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Original Article

Spectrophotometric and High Performance Liquid Chromatographic Determination of Cefpodoxime Proxetil and Azithromycin Dihydrate in Pharmaceutical formulation

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ABSTRACT

Introduction: UV spectrophotometric methods and High performance liquid chromatographic method were developed for the determination of the Cefpodoxime Proxetil and Azithromycin Dihydrate in tablet form. **Methods:** In UV Simultaneous equation method and Absorbance ratio method were used. The wavelength of maximum absorbance 232.40 nm for Cefpodoxime Proxetil and 218 nm for Azithromycin Dihydrate are used in simultaneous equation method and 220.60 nm (isoabsorptive point) and 232.40 nm (λ_{max} of Cefpodoxime Proxetil) are used for absorbance ratio method. A simple liquid chromatographic assay has been developed for the determination of Cefpodoxime Proxetil and Azithromycin Dihhydrate. A C_{18} (150×4.6 mm, 5 µm) column was used with a mobile phase consisting of Acetonitrile: Methanol: Phosphate buffer (40:40:20 v/v) at a flow rate of 1.0 ml min^{-1}. Quantitation of both drugs was achieved by using UV detector at 235 nm. **Results:** Calibration curves were linear in the range of 8-40 µg/ml for Cefpodoxime Proxetil and 10-50 µg/ml for Azithromycin Dihydrate in absorbance ratio method. Accuracy for both the drugs was in the range of 98-101 %. The retention time for Cefpodoxime Proxetil and Azithromycin Dihydrate was found to be 6.14 and 2.95 respectively. Beer's law was obeyed in a concentration range of 20-100 µg/ml for Cefpodoxine Proxetil and 25-150 µg/ml for Azithromycin Dihydrate and the regression line equation was derived with a correlation coefficient of 0.9984 and 0.9978 for Cefpodoxime Proxetil and Azithromycin Dihydrate respectively. The method was validated according to ICH guidelines for various parameters like accuracy, precision, specificity, linearity, robustness, LOD and LOQ. **Discussion:** The proposed procedures were successfully applied to the determination of Cefpodoxine Proxetil and Azithromycin Dihydrate in tablet form, with high percentage of recovery, good accuracy and precision.

Key words: Cefpodoxime Proxetil, Azithromycin Dihydrate, Simultaneous equation method, Absorbance ratio method, HPLC, Tablets.

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INTRODUCTION

Cefpodoxime, 1-(isopropoxycarbonyloxy) ethyl (6R,7R)-7-[2-(2-amino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-methoxymethyl-3-cephem-4-carboxylate (Figure 1a), is an oral third generation cephalosporin antibiotic. It is active against most Gram positive and Gram negative bacteria. It is commonly used to treat acute otitis media, pharyngitis, and sinusitis. The bactericidal activity of Cefpodoxime results from its inhibition of cell wall synthesis.[1-3]

Azithromycin Dihydrate, (2R, 3S, 4R, 5R, 8R, 10R, 11R, 12S, 13S, 14R)-2-ethyl-3, 4, 10-trihydroxy-3, 5, 6, 8, 10, 12, 14-heptamethyl-15-oxo- 11-{[3, 4, 6-trideoxy–3-(dimethylamino–β-D-xylo-] oxy}-1-oxa-6-azacyclopentadec-13-yl 2, 6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranoside (Figure 1b), is a semi-synthetic macrolide antibiotic of azalide class.[4-5] Cefpodoxime is official in IP-2010,[6] USP-30 NF-24[7] and describes Liquid chromatographic method for its estimation and a literature survey reveals that HPTLC,[8-9] HPLC[10-15] and Spectrophotometric[16-26] methods has been developed for its estimation in alone or in combination with other drugs.

Azithromycin Dihydrate is official in IP 2010,[27] BP 2012,[28] USP-30 NF-25[29] and describes Liquid Chromatographic method for its estimation and a literature survey reveals that HPLC[30-38] and Spectrophotometric[39-41] methods has been developed for its estimation in alone or in combination with other drugs.

Two spectrophotometric methods[42-43] have been published for simultaneous estimation of Cefpodoxime Proxetil and Azithromycin Dihydrate. The purpose of present study was to develop and validate new spectrophotometric and HPLC method for simultaneous determination of Cefpodoxime Proxetil and Azithromycin Dihydrate in tablet dosage form.

EXPERIMENTAL

Instrumentation

A double beam, UV–Visible Spectrophotometer (Simadzu–1800, Software–UV Probe, Version 2.42) with 1 cm matched quartz cell was used. The spectral band width was 2 nm and the wavelength scanning speed was medium.

The HPLC Model was JASCO 980 Pump. Column was Thermo Hypersil C_{18} (150×4.6 mm, 5 µm) Column. An auto injector, UV-Detector and Chem 32 software.

