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DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF CEFIXIME TRIHYDRATE AND AZITHROMYCIN DIHYDRATE IN COMBINED DOSAGE FORM

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ABSTRACT

A novel, simple, accurate, sensitive, reproducible, economical spectroscopic method was developed and validated for the determination of Azithromycin dihydrate and Cefixime trihydrate in combined dosage form. Second order derivative spectroscopy method is adopted to eliminate spectral interference. The method obeys Beer's Law in concentration ranges of 10-40 ppm for Cefixime trihydrate and 25-100 ppm of Azithromycin dihydrate. The method was validated for linearity, accuracy and precision as per ICH guidelines. The zero crossing point for Azithromycin dihydrate and Cefixime trihydrate was 328 nm and 231.6 nm, respectively in water. The LOD and LOQ value were found to be 0.13 and 0.38 ppm for Cefixime trihydrate and 0.80 and 2.44 ppm for Azithromycin dihydrate respectively. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form (ZIMNIC -AZ).

KEYWORDS : Azithromycin dihydrate, Cefixime trihydrate, Derivative Spectroscopy, Zero crossing point, combined dosage form.

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INTRODUCTION

Azithromycin [9-de-oxy-9a-aza-9a-methyl-9a-homoerythromycin A dihydrate] is an Azalide, a subclass of macrolide antibiotics[1]. It acts by inhibiting protein synthesis by binding reversibly to the 'P' site of the 50S ribosomal subunit of the bacteria[2, 3]. It is used for Adult and Pediatric[4, 5] infections. e.g. Respiratory tract infection[1,6,7,8], Skin, Soft tissue infections, Otitis media[1,9,10], Sinusitis, Pharyngitis, Acute bronchitis, Community-acquired Pneumonia[1], Cystic fibrosis[11,12], Tonsilitis[13], Anti-inflammatory in COPD Patient[14], in *P. Falciparum* Malaria with other Antimalarial drugs[15], Typhoid fever[16,17]. Cefixime (6R, 7R)-7-[2-(2-amino-4- thiazolyl) glyoxylamido]-8-oxo-3-vinyl-5-1 –azabicyclo [4.2.0] oct-2-ene-2-carboxylicacid,7-9z)-[o carboxymethyl)-oxime] trihydrate is third generation cephalosporin antibiotic. It is under the category of β-Lactam Antibiotics/Cell Wall inhibitor. It Acts by inhibiting an enzyme Transpeptidase, involved in the building of Bacterial Cell Walls[18]. It is used in Lower Respiratory Tract Infections[19,20,21], Acute Urinary Tract Infections[21,22], Biliary Tract Infections[23], Sinusitis[24], Acute Otitis Media[25], Peptic Ulcer[26]. Combination of Cefixime Trihydrate and Azithromycin Dihydrate has a Synergistic effect. The effect of Cefixime Trihydrate against *Neissaria gonorrhoeae* can be significantly enhanced in combination with Azithromycin Dihydrate[28]. This Combination is used in treatment of Uncomplicated Gonococcal Urethritis[29], Gonorrhoea[28], Typhoid Fever[31,32]. Both the drugs are official in Indian pharmacopoeia 2010[33,34]. Literature survey reveals that HPLC[35], RP-HPLC[36,37,38], UV-Visible Spectrophotometry[39,40,41,42], UPLC[43], methods were reported for the estimation of Azithromycin Dihydrate alone or in combination with other drugs except Cefixime Trihydrate and UV-Visible Spectrophotometry[44,45,46,47], HPLC[48,49,50], RP-HPLC[51,52], HPTLC[58.53], Voltametry[54,55], High Performance Capillary Electrophoresis[56] methods were reported for the estimation of Cefixime Trihydrate alone or in combination with other drugs except Azithromycin Dihydrate. As per literature survey, no analytical method has been reported for simultaneous estimation of Cefixime Trihydrate and Azithromycin Dihydrate in pharmaceutical dosage forms. Therefore the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Cefixime Trihydrate & Azithromycin Dihydrate in their combined dosage form in routine analysis.

