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High Performance Thin Layer Chromatographic Determination of Potassium Clavulanate and Cefadroxil in Combined Tablet Dosage Form

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ABSTRACT

A new simple High Performance Thin Layer Chromatographic (HPTLC) method for determination of Potassium Clavulanate and Cefadroxil in combined tablet dosage form has been developed and validated. The mobile phase selected was Methanol: Ethyl acetate: Formic acid (1.5: 8: 0.8, v/v/v) with UV detection at 230 nm. The retention factor for Potassium Clavulanate and Cefadroxil were found to be 0.77 ± 0.011 and 0.39 ± 0.007, respectively. The method was validated with respect to linearity, accuracy, precision and robustness as per the International Conference on Harmonisation (ICH) guidelines. Results found to be linear in the concentration range of 2000-12000 ng/band for Potassium Clavulanate and 500-3000 ng/band for Cefadroxil respectively. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean ± S.D.) was found to be 100. 06 ± 0.916 for Cefadroxil and 99.93 ± 0.996 for Potassium Clavulanate. The method can be used for routine analysis of these drugs in combined tablet dosage forms in quality-control laboratories.

Key Words: Potassium Clavulanate, Cefadroxil, Densitometry, Tablet dosage form.

INTRODUCTION

Cefadroxil, (CFL), (6R,7R)-7-[(2R)-2-Amino-2-(4-hydroxyphenyl)acetylamino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid is β-lactamantibiotic from cephalosporin group effective against urinary tract infection as well as pharyngitis and laryngitis [1].

Potassium Clavulanate, chemically, Monopotassium (PC) (2R,5R)-3-[(1Z)-2-hydroxy ethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate is a naturally occurring β-lactamase inhibitor.For the treatment of infection caused by β-lactamase producing bacteria [2].

Literature survey reveals High Performance Liquid Chromatographic (HPLC) methods for determination of CFL as single and in combination with other drugs [3-10]. Also spectrophotometric methods for determination of CFL as single and in combination with other drugs in dosage form have been also reported [11-14]. HPLC methods in human plasma, biological fluids in combination with other drugs have been reported for the determination of PC [15-25]. Densiometric method for simultaneous estimation of PC with other drugs have also been reported [26].

No reports were found for the simultaneous estimation of the CFL and PC in combined dosage form by HPTLC method. This paper describes a simple, accurate, sensitive and validated HPTLC method for simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [27].

MATERIALS AMD METHODS

Materials

Pharmaceutical grade working standards CFL and PC were kindly supplied by Okasa Pvt. Ltd. (Satara, India) and Maxim pharmaceuticals (Pune, India), respectively. Ethyl acetate, Methanol and Formic acid (all AR grade) were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). The pharmaceutical dosage form used in this study was Droxyl Clav 500 tablets (Torrent Ltd., Mehsana, India) labeled to contain 500 mg of Cefadroxil and 125 mg of Potassium Clavulanate were procured from the local market.

Instrumentation and chromatographic conditions

The samples were spotted in the form of bands of width of 6 mm with space between bands of 5 mm, with a 100 µl sample syringe (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium plate 60 F_{254} (10 ×10) with 250 µm thickness (E. MERCK, Darmstadt, Germany) using a CAMAG Linomat 5 sample applicator (Switzerland). The slit dimensions 5 mm × 0.45 mm and scanning speed of 20 mm/sec was employed.

The linear ascending development was carried out in 10 cm × 10 cm twin trough glass chamber (CAMAG, Muttenz, Switzerland) using Ethyl acetate: Methanol: Formic acid (8: 1.5: 0.8, v/v/v) as mobile phase. The optimized chamber saturation time for mobile phase was 20 min. The length of chromatogram run was 9 cm and development time was approximately 15 min. TLC plates were dried in a current of air with the help of a hair drier. Densitometric scanning was performed on CAMAG thin layer chromatography scanner at 230 nm for all developments operated by WINCATS software version 1.4.2. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 200 to 400 nm.

Preparation of standard stock solutions

Standard stock solution of CFL was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1 mg/ml from which 5 ml was further diluted to 10 ml to get solution having concentration 500 ng/µl. Standard stock solution of PC was prepared by dissolving 10 mg of drug in 10 ml of methanol to get final concentration of 1000 ng/µl.