Materials

Cefpodoxine Proxetil was gift sample by Sunrise Remedies Ltd, Ahmadabad. Azithromycin Dihydrate was supplied as gift sample by Vapi Care Ltd, Vapi. Combined tablet formulation (Cepodem-AZ & Gudcef-AZ) was purchased from local pharmacy. All other reagents used were AR grade and HPLC grade. The analytical grade methanol was purchased from SD Fine Chemical and NaOH (Rankem). Distilled water (HPLC grade-Fischer Scientific), Acetonitrile (HPLC grade-Fischer Scientific), Methanol (HPLC grade-Fischer Scientific), Disodium hydrogen phosphate (Rankem), Ortho Phosphoric acid (Rankem) were used for analysis.

Chromatographic conditions

The mobile phase was prepared by mixing Acetonitrile, Methanol and phosphate buffer in the ratio of (40:40:20 v/v) and pH 6.5 adjusted with ortho phosphoric acid. It was filtered through 0.45 µ membrane filter. All determinations were performed at ambient temperature (20°C) using C_{18}, (150×4.6 mm, 5 µm), reverse phase column (JASCO). The column effluent was monitored at 235 nm, which represents the wavelength of maximum absorbance of CEF and AZI. The injection volume was 20 µl with a flow rate of 1 ml/min.

Standard solutions and calibration graphs for spectrophotometric measurements

Simultaneous Equation Method

A stock solution was prepared by dissolving CEF and AZI and diluting with 0.2 N NaOH to obtain a concentration of 100 µg ml^{-1}. The standard solutions were prepared by dilution of the stock solution in 0.2 N NaOH to reach concentration ranges of 8–40 µg/ml and 10-50 µg/ml for CEF and AZI respectively for absorbance ratio method. Each solution was scanned between 200-400 nm. Wavelengths were selected from the overlay spectra of CEF and AZI. The absorbance of the solutions was measured at 232.40 nm and 218 nm against 0.2 N NaOH as a reagent blank. The concentrations versus their Absorbance were plotted in order to obtain the calibration graphs.

Absorbance Ratio Method

A stock solution was prepared by dissolving CEF and AZI and diluting with 0.2 N NaOH to obtain a concentration of 100 µg ml^{-1}. The standard solutions were prepared by dilution of the stock solution in 0.2 N NaOH to reach concentration ranges of 8–40 µg/ml and 10-50 µg/ml for CEF and AZI respectively for absorbance ratio method. Each solution was scanned between 200-400 nm. Wavelengths were selected from the overlay spectra of CEF and AZI. The absorbance of the solutions was measured at 232.40 nm and 220.60 nm against 0.2 N NaOH as a reagent blank. The concentrations versus their Absorbance were plotted in order to obtain the calibration graphs.

Standard solutions and calibration graphs for chromatographic procedure (HPLC)

Standard solutions of CEF and AZI containing concentration range of 20-100 µg/ml for CEF and 25-125 µg/ml for AZI were prepared in the mobile phase. Triplicate 20 µl injections were made for each concentration and the peak height ratio of each concentration were plotted against the corresponding concentrations to obtain the calibration graph.

Sample preparation

A total of 20 tablets containing CEF and AZI as the active ingredients were weighed and finely powdered. The powder equivalent to 200 mg of CEF and 250 mg of AZI was taken in 100 ml volumetric flask and dissolved in mobile phase. The volume was made up to mark and the solution was filtered through 0.45 micro membrane filter. The appropriately diluted solution was analyzed under optimized chromatographic conditions. The areas of resulting peak were measured at 235 nm. The peak-height ratios were used for the determination of CEF and AZI in each sample.

For Simultaneous equation method a 1 ml of this solution was diluted to 10 ml with 0.2 N NaOH and 2.4 ml of this solution was further diluted to 10 ml with 0.2 N NaOH. Absorbance of the resulting solution was measured at 232.40 nm and 218 nm against 0.2N NaOH. The concentration of CEF and AZI can be obtained as,

$$Cx = (A2\ ay1 - A1\ ay2)/(ax2\ ay1 - ax1\ ay2)$$

$$Cy = (A1\ ax2 - A2\ ax1)/(ax2\ ay1 - ax1\ ay2)$$

Where,

$A1$ and $A2$ are the absorbances of mixture at 232.40 nm and 218 nm respectively,

$aX1$ and $aX2$ are absorptivities of CEF at 232.40 nm and 218 nm respectively,

$aY1$ and $aY2$ are absorptivities of AZI at 232.40 nm and 218 nm respectively,

Cx is concentration of CEF,

Cy is concentration of AZI.

