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SIMULTANEOUS UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF AZITHROMYCIN AND CEFEXIME IN COMBINED DOSAGE FORM

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ABSTRACT

A UV SPECTROSCOPY method was developed and validated for quantitative determination of Azithromycin and Cefexime in pure and tablet dosage forms. Simple, accurate and reproducible spectrophotometric methods have been developed for the simultaneous estimation of Azithromycin and Cefexime in pharmaceutical tablet dosage forms. The method involved the determination using the Simultaneous equation method, the sampling wavelengths selected are 275 nm and 240 nm over the concentration ranges of 2.5-12.5µg/ml and 2-10 µg/ml for Azithromycin and Cefexime respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines.

Keywords: Azithromycin, Cefexime, UV Spectrophotometer, Methanol.

INTRODUCTION

Azithromycin

Azithromycin chemically (2R,3S,4R,5R,8R,10R, 11R,12S,13S,14R)-2-etyhl-3,4,10-trihydroxy-3,5,6,8, 10, 12,14-heptamethyl-15-oxo-11-{[3,4 ,6- trideoxy-3-(demetyhl amino)-D-xylo-hexopyr anos yl] oxy}-1-oxa-6azacyclopentadec-13-yl2,6-dideoxy- 3-C-methyl-3-Omethyl-a-L- ribo – hexopyranoside (Fig 1). It is antibiotic, antibacterial. Treatment of community acquired pneumonia and pelvic inflammatory disease caused by susceptible organisms. Includes- Staphylococcus aureus, Streptococcus pneumonia [1].

Cefexime

Cefexime chemically (6R,7R)-7-{[2-(2-amino-1,3-thiazol-4-yl)-2-(carboxy methoxy imino) acetyl] amino}-3-ethenyl-8-oxo-5-thia-1-azabicyclo[4.2.0] oct - 2-ene-2-carbo xylic acid (Fig 2). Cefixime is an antibiotic useful for the treatment of a number of bacterial infections. It is a third generation cephalosporin. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. Hence attempts were made to develop simultaneous estimation of Azithromycin and Cefexime in pharmaceutical dosage form by UV Spectroscopy.

MATERIALS AND METHODS

Pure drugs of Azithromycin and Cefexime were obtained from Chandra labs pvt ltd., Hyderabad. The commercial formulations of Azithromycin and Cefexime are available in the ratio of 5:2 (Cefolac-AZ) (500/200mg) as tablets. Analytical technologies limited UV/VIS spectrophotometer with 1cm matched quartz cells were used for all the special measurements. All the chemicals used were of A.R Grade. Standard stock solution was prepared. 25 mg standard Azithromycin was weighed accurately and transferred to a 25 ml volumetric flask and dissolved in methanol. The flask was shaken and volume was made up to the mark with methanol to give a solution of 1000 µg/ml. From this solution, 10ml of solution was pipette out and placed into 100 ml volumetric flask. The volume was made up to mark with double distilled water to give a solution containing 10 µg/ml. Standard solution of 10µg/ml of Azithromycin and Cefexime were scanned in the wavelength range of 200-400nm. Wavelengths of Azithromycin 275 nm, wavelength of Cefexime 245 nm were selected for formulation of the simultaneous equation method. The absorptivities (A1%, 1 cm) of both the

drugs at the wavelengths were determined. The absorbance and absorptivities values at the particular wavelength were substituted in the following to obtain the concentration [2-5].

$$C_{x} = \frac{A_{2} ay_{1} - A_{1} ay_{2}}{ax_{2} ay_{1} - ax_{1} ay_{2}}$$
(1)
$$C_{y} = \frac{A_{1} ax_{2} - A_{2} ax_{1}}{ax_{2} ay_{1} - ax_{1} ay_{2}}$$
(2)

Where,

$$a_{x_1}, a_{x_2} =$$
 Absorptivities of AZT at λ_1, λ_2
 $a_{y_1}, a_{y_2} =$ Absorptivities of CEF at λ_1, λ_2 ,
A₁, A₂ = Absorbances of the diluted
at λ_1, λ_2

sample at λ_1, λ_2 .

RESULTS AND DISCUSSION

Estimation of multiple drug formulations have advantage that the methods are time consuming and usage of solvent is minimized. Simple, rapid, precise and accurate UV spectrophotometric method were developed and validated for the estimation of Azithromycin and Cefexime in pure form and in combined tablet dosage form.

UV SPECTROPHOTOMETRIC METHOD

The solubility of Azithromycin and Cefexime was determined as per Indian Pharmacopoeia. Number of polar and non – polar solvents were tried to dissolve the drugs. From the solubility profile methanol was chosen as a common solvent followed by double distilled water for the estimation of Azithromycin and Cefexime [6-10].

The sample solutions of 10 μ g/ml of Azithromycin and Cefexime in methanol followed by double distilled water prepared individually and the solutions were scanned in UV region in the wavelength range from 200 to 400 nm by using water as blank. The overlain spectrum of Azithromycin and Cefexime was recorded as shown in Figure 3. From the spectrum 275 and 240 nm was selected for the estimation of Azithromycin and Cefexime without any interference. To estimate the amount of Azithromycin and Cefexime these wavelengths were used and the simultaneous equation method was applied to estimate titled drugs [11-14].

