

## Spectrophotometric and Spectrofluorimetric Studies on Azilsartan Medoxomil and Chlorthalidone to Be Utilized in Their Determination in Pharmaceuticals

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**ABSTRACT:** The recently approved angiotensin II receptor blocker, azilsartan medoxomil (AZL), was determined spectrophotometrically and spectrofluorimetrically in its combination with chlorthalidone (CLT) in their combined dosage form. The UV-spectrophotometric technique depends on simultaneous measurement of the first derivative spectra for AZL and CLT at 286 and 257 nm, respectively, in methanol. The spectrofluorimetric technique depends on measurement of the fourth derivative of the synchronous spectra intensities of AZL in presence of CLT at 298 nm in methanol. The effects of different solvents on spectrophotometric and spectrofluorimetric responses were studied. For, the spectrofluorimetric study, the effect of pH and micelle-assisted fluorescence enhancement were also studied. Linearity, accuracy, and precision were found to be satisfactory over the concentration ranges of 8–50  $\mu\text{g mL}^{-1}$  and 2–20  $\mu\text{g mL}^{-1}$  for AZL and CLT, respectively, in the spectrophotometric method as well as 0.01–0.08  $\mu\text{g mL}^{-1}$  for AZL in the spectrofluorimetric method. The methods were successfully applied for the determination of the studied drugs in their co-formulated tablets. The developed methods are inexpensive and simple for the quality control and routine analysis of the cited drugs in bulk and in pharmaceuticals.

**KEYWORDS:** spectrophotometry, spectrofluorimetry, derivative, synchronous, azilsartan medoxomil, chlorthalidone, pharmaceuticals

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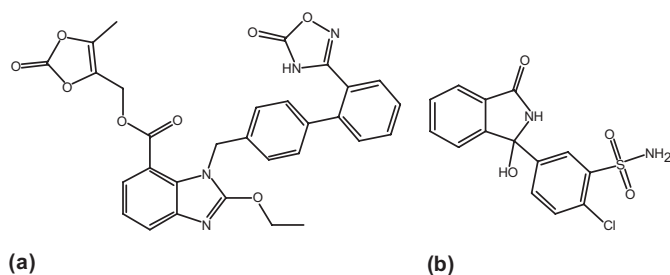
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### 1. Introduction

Azilsartan medoxomil (AZL) (5-methyl-2-oxo-1,3-dioxol-4-yl) methyl 2-ethoxy-1-[[2'-(5-oxo-4,5-dihydro-1,2,4-oxadiazol-3-yl) biphenyl-4-yl]methyl]-1H-benzimidazole-7-carboxylate—Figure 1a—is practically insoluble in water, freely soluble in methanol and dimethylsulfoxide, soluble in acetic acid, slightly soluble in acetone and acetonitrile, and sparingly soluble in tetrahydrofuran and 1-octanol.<sup>1</sup> Azilsartan, the active metabolite of AZL, is a novel non-peptide angiotensin II type 1 (AT<sub>1</sub>) receptor blocker (ARB) that was recently approved for treatment of hypertension.<sup>2</sup> It has a superior ability to control systolic blood pressure (BP) relative to other widely used ARBs. Greater antihypertensive effects of AZL might be in

part because of its unusually potent and persistent ability to inhibit binding of angiotensin II to AT<sub>1</sub> receptors.<sup>3</sup> Preclinical studies have indicated that AZL may also beneficially affect cellular mechanisms of cardio-metabolic disease and insulin sensitizing activity.<sup>4</sup> There is only one reported method<sup>1</sup> for determination of AZL alone, and it was applied to its analysis in plasma. Chlorthalidone (CLT) (2-chloro-5-(1-hydroxy-3-oxoisindolin-1-yl) benzenesulfonamide)—Figure 1b—is an orally taken thiazide-like diuretic for controlling hypertension and edema, including that associated with heart failure.<sup>5</sup> CLT has been determined in different matrices either alone or in combination with other medications using LC,<sup>6,7</sup> LC-MS/MS,<sup>8</sup> HPTLC,<sup>9,10</sup> chemiluminometry,<sup>11</sup> spectrophotometry,<sup>12</sup> and