For Absorbance ratio method a 1 ml of this solution was diluted to 10 ml with 0.2 N NaOH and 2.4 ml of this solution was further diluted to 10 ml with 0.2 N NaOH. Absorbance of the resulting solution was measured at 232.40 nm and 220.60 nm against 0.2 N NaOH. The concentration of CEF and AZI can be obtained as,

$$Cx = (Qm - Qy/Qx - Qy)*A1/ax1$$

$$Cy = (Qm - Qx/Qy - Qx)*A1/ay1$$

Qm = Absorbance of sample at 232.40 nm (A2)/Absorbance of sample at 220. 60 nm (A1)

Qx = Absorptivity of CEF at 232.40 nm/Absorptivity of CEF at 220.60 nm

Qy = Absorptivity of AZI at 232.40 nm/Absorptivity of AZI at 220.60 nm

Where,

Qx and Qy are value of CEF and AZI respectively, ax1 and ay1 are absorptivity value at isosbestic point of CEF and AZI

VALIDATION PROCEDURE

System suitability

The typical values for evaluating system suitability of a chromatographic procedure include the RSD <1%, tailing factor <2 and theoretical plates >2000. The determination of system suitability of analytical method was accomplished by assaying six samples of CEF and AZI. The sample concentration of CEF and AZI used in this analysis was 25-100 µg/ml and 25-125 µg/ml, respectively. The retention time, peak area, theoretical plates and tailing factor were evaluated for system suitability.

Sensitivity

The limit of detection (LOD) and quantification limit (LOQ) were determined by gradually diluting the sample and analysing by the proposed method. The signal/noise ratio (S/N) was determined for each tested strength. The typical S/N ratio recommended by the International Conference on Harmonisation (ICH) is 3/1 and 10/1 for LOD and LOQ, respectively.

Calibration curve

The above-mentioned calibration standards were analysed for determining linearity. The sample strengths ranged from 20-100 µg/ml and 25-125 µg/ml for Cefpodoxime Proxetil and Azithromycin Dihydrate, respectively for HPLC method and 8-40 µg/ml and 10-50 µg/ml of CEF and AZI for Simultaneous equation method and Absorbance ratio method. The regression analysis was accomplished by slope, intercept and correlation coefficient (r^2).

Accuracy and precision

The accuracy was determined by percent recovery method. Furthermore, precision (inter-day variance and intra-day variance) were determined by assaying samples over a period of 1 day and 3 days, respectively. The standard concentrations used for these study were 20, 60, and 100 µg/ml for Cefpodoxime Proxetil and 25, 75, and125 µg/ml for Azithromycin dihydrate for HPLC method and for Absorbance ratio method the concentrations used were 8, 24, 40 µg/ml and 10, 30 and 50 µg/ml for Cefpodoxime Proxetil and Azithromycin Dihydrate.

Robustness

The influence of slight deliberate changes in chromatographic conditions such as column temperature, flow rate of mobile phase and pH of mobile phase on the retention time and peak area were observed one by one. The test was performed in triplicate for each set of conditions. The standard concentrations of Cefpodoxime Proxetil and Azithromycin Dihydrate used in this analysis were 40 µg/ml and 50 µg/ml, respectively.

RESULTS AND DISCUSSION

Simultaneous Equation Method

To determine wavelength for measurement, standard spectra of CEF and AZI were scanned between 200-400 nm against 0.2N NaOH. Absorbance maxima were obtained at 232.40 nm and at 218 nm for CEF and AZI respectively. Overlay spectra of CEF and AZI are presented in Figure 2.

Absorbance ratio method

Tablet powder was dissolved in methanol and 0.2 N NaOH (Figure 4). shows the absorption (zero-order) UV spectra of (a) CEF and AZI standard solution. However, the application of the absorbance ratio spectrophotometric technique allowed complete elimination of the back-ground absorption due to the excipients.

Chromatographic procedure (HPLC)

A reversed phase HPLC method was developed to provide a specific procedure suitable for rapid quality control of CEF and AZI tablet dosage form. A mobile phase consisting of Acetonitrile: Methanol: Phosphate buffer and in the ratio of (40: 40: 20v/v) and pH 6.5 adjusted with ortho phosphoric acid, was chosen after several trials with acetonitrile: water and methanol: water. The apparent pH of the aqueous phase was adjusted to 6.5 using orthophosphoric acid. The above described chromatographic system allowed an adequate resolution (R_s 12.02) between CEF (t_r 6.14) and AZI (t_r 2.95) in a reasonable time (Figure 4) (R_s, resolution; t_r, retention time). The applied analytical conditions produced the peaks with suitable peak symmetry (<2).