Different aliquots of Azithromycin and Cefexime in methanol followed by water were prepared in the concentration range of $2.5 - 12.5 \ \mu g/ml$ and $2-10 \ \mu g/ml$

respectively. The absorbances of solutions were measured at 275 and 240 nm. The calibration curve was plotted using concentration against absorbance. The preparation of calibration curve was repeated for six times for each drug at their selective wavelengths. Absorbance was measured for Azithromycin and Cefexime for 6 times and average absorbance. The optical parameters like, Sandell's sensitivity, Molar absorptivity, correlation coefficient, slope, intercept, LOD, LOQ and Standard error were calculated. The correlation coefficient for the two drugs was found to be above 0.998. This indicates that the drugs obey Beer's law in the selected concentration range. Hence the concentrations were found to be linear. The amount of Azithromycin and Cefexime present in tablet formulation was found to be 100.533 and 99.8 respectively. The amount present in tablet formulation was in good concord with the label claim and the % RSD values were found to be 0.73269 and 0.54297 for Azithromycin and Cefexime respectively. The low % RSD values indicate that the method has good precision.

Further the precision of the method was confirmed by Intraday and Interday analysis. The analysis of formulation was carried out for three times in the same day and one time in the three consecutive days. The SD values of intraday and interday analysis were found to be 0.734314 and 1.052141 for Azithromycin. The SD values of intraday and interday analysis were found to be 0.632456 and 0.614546 for Cefexime. The % RSD values of intraday and interday analysis were found to be 0.71372 and 1.04784 for Azithromycin. The % RSD values of intraday and interday analysis were found to be 0.62959 and 0.601532 for Cefexime. The results showed that the precision of the method was confirmed.

The developed method was validated for Ruggedness. It refers to the specific of one lab to multiple days which may include analysts, different instruments and different sources of reagents and so on. In the present work it was confirmed by analysts and different instruments. The % RSD value by analyst 1 and analyst 2 were found to be 0.6370 and 0.9271 for Azithromycin, 0.7253 and 0.9047 for Cefexime. The low % RSD values indicate that the developed method was more rugged.

The accuracy of the method was performed by recovery studies. To the 50% preanalyzed formulation, a known quantity of Azithromycin and Cefexime raw material solutions were added at different levels. The absorbance of the solutions was measured and the percentage recovery was found to be in the range of 100.6 - 99.96% for Azithromycin, 100.12 - 98.6% for Cefexime. The low % RSD value for two drugs indicates that this method is very accurate [14-19].

Table 1. Results of Analysis of Formulations

Pharmaceutical formulation	Laballed amount tablet* (mg)	Amont found*(mg)	% label claim*(mg)	<u>+</u> S.D	RSD
AZT	500	500.2	100.10	0.73663	0.73269
CEF	200	199.4	99.92	0.54497	0.54297

Table 2.Average Absorbance of Azithromycin for Azithromycin at 275 nm

Sl.no	Conc. µg/ml	Abs -1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	2.5	0.075	0.074	0.075	O.076	0.073	0.074	0.075
2	5	0.162	0.164	0.165	0.163	0.162	0.164	0.162
3	7.5	0.255	0.254	0.253	0.255	0.253	0.252	0.255
4	10	0.342	0.343	0.344	0.342	0.343	0.344	0.342
5	12.5	0.435	0.434	0.435	0.433	0.435	0.434	0.435

Table 3. Average Absorbance of Azithromycin for Azithromycin at 240 nm

Sl.no	Conc µg/ml	Abs -1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	2.5	0.098	0.096	0.098	0.097	0.098	0.097	0.098
2	5	0.184	0.182	0.184	0.182	0.181	0.181	0.184
3	7.5	0.264	0.267	0.265	0.264	0.267	0.267	0.267
4	10	0.352	0.351	0.353	0.352	0.353	0.352	0.352
5	12.5	0.435	0.433	0.435	0.434	0.435	0.434	0.434

Table 4. Average Absorbance of Cefexime for Cefexime at 240 nm

Sl.no	Conc (µg/ml)	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	2	0.127	0.125	0.129	O.127	0.125	0.125	0.129
2	4	0.272	0.275	0.274	0.273	0.274	0.272	0.274
3	6	0.412	0.411	0.410	0.413	0.412	0.412	0.411
4	8	0.536	0.538	0.536	0.535	0.536	0.537	0.538
5	10	0.649	0.648	0.649	0.647	0.647	0.648	0.649

Table 5. Average Absorbance of Cefexime for Cefexime at 275 nm

Sl.no	Conc (µg/ml)	Abs-1	Abs-2	Abs-3	Abs-4	Abs-5	Abs-6	Avg. Abs
1	2	0.130	0.129	0.129	0.130	0.128	0.129	0.130
2	4	0.280	0.279	0.282	0.280	0.281	0.282	0.280
3	6	0.417	0.419	0.418	0.419	0.418	0.417	0.418
4	8	0.542	0.541	0.540	0.542	0.541	0.543	0.542
5	10	0.654	0.656	0.654	0.655	0.654	0.655	0.656

