American-Eurasian Journal of Scientific Research 5 (2): 88-93, 2010
ISSN 1818-6785

Spectrophotometric and RP-HPLC Methods for Simultaneous Determination of Cefpodoxime Proxetil and Clavulanate Potassium in Combined Tablet Dosage Form

[1]Sunil Singh, [1]Nitin Dubey, [1]Dinesh Kumar Jain,
[2]Lalit Kumar Tyagi and [3]Mahendra Singh

[1]College of Pharmacy, IPS Academy, Rajender Nagar, Indore, Madhya Pradesh, India
[2]Geetanjali College of Pharmaceutical Studies, Udaipur, Rajasthan, India
[3]Vel's College of Pharmacy, P.V. Vaithiyalingam Road, Pallavaram, Chennai, India

Abstract: Simple, accurate, precise and sensitive ultraviolet spectrophotometric and reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of clavulanate potassium (CLA) and cefpodoxime proxetil (CEF) in combined tablet dosage form have been developed and validated. Beer's law is obeyed in the concentration range of 15-150 and 5-50 µg/mL in methanol at 270 nm and 235 nm for CLA and CEF, respectively for simultaneous equation method. The RP-HPLC method uses a Shimadzu LC 10 AT VP system with Luna C18 column and methanol: acetonitrile: water: tetrahydrofuran (THF) (40:30:20:10 v/v/v/v) as the mobile phase. The detection was carried out using a diode array detector set at 220 nm. Linearity of LC method in the concentration range of 15-200 and 5.0-50 µg/mL for CLA and CEF, respectively. The recoveries were in the range of 99.56±0.32 and 99.67±0.46 for CEF and 99.89±0.27 and99.7±0.45 for CLA in simultaneous equation method and HPLC method, respectively. In conclusion, spectrophotometric and RP-HPLC methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation and results of analysis were validated statistically and by recovery studies.

Key words: Cefpodoxime Proxetil · Clavulanate Potassium · HPLC · Validation

INTRODUCTION

Cefpodoxime proxetil (CEF) is chemically known as (6R,7R)-7-{[(2Z)-2-(2-amino-1,3-thiazol- 4-yl)-2methoxyimino-acetyl] amino}-(3methoxymethyl)-8-oxo-5-thia-1azabicyclo [4.2.0] oct-2-ene-2corboxylic acid and belongs to the class of compounds known as third generation cephalosporins [1, 2] and have antibacterial action. It is official in British Pharmacopoeia and United States Pharmacopoeia. Cefpodoxime proxetil inhibits the proper formation of bacterial cells walls in the last stage of cell wall synthesis. Because cefpodoxime is stable against many beta-lactamases, many organisms that are resistant to penicillins and cephalosporins, due to their beta-lactamase production, may be susceptible to cefpodoxime.

Clavulanate potassium (CLA) is chemically known as (2R,5R,Z)-3-(2-ydroxyethylidene)-7-oxo-4-oxo-1-aza-bicyclo[3.2.0] heptane-2-corboxylic acid and official in British Pharmacopoeia and United state pharmacopoeia. CLA is beta-lactamase inhibitor [1, 2], the molecule to act as a competitive inhibitor of beta-lactamases secreted by certain bacteria to confer resistance to beta-lactam antibiotics. The chemical structures of CEF and CLA are shown in Fig. 1:

Literature survey revealed that the assay of the CEF in pure and dosage forms is official in British Pharmacopoeia and United State Pharmacopoeia apart from Pharmacopeias several analytical methods that have been reported for the determination of CEF in biological fluids and urine [3] including column high-performance liquid chromatography (HPLC), HPLC, HPLC/MS and HPLC-NMR.

HPLC method for determination of CEF from tablet formulation is official in USP (2007). Several analytical methods that have been reported for the determination of CLA in biological fluids and in bulk as well as pharmaceutical formulations [4-8] include HPLC, UV absorption spectrophotometry, fluorometry and Fourier transform Raman and infrared spectrophotometry.

