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ОРИГИНАЛЬНЫЕ СТАТЬИ

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DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF POTASSIUM CLAVULANATE AND CEFADROXIL IN SYNTHETICALLY PREPARED TABLETS

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A simple, sensitive and rapid high performance liquid chromatographic method was developed and validated for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically prepared tablets. Chromatographic separation and detection was carried out on a C-18 column using 0.05 M potassium dihydrogen phosphate buffer (pH 5.0) and acetonitrile in the ratio of 94 : 06 (v/v) as mobile phase at wavelength of 225 nm. The method was linear in the concentration range of 3.75–22.5 μg/mL for potassium clavulanate and 15–90 μg/mL for cefadroxil. The flow rate was 1.0 mL/min and the total analysis time was less than 10 min. The mean recoveries was found to be greater than 99% with RSD less than 1.0%. The proposed method was validated by performing linearity, recovery, specificity, robustness, LOD/LOQ and within-day and between-day precision. The chromatographic results obtained from the synthetically prepared tablets show that the method is highly precise and accurate for the simultaneous quantitation of clavulanate potassium and cefadroxil.

Key words*:* high performance liquid chromatografia, potassium clavulanate, cefadroxil.

Cefadroxil (Fig. 1) is a semi-synthetic cephalosporin antibiotic for oral administration with a chemical name (6*R*,7*R*)-7-[[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate. It is used to treat different types of bacterial infections such as bronchitis, tonsillitis, ear and skin infections, gonorrhea, and urinary tract infections [1]. Potassium clavulanate (Fig. 2) is a white to off-white powder produced by fermentation of streptomyces clavuligerus and chemically designated as (2R,3Z,5R)-3-(2-hydroxyethylidene)-7-oxa-1-azabicyclo [3.2.0] heptane-2-carboxylate. It is potent inhibitor of β-lactamase enzyme and is mostly formulated in combination with antibiotics and is usually supplied mixed with Avicel (Microcrystalline cellulose), Syloid 244 (Colloidal silicon dioxide) [2]. The combination of potassium calvulanate with either cefixime or cefadroxil has recently been approved by Central Drugs Standard Control Organization India [3]. Because of additive effects, some pharmaceutical companies are pursuing to launch combination of potassium calvulanate with either cefixime or cefadroxil. In the first phase of our study, we reported an HPLC method for a synthetic binary mixture of cefixime and potassium clavulanate [4]. In the second phase we analysed binary synthetically prepared tablets of cefadroxil and potassium calvulanate by HPLC and its method development and validation studies are reporting here.

A large number of analytical methods have already been published for both the drugs either alone or in combination with other drugs. These include [5–13] for cefadroxil and [5, 14–24] for clavulanate potassium. According to our information, no method has yet been reported for simultaneous determination of potassium clavulanate and cefadroxil. The present work is therefore, focused on to achieve the optimum chro-

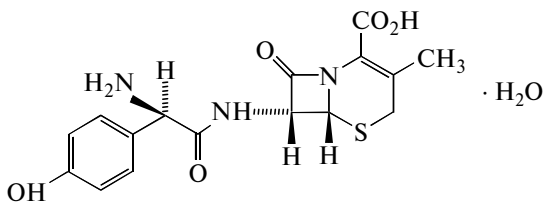

Fig. 1. Chemical structure of cefadroxil.

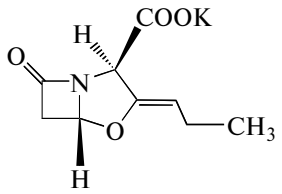

Fig. 2. Chemical structure of potassium clavulanate.

matographic conditions for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically prepared tablets.

We describe herein a simple, sensitive and validated HPLC method with isocratic elution for the simultaneous determination of potassium clavulanate and cefadroxil. The developed method can be applied successfully to quality control and for other analytical purposes.

EXPERIMENTAL

Chemicals and reagents. Reference potassium clavulanate and Cefadroxil with claimed purity of 99.65% and 99.75% respectively were obtained from Ideal Pharmaceutical & Pharmagen Ltd., (Lahore, Pakistan). Potassium dihydrogen phosphate and Potassium hydroxide were of analytical reagent grade (Merk, Rahway, NJ) whereas acetonitrile (Fisher Scientific, Pittsburgh, PA) was of HPLC grade. All excipients used were of pharmaceutical grade. Starch was purchased from Rafhan (Faisalabad, Pakistan), Lactose from Borculo Domo (Borculo, Netherland), magnesium stearate from Coin Chen (Taiwan, China), and Avicel from JRS Pharma (Rosenberg, Germany). Water for injection was used throughout the experiment. Mobile phase was degassed by Sonicator PSO 13000 A and filtered using 0.45 μm nylon filters made by Sartorius (Germany); Whatmann filter papers No. 41 (purchased from the local market) were used in the preparation of sample solution in section 2.7.