MATERIALS AND METHODS

Reagents and Chemicals

Methanol (AR Grade) was used as solvent. Pure Standard gift sample of Cefixime Trihydrate (CEF) and Azithromycin Dihydrate (AZI) provided by Alicon Pharmaceuticals. Tablets of ZIMNIC-AZ (Cefixime Trihydrate-200 mg, Azithromycin Dihydrate- 500 mg) were purchased from local market.

Instruments

Shimadzu UV/Vis-2450 and UV/Vis-1800 double beam UV/Vis spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used for recording derivative spectra of standard and test samples. Sartorius CD2250 balance was used for weighing the samples. Class 'A' volumetric glassware were used.

Preparation of stock solution

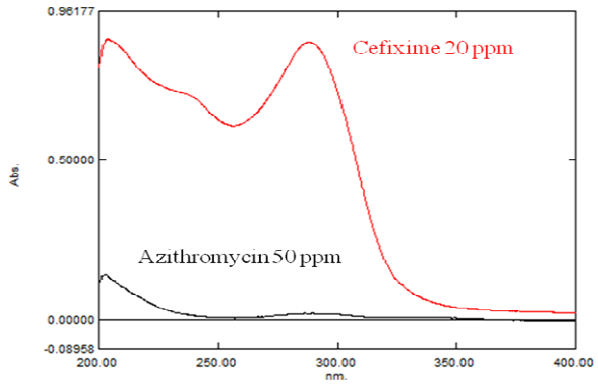
The standard stock solutions of 100 µg/ml of CEF and 100 µg/ml of AZI were prepared. 10 mg of both the drugs were weighed, taken in 100 ml volumetric flask and dissolved in Methanol and then make up to the mark with Methanol. Further dilutions were made in Methanol to obtain concentrations ranging from 10-40 µg/ml for CEF and 25-100 µg/ml for AZI.

Determination of Absorption Maxima

By appropriate dilution of two standard drug solutions with Methanol, solutions

containing 10 µg/mL of CEF and 25 µg/mL of AZI were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. CEF showed absorbance maxima at 288 nm and AZI at 217 nm (Fig. 1).

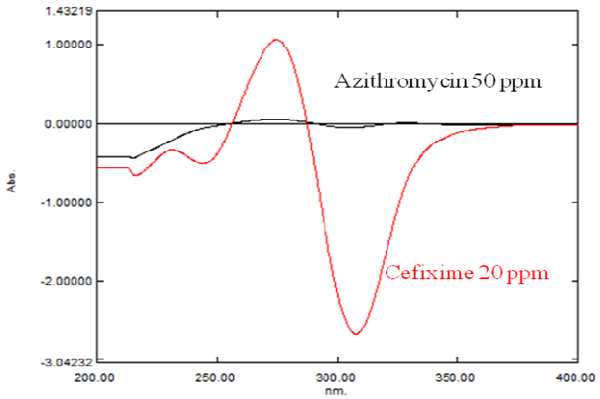
Figure 1
Overlain zero order spectra of CEF and AZI



Derivative spectroscopy

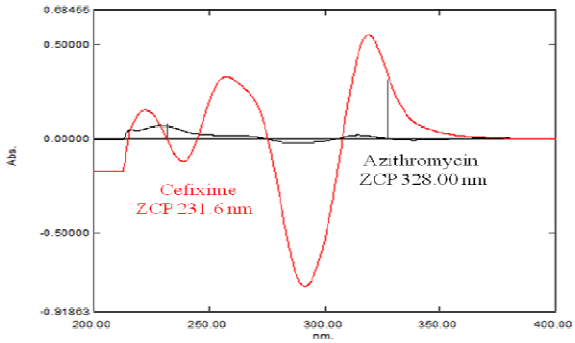
The Overlain spectra in Fig.1 reveal that no method was possible in zero order. To overcome this, solutions of CEF (20 µg/mL) and AZI (50 µg/mL), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The Zero order spectra of both the drugs were derivatised to first order (Fig. 2).