Selection of Detection Wavelength

After chromatographic development bands were scanned over the range of 200-400 nm and the spectra were overlain. It was observed that both drugs showed considerable absorbance at 230 nm. So, 230 nm was selected as the wavelength for detection as shown in Figure 1.

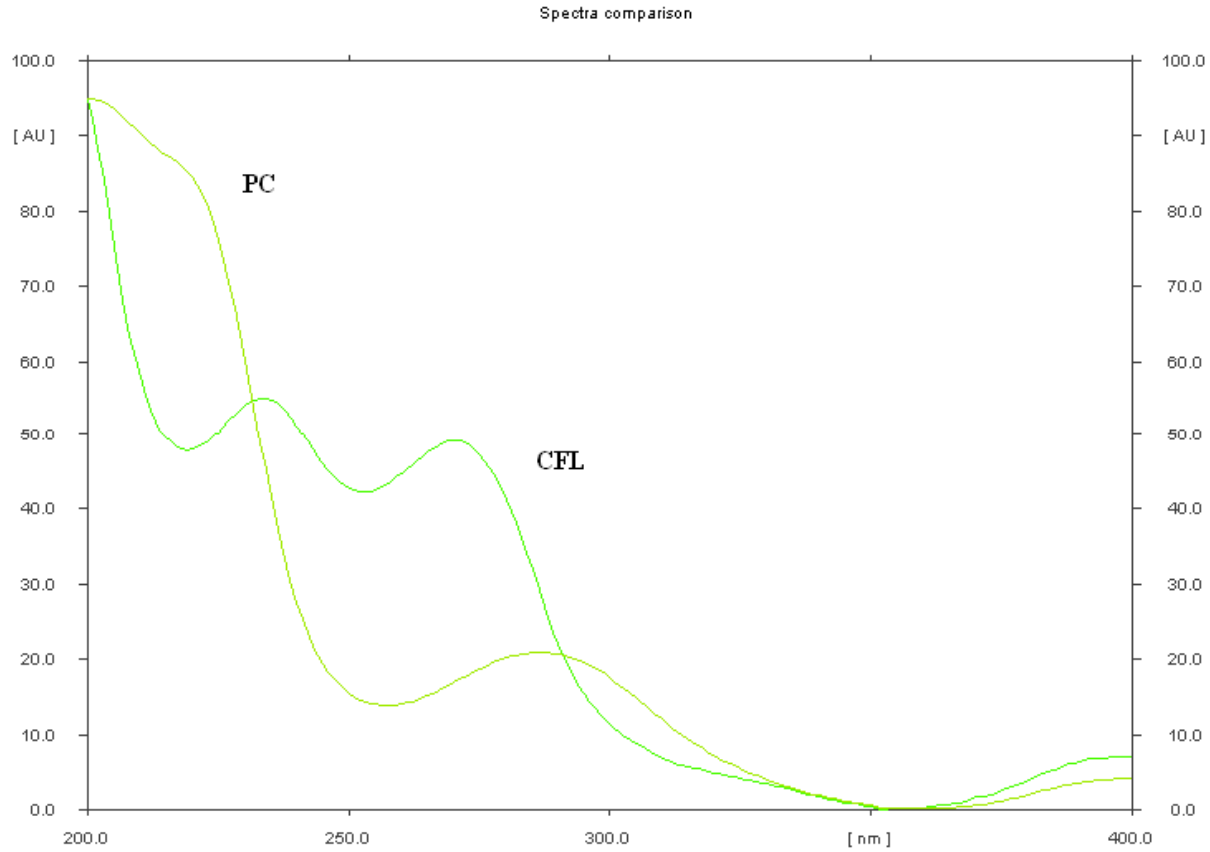

Figure 1: Overlain spectra of CFL and PC

Analysis of Tablet formulation

Twenty tablets were weighed accurately and finely powdered. A quantity of powder equivalent to 10 mg of CFL was weighed and dissolved in 10 ml of methanol. The solution was filtered using Whatman paper No. 41 and one microlitre volume of this solution was applied on TLC plate to obtain final concentration of 1000 ng/band.