The typical conditions for system suitability of an analytical method encompass the relative standard deviation (RSD) < 2%, peak symmetry <2 and theoretical plates >2000. The results of system suitability of present chromatographic method are described in Table 1. The peak area, retention time, tailing factor and theoretical plates were within the recommended limits. Therefore, the method was considered as suitable for quantitative determinations a linear calibration graph (Y=318450.2267x+12119543.8, r^2=0.9984; n=3 for CEF and Y=36493.6520x+1070413.0333, r^2=0.9978; n=3) was obtained over the working concentration range of 20-100 µg/ml for CEF and 25-125 µg/ml for AZI.

The specificity and selectivity of the HPLC system were ascertained by a separate chromatographic analysis of either the excipient mixtures or sample; no interfering peaks at the retention times of CEF and AZI peaks were observed.The LOD and LOQ in accordance with the ICH guidelines is 3/1 and 10/1, respectively. LOD and LOQ values for Cefpodoxime proxetil and Azithromycin dihydrate for Simultaneous equation method, Absorbance ratio method and HPLC method are given in Table 2.

Each calibration curve was constructed with five standard strengths. For Simultaneous equation method, the calibration curve of CEF was made with 8, 16, 24, 32 and 40 µg/ml concentrations (Figure 3a and 3b). Similarly, the concentrations used in the formation of calibration curve of azithromycin dihydrate were 10, 20, 30, 40 and 50 µg/ml (Figure 3c and 3d). For Absorbance ratio method (Figure 4), the calibration curve of CEF was made with 8, 16, 24, 32 and 40 µg/ml concentrations

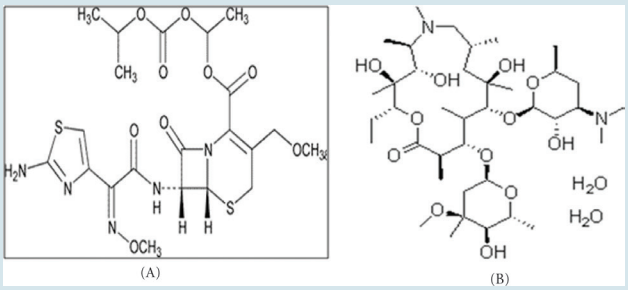

Figure 1: Chemical structures of (A) Cefpodoxime Proxetil and (B) Azithromycin Dihydrate.

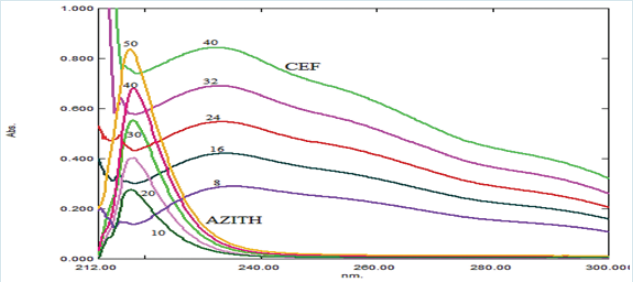

Figure 2: Overlay spectra of CEF and AZI.

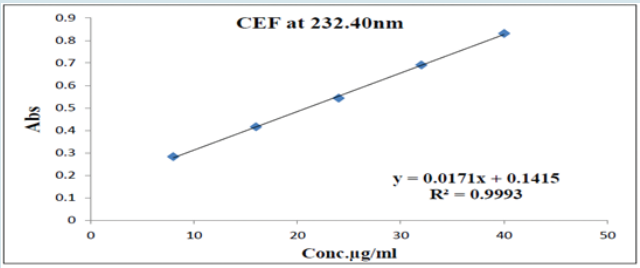

Figure 3a: Calibration curve for CEF at 232.40 nm in methanol: 0.2N NaOH.

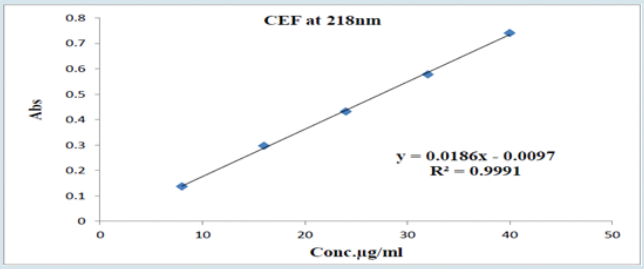

Figure 3b: Calibration curve for CEF at 218 nm in methanol: 0.2N NaOH.

