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Simultaneous Estimation of Cefpodoxime Proxetil and Clavulate Potassium Combined Dosage Form Using UV-Spectroscopy and Reverse Phase Liquid Chromatography

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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 was obeyed in the concentration range of 5-70 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 C_{18} 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 the LC method was in the concentration range of 5-70 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 and 99.7 ± 0.45 for CLA in simultaneous equation method and HPLC method, respectively. Both methods have been successfully applied for the analysis of CEF and CLA in a pharmaceutical formulation. Results of analysis were validated statistically and by recovery studies.

Keywords: absorptivity, robustness, simultaneous equation, validation
Abbreviations: CEF, cefpodoxime proxetil; **CLA**, clavulanate potassium; **RP-HPLC**, reverse phase high performance liquid chromatography

INTRODUCTION

Cefpodoxime proxetil (CEF) is chemically known as (6R, 7R) -7- {[(2Z) – 2 - (2-amino-1,3 – thiazol – 4 - yl) – 2 methoxyimino - acetyl] amino} - (3 methoxymethyl) – 8 – oxo – 5-thia -1 azabicyclo [4.2.0] oct – 2 – ene – 2 corboxylic acid and belongs to the class of compounds known as third generation cephalosporins (British Pharmacopoeia 2002; United States Pharmacopoeia 2007) and also having anti-bacterial 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 β-lactamases, many organisms that are resistant to penicillins and cephalosporins, due to their β-lactamase production, may be susceptible to cefpodoxime (Tsou *et al.* 1997; Hesham 2004).

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 States Pharmacopoeia. CLA is β-lactamase inhibitor (Anonymous 2002, 2007), the molecule to act as a competitive inhibitor of β-lactamases secreted by certain bacteria to confer resistance to β-lactam antibiotics. The chemical structures of CEF and CLA are shown in **Fig. 1**.

A 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 (Abdel-Moety *et al.* 1989; Camus *et al*. 1994) including column high-performance liquid chromatography (HPLC), HPLC/MS, and HPLC-NMR.

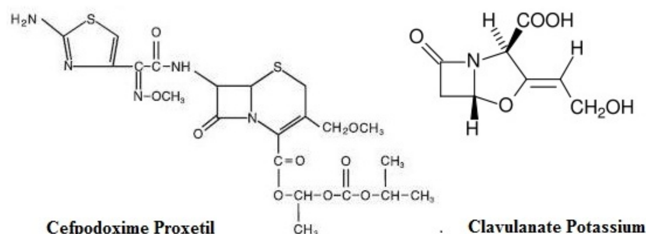

Fig. 1 Chemical structure of CEF and CLA.

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 (Watson *et al.* 1985; Abounassif *et al.* 1991; Eckers *et al*. 1994; Tsou *et al.* 1997; Hesham *et al.* 2004), including HPLC, UV absorption spectrophotometry, fluorometry, and Fourier transform Raman and infrared spectrophotometry.

This paper describes a simple, accurate, precise, and sensitive simultaneous equation method (Hoizey *et al.* 2002) and a reversed-phase (RP)-HPLC method for simultaneous determination of CEF and CLA in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines (Anonymous 1996).

MATERIALS AND METHODS

Drugs and chemicals

Acetonitrile, methanol and THF (Tetra Hydro Furon) were pur-

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chased 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 (99.86% pure) were provided by FDC Ltd. (Aurangabad, India) and (99.79% pure) were provided by Ranbaxy Laboratory Ltd. (Dewas, India) as gratis sample. The pharmaceutical dosage form used in this study was tablets labeled to contain CEF 200 mg and CLA 125 mg/tablet (FDC Ltd.).

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 C_{18} (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 **Fig. 2** determinations were carried out at 235 and 270 nm, the maximum absorbance wavelength (λ_{max}) of CEF and CLA, respectively. Appropriate dilutions 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 µg/ml. 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.

Quantitation of the tablet formulation was achieved by accurately weighing 20 tablets 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 (Davidson *et al.* 2004; Fukutsu *et al.* 2006). The contents of CEF and CLA were calculated by solving the following equations (Davidson *et al*. 2004):

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

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

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. The mobile phase, consisting of methanol: acetonitrile: water: tetrahydrofuran (THF) (40: 30: 20: 10, v/v/v/v; filtered through a 0.2 µm membrane filter, degassed and sonicated), was used at a flow rate of 1 mL/min.