For PC, powder equivalent to 10 mg was weighed and dissolved in 10 ml of methanol. The solution was filtered using Whatman paper No. 41. Four microlitre volume of this solution was applied on TLC plate to obtain final concentration of 4000 ng/band. After chromatographic development peak areas of the bands were measured at 230 nm and the amount of each drug present in sample was estimated from the respective calibration curves. Procedure was repeated six times for the analysis of homogenous sample.

Method Validation
The method was validated for linearity, accuracy, intra-day and inter-day precision and robustness, in accordance with ICH guidelines [27].

Preparation of Calibration Curve
The standard stock solutions of CFL (500 ng/µl) and PC (1000 ng/µl) were applied by overspotting on TLC plate in range of 1, 2, 3, 4, 5, 6 µl and 2, 4, 6, 8, 10, 12 µl, respectively with the help of CAMAG 100 µL sample syringe, using Linomat 5 sample applicator. The plate was developed and scanned under above established chromatographic conditions. Each standard in six replicates was analyzed and peak areas were recorded. Calibration curves of CFL and PC were plotted separately of peak area vs respective concentration of CFL and PC.

Precision
Set of three different concentrations in three replicates of mixed standard solutions of CFL and PC were prepared. All the solutions were analyzed on the same day in order to record any intra day variations in the results. For Inter day variation study, three different concentrations of the mixed standard solutions in linearity range were analyzed on three consecutive days.

Limit of detection (LOD) and Limit of quantitation (LOQ)
LOD and LOQ for both the drugs were calculated by using the values of slopes and intercepts of the calibration curves.

Robustness Studies
In the robustness study, the influence of small, deliberate variations of the analytical parameters on peak area of the drugs was examined. Factors varied were mobile phase composition (± 2 %), mobile phase saturation (± 10 %), time from application to development (0, 10, 20, and 30 min) and from development to scanning (0, 30, 60, and 90 min). One factor at a time was changed to estimate the effect. Robustness of the method was checked at a concentration level of 1500 ng/ band for CFL and 6000 ng/band for PC. The result are given in Table 1.

Table 1: Robustness Data in Terms of Peak Area (% RSD)

Sr. No.	Parameter	(% RSD)*	
		CFL	PC
1	Mobile phase composition (± 2 %),	0.013	0.007
2	Mobile phase saturation (± 10 %)	0.021	0.012
3	Time from application to development (min)	0.033	0.017
4	Development to scanning (min)	0.034	0.009

**Average of three determinations .*

Recovery Studies
To check the accuracy of the 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 %. Chromatogram was developed and the peak areas were noted. At each level of the amount, three determinations were carried out. The results of recovery studies were expressed as percent recovery and are shown in Table 2.

Table 2: Recovery Studies of CFL and PC

Drug	Amount taken (ng/band)	Amount added (ng/band)	Total amount found (ng/band)	% Recovery	% RSD[a]
CFL	1000	500	1512.39	100.82	0.393
	1000	1000	2020.78	101.03	0.611
	1000	1500	2500.79	100.03	0.280
PC	4000	2000	6041.10	100.68	0.214
	4000	4000	7988.26	99.85	0.898
	4000	6000	9936.37	99.36	0.937

[a] *Average of three determinations*

RESULTS AND DISCUSSION

Different mobile phases containing various ratios of Formic acid, Ethyl acetate, Methanol, Chloroform, Toluene and Glacial acetic acid were examined (data not shown). Finally the mobile phase containing Ethyl acetate: Methanol: Formic acid (8: 1.5: 0.8, v/v/v) was selected as optimal for obtaining well defined and resolved peaks. The optimum wavelength for detection and quantitation used was 230 nm. The retention factors for CFL and PC were found to be 0.39 ± 0.007 and 0.77 ± 0.011 respectively. Representative densitogram of mixed standard solution of CFL and PC is shown in Figure 2.