**Figure 1.** Chemical structures for (a) AZL and (b) CLT.

CE<sup>13</sup> techniques. Binary combination therapies of AZL and CLT proved to induce significant reductions in systolic and diastolic BP in patients with mild to severe hypertension.<sup>14</sup> Accordingly, it is desirable to develop validated simple analytical methods for assay of AZL in its combination with CLT either in their laboratory prepared mixtures or in their combined pharmaceutical preparations. Regarding simultaneous determination of CLT and AZL, there is only one LC reported method<sup>15</sup> that was done on a symmetry C18 column with a mobile phase consisting of methanol:water:acetonitrile:0.1% ortho phosphoric acid (30:35:15:5, v/v/v/v) at detection  $\lambda$  of 251 nm. To the best of our knowledge, there is no reported spectrophotometric or spectrofluorimetric methods for determination of AZL either alone or in its combination with CLT. Hence, the aim of these studies was to develop and validate simple, rapid, and inexpensive analytical methods using the spectrophotometric and spectrofluorimetric techniques for determination of AZL in its combination with CLT to be applied to their determination in pharmaceuticals. These newly developed and validated methods can be used for routine analysis and quality control of the cited medications either separately or in combination without pre-separation.

## 2. Experimental

**2.1. Materials and reagents.** All chemicals used were of analytical reagent grade, and solvents were of HPLC grade. CLT (certified to contain 98%) was supplied by Ark Pharm. Inc., IL 60048 USA. AZL (certified to contain 98%) was supplied by D-L Chiral Chemicals, LLC, NJ 08540, USA. Edarbyclor<sup>®</sup> 40/25 mg tablets, batch NDC 64764-0994-30 (Takeda Pharmaceutical Company Limited, Tokyo, Japan), each labeled to contain 42.68 mg of azilsartan kamedoxomil (equivalent to 40 mg AZL) and 25 mg CLT were purchased from commercial sources in the local market. Methanol, sodium dodecyl sulphate (SDS), cetyltrimethylammonium bromide (CTAB), and hydroxymethylcellulose (HMC) were obtained from Sigma-Aldrich, MO, USA, and  $\beta$ -cyclodextrin ( $\beta$ -CD) from TCI, Tokyo Kasei, Japan. *O*-phosphoric acid 85% and hydrochloric acid (Fisher Scientific, NJ, USA) were used. Sodium hydroxide and sodium dihydrogen phosphate were purchased from J.T. Baker, NJ, USA. Phosphate buffer solutions (PBSs) (0.02 M) were freshly prepared. SDS,

CTAB,  $\beta$ -CD, and HMC were prepared as 1% w/v aqueous solutions. Also, 0.1 N NaOH and 0.1 N HCl were prepared.

**2.2. Instrument.** A lambda 20 UV/Vis spectrometer (Perkin Elmer, MA, USA) was used for the spectrophotometric measurements, whereas a LS 55 fluorescence spectrometer, 120 V (Perkin Elmer, MA, USA), was used for the spectrofluorimetric measurements. Spectrophotometric measurements and data handling were performed using UV WinLab 1.22 software, whereas fluorescence spectra measurements and data handling were performed using FL WinLab<sup>™</sup> software. Quartz cells (1 cm) were used. A VWR symphony (SB20) pH meter (Thermo Orion, Beverly, MA, USA) was used for pH measurements. Deionized water was prepared using a Barnstead NANO pure diamond analytical ultrapure water system (Thermo Fischer Scientific, Waltham, MA, USA).

**2.3. Standard stock solutions preparation.** A total of 10 mg of AZL and CLT were accurately weighed and transferred separately into 10 mL of volumetric flasks. Then, they were dissolved and made up to volume with methanol to give concentrations of 1000  $\mu\text{g mL}^{-1}$  for each. Further dilutions were made separately using the same solvent to prepare 1  $\mu\text{g mL}^{-1}$  solutions for each.

### 2.4. Procedures.

**2.4.1. Linearity and construction of calibration graphs for the spectrophotometric technique.** Aliquots of AZL and CLT standard stock solutions (1000  $\mu\text{g mL}^{-1}$ ) were separately transferred into two series of 10 mL Fisherbrand disposable tubes to give final concentrations of 8–50  $\mu\text{g mL}^{-1}$  and 2–20  $\mu\text{g mL}^{-1}$  for AZL and CLT, respectively, then completed to 5 mL with methanol, and the contents were mixed well. The absorbance spectra were recorded against a methanol blank and then the first derivative spectra were obtained (number of points = 18). The amplitudes at 286 and 257 nm for AZL and CLT, respectively, were measured and then plotted versus the corresponding final drug concentration of AZL and CLT ( $\mu\text{g mL}^{-1}$ ) to obtain the calibration graphs and regression equations.

