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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS DETERMINATION OF CEFUROXIME AXETIL AND POTASSIUM CLAVULANATE IN TABLET DOSAGE FORM

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ABSTRACT: A simple, specific, accurate, and precise reverse phase high-performance liquid chromatographic method for analysis of Cefuroxime axetil and Potassium clavulanate has been developed. Separation of drugs was carried out on Jasco HPLC system with Hypersil Gold C-18 column (250 mm × 4.6 mm i.d.), using 0.01M Potassium dihydrogen phosphate: methanol 60: 40 (*v/v)* as mobile phase. Quantitation of drugs was carried out at wavelength 225 nm. Results were found to be linear in the concentration range of 5-50 µg/ml for CA and 5-30 µg/ml for PC. Mean retention times for Potassium clavulanate (PC) and the two isomers of Cefuroxime axetil (CA_1 and CA_2) were found to be 2.573, 8.293 and 9.987 min, respectively. Intra-day variation, as RSD (%), was 0.328 for Cefuroxime axetil and 0.382 for Potassium clavulanate. Interday variation, as RSD (%) was 0.545 for Cefuroxime axetil and 0.552 for Potassium clavulanate. The % assay was found to be 100.976 ± 0.439 for Cefuroxime axetil and 101.053 ± 0.423 for Potassium clavulanate (Mean ± S.D., n = 6).

KEYWORDS: RP-High performance liquid chromatography, Cefuroxime axetil, Potassium clavulanate

INTRODUCTION

Cefuroxime Axetil (CA), (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,7^2-(Z)-(O-methyl-oxime),1-acetate3-carbamate) is second generation cephalosporin used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria[1]. Clavulanic acid administered as potassium salt, 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[2].

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Literature survey reveals spectrophotometric[3] and HPTLC methods[4-6] for CA determination in combination with other drugs. Stability indicating[7] and bioanalytical chromatographic methods [8, 9] for quantification of CA are also reported. CA is official in USP which dictates RP-HPLC method for CA determination as single drug[10]. RP-HPLC[11, 12] determination of PC with other drugs and bioanalytical methods [13, 14] for determination of PC as single drug are reported. No reports were found for simultaneous determination of CA and PC by RP-HPLC method. Aim of present work was to develop simple, economical, rapid, precise and accurate RP-HPLC method for simultaneous determination of binary drug formulation.

EXPERIMENTAL

CHEMICALS AND REAGENTS

Potassium dihydrogen orthophosphate (AR grade, S.d. fine - Chem Laboratories Pvt. Ltd., Mumbai, India), Methanol (HPLC grade, Merck Specialities Pvt.

Ltd., Mumbai, India) and Water (HPLC grade, Loba Chemie, Mumbai, India) were used in analysis.

Analytically pure sample of CA was kindly supplied by Maxim Pharmaceuticals Pvt. Ltd. (Pune, India) and that of PC was supplied by Medriech Pharmaceuticals (Bangalore, India) used as such without further purification. The pharmaceutical dosage form used in this study was Covatil CV 250 film coated tablets (Macleods Pharmceuticals, Mumbai India) labeled to contain Cefuroxime axetil USP equivalent to Cefuroxime 250 mg and diluted Potassium clavulanate BP equivalent to Clavulanic acid 125 mg per tablet.

INSTRUMENTS AND CHROMATOGRAPHIC CONDITIONS

Jasco HPLC system consisting of Jasco PU-2080 plus HPLC pump, MD 2010 PDA detector and BORWIN-PDA (Version 1.50) software was used for analysis. Separation was carried out on Hypersil Gold C_{18} (250 x 4.6 mm i.d) column using 0.01 M Potassium dihydrogen orthophosphate: methanol in ratio 60: 40 as mobile phase at a flow rate of 1.0 ml/min. Samples were injected using Rheodyne injector with 20 µl loop. Detection wavelength selected was 225 nm. All weighing were done on Shimadzu balance (Model AY-120).

PROCEDURE

PREPARATION OF STANDARD STOCK SOLUTION

Standard stock solution of CA was prepared by dissolving 10 mg of drug in 10 ml methanol to get concentration of 1 mg/ml, which was used as a working standard solution. Standard stock solution of PC was prepared by dissolving 10 mg of drug in 10 ml methanol to get concentration of 1 mg/ml. From this solution 0.5 ml was further diluted to 10 ml with methanol to get working standard stock solution of concentration 50 µg/ml.

PREPARATION OF CALIBRATION CURVE

Aliquots of working standard solution of CA and PC were transferred to separate 10 ml volumetric flasks and volume was made up to mark with mobile phase. Each solution was injected and chromatogram was recorded. The peak areas of CA (CA_1 + CA_2) and PC were calculated and respective calibration curves were plotted of response factor against concentration of drug.