Figure 2
Overlain 1st order spectra of CEF and AZI



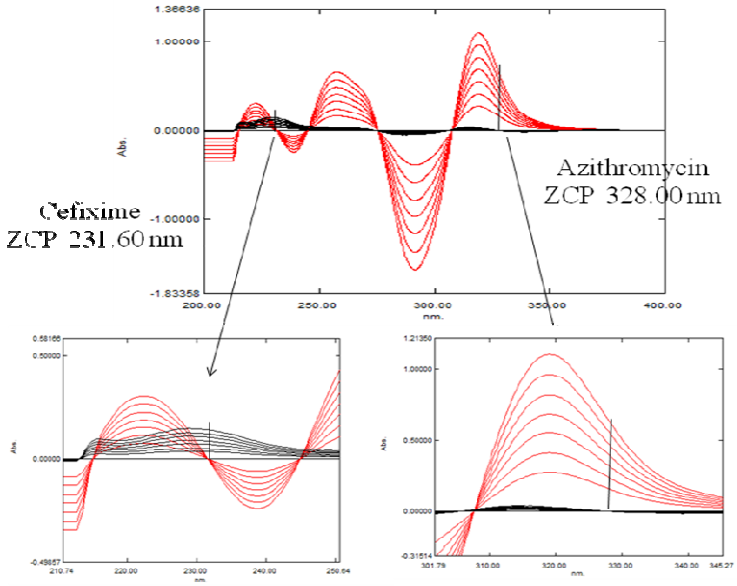
The Overlain spectra in Fig.2 reveal that at the Zero Crossing Point (ZCP) of CEF, there is difficulty in recording absorbance of AZI. So, again the spectra were derivatised to 2nd order between 400-200 nm (Fig. 3).

Figure 3
Overlain 2nd order spectra of CEF and AZI.



Second order derivative spectra were selected for analysis of both drugs. From the overlain spectra of both drugs (Fig. 3), wavelength selected for quantitation were 326.4 nm for CEF (zero cross for AZI) and 226.8 nm for AZI (zero cross for CEF). The calibration curves for CEF and AZI were plotted in the concentration range of 10 – 100 µg/ml at wavelength 326.4 nm and 226.8 nm, respectively (Fig. 4). The concentration of the individual drug present in the mixture was determined against the calibration curve in quantitation mode
.

Figure 4
Overlain Linear 2nd order spectra of CEF and AZI.



Validation

The methods were validated with respect to linearity, precision, accuracy, robustness, LOD & LOQ and assay.

Linearity:

Standard stock solutions were prepared by dissolving 25 mg AZI and 10 mg of CEF in 100 ml volumetric flasks in Methanol and the volume was made up with Methanol to get a concentration of 250 µg/ml of AZI and 100 µg/ml of CEF. From this, suitable dilutions were made in Methanol to get the working standard solutions of 25-100 µg/ml for AZI and 10-40 µg/ml for CEF. The absorbances of the derivatised spectra were measured at 226.8 nm and 326.4 nm for AZI and CEF, respectively. Five replicate analysis were carried out.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The system precision is a measure of the method variability that can be expected for a given analyst performing the analysis and was determined by performing three replicate analyses of the same working solution.

The intra-day, inter-day, reproducibility was done to determine precision of the developed method. The intra-day precision of the developed UV method was determined by preparing the samples of the same batch in nine determinations with three concentrations (10, 20, 40 µg/ml for CEF and 25, 50, 100 for AZI) and three replicate (n=3) each on same day. The Percentage R.S.D. of the results was used to evaluate the method precision. The inter-day precision was determined by assaying the samples in triplicate (n=3) per day for consecutive 3 days. The reproducibility was determined by assaying the samples in triplicate (n=3) in another laboratory.

Accuracy

Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 10 mg CEF was transferred to 100.0 ml volumetric flask. Methanol was added to dissolve the drugs and then volume was made up to the mark with Methanol and sonicated for 10 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 1.0 ml was transferred to three 10.0 ml volumetric flasks and add 0.8 ml (Flask 1), 1.0 ml (Flask 2), and 1.2 ml (Flask 3) of stock solution of API and then made up to the mark with Methanol to made them 80%, 100% and 120% spiking.

