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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 326.4 nm and 226.8 nm, respectively in water. The LOD and LOQ value were found to be 0.54 and 1.64 ppm for Cefixime trihydrate and 0.77 and 2.34 ppm for Azithromycin dihydrate respectively. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form (ZIMNIC -AZ).

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) and Distill Water were 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 25 ml Methanol and then make up to the mark with Distill Water. Further dilutions were made in Distill Water 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 Distill Water, 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**).

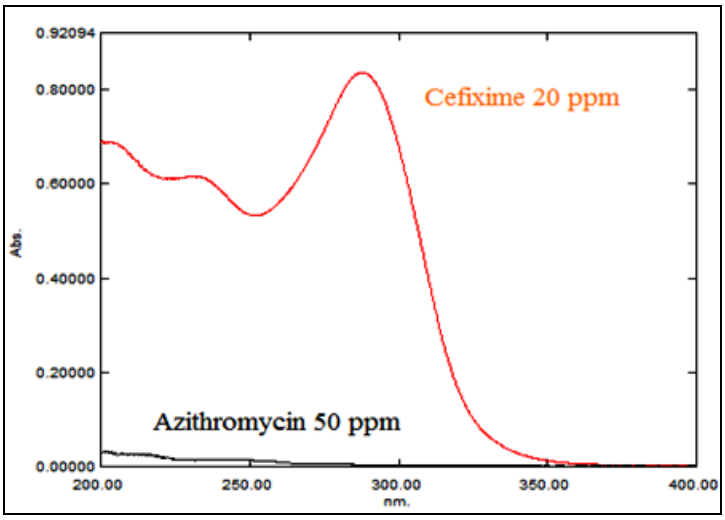

FIG. 1: OVERLAIN ZERO ORDER SPECTRA OF CEF AND AZI

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**).

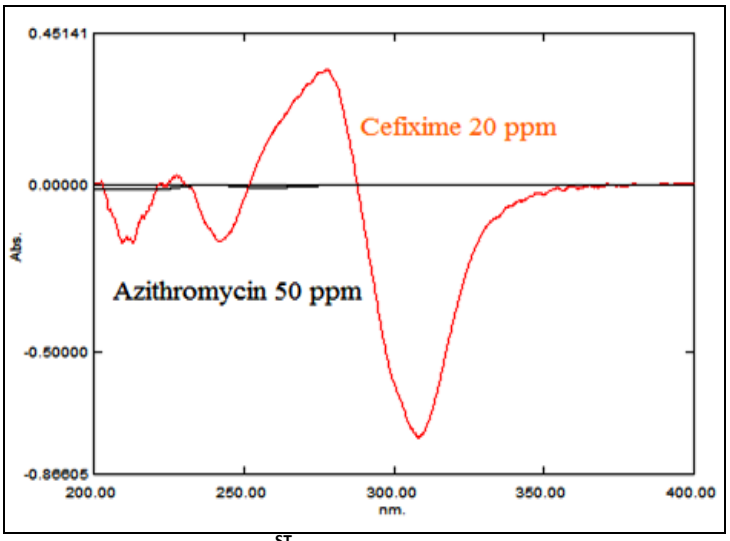

FIG. 2: OVERLAIN 1ST ORDER SPECTRA OF CEF AND AZI

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

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).

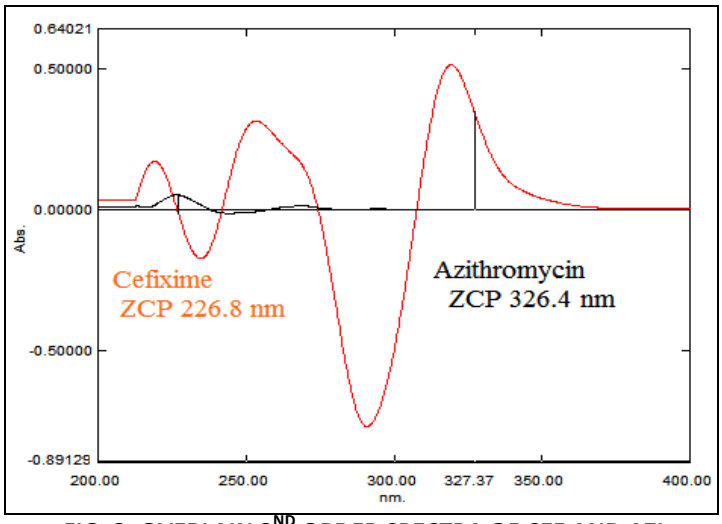

FIG. 3: OVERLAIN 2ND ORDER SPECTRA OF CEF AND AZI

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.