(Figure 5a and 5b). Similarly, the concentrations used in the formation of calibration curve of azithromycin dihydrate were 10, 20, 30, 40 and 50 µg/ml (Figure 5c and 5d). Typical chromatogram of Cefpodoxime proxetil and Azithromycin dihydrate (Figure 6). For HPLC method, the calibration curve of CEF was made with 20, 40, 60, 80 and 100 µg /ml concentrations (Figure 7a). Similarly, the concentrations used in the formation of calibration curve of azithromycin dihydrate were 25, 50, 75, 100 and 125 µg/ml (Figure 7b). The regression

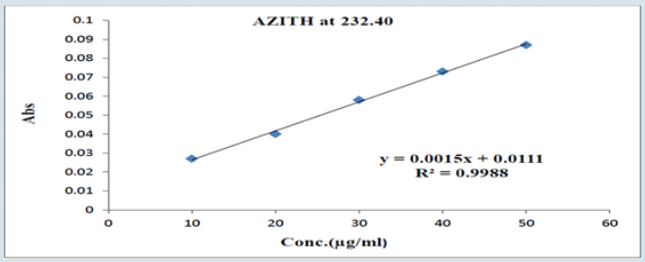

Figure 3c: Calibration curve for AZI at 232.40 nm in methanol: 0.2N NaOH.

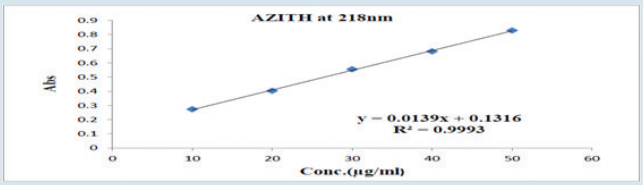

Figure 3d: Calibration curve for AZI at 218 nm in methanol: 0.2 N NaOH.

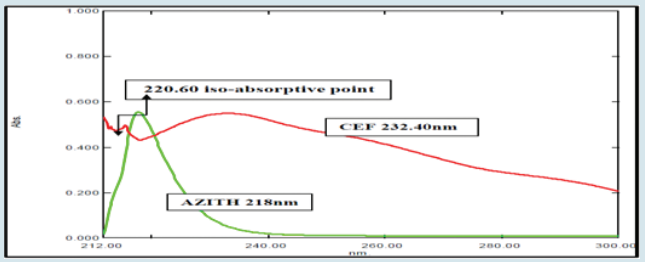

Figure 4: Overlay spectra of CEF and AZI showing Wavelength.

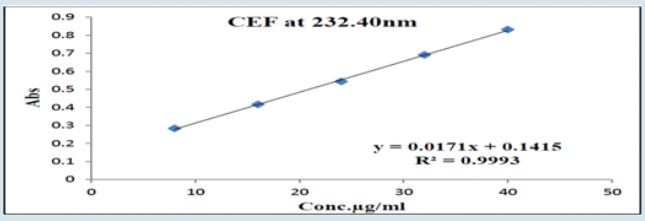

Figure 5a: Calibration curve for CEF at 232.40 nm in methanol: 0.2N NaOH.

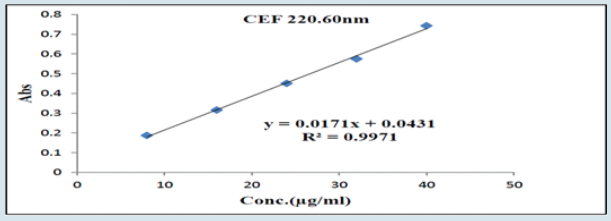

Figure 5b: Calibration curve for CEF at 220.60 nm in methanol: 0.2N NaOH.

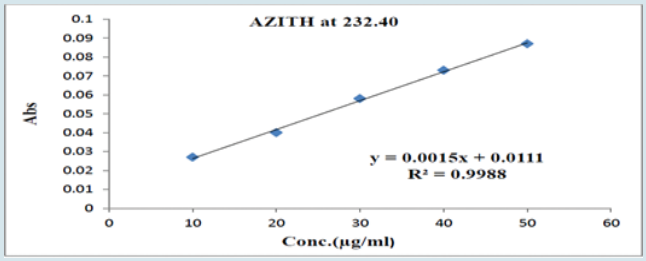

Figure 5c: Calibration curve for AZI at 232.40 nm in methanol: 0.2N NaOH.

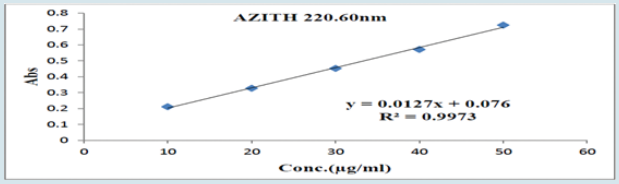

Figure 5d: Calibration curve for AZI at 220.60 nm in methanol: 0.2N NaOH.

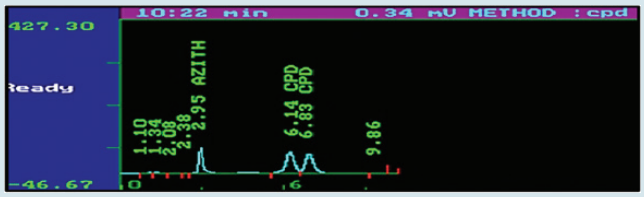

Figure 6: Typical Chromatogram of Cefpodoxime proxetil and Azithromycin dihydrate.