Apparatus and chromatographic conditions. The Simultaneous determination of potassium clavulanate and cefadroxil was performed on HPLC Shimadzu (Japan) LC-20AT system equipped with SPD-20A UV detector and SIL-20A auto injector. Shimadzu LC Solution software was used to record the chromatograms and to calculate the chromatographic parameters. Isocratic separation of both the components was achieved using C-18 column, 250 * 4.6 mm, 5 μ Hypersil (UK). Injection volume was set 20 μL by auto injector. The flow rate of the mobile phase was 1 mL/min. UV detection was performed at 225 nm. Peak identity was confirmed by spectrum and retention time comparison. All the analysis was performed at room temperature.

Preparation of mobile phase. The mobile phase was prepared by mixing 0.05 M potassium dihydrogen phosphate buffer (pH 5.0) and acetonitrile in the ratio of 94 : 06 (v/v). The pH of the potassium dihydrogen phosphate was adjusted to 5.0 ± 0.1 with 10% potassium hydroxide. The mobile phase was filtered through 0.45 μm nylon filters and degassed before use.

Preparation of standard solution. The standard stock solution of potassium clavulanate and cefadroxil (0.0625 mg/mL and 0.25 mg/mL respectively) was prepared by dissolving 12.5 mg potassium clavulanate and 25 mg cefadroxil to a small amount of mobile phase in a 100 mL volumetric flask and then raising the volume to the mark with mobile phase. The working standard solution 11.25 μg/mL for potassium clavulanate and 45 μg/mL for cefadroxil were prepared by diluting the stock solution with mobile phase.

Linearity. The Linearity of the developed method was checked by analyzing five solutions in the range of 3.75–22.5 μg/mL for potassium clavulanate and 15–90 μg/mL for cefadroxil. Each concentration was made and analyzed in triplicate.

Accuracy. To check accuracy of the developed method, known amounts of potassium clavulanate and cefadroxil were added to the placebo solution. The actual and experimental concentrations were then compared. From the stock standard solutions of potassium clavulanate and cefadroxil, aliquots of 2.25, 4.5 and 6.75 mL were transferred separately to three 25 mL volumetric flasks containing placebo solution. Mobile phase was then added to make up the volume to get the final concentration of 5.62, 11.25, 16.87 μg/mL and 22.5, 45.0, 67.5 μg/mL for potassium clavulanate and cefadroxil respectively. These concentrations correspond to 50, 100 and 150 % of the nominal analytical concentration which is 11.25 μg/mL for potassium clavulanate and 45 μg/mL for cefadroxil (same as of working standard solution section 2.4).

Preparation of synthetic tablets and their analysis. The recovery of the proposed method was determined by analyzing the synthetically prepared tablets of both the analytes with commonly occurring excipients that are found in most of the tablet formulations such as starch, lactose, magnesium stearate and avicel and then measuring the percentage recovery of each component. Synthetically prepared tablets were accurately weighed and then crushed physically using pestle and mortor. An amount of crushed tablets equivalent to 12.5 mg of potassium clavulanate and 25 mg cefadroxil were accurately weighed and transferred to a 100 mL volumetric flask. To this 70 mL of mobile phase was added to dissolve the active substances completely. The mixture was dissolved well by manual shaking. After making the volume up to the mark, with mobile phase, the solution was filtered with Whatmann filter paper No. 41. A quantity equal to 9 mL of this filtrate was diluted to 50 mL with mobile phase to get a final concentration of 11.25 μg/mL of potassium clavulanate and 45 μg/mL of cefadroxil.