This paper described simple, accurate, precise and sensitive simultaneous equation method [9] and reversed-phase (RP)-HPLC methods for simultaneous determination of CEF and CLA in a combined tablet

Corresponding Author: Sunil Singh,College of Pharmacy, IPS Academy, Rajender Nagar, Indore, Madhya Pradesh, India
E-mail: rssunil29@rediffmail.com; lktyagi13@yahoo.co.in, Tel: +91-9602989378

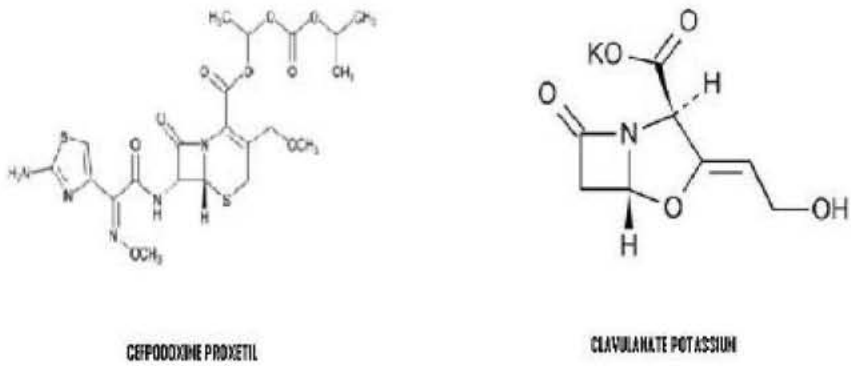

Fig. 1: Chemical structures of CEF and CLA

dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines.

Experimental

Drugs and Chemicals: Acetonitrile, methanol and THF (Tetra Hydro Furon) were purchased from Merck (Mumbai, India). All other reagents used were of analytical grade for the spectrophotometric determination and of HPLC grade for the HPLC method. Standard bulk drug samples of CEF (99.86% pure) were provided by FDC limited (Aurangabad, India) and CLA (99.79% pure) were provided by Ranbaxy laboratory limited (Dewas, India) as gratis sample. The pharmaceutical dosage form used in this study was ZIPOD CV tablets labeled to contain CEF 200 mg and CLA 125 mg/tablet (FDC Ltd. Aurangabad, India).

Instruments: A UV-visible (UV-Vis) double beam spectrophotometer (Model 1601; Shimadzu, Japan) with 1 cm matched quartz cells and UV probe software was used for the spectrophotometric method. For the HPLC method, an HPLC system consisting of LC 10 AT VP pump equipped with diode array detector (Shimadzu, Japan) and Luna C18 (4.6 mm id) column and class M10A software was used. A Rheodyne (Rohnert Park, CA) injector with 20 µL loop was used for injecting the sample.

Method -I (Simultaneous Equation Method): A stock solution of each drug having a concentration of 1 mg/mL (i.e.1000 µg/mL) was prepared by dissolving CEF and CLA separately in 100 ml methanol. Aliquots of the stock solutions were further diluted in methanol and were scanned in the wavelength range of 400-200 nm. Zero order overlain spectra are presented in Figure 2. Determinations were carried out at 235 and 270 nm, the maximum absorbance wavelength (λ_{max}) of CEF and CLA, respectively. Appropriate dilution were prepared using methanol from the stock solutions 1000 µg/ml of CEF and CLA to get aliquots of the concentration of 5, 10, 15, 20, 25, 30 and 35. The calibration curves were plotted from mean absorbance values of observation of the six replicate. The absorptivity values for both the drugs were determined at their respective λ_{max} by measuring absorbance values for working standards of CEF and CLA.

Procedure for Analysis of Tablet Formulation: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg CEF and 62.5 mg CLA was transferred to a 100 mL volumetric flask, 70 mL methanol was added and the flask was shaken vigorously for 5 min and sonicated for 10 min. The volume was made up to the mark with methanol. The solution was filtered and further diluted with methanol to obtain a concentration within the Beer's law range. The absorbance of sample was measured at 235 and 270 nm. The contents of CEF and CLA were calculated by solving the following equations.

$$A_1 = a_{x1}.b.C_x + a_{y1}.b.C_y$$
$$A_2 = a_{x2}.b.C_x + a_{y2}.b.C_y$$

Where, a_{y1} and a_{y2} are the absorptivity of drug Y at λ_1 and λ_2,

A_1 and A_2 are the absorbencies of the diluted sample at λ_1 and λ_2, b is the path length

C_x and C_y is the concentration of CEF and CLA respectively in diluted sample.