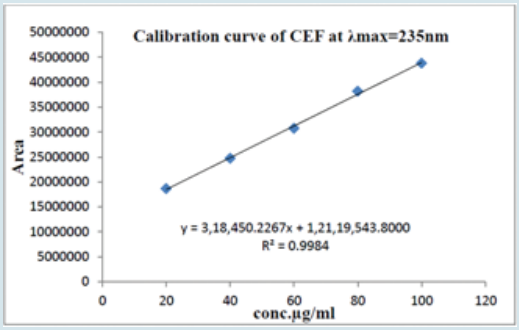

Figure 7a: Calibration plot of CEF (20-100 µg/ml).

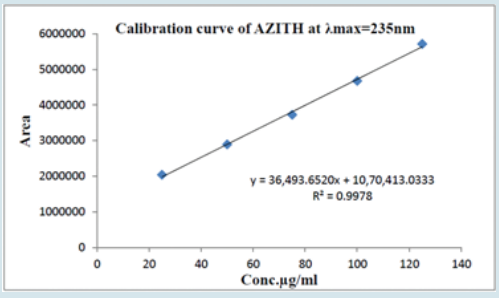

Figure 7b: Calibration plot of AZI (25-125 µg/ml)

Table 1: Results for system suitability test

Parameters	Data obtained	
	CEF	AZITH
Theoretical Plates per column	8674	3233
Symmetry factor/Tailing factor	1.02	0.42
Resolution	12.02	

Table 2a: Regression and analytical parameters for estimation of two drugs by Simultaneous equation method and absorbance ratio method

Parameter	Simultaneous equation method				Absorbance ratio method			
	CEF		AZI		CEF		AZI	
Wavelength (nm)	232.40	218	232.40	218	232.40	220.60	232.40	220.60
Concentration range(µg/ml)	8-40	8-40	10-50	10-50	8-40	8-40	10-50	10-50
Intercept	0.1415	0.0097	0.0111	0.1316	0.1415	0.0431	0.0111	0.076
Slope	0.0171	0.0186	0.0015	0.0139	0.0171	0.0171	0.0015	0.0127
Correlation coefficient (r^2)	0.9993	0.991	0.9988	0.9993	0.9993	0.9971	0.9988	0.9973
Regression equations	0.0171x+ 0.1415	0.0186x+ 0.0097	0.0015x+ 0.0111	0.0139x+ 0.1316	0.017x+ 0.1415	0.0171x+ 0.0431	0.0015x+ 0.0111	0.0127x+ 0.076
Repeatability (%RSD, n=6)	0.2795	0.340	1.7928	0.3901	0.2795	0.3809	1.7928	0.3250
Intraday precision (%RSD, n=3)	0.7092-0.183	1.1204-0.2710	0.740-1.259	0.5629-0.30	0.7092-0.18	0.5347-0.2695	0.740-1.259	0.9523-0.4223
Interday precision (%RSD, n=3)	1.843-0.4877	1.9598-0.6234	1.139-2.015	1.4869-0.57	1.843-0.487	1.1211-0.4872	1.139-2.015	2.0008-0.9264
Accuracy (% recovery)	98.95-101.73	98.20-101.5	99.63-101.5	100.04-100.9	98.95-101.7	99.48-101.47	99.63-101.58	98.29-99.36
LOD (µg/ml)	0.249	0.398	2.202	0.522	0.249	0.212	2.202	0.571
LOQ (µg/ml)	0.755	1.208	6.670	1.582	0.755	0.640	6.670	1.732

Table 2b: Regression and analytical parameters for estimation of two drugs by HPLC Method

Parameter	HPLC Method	
	CEF	AZI
Wavelength (nm)	235	235
Concentration range (µg/ml)	20-100	25-125
Intercept	12119543.80	1070413.03
Slope	318450.226	36493.6520
Correlation coefficient (r^2)	0.9984	0.9978
Regression equations	318450.226x+12119543.8	36493.6520x+1070413.03
Repeatability (%RSD, n=6)	0.4560	1.3713
Intraday precision (%RSD, n=3)	1.1633-0.926	1.222-1.2896
Interday precision (%RSD, n=3)	1.9915-1.7953	1.8088-1.8315
Accuracy (%recovery)	99.78-100.04	99.34-100.07
LOD (µg/ml)	2.346	2.121
LOQ (µg/ml)	7.110	6.429

Table 3a: Data for Intraday and Interday precision for Simultaneous equation method and Absorbance ratio method