1. Standard stock solutions

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

2. Preparation of the calibration curves

Aliquots of 1, 2, 4, 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.

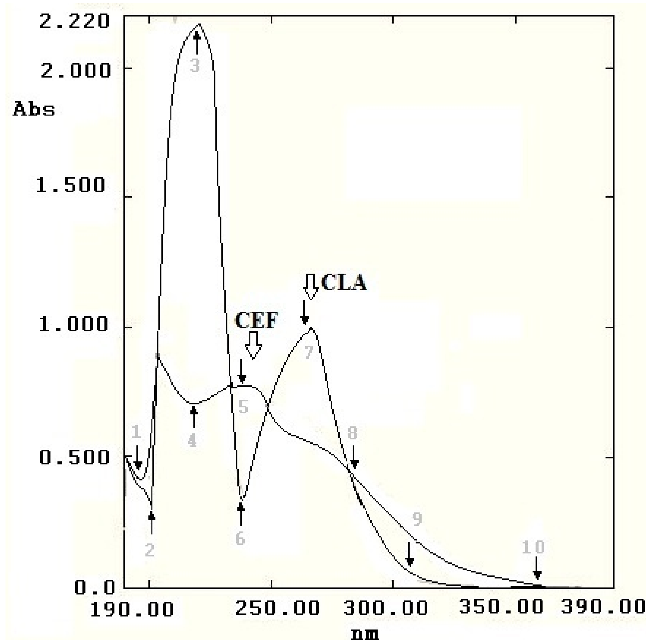

Fig. 2 Zero order overlain spectra of CEF and CLA.

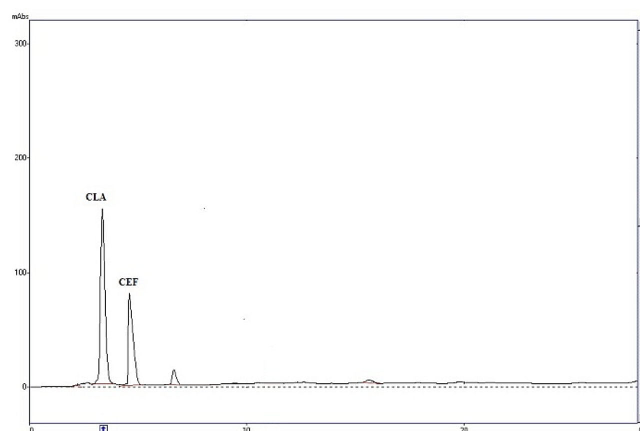

Fig. 3 Chromatogram of CEF and CLA in tablet dosage form.

3. 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 **Fig. 3**. Form the peak area the both the drugs concentration of each drug/tablet was estimated from the respective calibration curves.

4. Robustness studies

The influence of small, deliberate variations of the analytical para-

meters 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, +).

5. 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%.

6. Precision

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

Statistical analysis

Means, standard deviation (SD), relative standard deviation (RSD), and linear regression analyses were calculated 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 tetrahydrofuron 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 use 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 SD values ranging from 0.0004 to 0.008 and 0.009 to 0.005%, respectively (**Table 5**).

CONCLUSIONS

The proposed spectrophotometric and RP-HPLC methods were found to be simple, fast, accurate, precise, and sensitive. Thus, they may be used for routine analysis of CEF and CLA in combined tablet dosage form.

Table 1 Regression analysis of calibration curves of methods I and II.

Parameters	Method I		Method II	
	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	CEF	CLA
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 PCM and NAB [a].

Level	Wavelength [b]		Flow rate [c]	
	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 ± 0.02 mL/min.

Table 4 Results of analysis of commercial formulation.

Method	Label claim, mg/tablet		% claim, estimated [a]	
	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] Mean ± Relative standard deviation, n = 6.

Table 5 Recovery studies of PCM and NAB by methods I and II.

Drug	Concentration taken, µg/ml for methods	Concentration added, µg/ml for methods	Total concentration found µg/ml		Recovery, % [a]	
			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

[a] mean ± relative standard deviation (n = 3).

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