**2.4.2. Linearity and construction of calibration graphs for the spectrofluorimetric technique.** Aliquots of AZL standard stock solution (1  $\mu\text{g mL}^{-1}$ ) were transferred into a series of 10 mL Fisherbrand disposable tubes to give final concentrations in the range of 0.01–0.08  $\mu\text{g mL}^{-1}$ . Then, completed to 5 mL with methanol and the contents were mixed well. Relative fluorescence intensity (RFI) of the synchronous spectrofluorimetric spectra was recorded against a solvent blank using  $\Delta\lambda = 110$  nm (slit width for excitation and emission = 10 nm), and then the fourth derivatives were obtained (number of points = 90). The amplitudes of the fourth derivative of the synchronous spectra at 298 nm were measured and plotted versus the corresponding final drug concentration of AZL ( $\mu\text{g mL}^{-1}$ ) to obtain the calibration graphs and regression equations.

**2.4.3. Preparation of AZL and CLT laboratory prepared mixtures.** For the spectrophotometric method, aliquots of AZL and CLT standard stock solutions (1,000  $\mu\text{g mL}^{-1}$ )

were accurately transferred into a series of 10 mL Fisherbrand disposable tubes to give final concentrations of 9.6, 16.0, and 24.0  $\mu\text{g mL}^{-1}$  for AZL and 6.0, 10.0, and 15.0  $\mu\text{g mL}^{-1}$  for CLT. The procedure described under section "2.4.1" was then applied. For the spectrofluorimetric method, aliquots of AZL and CLT standard stock solutions (1  $\mu\text{g mL}^{-1}$ ) were accurately transferred into a series of 10 mL Fisherbrand disposable tubes to give final concentrations of 0.0384, 0.0576, and 0.0768  $\mu\text{g mL}^{-1}$  for AZL and 0.0240, 0.0360, 0.0480  $\mu\text{g mL}^{-1}$  for CLT. The procedure described under section "2.4.2" was then applied. For both methods, the recovery percentage was calculated using the corresponding regression equation.

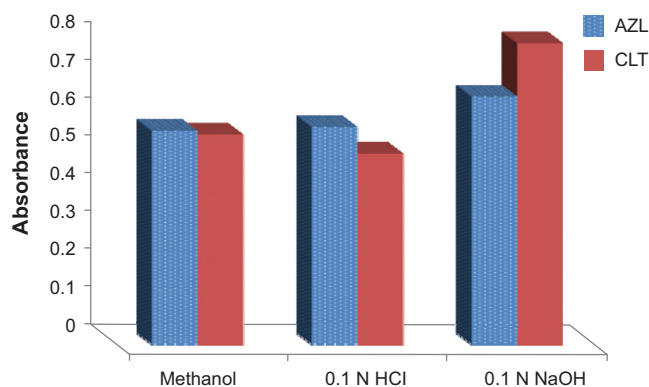
**2.4.4. Sample preparation.** 10 tablets of Edarbicyclor® were weighed and finely powdered after removal of the coat using a tissue moistened with methanol. A portion of the powder equivalent to AZL (40 mg) and CLT (25 mg) was introduced into a 25 mL volumetric flask to which 20 mL methanol were added and sonicated for 30 min. The solution was made up to volume using the same solvent and filtered through a Whatman filter paper, and then filtered again using a 0.2  $\mu\text{m}$  Whatman inorganic membrane filter. For spectrophotometric analyses, different aliquots from the prepared sample solutions were scanned and manipulated using the same procedure described under section "2.4.1." For the spectrofluorimetric analyses, further dilutions were prepared and different aliquots from the prepared sample solutions were scanned and manipulated using the same procedure described under section "2.4.2." The percentage of recoveries were calculated by referring to the corresponding regression equations.

**2.5. Validation.** The suggested analytical method was validated according to ICH guidelines<sup>16</sup> with respect to certain parameters such as linearity, limit of detection (LOD) and limit of quantitation (LOQ), accuracy, precision, specificity, and stability.