Simultaneous equation method						Absorbance ratio method				
Drug	Amt. (µg/ml)	% RSD				Amt. (µg/ml)	% RSD			
		Intraday		Interday			Intraday		Interday	
		232.40 nm	218 nm	232.40 nm	218 nm		232.40 nm	220.60 nm	232.40 nm	220.60 nm
CEF	8	0.709	1.120	1.843	1.959	8	0.7092	0.5347	1.843	1.1211
	24	0.3690	0.465	0.7488	0.974	24	0.3690	0.3407	0.7488	0.9068
	40	0.1836	0.271	0.4877	0.623	40	0.1836	0.2695	0.4877	0.4872
AZI	10	0.7400	0.562	1.139	1.486	10	0.7400	0.9523	1.139	2.0008
	30	0.8620	0.362	1.744	0.919	30	0.8620	0.5571	1.744	1.3970
	50	1.259	0.304	2.015	0.574	50	1.259	0.42235	2.015	0.9264

Table 3B: Data for Intraday and Interday precision for HPLC Method

HPLC method			
Drug	Amt. (µg/ml)	% RSD	
		Intraday	Interday
CEF	20	1.1633	1.9915
	60	0.8081	2.0095
	100	0.9262	1.7953
AZI	25	1.222	1.8088
	75	0.8601	1.9587
	125	1.2896	1.8315

Table 4: Results of recovery study by HPLC and UV methods. (n=3)

Method	Drug	Amt. present (µg/ml)	Amt. added (µg/ml)	Amt. recovered (µg/ml)	% Recovery
HPLC Method	CEF	60	48	47.77	99.78
		60	60	61.27	101.06
		60	72	72.05	100.04
	AZI	75	60	60.46	100.34
		75	75	74.23	99.48
		75	90	90.11	100.07
Simultaneous equation method	CEF 232.40 nm	24	19.2	19.05	98.95
		24	24	24.20	102.00
		24	28.8	29.10	101.73
	AZI 232.40 nm	30	24	25.02	101.58
		30	30	30.45	99.36
		30	36	36.51	99.63
	CEF 218 nm	24	19.2	20.00	101.50
		24	24	23.90	98.20
		24	28.8	29.20	99.65
	AZI 218 nm	30	24	24.25	100.04
		30	30	30.38	100.46
		30	36	36.57	100.91
Absorbance ratio method	CEF 232.40 nm	24	19.2	19.05	98.95
		24	24	24.20	102.00
		24	28.8	29.10	101.73
	AZI 232.40 nm	30	24	25.02	101.58
		30	30	30.45	99.36
		30	36	36.57	99.63
	CEF 220.60 nm	24	19.2	19.80	99.48
		24	24	24.90	100.45
		24	28.8	30.00	101.97
	AZI 220.60 nm	30	24	24.68	98.29
		30	30	30.83	99.13
		30	36	36.86	99.36

Table 5a: Robustness data for Cefpodoxime proxetil

Parameters	Mean area	%RSD
Flow rate + 2	30922198.3	0.4315
Flow rate–2	30675379	0.2583
Mobile phase + 2	30897403.7	0.6412
Mobile phase–2	30529443.7	0.7291
pH + 2	30768830	0.6230
pH–2	30656780	1.222

Table 5b: Robustness data for Azithromycin Dihydrate

Parameters	Mean area	%RSD
Flow rate + 2	3756537	0.7096
Flow rate–2	3734082.33	0.4597
Mobile phase + 2	3721388.33	0.6861
Mobile phase–2	3737274.67	0.9281
pH + 2	3727952.33	0.81610.7066
pH–2	3731897	0.7066

Table 6: Analysis of Marketed Formulation

Assay	Formulation	Drug	Label claimed (mg/tab)	Amt. found (mg/tab)	% Label claimed	±SD
Simultaneous equation method	GUDCEF-AZ	CEF	200	199.83	99.91	±0.3055
		AZI	250	250.08	100.03	±0.8207
	CEPODEM-AZ	CEF	200	201.33	100.66	±0.3629
		AZI	250	253.41	101.36	±0.5609
Absorbance ratio method	GUDCEF-AZ	CEF	200	201.66	100.83	± 0.3763
		AZI	250	248.08	99.23	±0.6981
	CEPODEM-AZ	CEF	200	203.16	101.58	± 0.4521
		AZI	250	249.41	99.76	± 0.6502
HPLC method	GUDCEF-AZ	CEF	200	200.60	100.3	± 1.1875
		AZI	250	250.43	100.17	± 0.6658
	CEPODEM-AZ	CEF	200	199.00	99.6	± 0.4109
		AZI	250	250.20	100.08	± 0.7681

analysis is displayed in Table 2a and 2b. The correlation coefficient (r^2) was close to 0.9999 for both of CEF and AZI. The results of accuracy and precision (inter-day variance and intra-day variance) are shown in (Table 3 and 4). For accuracy, all the recovery values were within ± 5%. By Absorbance ratio method, the mean recovery value of CEF and AZI was 98.9-101.7% and 99.63-101.58% at 232.40 nm and 99.48-101.47% and 98.29-99.36% at 220.60 nm. In HPLC method the recovery values of CEF and AZI was 99.78% and 99.34%. For inter-day and intra-day variance assessment % RSD was calculated. All the samples exhibited RSD values <1% confirming that the analytical method was precise.