PROCEDURE FOR ANALYSIS OF TABLET FORMULATION

Twenty tablets were weighed accurately; the average weight was determined and then ground to a fine powder. Powder equivalent to 10 mg of PC was weighed; transferred to a 10 ml volumetric flask containing about 5 ml of mobile phase and sonicated for 5 min. Then volume was made up to the mark with the mobile phase; filtered through Whatman filter paper no. 41.From this solution 0.1 ml was taken and transferred to 10 ml volumetric flask. This sample solution having concentration 10 µg/ml of PC was injected and chromatogram was obtained. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in Fig. 1.

RECOVERY STUDIES

To study the accuracy and precision of the proposed method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50 %, 100 % and 150 %.

ROBUSTNESS

Robustness of the developed method was determined by small but deliberate changes in chromatographic conditions such as flow rate (± 0.02 ml/min), wavelength (± 1 nm), and mobile phase composition (± 2 %). It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is robust.

RESULTS AND DISCUSSION

For RP-HPLC method different mobile phases were tried and the mobile phase containing 0.01 M Potassium dihydrogen orthophosphate and methanol in ratio 60: 40 (% *v/v*) was found to be optimal for obtaining well defined and resolved peaks with retention time 2.573 ± 0.047 min for PC, 8.273 ± 0.055 min for CA_1 and 9.987 ± 0.056 min for CA_2 (mean ± S.D.). The calibration plots were found to be linear over the ranges 5–50 µg/ml and 5–30 µg/ml for CA and PC, respectively, with correlation coefficients of 0.995 ± 0.332 and 0.991 ± 0.095 respectively. System suitability parameters for RP-HPLC method are listed in Table 1.

The LOD and the LOQ for the CA were found to be 2.409 and 7.951 µg/ml, respectively, and for PC were found to be 0.538 and 1.778 µg/ml, respectively. The % recovery was found to be 100.598 ± 0.371 for CA and 100.741 ± 0.418 for PC (Mean ± % RSD, n = 6). Results of recovery studies are represented in Table 2. The % RSD values were satisfactorily low indicating reproducibility of the method. The % assay was found to be 100.976 ± 0.439 for Cefuroxime axetil and 101.053 ± 0.423 for Potassium clavulanate (Mean ± S.D., n = 6).

CONCLUSION

This work describes a simple, accurate and sensitive validated RP-HPLC method for simultaneous determination of both the drugs and method can be used conveniently for quality control purposes.

Table 1: System suitability parameters for RP-HPLC method

Parameter	PC	CA_1	CA_2
Theoretical plates	4020	4071	4502
Asymmetry Factor	1.26	1.15	1.11
HETP (cm)	0.0062	0.0061	0.0055
Resolution*	_	2.07	2.128

* With respect to previous peak

Table 2: Recovery studies of CA and PC

Drug	Amount taken,(µg/ml)	Amount added,(µg/ml)	Total amount found (µg/ml)	%Recovery*	% RSD*
CA	20	10	30.068	100.23	0.312
	20	20	40.464	101.16	0.408
	20	30	50.205	100.41	0.394
PC	10	05	15.062	100.41	0.386
	10	10	20.070	100.35	0.474
	10	15	25.364	101.46	0.396

* Average of three determinations

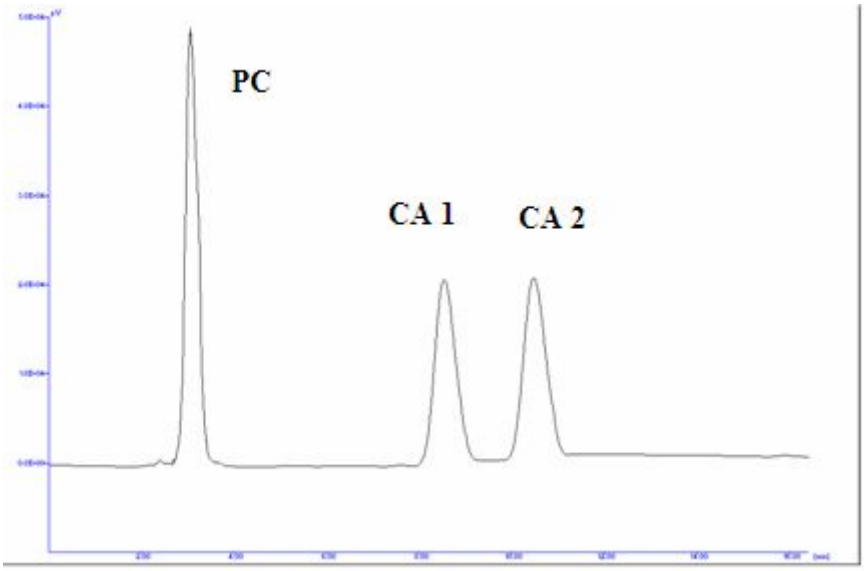

Figure 1: Chromatogram of standard mixture contaning PC 10 µg/ml (2.573 min) and CA 20 µg/ml (CA_1 8.293 min; CA_2 9.987 min).

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