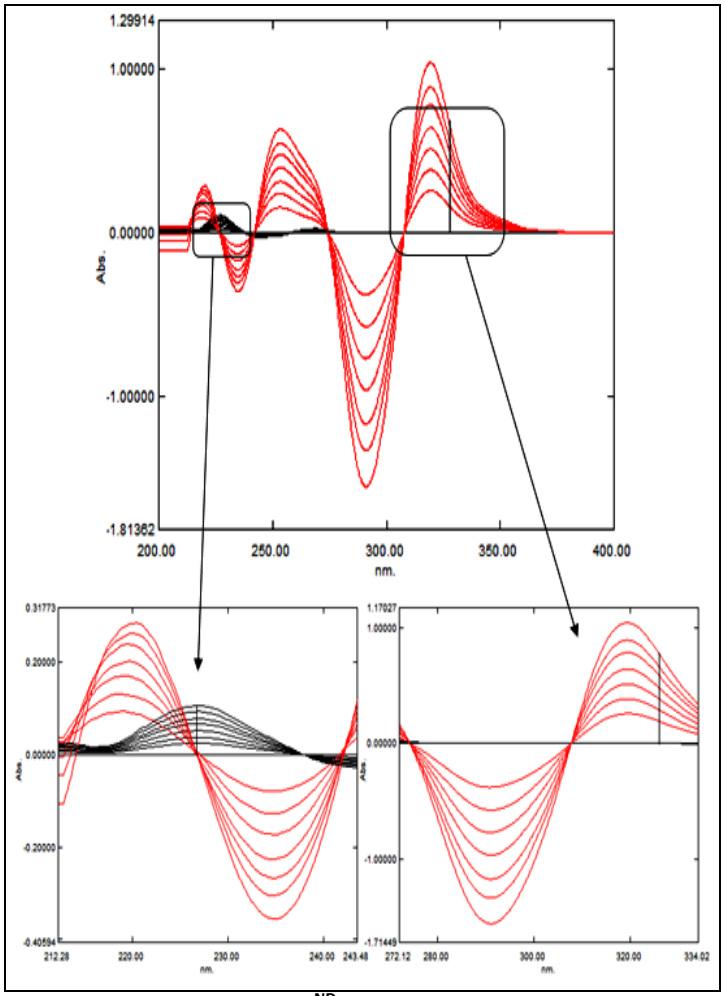

FIG. 4: OVERLAIN LINEAR 2ND ORDER SPECTRA OF CEF AND AZI

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 25 ml Methanol and the volume was made up with Distill Water to get a concentration of 250 µg/ml of AZI and 100 µg/ml of CEF. From this, suitable dilutions were made in Distill Water 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. 25 ml Methanol was added to dissolve the drugs and then volume was made up to the mark with Distill Water 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 Distill Water 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 x SD) / Slope

LOQ = (10 x 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 Distill Water. 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) (**Fig. 5, Table 1**). 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**.

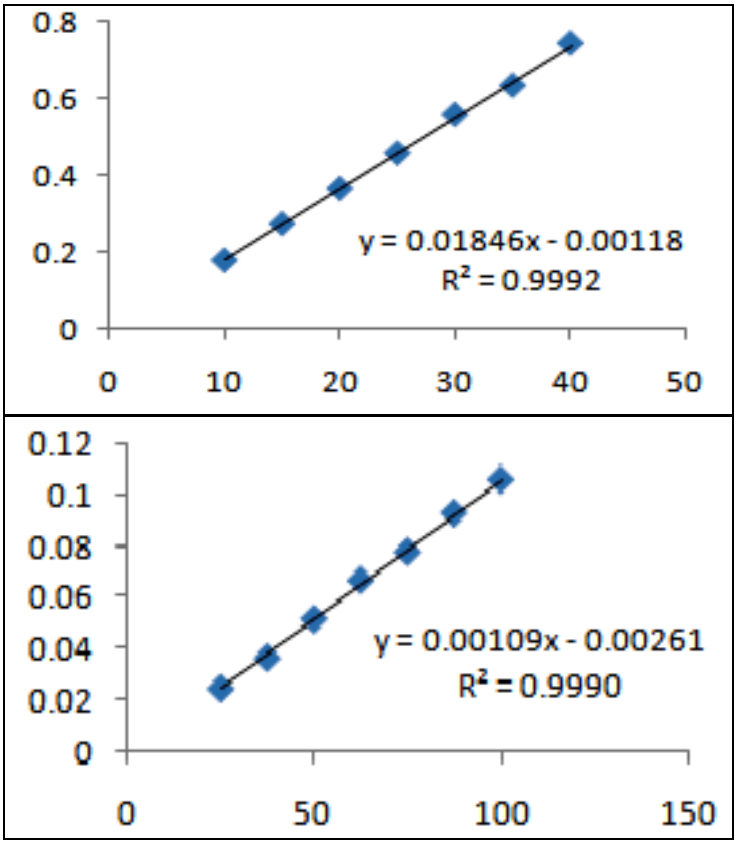

FIG. 5: CALIBRATION CURVE OF CEF AND AZI

TABLE 1: LINEARITY (n=5)