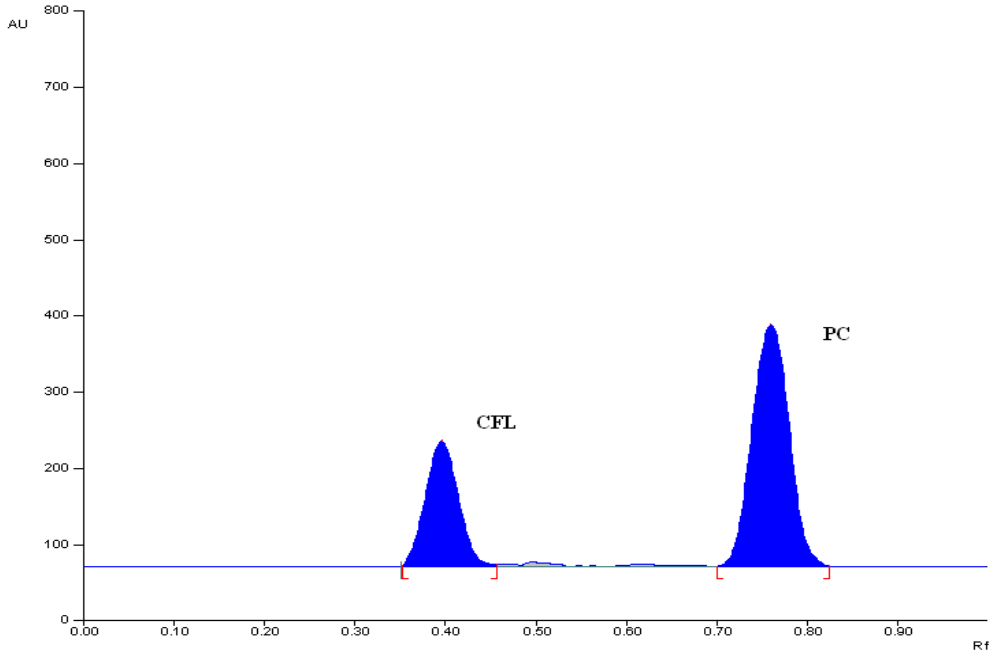

Figure 2: Representative chromatogram of mixed standard solution of CFL (1500 ng/band, R_f = 0.39 ± 0.007) and PC (6000 ng/band, R_f = 0.77 ± 0.011)

Straight-line calibration graphs were obtained for CFL and PC in the concentration range 500-3000 ng/band for CFL and 2000-12000 ng/band for PC with high correlation coefficient. The proposed method was also evaluated by the assay of commercially available tablets containing CFL and PC. The % assay (Mean ± S.D.) was found to be 100.06 ± 0.916 for CFL and 99.93 ± 0.996 for PC. Robustness of the method checked after deliberate alterations of the analytical

parameters showed that areas of peaks of interest remained unaffected by small changes of the operational parameters (% RSD < 2).

For CFL, the recovery study results ranged from 100.03 to 101.03 % with % RSD values ranging from 0.280 to 0.611. For PC, the recovery results ranged from 99.36 to 100.68 % with % RSD values ranging from 0.214 to 0.937. The method was found to be accurate and precise, as indicated by recovery studies as recoveries were close to 100 % and % RSD not more than 2. Intra-day variation, as RSD (%), was found to be in the range of 0.50–1.19 for CFL and 0.33–0.86 for PC. Interday variation, as RSD (%) was found to be in the range of 0.22–0.85 for CFL and 0.10–0.72 for PC. The summary of validation parameters of proposed method are given in Table 3.

Table 3: Summary of validation parameters of proposed method

Parameters	CFL	PC
Linearity range (ng/band)	500 - 3000	2000 -12000
Correlation coefficient (r)	0.992	0.993
LOD[a] (µg/ml)	47.44	78.48
LOQ[b] (µg/ml)	143.75	843.90
Accuracy (% Recovery)	100.03-101.03	99.36-100.68
Precision (% RSD)[c]		
Intra day (n[d] = 3)	0.50-1.19	0.33-0.86
Inter day (n = 3)	0.22-0.85	0.10-0.72

[a]LOD = Limit of detection.
[b]LOQ = Limit of quantitation.
[c]RSD = Relative standard deviation.
[d]n = Number of determinations

CONCLUSION

The validated HPTLC method employed here proved to be simple, fast, accurate, precise and robust, thus can be used for routine analysis of CFL and PC in combined tablet dosage form.

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