Robustness

The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. The parameters were change of volumetric flasks (10 ml, 50 ml and 100 ml) and Change in instrument (UV-Vis Spectrophotometer model no. 1800 and 2450). Three replicates were made for the same conc. (10 µg/ml of CEF and 25 µg/ml of AZI) in 10 ml, 50 ml and 100 ml volumetric flasks and the recording of absorbances were done on both the UV-Vis spectrophotometer. The result is expressed in Percentage RSD.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same conc. (10 µg/ml of CEF and 25 µg/ml of AZI), standard deviation (SD) of the responses was calculated. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation were determined on the basis of standard deviation and slope of the regression equation.

$$LOD = (3.3 \times SD) / Slope$$

$$LOQ = (10 \times SD) / Slope$$

Assay

Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to the weight of 10 mg of CEF was accurately weighed into 100 ml A-grade volumetric flask and 25 ml Methanol was added. The volumetric flask was sonicated for 20 min to effect complete dissolution of the AZI and CEF, the solution was then made up to volume with Methanol. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 25 µg/ml of AZI and 10 µg/ml of CEF. The % assay of the drugs was calculated.

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of CEF and AZI. In second order derivative spectroscopy, wavelengths selected for quantitation were 326.4 nm for CEF (zero cross for AZI) and 226.8 nm for AZI (zero cross for CEF). Both the drugs obey the Beer's law with the concentration range (CEF: 10 – 40 ppm, AZI: 25 – 100 ppm) with R^2 value of 0.9992 and 09990 for CEF and AZI, respectively (n=5). The Percentage RSD was found in the range of 0.13 – 0.78 for intra-day precision (Table 2), 0.12 – 0.75 for inter-day precision (Table 3) and 0.11 – 0.24 for reproducibility (Table 4) (n=3). The mean % assay was found to be 100.37 % and 95.65 % for CEF and AZI, respectively (Table 5) (n=3). The mean % recovery was found to be 100.17 % and 99.81 % for CEF and AZI, respectively (Table 6) (n=3). The mean Limit of Detection (LOD) and Limit of Quantitation (LOQ) value were found to be 0.54 and 1.64 ppm for CEF and 0.77 and 2.34 ppm for AZI, respectively (Table 7) (n=3). The overall results of various validation parameters were summarized in table 8.

Figure 5
Calibration curve of CEF and AZI.

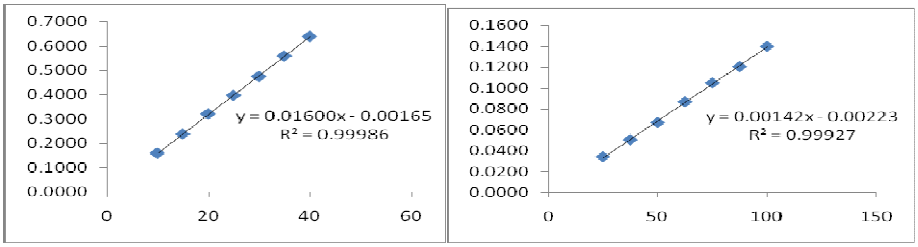


TABLE 1
Linearity (n=5)

Sr. No.	Concentration (µg/ml)		Absobance (328 nm)	Absobance (231.6 nm)
	CEF	AZI		
1	10	25	0.1593	0.0342
2	15	37.5	0.2374	0.0505
3	20	50	0.3205	0.0670
4	25	62.5	0.3967	0.0868
5	30	75	0.4747	0.1050
6	35	87.5	0.5592	0.1207
7	40	100	0.6398	0.1401

TABLE 2
Intraday precision (repeatability) (n=3)

Conc. (µg/ml)		Absorbance (328 nm)	SD	% RSD	Absorbance (231.6 nm)	SD	% RSD
CEF	AZI						
		0.15986			0.01854		
10	**25**	0.15932	0.000312	**0.20**	0.01829	0.000144	**0.78**
		0.15986			0.01854		
Avg.		0.15968			0.01845		
		0.31973			0.03603		
20	**50**	0.31978	0.000563	**0.18**	0.03569	0.000247	**0.69**
		0.31878			0.03555		
Avg.		0.31943			0.03575		
		0.62398			0.07236		
40	**100**	0.62298	0.000931	**0.15**	0.07264	0.000260	**0.36**
		0.62212			0.07212		
Avg.		0.62302			0.07237		