### 3. Results and Discussion

The goal of the present investigations was to do some spectrophotometric and spectrofluorimetric studies on AZL and CLT in order to develop validated, rapid, sensitive, simple, inexpensive, and selective spectrophotometric and spectrofluorimetric analytical methods for quantification of AZL in its combination with CLT. These studies focused on factors that enhance the absorbance or the intensities for the emission bands of each analyte.

**3.1. Optimization of the experimental conditions for the spectrophotometric technique.** For the spectrophotometric technique, the effect of different diluting solvents such as methanol, 0.1 N HCl, and 0.1 N NaOH on absorbance was studied. 0.1 N NaOH showed a slightly higher response for both drugs (Fig. 2). This solvent can be used for ordinary UV measurements of AZL or CLT separately at 260 and 232 nm, respectively. Methanol was used as a convenient diluting solvent with satisfactory linearities in the range of 8–50 and 2–20  $\mu\text{g mL}^{-1}$  for AZL and CLT at 266 and 242 nm,



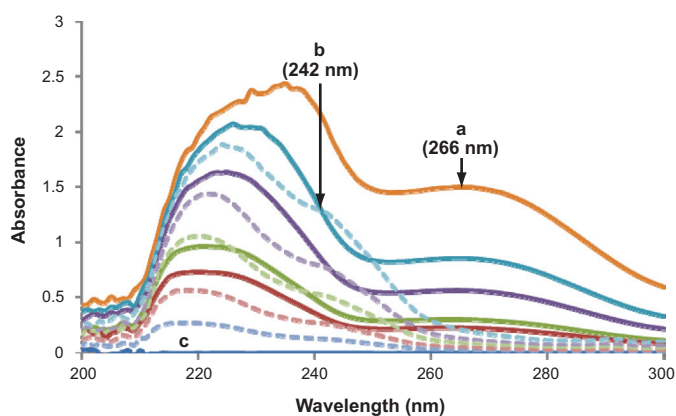
**Figure 2.** Effect of different diluting solvents on the zero-order UV-absorbance spectra of (a) 20  $\mu\text{g mL}^{-1}$  of AZL at 260 nm and (b) 8  $\mu\text{g mL}^{-1}$  of CLT at 232 nm.

respectively (Fig. 3). The spectra of AZL and CLT in all of the studied solvents showed severe overlap (Fig. 3). Obtaining the first derivative for the absorbance spectra of the analyzed drugs was able to resolve such overlapping between AZL and CLT, only in methanol, to be utilized for their simultaneous quantitative determination at 286 and 257 nm, respectively (Fig. 4).

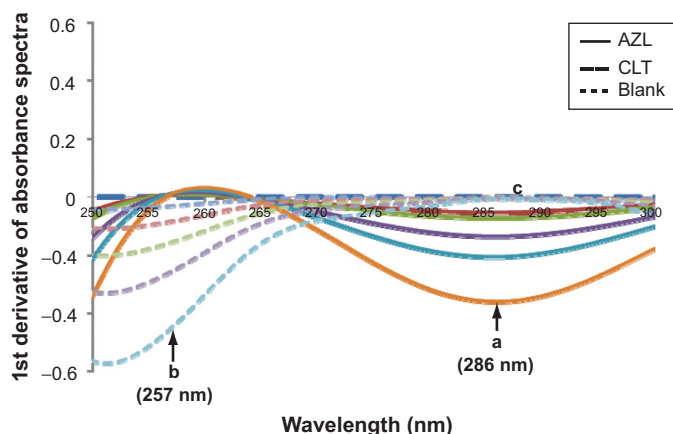
#### 3.2. Optimization of the experimental conditions for the spectrofluorimetric technique.

**3.2.1. Effect of diluting solvent.** AZL is freely soluble in methanol, so it was added to the solvent media in at least 10% of the final volume and then either methanol, 0.1 N HCl, 0.1 N NaOH, or PBS was added to complete the volume. The influence of these different diluting solvents on the RFI of AZL and CLT was studied, and methanol was chosen as it showed the highest RFI (Fig. 5).