Table 6. Optical Characteristics of Azithromycin by Simultaneous Equation Method

Parameters	At 275 nm	At 240 nm
Beers law limit (µg/ml)	2.5 - 12.5	2.5 - 12.5
Molar absorptivity (L mol ⁻¹ cm ⁻¹⁾	25750.069	26289.33
Sandell's sensitivity (µg/cm ² /0.001 A.U)	0.02777	0.029761
Correlation coefficient (r)	0.999933	0.9999
Slope (m)	0.2898	0.3006
Intercept (c)	0.0162	0.015
LOD (µg/ml)	0.034388	0.05583
LOQ (µg/ml)	0.104208	0.16919
Standard Error	0.0018973	0.0011547

Parameters	At 275 nm	At 240 nm
Beers law limit (µg/ml)	2-10	2-10
Molar absorptivity (L mol ⁻¹ cm ⁻¹⁾	30290.593	29973.177
Sandell's sensitivity (µg/cm ² /0.001 A.U)	0.0152207	0.0153374
Correlation coefficient (r)	0.99847	0.99970
Slope (m)	0.4709	0.4654
Intercept (c)	0.011	0.009
LOD (µg/ml)	0.032585	0.040414
LOQ (µg/ml)	0.098742	0.122467
Standard Error	0.0132815	0.0121271

Table 7. Optical Characteristics of Cefexime by Simultaneous Equation Method

Table 8. Analysis of Tablet Formulation (Cefolac-AZ)

Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained [*]	Average (%)	S.D	% R.S.D.	S.E.
	1	500	500.02	100.2				
	2	500	500.08	100.8				
	3	500	500.06	100.6				
	4	500	499.93	99.3	100.533	0.73663	0.73269	0.26127
AZT	5	500	499.15	101.5				
	6	500	500.08	100.8				
	1	200	199.95	99.5				
	2	200	200.05	100.5				
CEF	3	200	200.12	101.2				
	4	200	200.02	100.2	99.8	0.54497	0.54297	0.23420
	5	200	200.07	99.7				
	6	200	200.05	100.5				

Table 9. Intra Day and Inter Day Analysis of Formulation (Cefolac-AZ) by Simultaneous Equation Method

	Sampla	Labeled	Percenta	ge obtained*	<u>S.D</u>		% R.S.D.	
Drug	No.	amount (mg/tab)	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
	1	500	98.5	98.8				
	2	500	99.9	99.1				
	3	500	99.8	99.3	0 724214		0 71272	
AZT	4	500	100.4	101.4	0.754514	1.052141	0.71372	1.04794
	5	500	100.6	100.9				1.04/84
	6	500	99.91	100.2				
	Mean		99.65	99.87				
	1	200	100.3	100.5				
	2	200	98.9	98.9				
	3	200	99.9	99.5				
CEF	4	200	100.2	99.7	0.632456	0.614546	0 62050	0 601522
	5	200	100.8	100.5			0.02939	0.001332
	6	200	99.9	99.8				
	Mean		99.93	99.80				

Drug	Condition	Average % Obtained*	S.D	% R.S.D	S.E.
	Analyst 1	100.22	0.6384	0.6370	0.3685
AZT	Analyst 2	99.53	0.9228	0.9271	0.5327
	Instrument 1	100.58	0.3200	0.3181	0.1306
	Analyst 1	99.57	0.7940	0.7253	0.2524
CEF	Analyst 2	99.47	0.9000	0.9047	0.5276
	Instrument 1	100.65	0.2756	0.2754	0.1252

Table 11. Recovery Study Data of 50% Preanalyzed Formulation (Cefolac-AZ)

Drug	Amount present (mcg/mL)	Amount added (mcg/mL)	Amount estimated (mcg/mL)	Amount recovered (mcg/mL)	% Recovery	S.D	% R.S.D	S.E.
	5	2.5	7.4266	2.443	100.6			
AZT	5	5.0	9.8466	4.963	99.96	0.2140	0.2364	0.1237
	5	7.5	12.4563	7.467	100.2			
	5	2	6.8233	2.023	98.6			
CEE	5	4	8.997	3.940	99.76	0.0132	0.01240	0.0080
CEF	5	6	10.912	5.943	100.1			





CONCLUSION

One simple, rapid and accurate analytical method was developed for the determination of Azithromycin and Cefexime in bulk and in tablet formulation by using UV spectrophotometry. The method showed excellent sensitivity, reproducibility, accuracy and repeatability, which is evidenced by low percentage relative standard deviation. The results obtained in recovery studies were indicating that there is no interference from the excipients used in the formulation. Hence it is suggested that the proposed UV spectrophotometric method can be effectively applied for the routine analysis of Azithromycin and Cefexime in bulk and in tablet formulation and the obtained results will be presented elsewhere.

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