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Research Article

SIMULTANEOUS DETERMINATION OF CEFUROXIME AXETIL AND POTASSIUM CLAVULANATE IN PHARMACEUTICAL DOSAGE FORM BY RP- HPLC

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ABSTRACT

Objective: To develop a new validated simple, precise, specific and accurate reverse phase High Pressure Liquid Chromatographic method for the simultaneous determination of Cefuroxime axetil (CA) and Potassium clavulanate (PC) in tablet dosage form.

Method: Chromatographic separation was achieved on reverse phase Microsorb-MV 100-5 C-18 (250x4.6mm, 5 µm) column with a mobile phase consisting of HPLC grade methanol:water in the ratio of 90:10 (v/v) at a flow rate of 1.0 mL/min with UV detection at 230 nm.

Result: The retention time for Cefuroxime axetil and Potassium clavulanate were 2.46 & 3.33 min. respectively. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness. The regression value for both the drugs was found to be 0.996 and 0.992, the S.D. & R.S.D. values were found to be well within the acceptable limit of 2.0%.

Conclusion: Proposed HPLC method is specific, accurate and precise for the simultaneous determination of Cefuroxime axetil and Potassium clavulanate from pharmaceutical dosage form. The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

Keywords: Cefuroxime axetil, Potassium clavulanate, HPLC, Simultaneous determination; 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 is a second-generation cephalosporin that contains the classic β-lactam ring structure.

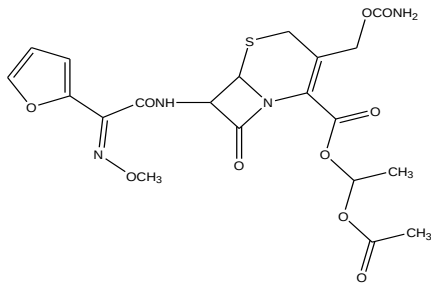

Fig. 1: Structure of Cefuroxime axetil

Potassium clavulanate 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 [1-3].

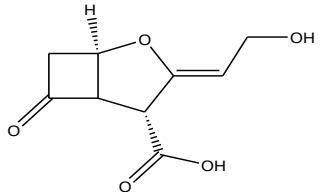

Fig. 2: Structure of Potassium clavulanate

Literature survey reveals; spectrophotometric, mercurimetric, DSC, 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. One RP-HPLC method for simultaneous determination of CA & PC is also reported still yet.

This paper describes the development and validation of a method to simultaneously quantify CA and PC by liquid chromatography in a more simpler manner.

MATERIALS AND METHODS

Instrumentation

HPLC system (Cyberlab LC 100) consisting of binary gradient pump, Microsorb-MV 100-5 C-18 column (250x4.6mm, 5 µm), UV detector was employed for analysis. Chromatographic data was acquired using WS-100 Workstation software.

Reference substances, sample, reagents and chemicals

Active pharmaceutical ingredient (API) working standards of cefuroxime axetil (CA), potassium clavulanate (PC) were obtained as gift sample from Lupin Pharmaceuticals Ltd. and test samples (tablets with composition CA-500 mg and PC-125mg) were obtained from MACLEODS PHARMACEUTICALS LTD., India. HPLC grade acetonitrile, methanol and orthophosphoric acid were obtained from Merck, Mumbai, India Limited. HPLC grade water was obtained from MOLYCHEM, Thane, India.

Chromatographic conditions

Microsorb MV 100-5 C-18 column (250mm×4.6 mm, 5µm) was used as a stationary phase. The isocratic mobile phase consisting of a mixture of methanol: water in the ratio of 90:10 (v/v) was used throughout the analysis. The flow rate of the mobile phase was 1.0 ml/ min. Detector signal was monitored at a wavelength of 230 nm. The column temperature was kept ambient and injection volume was 20µl.

Solution preparation

CA stock solution

CA standard stock solution was prepared by transferring 10mg of cefuroxime axetil working standard into a 100ml volumetric flask. A

20ml portion of methanol was added, sonicated and cooled to room temperature. The solution was diluted to the mark with the same solvent to give a stock solution of 100µg/ml.

PC stock solution

PC standard stock solution was prepared by transferring 10 mg of potassium clavulanate working standard into a 100ml volumetric flask. A 20ml portion of methanol was added, sonicated and cooled to room temperature. The solution was diluted to the mark with the same solvent to give a stock solution of 100µg/ml.

Sample solution

Twenty tablets, labeled as containing 500 mg of CA, and 125 mg of PC together with excipients, were accurately weighed, transferred to a clean and dry mortar and ground into a fine powder.

A weight of the powder equivalent to one tablet content (1345 mg) was accurately weighed, then transferred to a clean 50 ml volumetric flask, 20 ml of methanol was added. The mixture was then sonicated for 10 min and diluted to volume with mobile phase to give a solution containing 1000 µg/ml. This solution was filtered through a 0.45µm pore size Nylon 66 membrane filter.

Validation procedure

The specificity of the method was determined by injecting the sample solution containing excipients without drug having concentration same as that of the sample. [4]

Linearity solutions were prepared at 10 concentration levels from 10% to 400% of analyte concentration.

The accuracy of the method was carried out by adding known amount of each drug corresponding to three concentration levels 80%, 100% and 120% of the label claim along with the excipients in triplicate [4].The samples were given the same treatment as described in Section 2.4.4

Precision of the method was checked by carrying out six independent assays of CA and PC test samples against qualified working standard [4].

Intermediate precision was performed by analyzing the samples by two different analysts on different days [4].

Robustness was performed by deliberately changing the chromatographic conditions [4].

The flow rate of the mobile phase was changed from 1.0 mL/min to 1.1 mL/min. The organic strength was varied by ±5%. Standard solution was injected six times in replicate for each change.