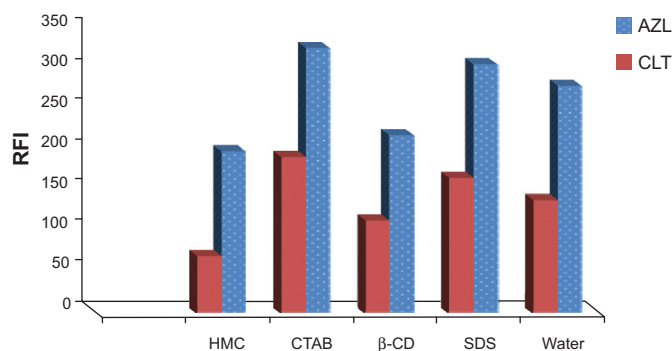
**3.2.2. Effect of pH.** The effect of pH on RFI of AZL and CLT was investigated and pH 7.5 was found to give slightly higher RFI for AZL as its pKa value is 6.1, and it will have higher ionization percentage at this pH (Fig. 6). For CLT (pKa = 9.4), it is ionized at all of the studied pHs, so the effect of the studied pH values was not significant (Fig. 6).



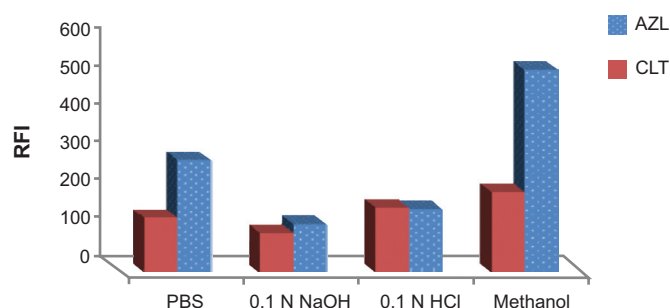
**Figure 3.** Zero-order UV-absorbance spectra of (a) 8, 12, 20, 30, and 50  $\mu\text{g mL}^{-1}$  AZL and (b) 2, 4, 8, 12, 20  $\mu\text{g mL}^{-1}$  CLT against (c) methanol blank.



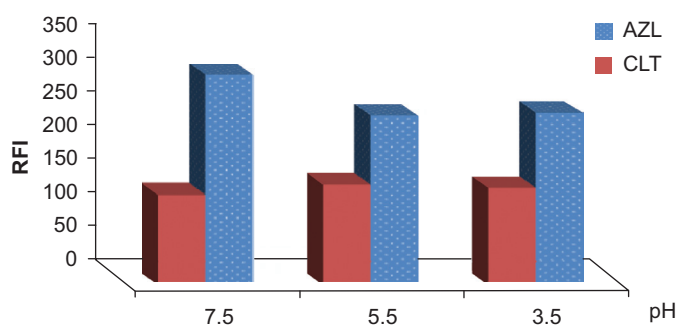
**Figure 4.** First derivative for the spectra of (a) 8, 12, 20, 30, and 50  $\mu\text{g mL}^{-1}$  of AZL and (b) 2, 4, 8, 12, 20  $\mu\text{g mL}^{-1}$  of CLT against (c) methanol blank.



**Figure 7.** Effect of different surfactants (1 mL of 1% w/v solution for each) on the relative conventional fluorescence intensities for 0.02  $\mu\text{g mL}^{-1}$  of AZL and CLT at 381 nm upon excitation at 260 nm using the described parameters.



**Figure 5.** Effect of different diluting solvents on the relative conventional fluorescence intensities for 0.02  $\mu\text{g mL}^{-1}$  of AZL and CLT at 381 nm upon excitation at 260 nm using the described parameters.



**Figure 6.** Effect of pH of PBS on the relative conventional fluorescence intensities for 0.02  $\mu\text{g mL}^{-1}$  of AZL and CLT at 381 nm upon excitation at 260 nm using the described parameters.

**3.2.3. Effect of different organized media.** The fluorescence properties of AZL and CLT in various micellar media were studied using anionic surfactant (SDS), cationic surfactant (CTAB), as well as different macromolecules such as ( $\beta$ -CD) and (HMC). Micelles or reversed micelles are able to stabilize the excited singlet state and delay the decay process, and thus may enhance the intensity of the emission bands.<sup>17</sup> PBS of pH