Specificity. Accelerated degradation study was performed to check the specificity of the proposed method. For this purpose both the active ingredients were

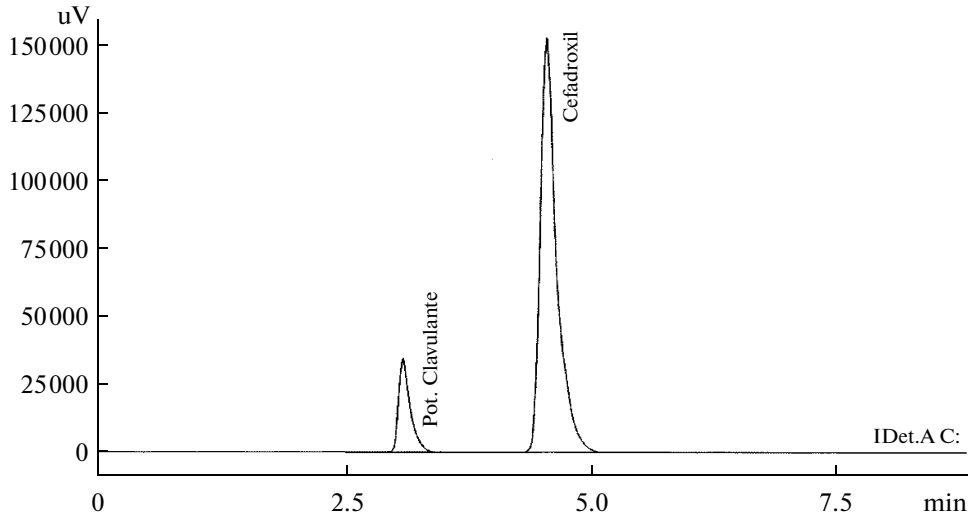

Fig. 3. Chromatograms of potassium clavulanate and cefadroxil reference standard.

subjected to acidic, basic, oxidative and thermal stress. For acidic degradation, 1mL of 0.1 M HCl was added to 4.5 mL of the stock standard solution and let it for 30 min at 25°C. The solution was then subsequently diluted to 25 mL with mobile phase. For basic degradation 1mL of 0.2 M KOH was added to 4.5 mL of standard stock solution and kept it for 45 min at 25°C. The solution was then diluted to 25 mL with mobile phase. For thermal degradation 4.5 mL of the standard stock solution was heated to 80°C for 25 min and then diluted to 25 mL with mobile phase. For oxidative degradation 0.5 mL of 0.1% H_2O_2 was added to 4.5 mL of stock standard solution, let it for 30 min at 25°C and then diluted to 25 mL.

Robustness. Robustness of the method was evaluated by deliberately changing the chromatographic conditions such as composition of the mobile phase, flow rate and pH of the buffer solution. The percentage recovery of each analyte along with the chromatographic parameters like retention time, tailing factor, resolution and number of theoretical plates were measured at each changed conditions.

RESULTS AND DISCUSSION

Development and optimization of the method. Both potassium clavulanate and cefadroxil are listed as official drugs in United States Pharmacoepia and found in individual monographs. The present research pursuit aims at the development of an HPLC method for the simultaneous determination of potassium clavulanate and cefadroxil in synthetically prepared tablets.

During development of the method, number of mobile phases and stationery phases were attempted to elute both the components simultaneously. Method development was started with methanol and phosphate buffer used in different proportions and with different pH. Although elution of potassium clavulanate occurred at phosphate buffer pH 6.5 and methanol in the ratio of 84 : 16, but cefadroxil was not eluted well. Some other pH and ratios were tried but both drugs were not eluted as a true gaussians curves. The mobile phase was then replaced from methanol to acetonitrile as the organic phase of the mobile phase. At the composition of 10 : 90 for acetonitrile and phosphate buffer pH 5.0, the elution of both the components occurred but tailing factor for cefadroxil was greater than 1.5. By slightly increasing the buffer contents the tailing was considerably reduced with good resolution between the two peaks. The optimum ratio of acetonitrile and phosphate buffer pH 5.0 in the mobile phase was found to be 06 : 94, v/v giving resolution of 5.8 and tailing factor less than 1.5. Upon application of the developed method, well-separated peaks were obtained for both clavulanate potassium and cefadroxil. The representative chromatograms of clavulanate potassium and cefadroxil are given in Fig. 3 and Fig. 4.

Method validation. The developed chromatographic method was validated using ICH guidelines [26] before implementation. Validation parameters performed include linearity, limit of detection and quantitation, specificity, robustness, accuracy and repeatability.

