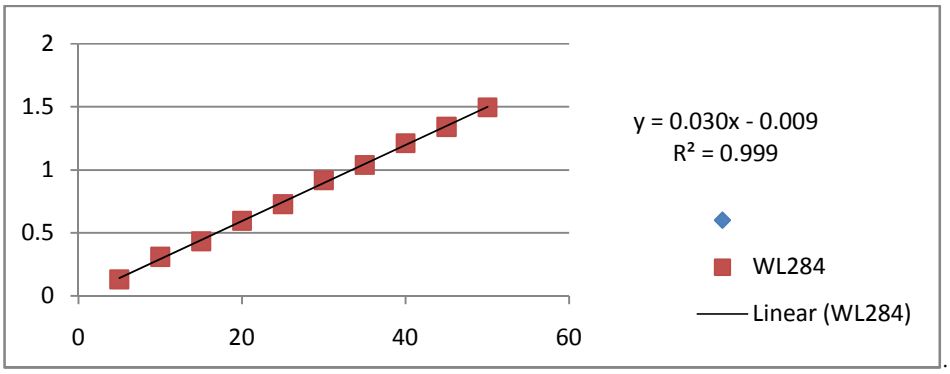


Fig. No. 4: Standard Calibration curve of PC at 271nm

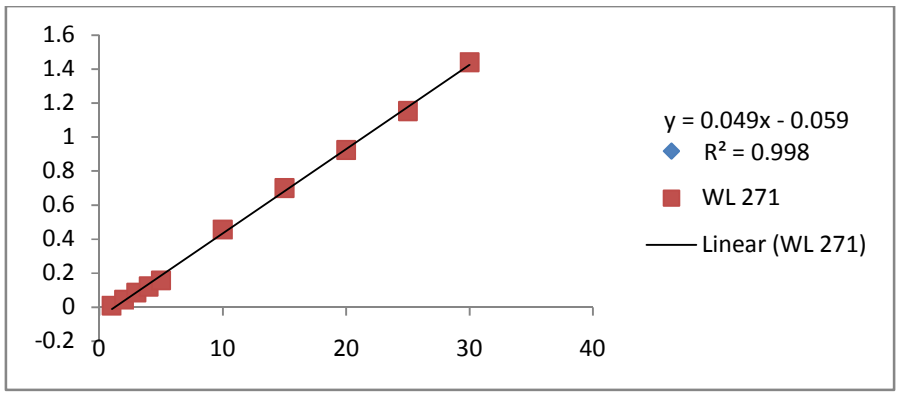


Table 1: Standard Calibration data for CA and PC

	CA		PC	
Sr. no.	**Conc (µg/ml)**	**ABS*(284nm)**	**Conc (µg/ml)**	**ABS*(271nm)**
1	5	0.132	1	0.01
2	10	0.311	2	0.044
3	15	0.435	3	0.087
4	20	0.597	4	0.122
5	25	0.729	5	0.158
6	30	0.918	10	0.456
7	35	1.039	15	0.701
8	40	1.213	20	0.924
9	45	1.343	25	1.153
10	50	1.497	30	1.439

**Average of six determinations*

Determination of Absorptivity value of CA & PC:

Appropriate dilutions of the standard stock solution were done to get 10µg/ml of each CA & PC, respectively. The absorbances were measured for CA and PC at 284nm (λmax of CA), 271nm (λmax of PC). The absorptivity values of the drugs were determined at the selected wavelengths. These absorptivity values are the mean of six determinations.

Application of the proposed method for the determination of CA & PC in tablets:

Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 500mg of CA and 125mg of PC was transferred to 100.0 ml volumetric flask, methanol

added, sonicated for 10 minutes and volume was made-up to the mark with methanol. The solution was then filtered through a Whatmann filter paper (No. 45). The filtrate was further diluted with methanol to obtain 40µg/ml of CA and 10µg/ml of PC. The concentration of both CA & PC were determined by measuring the absorbance of the sample at 284nm, 271nm. Concentration of sample solution was determined by Simultaneous equation method. The readings for analysis of mixed standards are in Table No. 3.