Method-II (RP-HPLC Method): In the RP-HPLC method, separation and analysis of CEF and CLA were carried out on a Luna C18 column (4.6 mm id) with the diode array detector set at 220 nm. Mobile phase consisting of methanol: acetonitrile: water: thf (40:30:20:10 v/v/v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated) was used with a flow rate of 1 mL/min.

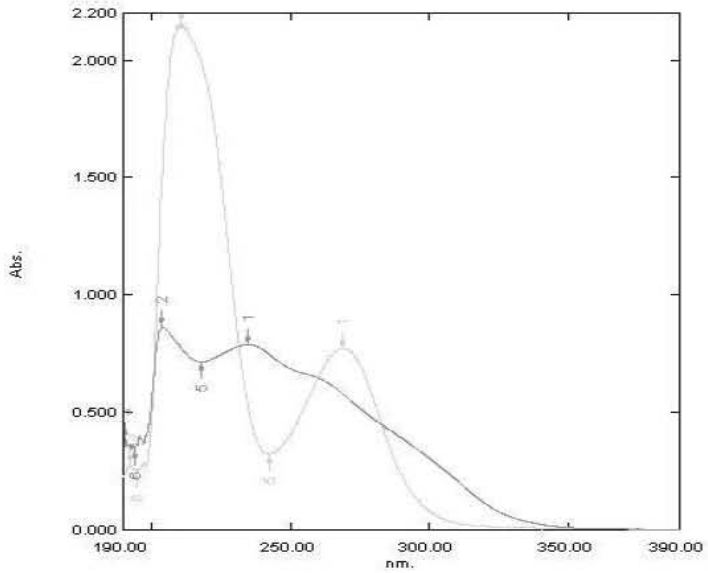

Fig. 2: Zero order overlain spectra of CEF (15 µg/mL) and CLA (15 µg/mL)

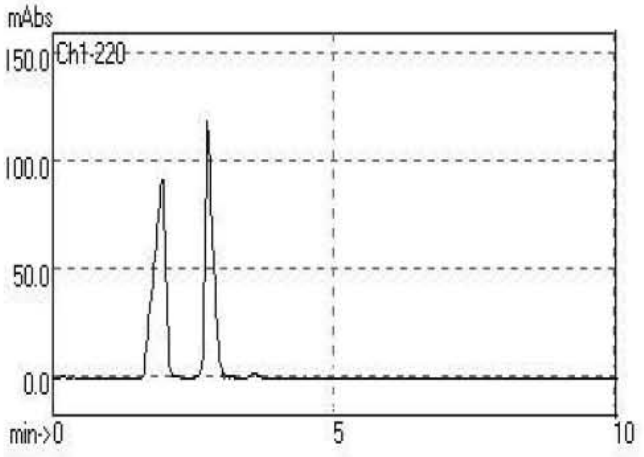

Fig. 3: Chromatogram of CLA (20 µg/mL, retention time 1.924 min); CEF (20 µg/mL, retention time 3.025 min).

Standard Stock Solutions: Standard stock solutions containing 100 µg/mL CEF, 100 µg/mL CLA were prepared by dissolving the pure drugs separately in the mobile phase.

Preparation of the Calibration Curves: Aliquots of 1, 2,,6,8 and 10 mL stock solution of CEF and 15,20,25,30 and 35 mL stock solution of CLA were transferred into a series of 10 mL volumetric flasks and the volume was made up to the mark with the mobile phase. Each solution was injected and chromatogram was recorded. Mean retention times for CEF and CLA were found to be 3.025 and 1.924 min, respectively. The peak area of CEF and CLA were noted and respective calibration curves were plotted as peak area against concentration of each drug.

Procedure for Analysis of Tablet Formulation: Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 100 mg CEF and 62.5 mg CLA weighed and transferred to a 50 mL volumetric flask containing about 70 mL mobile phase, ultrasonicated for 10 min and the volume was made up to the mark with the mobile phase. The solution was filtered through Whatman (Florham Park, NJ) No. 41 paper, 0.2 mL filtrate was transferred to a 10 mL volumetric flask and the volume was made up to the mark with the mobile phase. The tablet sample solution was injected, the chromatogram was obtained and the peak areas were recorded. A representative chromatogram is given in Figure 3. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.