Respective peak areas, dilution factors, sample and standard weights were taken into account to quantitate the amounts of CA and PC in mg per tablet.

RESULTS & DISCUSSION

Optimization of chromatographic conditions

In order to achieve simultaneous elution of the two components the chromatographic condition i.e. stationary phase like C18 was used in different mobile phase compositions. Optimization of the mobile phase was performed based on resolution, asymmetric factor and peak area obtained for both CA and PC. The mobile phase combination of methanol:water (90:10, 70:30 and 80:20 (v/v)) were tried. Methanol: Water (90:10) at a flow rate of 1.0 ml/min found to be satisfactory and gave two symmetric and well resolved peaks for CA and PC. The chromatogram was recorded at 230 nm as a spectrum of CA and PC showed maximum response at this wavelength. The retention time for CA and PC were 2.4 and 3.3, respectively. A chromatogram of tablet extract was recorded and shown in Fig. 3.

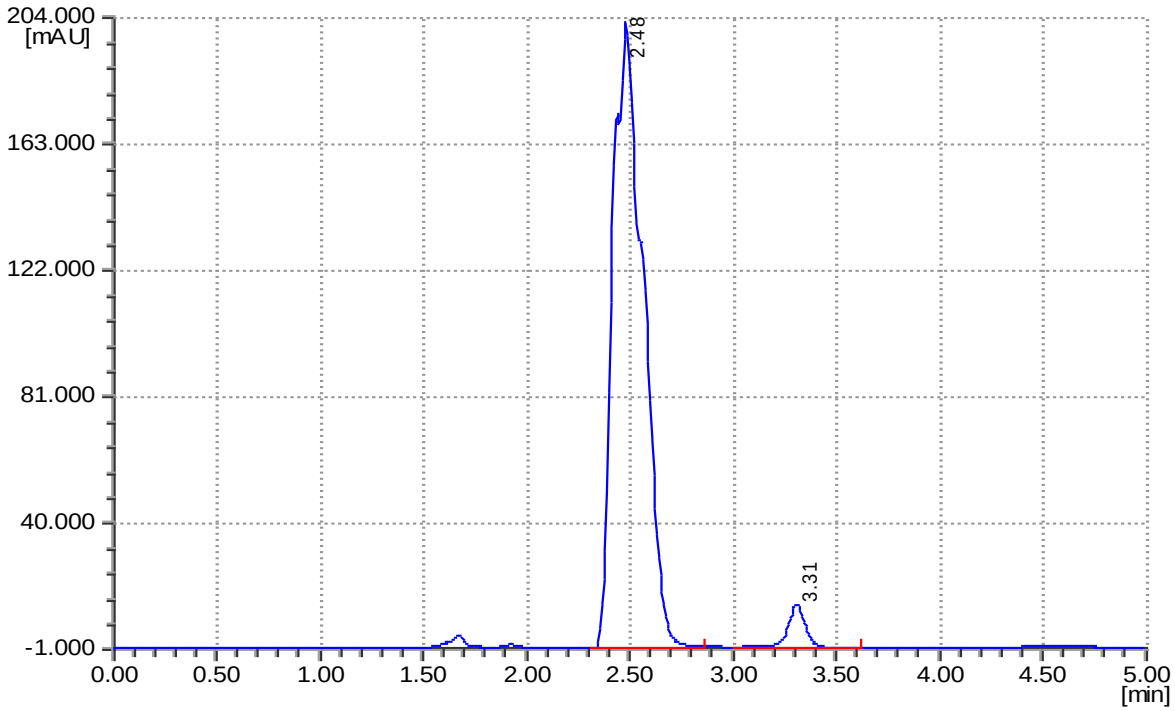

Fig. 3: Chromatogram of Cefuroxime axetil (peak at RT 2.4) and Potassium clavulanate (peak at RT 3.3)

Table 1: System suitability parameters for CA & PC

Component (*n* = 6)	Area Peak	Tailing Factor	Theoretical plates	Capacity factor	Resolution (Rs)
Cefuroxime axetil	23108.35	1.51	2772.72	4.25	2.35
Potassium clavulanate	34058.65	0.76	5476.71	1.75	2.77

Method validation

The newly developed method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, and robustness [4]. System suitability was established by injecting standard solution and results are shown in Table 1.

Specificity

The chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from the tablet excipients was found. Peak purity was verified by confirming homogeneous spectral data for CA and PC.

Linearity

CA and PC showed linearity in the range of 5-50 µg/ml and 5-30µg/ml, respectively. Linear regression equations and correlation coefficient (r^2) are: Y_{CA} = 562.99x+459.95

(R^2 = 0.996) and Y_{PC} = 2915.1x + 3257.6 (r^2 = 0.992).

Accuracy

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is highly accurate (Table 2).

Table 2: Results of Accuracy of CA & PC

Theoretical (% of target level)	Amount added (mg)	Amount recovered (mg)	Recovery (%)
Cefuroxime axetil			
80	16	16.17	101.06
100	20	20.1	100.5
120	24	24.05	100.2
Potassium clavulanate			
80	8	7.91	98.87
100	10	10.79	107.9
120	12	11.97	99.75

n = 3 determinations

Precision

The relative standard deviations (R.S.Ds.) were 1.383% for CA and 1.235% for PC, which are well within the acceptable limit of 2.0%. The R.S.D's. for intermediate precision were found to be 1.613% for CA and 1.004% for PC.

Robustness

In all deliberately varied conditions, the RSD of peak areas of CA and PC were found to be well within the acceptable limit of 2%. The tailing factor for both the peaks was found to be <2.

CONCLUSION

Proposed HPLC method is specific, accurate and precise for the simultaneous determination of cefuroxime axetil and potassium clavulanate from pharmaceutical dosage form. The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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