Linearity. The linearity of the method was done by analyzing five solutions in the range of 3.75–22.5 μg/mL for potassium clavulanate and 15–90 μg/mL for cefadroxil. Each concentration was made and analyzed in triplicate. The peak areas obtained against each concentration of the analytes were used to build a linear regression equation and to determine value of correlation coefficient. Good linearity was observed over the above-mentioned range with linear regression equation $Y = 21059.323X + 1469.270$ for clavulanate potassium and $Y = 3358.912X - 799.06$ for cefadroxil (X is concentration of analytes in μg/mL and Y is peak area). The val-

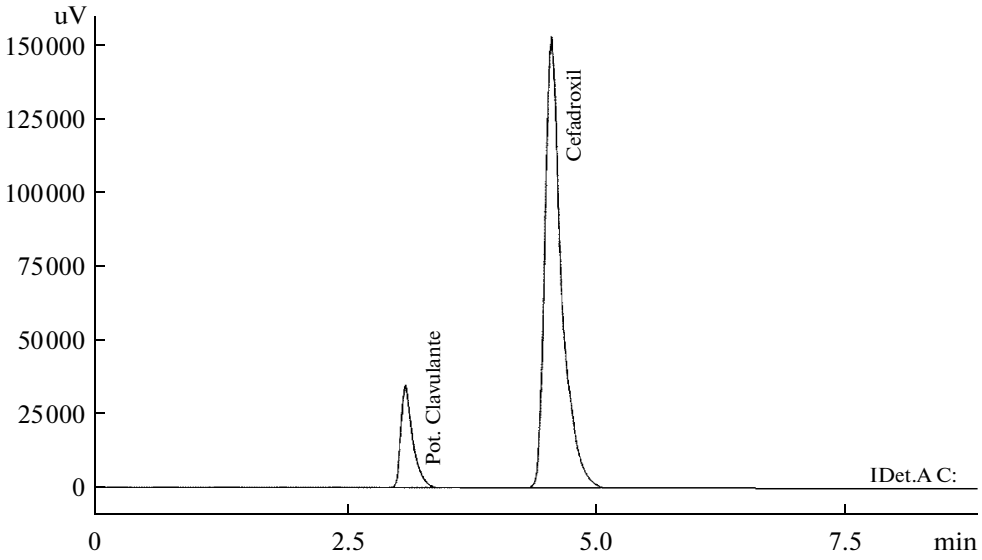

Fig. 4. Chromatograms of potassium clavulanate and cefadroxil in a synthetically prepared tablets.

ue of correlation coefficient was found to be 0.9997 for potassium clavulanate and 0.9994 for cefadroxil.

Limits of detection and quantitation. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated by using the following equations.

$$DL = 3\alpha/S,$$

$$QL = 10\alpha/S,$$

where DL = Limit of detection, QL = Limit of quantitation, α = the standard deviation of the response, S = Slope of the calibration curve.

The LOD values were found to be 0.05 μg/mL for clavulanate potassium and 0.21 μg/mL for cefadroxil. The LOQ values were 0.17 μg/mL and 0.7 μg/mL for clavulanate potassium and cefadroxil respectively.

Accuracy. The accuracy of the method was performed by adding known amounts of potassium clavulanate and cefadroxil to the placebo solution . Three level of solutions were made having concentrations of 5.62, 11.25, 16.87 μg/mL for potassium clavulanate and 22.5, 45.0, 67.5 μg/mL for cefadroxil which correspond to 50, 100, and 150% of the nominal analytical concentration. The recovery range for each of the analytes was found to be 98.96. to 101.42 % and the relative standard deviation ranged from 0.41 to 1.15% (Table 1).

Precision. To check the precision of the proposed method three different concentrations were made and analyzed. The within-day precision was determined by calculating the relative standard deviation of five replicate analysis of samples on the same day. The between day precision was determined by calculating the relative standard deviation of the results from the same samples analyzed on five consecutive days. From the five replicates for both within day and between days, calibration curves were constructed and correlation coefficients were calculated. For within day the value of correlation coefficients were 0.9995–0.9998 for potassium clavulanate and 0.9993–0.9999 for cefadroxil whereas for between days it was found to be 0.9991–0.9995 for potassium clavulanate and 0.9990–0.9996 for cefadroxil. In addition the percentage relative standard deviation was calculated from five replicates of three different concentrations for both within days and between day. The results are given in Table 2.