Robustness Studies: The influence of small, deliberate variations of the analytical parameters on the retention time of the drugs was examined. The following factors were selected for change: the wavelength at which the drugs were recorded (220 ± 1 nm) and the flow rate of the mobile phase (1.0 ± 0.02 mL/min). One factor at the time was changed to estimate the effect. The solutions containing 20 µg/mL CEF and 20 µg/mL CLA were applied onto the column. Six replicate analyses (n = 6) were conducted at 3 levels of the factor (-, 0, +).

Recovery Studies: To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100 and 120%.

Precision: Precision of the method was checked by 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

Statistical Analysis: The statistical analysis was performed using Microsoft Excel 2003.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for CEF and CLA. Different mobile phases containing methanol, acetonitrile, water and thf were examined and the mobile phase methanol: acetonitrile: water: thf (40:30:20:10 v/v/v/v) was selected as optimal for obtaining well-defined and resolved peaks. The optimum wavelength for detection and quantitation was 220 nm, at which the best detector response for both the substances was obtained. Straight line calibration curves were obtained for CEF and CLA in the spectrophotometric and RP-HPLC methods. Table 1 summarizes the Beer's law limit, linear regression equation, correlation coefficient, standard deviations (SD) and limit of detection (LOD) and limit of quantitation (LOQ) values for both methods. System suitability parameters for the RP-HPLC method are listed in Table 2.

Robustness studies of the HPLC method, carried out after deliberate alterations of the analytical wavelength and flow rate of mobile phase, showed that small changes of these operational parameters did not lead to changes of retention times for the peaks of interest. The effect of a single factor at two levels indicated that the selected factors remained unaffected by small variations of these parameters. Therefore, this method is suitable for in routine analysis (Table 3).

The proposed methods were also evaluated in the assay of commercially available tablets containing CEF and CLA. Six replicate determinations were performed on the accurately weighed amounts of tablets. For CEF, recovery (mean,%, ± SD, n = 6) was found to be 99.56 ± 0.32 and 99.67 ± 0.46 for Methods I and II, respectively. For CLA, recovery was found to be 99.89 ± 0.27 and 99.7 ± 0.45 for Methods I and II, respectively (Table 4), for CEF, the recovery study results ranged from 99.56 to 100.05% and 99.49 to 100.05% for Methods I and II, respectively, with relative standard deviation (RSD) values ranging from 0.002 to 0.007 and 0.003 to 0.006%, respectively. For CLA, the recovery results ranged from 99.89 to 99.71 and 99.49 to 100.08% for Methods I and II, respectively, with RSD values ranging from 0.0004to 0.008 and 0.009 to 0.005%, respectively.

Table 1: Regression analysis of calibration curves of method I and II

	Method I		Method II	
Parameters	CEF	CLA	CEF	CLA
λ_{max}	235	270	220[a]	220[a]
Beer's law limit µg/mL	5-50	15-200	5-60	5-100
Correlation coefficient	0.9995	0.999	0.999	0.9991
Molar absorptivity	0.089	0.049	-	-
Linear regression equation[b]				
Intercept	0.0069	0.0064	49112	352
Slope	0.021	0.024	34775	19584
SD[c]	0.0032	0.0229	8316.7	6694.96
Detection limit, µg/mL	0.53	15.74	0.789	1.128
Quantitation limit, µg/mL	1.52	47.7	2.391	3.41

[a] Detection wavelength for HPLC method

[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL)

[c] SD = standard deviation

Table 2: System suitability parameters for RP-HPLC method

Parameters	Cefpodoxime proxetil	Clavulanate potassium
Calibration range, µg/ml	5-60	5-100
Theoretical plate number	2579	3856
HETP[a]	0.0091	0.0069
Asymmetric factor	1.17	0.98
Tailing factor	1.06	1.29
Capacity factor (k')	1.42	1.56
Resolution	2.06	2.92