TABLE 3
Interday precision (intermediate precision) (n=3)

Conc. (µg/ml)		Absorbance (328 nm)	SD	% RSD	Absorbance (231.6 nm)	SD	% RSD
CEF	AZI						
		0.15960			0.01883		
10	**25**	0.15977	0.000185	**0.12**	0.01894	0.000082	**0.43**
		0.15997			0.01899		
Avg.		0.15978			0.01892		
		0.31354			0.03655		
20	**50**	0.31395	0.001747	**0.56**	0.03628	0.000226	**0.62**
		0.31675			0.03673		
Avg.		0.31474			0.03652		
		0.62467			0.07289		
40	**100**	0.62356	0.000913	**0.15**	0.07212	0.000386	**0.53**
		0.62537			0.07245		
Avg.		0.62453			0.07248		

TABLE 4
Reproducibility (n=3)

Conc. (µg/ml)		Absorbance (328 nm)	SD	% RSD	Absorbance (231.6 nm)	SD	% RSD
CEF	AZI						
		0.15995			0.01889		
10	**25**	0.15929	0.000338	**0.21**	0.01898	0.000045	**0.24**
		0.15949			0.01893		
Avg.		0.15957			0.01893		
		0.31293			0.03620		
20	**50**	0.31250	0.000376	**0.12**	0.03609	0.000070	**0.19**
		0.31325			0.03607		
Avg.		0.31289			0.03612		
		0.62329			0.07243		
40	**100**	0.62376	0.000872	**0.14**	0.07267	0.000250	**0.35**
		0.62498			0.07217		
Avg.		0.62401			0.07242		

TABLE 5
Assay (n=3)

Sr. No.	Formulation (ZIMNIC-AZ)		Absorbance (328 nm)	% Assay	Absorbance (231.6 nm)	% Assay
	CEF	AZI				
1	10	25	0.15987	100.95	0.03278	98.62
2			0.15912	100.48	0.03196	96.31
3			0.15888	100.33	0.03295	99.10

TABLE 6
Accuracy (recovery study)

Sr. No.	Formulation		API % (CEF + AZI)	Conc. (CEF)	% Recovery	Conc. (AZI)	% Recovery
	CEF	AZI					
1	10	25	80(8 + 20)	18.24	101.36	44.95	99.88
2			100(10 + 25)	19.86	99.32	49.62	99.24
3			120(12 + 30)	22.12	100.54	55.03	100.06

TABLE 7
LOD and LOQ

Sr. No	Conc. (µg/ml)		Absorbance (328 nm)	Absorbance (231.6 nm)
	CEF	AZI		
1	10	25	0.15986	0.01854
2			0.15930	0.01889
3			0.15986	0.01854
4			0.15972	0.01830
5			0.15958	0.01805
6			0.15803	0.01898
7			0.15851	0.01840
8			0.15824	0.01850
9			0.15906	0.01848
10			0.15986	0.01854
	SD		**0.000710**	**0.000266**
	LOD(µg/ml)		**0.13**	**0.80**
	LOQ(µg/ml)		**0.38**	**2.44**

TABLE 8
RESULT

Sr. No	Parameter	Cefixime Trihydrate	Azithromycin Dihydrate
1	Zero crossing point	231.6 nm	328 nm
2	Range(µg/ml)	10 – 40	25 – 100
3	Linearity	0.9998	0.9992
4	Precision (% RSD)		
	(a) Intraday	0.15 – 0.20	0.36 – 0.78
	(b) Interday	0.12 – 0.56	0.43 – 0.62
	(c) Reproducibility	0.12 – 0.21	0.19 – 0.35
5	Accuracy	100.41 %	99.72 %
6	Robustness	0.09 – 0.21	0.24 – 0.48
7	LOD(µg/ml)	0.13	0.80
8	LOQ(µg/ml)	0.38	2.44
9	Assay	100.59%	98.01 %

CONCLUSION

The developed method was novel, simple, accurate, precise reproducible, economical, which would be used to estimate CEF & AZI in their combined dosage form in routine analysis.

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