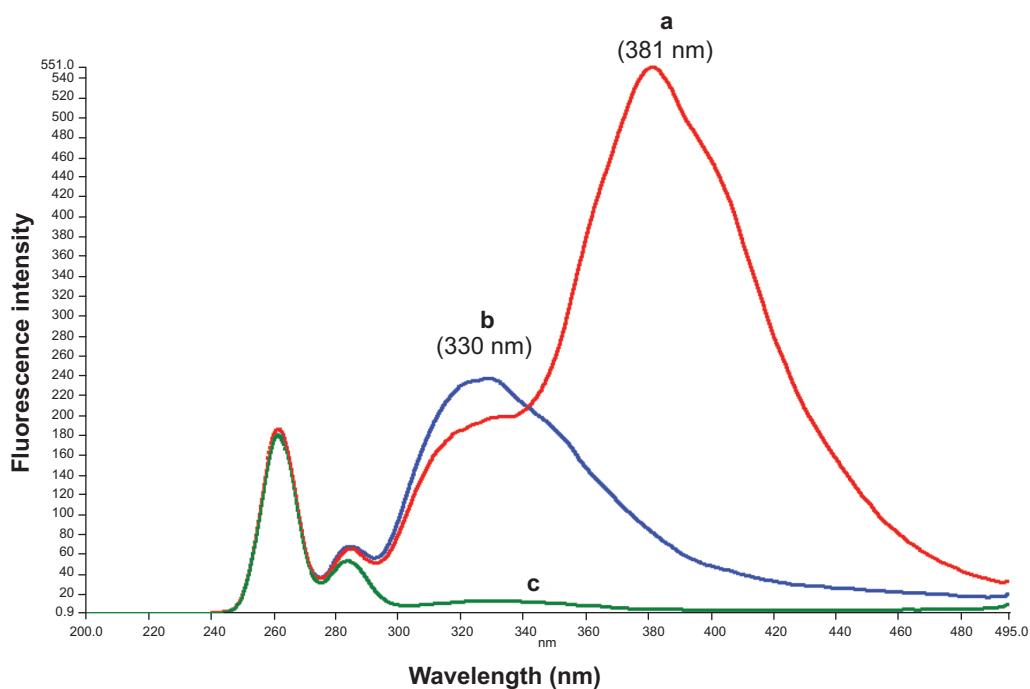
7.5 was used through this study to allow proper ionization of both analytes. None of the examined surfactants showed significant enhancement in RFI of AZL and CLT (Fig. 7).

**3.2.4. Techniques and parameters settings.** Satisfactory linearity was obtained for AZL and CLT in methanol at 381 and 330 nm, upon excitation at 260 nm, respectively, at slit width of excitation and emission of 10 nm (Fig. 8, Table 1). However, the spectra of AZL and CLT in all of the studied solvents showed severe overlap, so they cannot be used for their simultaneous determination (Fig. 8). Also, synchronous fluorescence was not able to resolve such overlap at  $\lambda = 10\text{--}300$  nm (slit width for excitation and emission = 5–10 nm) (Fig. 9). Obtaining the fourth derivative for the synchronous fluorescence spectra ( $\lambda = 110$  nm, slit width for excitation and emission = 10 nm) of the analyzed drugs was able to resolve such overlap between AZL and CLT, only in methanol, to be utilized for quantitative determination of AZL in presence of CLT at 298 nm in their medicinally recommended ratio of 1.6:1, respectively (Fig. 10).

### 3.3. Validation of the methods.

**3.3.1. Linearity and range.** Linear relationship between the responses of AZL or CLT and their corresponding concentrations was obtained. The regression equation  $y = bC \pm a$  for each drug was also computed. In these studies, at least five concentrations for AZL or CLT were used. The linearity of the calibration curves was validated by the high value of correlation coefficients ( $r$ ) of the regression equation, small values of the standard deviation of residuals ( $S_{y/x}$ ), and small value of the percentage relative error (Table 1). The analytical data of the calibration curves including standard deviations for the slope and intercept ( $S_b, S_a$ ) are summarized in Table 1. These data indicate the linearity of the calibration graphs.

**3.3.2. Limit of detection and limit of quantification.** LOQ and LOD were calculated according to ICH recommendations.<sup>17</sup> LOD was considered as the minimum concentration with a signal to noise ratio of at least 3 ( $S/N \sim 3$ ), whereas LOQ



**Figure 8.** Conventional emission spectra for  $0.06 \mu\text{g mL}^{-1}$  of (a) AZL and (b) CLT upon excitation at 260 nm against (c) methanol blank using the described parameters.