[a] HETP = Height equivalent to theoretical plate, cm

Table 3: Robustness data in terms of retention time for CEF and CLA

	Wavelength[b]		Flow rate[c]	
Level	CEF	CLA	CEF	CLA
-	1.916 ± 0.069	3.022 ± 0.12	1.917 ± 0.092	3.022 ± 0.095
0	1.917 ± 0.096	3.022 ± 0.19	1.917 ± 0.072	3.022 ± 0.015
+	1.919 ± 0.120	3.024 ± 0. 11	1.917 ± 0.14	3.022 ± 0.049

[a] Mean ± SD, n = 6

[b] 220 ± 1 nm

[c] 1 ± 0.02 mL/min

Table 4: Results of analysis of commercial formulation

	Label claim, mg/tablet		% claim, estimated[a]	
Method	CEF	CLA	CEF	CLA
I	200	125	99.79±0.019	99.53±0.0025
II	200	125	99.90±0.0059	100.03±0.0044

[a] Average of 6 determinations

Table 5: Recovery studies of CEF and CLA by Methods I and II

			Total Conc. found µg/ml		Recovery,%[a]	
Drug	Conc. µg/ml for methods	Conc. µg/ml for methods	Method I	Method II	Method I	Method II
CEF	2	1.6	3.6	3.6	99.56±0.0042	99.61±0.0096
	2	2	4	4	99.47±0.0038	99.36±0.0068
	2	2.4	4.4	4.4	100.05±0.0021	100.05±0.0046
CLA	1.25	1	2.25	2.25	99.89±0.0013	99.49±0.0044
	1.25	1.25	2.50	2.50	99.88±0.0011	99.53±0.0046
	1.25	1.50	2.75	2.75	99.71±0.0061	100.08±0.0059

Mean ± standerd deviation (n=3)

CONCLUSIONS

The validated spectrophotometric and RP-HPLC methods developed here proved to be simple, fast, accurate, precise and sensitive. Thus, they can be used for routine analysis of CEF and CLA in combined tablet dosage form without prior separation.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to FDC limited. (Aurangabad, India) and Ranbaxy laboratory limited, (Dewas, India) for the samples of pure cefpodoxime proxetil and clavulanate potassium. The authors are also thankful to college of pharmacy, IPS academy, Indore, for providing the necessary facilities and constant encouragement.

REFERENCES

1. Anonymous, 2002. British Pharmacopoeia. British Pharmacopoeia Commission Office, London.
2. Anonymous, 2007. The United States Pharmacopeial Convention, 29th Edition, Rockville.

3. Campus, F., A. Deslandes, L. Harcouet and R. Farinotti, 1994. High-performance liquid chromatographic method for the determination of cefpodoxime levels in plasma and sinus mucosa. J. Chromatography B Biomedical Applications, 656(2): 383-388.
4. Christine, E., K.A. Hutton, V. Biasi, P.B. Eastc, N.J. Haskins and V.W. Jacewiczd, 1994. Determination of clavam-2-carboxylate in clavulanate potassium and tablet material by liquid chromatography: tandem mass spectrometry. J. Chromatography A, 686(2): 213-218.
5. Hesham Salem, 2004. Selective spectrophotometric determination of phenolic Lactam antibiotics in pure forms and in their pharmaceutical formulations. Analytica Chimica Acta, 515(2): 333-341.
6. Watson, I.D., 1985. Clavulanate Potentiated Ticarcillin High-Performance liquid Chromatographic assays for clavulanic acid and ticarcillin isomers in serum and urine. J. Chromatography, 337(2): 301-309.
7. Abounassif, M.A., E.M. Abdel-Moety, E.M. Mohamed and R.A. Gad-Kariem, 1991. Liquid chromatographic determination of Amoxicillin and clavulanic acid in pharmaceutical preparations. J. Pharmaceutical & Biomedical Analysis, 9(9): 731-735.
8. Tsou, T.L., J.R. Wu, C.A. Young and T.M. Wang, 1997. Simultaneous determination of amoxycillin and clavulanic acid in pharmaceutical products by HPLC with fl-cyclodextrin stationary phase. J. Pharmceutical and Biomedical Analysis, 15(8): 1197-1205.
9. Hoizey, G., D. Lamiable, C. Frances, T. Trenque, M. Kaltenbach, J. Denis and H. Millart, 2002. Simultaneous determination of Amoxicillin and clavulanic acid in human plasma by HPLC with UV detection. J. Pharmaceutical and Biomedical Analysis, 30(3): 661-666.