Concentration (µg/ml)		Absorbance (326.4 nm)	Absorbance (226.8 nm)
CEF	AZI		
10	25	0.183	0.025
15	37.5	0.277	0.037
20	50	0.368	0.052
25	62.5	0.459	0.067
30	75	0.559	0.078
35	87.5	0.634	0.093
40	100	0.743	0.106

TABLE 2: INTRADAY PRECISION (REPEATABILITY) (n=3)

Conc. (µg/ml)		Absorbance (326.4 nm)	SD	% RSD	Absorbance (226.8 nm)	SD	% RSD
CEF	AZI						
10	25	0.18086	0.001409	0.78	0.02254	0.000144	0.64
		0.18330			0.02229		
		0.18086			0.02254		
Avg.		0.18167			0.02245		
20	50	0.35699	0.001099	0.31	0.05534	0.000131	0.24
		0.35678			0.05509		
		0.35878			0.05515		
Avg.		0.35751			0.05519		
40	100	0.74398	0.000931	0.13	0.10536	0.000260	0.25
		0.74298			0.10564		
		0.74212			0.10512		
Avg.		0.74302			0.10537		

TABLE 3: INTERDAY PRECISION (INTERMEDIATE PRECISION) (n=3)

Conc. (µg/ml)		Absorbance (326.4 nm)	SD	% RSD	Absorbance (226.8 nm)	SD	% RSD
CEF	AZI						
10	25	0.15560	0.000994	0.64	0.02630	0.000195	0.75
		0.15577			0.02594		
		0.15397			0.02599		
Avg.		0.15511			0.02607		
20	50	0.36354	0.002316	0.64	0.06155	0.000366	0.60
		0.36395			0.06108		
		0.36975			0.06083		
Avg.		0.36241			0.06115		
40	100	0.74467	0.000913	0.12	0.10489	0.000668	0.64
		0.74356			0.10412		
		0.74537			0.10545		
Avg.		0.74453			0.10482		

TABLE 4: REPRODUCIBILITY (n=3)

Conc. (µg/ml)		Absorbance (326.4 nm)	SD	% RSD	Absorbance (226.8 nm)	SD	% RSD
CEF	AZI						
10	25	0.18295	0.000338	0.19	0.02289	0.000045	0.20
		0.18229			0.02298		
		0.18249			0.02293		
Avg.		0.18257			0.02293		
20	50	0.35293	0.000376	0.11	0.05420	0.000070	0.13
		0.35250			0.05409		
		0.35325			0.05407		
Avg.		0.35289			0.05412		
40	100	0.74329	0.000872	0.12	0.10543	0.000250	0.24
		0.74376			0.10567		
		0.74498			0.10517		
Avg.		0.74401			0.10542		

TABLE 5: ASSAY (n=3)

Formulation (ZIMNIC-AZ)		Absorbance (326.4 nm)	% Assay	Absorbance (262.8 nm)	% Assay
CEF	AZI				
10	25	0.18293	99.73	0.02445	99.30
		0.18345	100.01	0.02529	102.38
		0.18270	99.61	0.02479	100.55

TABLE 6: ACCURACY (RECOVERY STUDY)

Formulation (ZIMNIC-AZ)		API % (CEF + AZI)	Conc. (CEF)	% Recovery	Conc. (AZI)	% Recovery
CEF	AZI					
10	25	80(8 + 20)	17.92	99.57	44.87	99.17
		100(10 + 25)	20.05	100.27	49.95	99.90
		120(12 + 30)	22.15	100.67	54.90	99.82

TABLE 7: LOD and LOQ

Sr. No.	Concentration (µg/ml)		Absorbance (326.4 nm)	Absorbance (226.8 nm)
	CEF	AZI		
1	10	25	0.18086	0.02254
2			0.18330	0.02189
3			0.18086	0.02254
4			0.18672	0.02230
5			0.18458	0.02205
6			0.18403	0.02198
7			0.18751	0.02240
8			0.18824	0.02250
9			0.18806	0.02248
10			0.18086	0.02254
SD			**0.00302**	**0.000255**
LOD(µg/ml)			**0.54**	**0.77**
LOQ(µg/ml)			**1.64**	**2.34**

TABLE 8: RESULT

Sr. No	Parameter	Cefixime Trihydrate	Azithromycin Dihydrate
1	Zero crossing point	226.8 nm	326.4 nm
2	Range(µg/ml)	10 – 40	25 – 100
3	Linearity	R^2 – 0.9992	R^2 – 0.9990
	Precision (% RSD)		
4	(a) Intraday	0.31 – 0.78	0.24 – 0.86
	(b) Interday	0.64	0.60 – 0.75
	(c) Reproducibility	0.13 – 0.27	0.11 – 0.68
5	Accuracy	100.17 %	99.81 %
6	Robustness	0.12 – 0.19	0.16 – 0.27
7	LOD(µg/ml)	0.54	0.77
8	LOQ(µg/ml)	1.64	2.34
9	Assay	100.37 %	95.65 %

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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