Robustness study was carried out by making minor changes in conditions like composition of mobile phase, flow rate of mobile phase and pH of mobile phase, data of robustness study is given in Table 5a and 5b. No substantial variances were observed in the retention time and peak area of each component when the chromatographic conditions were slightly changed one by one. Moreover, the RSD for each value was <2%. Thus, the proposed method was considered as robust. These methods were applied for estimation of Cefpodoxime Proxetil and Azithromycin Dihydrate in marketed formulation. Results of assay are given in Table 6.

Statistical evaluation of the developed procedures

The HPLC method was chosen as the analytical reference method. Simultaneous equation method and Absorbance ratio spectrophotometric procedures were compared with HPLC. The slopes, intercepts and linearity of each calibration graph were calculated and summarized in Table 2. The order of linearity for the calibration graphs in the ranges stated in Table 2 for the different analytical method was: Absorbance ratio, Simultaneous equation and HPLC. The concentration ranges, detection limits and quantitation limits are summarized in Table 2. The lowest detection limit calculated was obtained for absorbance ratio method indicating the highest sensitivity. Relative sensitivities, based on detection limits, were calculated with respect to the chromatographic method. The order of sensitivity for this method was: Absorbance ratio, Simultaneous equation and HPLC. Commercially available tablets were analyzed using the HPLC, Simultaneous equation and the Absorbance ratio spectrophotometric methods. The results obtained were summarized in Table 5. No significant differences were found between the results obtained by the HPLC and the spectrophotometric procedures, for the same batch at the 95% confidence level. Statistical comparison was done on assay results obtained from UV and HPLC methods for marketed formulation (CUDCEF-AZ) by using student's t-test. Calculated values for t-test were-1.02 and 0.42 for CEF and AZI respectively which is less than $t_{critical}$ value (12.706) indicating that there was no significant difference between the HPLC method.

CONCLUSION

The HPLC method and the spectrophotometric (Simultaneous equation and Absorbance ratio) methods were found to be reproducible and accurate in the analysis of Cefpodoxime proxetil and Azithromycin dihydrate in pharmaceutical tablets. Under the experimental conditions, mentioned above, the Simultaneous equation and Absorbance ratio method was the most sensitive method; however, better selectivity was obtained with the HPLC method. All the proposed methods were linear with good reproducibility and sensitivity. In general, all the proposed methods can be

used for the routine analysis of Cefpodoxime proxetil and Azithromycin dihydrate in bulk and tablet dosage form.

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CONFLICTS IF INTEREST MISSING

No conflicts of interest.

ABBREVIATIONS USED

HPLC: High Performance Liquid Chromatography; **UV:** Ultra violet; **HPTLC:** High performance thin layer chromatography; **CEF:** Cefpodoxime proxetil; **AZI:** Azithromycin Dihydrate; **ICH:** International Conference of Harmonization; **S/N:** Signal to noise; **RSD:** Relative standard deviation.

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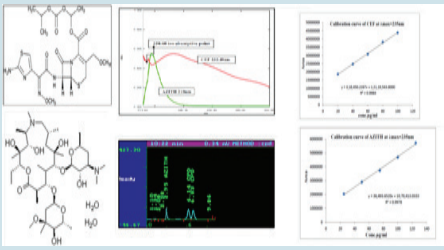
PICTORIAL ABSTRACT



SUMMARY

- Simultaneous equation and Absorbance ratio spectrophotometric methods are developed for the determination of Cefpodoxime Proxetil And Azithromycin Dihydrate in tablet dosage form.
- A simple liquid chromatographic assay method has been developed for the determination of Amlodipine Besylate And Nebivolol Hydrochloride. C_{18} (150×4.6 mm, 5 µm) column was used with a mobile phase consisting of Acetonitrile : Methanol : Phosphate buffer (40:40:20 v/v) at a flow rate of 1.0 ml min^{-1}. UV detection was at 235 nm based on the peak height ratios.
- The proposed procedures were successfully applied to the determination of Cefpodoxime Proxetil And Azithromycin Dihydrate in bulk and tablet form, with high percentage of recovery, good accuracy and precision.

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