Table 2: Optical and regression characteristics

	Simultaneous Equation Method	
Parameters	**CA**	**PC**
Absorbance maxima(nm)	284	271
Linearity range (µg/ml)	5-50	1-30
Correlation coefficient	0.9992	0.9987
Slope	0.0302	0.0495
Intercept	0.0091	0.0595
Precision:		
Intra-day (% RSD)	0.355	0.583
Inter-day (% RSD)	0.329	0.477
Mean % recovery	99.54	99.25

Table 3: Analysis of Mixed Standards

Conc. of drugs taken (µg/ml)		Conc. of drugs found (µg/ml)		*% Drug found	
CA	PC	CA	PC	CA	PC
8	2	7.93	2.01	99.12	100.5

**Average of six determinations*

Cx = A1ay2-A2ay1/ ax1ay2-ax2ay1……. Eq (1)

Cy = A1ax2-A2ax1/ ay1ax2-ay2ax1……. Eq (2)

Where; A1 and A2 are absorbances of the formulation at 284nm and 271nm respectively, ax1 and ax2 are the absorptivity values of CA at 284nm and 271nm respectively. Similarly, ay1 and ay2 are the absorptivity values of PC at 284nm and 271nm respectively. Cx is the concentration of CA and Cy is the concentration of the PC.

RESULTS AND DISCUSSION

The sensitivity of the method was determined with respect to limit of detection (LOD) and limit of quantification (LOQ). The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level. Recovery studies were done at three different levels. The pre-analyzed samples were spiked with the standard drugs and the mixtures were reanalyzed by the proposed method. At each level, three determinations were performed to check the recovery of the drug at different levels in the bulk drug.

Table 4: Data of Precision studies

Sr. No.	Sample	Repeatability			Inter-day precision			Intra-day precision		
		% Purity	S.D.	R.S.D	% Purity	S.D	R.S.D	% Purity	S.D	R.S.D
1	CA	99.54	0.352	0.354	98.58	0.324	0.329	98.25	0.349	0.355
2	PC	99.25	0.477	0.480	99.01	0.472	0.477	98.97	0.577	0.583

Table 5: Data of recovery studies

Level of % recovery	% Mean recovery		S.D		R.S.D	
	CA	PC	CA	PC	CA	PC
80	100.49	98.58	0.169	0.623	0.170	0.629
100	100.53	98.75	0.375	0.948	0.378	0.964
120	100.46	98.5	0.844	0.884	0.854	0.892

Under the established conditions, Cefuroxime axetil and Potassium clavulanate showed good correlation with Beer's law over the concentration range from 5-50µg/ml and 1-30µg/ml at excitation wavelengths 284nm and 271nm. The

linearity was shown in the concentration range of 5-50µg/ml and 1–30µg/ml is y = 0.0302 x -0.0091 and y = 0.0495 x -0.0595 and; correlation coefficient r^2 = 0.999 and 0.998 at wavelengths 284nm and 271nm respectively. A relative standard deviation of 0.352 % and 0.477 % was observed on analysis of six replicate samples at excitation wavelengths 284nm and 271nm respectively. The percent recovery studies revealed that the values lay between 97.56 % - 102.70 % and 98.22 % -101.22 % at wavelengths 284nm and 271nm respectively. Results of recovery studies demonstrated that the proposed method was highly accurate. (Table no.5) Both inter-day as well as intra-day precisions were carried out in different concentration of the solutions and the relative standard deviation (RSD) was found to be less than 2.0. (Table no.4) Results obtained confirmed ruggedness of the method.

CONCLUSION

The proposed method was validated with respect to linearity, sensitivity, accuracy, reproducibility and precision. The developed method was found to be accurate, precise and reproducible, which indicated that this method can be used for the routine analysis of Cefuroxime axetil and Potassium clavulanate.

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