Table 1. Accuracy of the proposed HPLC method

Drug	Level, %	*n*	Concentration, μg/mL	Amount recovered, μg/mL	% Recovery	% RSD
Potassium clavulanate	50	5	5.62	5.70	101.42	1.15
	100	5	11.25	11.18	99.38	0.76
	150	5	16.87	16.71	99.05	0.85
Cefadroxil	50	5	22.5	22.76	101.16	0.41
	100	5	45.0	45.62	101.38	0.65
	150	5	67.5	66.80	98.96	0.83

Table 2. Within-day and between-day precision of the proposed HPLC method

Compound	Conc.	*n*	Within-day precision		Between-day precision	
	μg/mL		Mean	RSD, %	Mean	RSD, %
Potassium clavulanate	11.25	5	11.32	1.22	11.22	0.55
	16.87	5	16.75	1.05	16.82	0.91
	22.5	5	22.36	0.42	22.31	0.77
Cefadroxil	45.0	5	45.15	0.65	44.80	0.22
	67.5	5	66.90	0.59	66.75	0.35
	90.0	5	89.25	0.88	89.52	0.98

Table 3. Robustness study of potassium clavulanate

Conditions	Assay, %	RT, min	Theoretical plate	Tailing
Acetonitrile : buffer (06 : 94)	100.21	3.39	22629	1.30
Acetonitrile : buffer (04 : 96)	99.69	3.96	21780	1.36
Acetonitrile : buffer (08 : 92)	100.52	2.86	18519	1.43
Flow rate, 1.1 mL/min	100.21	3.11	21904	1.34
Flow rate, 0.9 mL/min	99.82	3.74	23201	1.30
Buffer (pH 5.5)	99.45	3.31	20800	1.38
Buffer (pH 4.5)	100.12	3.42	22850	1.38

Specificity. Specificity of the method was evaluated by accelerated degradation of both the components in the presence of each other. For this purpose the analytes were degraded using acidic, basic, thermal and oxidative stress. The samples treated with HCl showed almost about 60% degradation for potassium clavulanate and negligible degradation for cefadroxil, whereas in case of oxidative and basic stress very little degradation occurred for both the analytes. The thermal stress degraded cefadroxil to about 30% with little degradation for potassium clavulanate. Under all the stress conditions the degradation products were well separated from the analyte peaks which showed the specificity of the method in the presence of the degraded products.

Robustness. The Robustness of the method was evaluated by deliberately changing the chromatographic conditions. The results showed that varying the chromatographic conditions had no appreciable effects on the chromatographic parameters. The results of the robustness study are given in Tables 3 & 4.

Stability of solution. In addition, the stability of each component in the presence of other in solution form was determined by calculating the percent deviation of the results obtained after 72 h compared with the data at zero time. The percent deviation of both the analytes was less than 2% after 72 h.

Application of the method in synthetically prepared tablets. The recovery of the developed method was evaluated by analyzing the synthetically prepared tablets of both the analytes with commonly occurring excipients that are found in most of the tablet formulations such as starch, lactose, magnesium stearate and avicel and then measuring the percentage recovery of each component. The recovery was found to be from 99.65 to 100.33 % (Table 5).

* * *

In this study an isocratic HPLC method was developed and validated for the simultaneous determination of clavulanate potassium and cefadroxil. The developed method is simple, precise and economical as evident from retention time and recovery study. The commonly found excipients do not interfere with the elution of both the analytes. Therefore, the developed method can be used for the analysis of potassium clavulanate and cefadroxil both in individual dosage forms and in combined pharmaceutical formulations. One draw back of this method is the use of very polar mobile phase and the excipients with low polarity may be trapped in column, thereby reducing column performance gradually. However this could be minimized

Table 4. Robustness study of cefadroxil

Conditions	Assay, %	RT, min	Theoretical plate	Tailing	Resolution
Acetonitrile : buffer (06 : 94)	99.75	4.64	25529	1.32	5.8
Acetonitrile : buffer (04 : 96)	100.51	6.78	26420	1.34	8.3
Acetonitrile : buffer (08 : 92)	100.11	3.33	16270	1.45	2.4
Flowrate, 1.1mL/min	99.68	4.06	26560	1.31	5.2
Flow rate, 0.9 mL/min	100.46	5.21	25900	1.37	6.9
Buffer (pH 5.5)	99.98	4.70	22500	1.35	5.7
Buffer (pH 4.5)	100.28	4.75	23680	1.38	5.9

Table 5. Recovery study of the proposed HPLC method in synthetically prepared tablets

Potassium clavulanate			Cefadroxil		
Actual, μg/mL	Recovered, μg/mL	% recovery	Actual, μg/mL	Recovered, μg/mL	% recovery
11.5	11.56	100.52	45.0	45.35	100.78
11.5	11.43	99.39	45.0	45.21	100.47
11.5	11.46	99.65	45.0	44.88	99.73
11.5	11.40	99.13	45.0	45.15	100.33
Mean % recovery = 99.65%			Mean % recovery = 100.33%		
RSD = 0.76%			RSD = 0.59%		

by thorough washing the column after work with water and an organic solvent such as methanol.

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