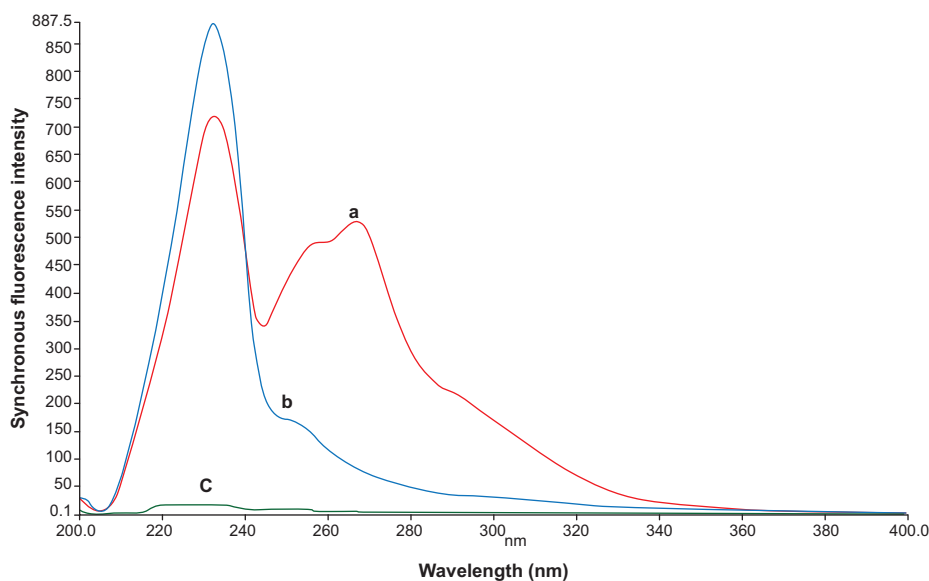
was taken as a minimum concentration with a signal to noise ratio of at least 10 ( $S/N \sim 10$ ). Results are given in Table 1.

**3.3.3. Accuracy and precision.** The satisfactory recovery percentage results for the assay of AZL and/or with CLT in their laboratory prepared mixtures indicate the accuracy of the method (Table 2). Repeatability (intra-day) and intermediate precision (inter-day) were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the proposed method (Table 3).

**3.3.4. Specificity.** The specificity of the method was investigated by observing any interference encountered from common tablet excipients, and it was confirmed that the signals measured was caused only by the analytes. The inactive ingredients in Edarbyclor<sup>®</sup> are mannitol, microcrystalline cellulose, fumaric acid, sodium hydroxide, hydroxypropyl cellulose, crospovidone, magnesium stearate, hypromellose 2910, talc, titanium dioxide, ferric oxide red, polyethylene glycol 8000, and printing ink gray F1. It was found that the excipients did not interfere with the results (Table 2). For the spectrophotometric method, it was found that AZL and CLT can be determined

**Table 1.** Performance data and results of the proposed spectroscopic methods.

ITEM	SPECTROPHOTOMETRIC TECHNIQUE		SPECTROFLUORIMETRIC TECHNIQUE		
	AZL (D1)	CLT (D1)	AZL (D4)	AZL (381 nm)	CLT (330 nm)
Limit of detection, LOD ( $\mu\text{g mL}^{-1}$ )	0.977	0.296	0.001	0.001	0.002
Limit of quantitation, LOQ ( $\mu\text{g mL}^{-1}$ )	2.960	0.897	0.003	0.004	0.005
Intercept (a)	0.0064	-0.0154	0.7876	41.996	3.5339
Slope (b)	0.0075	0.0224	789.1	6289.8	3922.4
Correlation coefficient ( $r$ )	0.9998	0.9999	0.9998	0.9991	0.9996
S.D of residuals ( $S_{y/x}$ )	0.003	0.003	0.478	4.396	2.576
S.D of intercept ( $S_a$ )	0.002	0.002	0.267	2.469	2.043
S.D of slope ( $S_b$ )	0.001	0.001	8.048	74.453	61.598
Percentage relative error	0.75	0.55	0.76	0.94	1.23
Linearity range ( $\mu\text{g/mL}$ )	8.00–50.00	2.00–20.00	0.01–0.08	0.01–0.08	0.01–0.06
Mean recovery $\pm$ %R.S.D	99.55 $\pm$ 1.69	100.62 $\pm$ 1.24	99.94 $\pm$ 0.80	99.54 $\pm$ 2.09	100.61 $\pm$ 2.75



**Figure 9.** Synchronous fluorescence spectra for  $0.06 \mu\text{g mL}^{-1}$  of (a) AZL and (b) CLT upon excitation at 260 nm against (c) methanol blank using the described parameters.

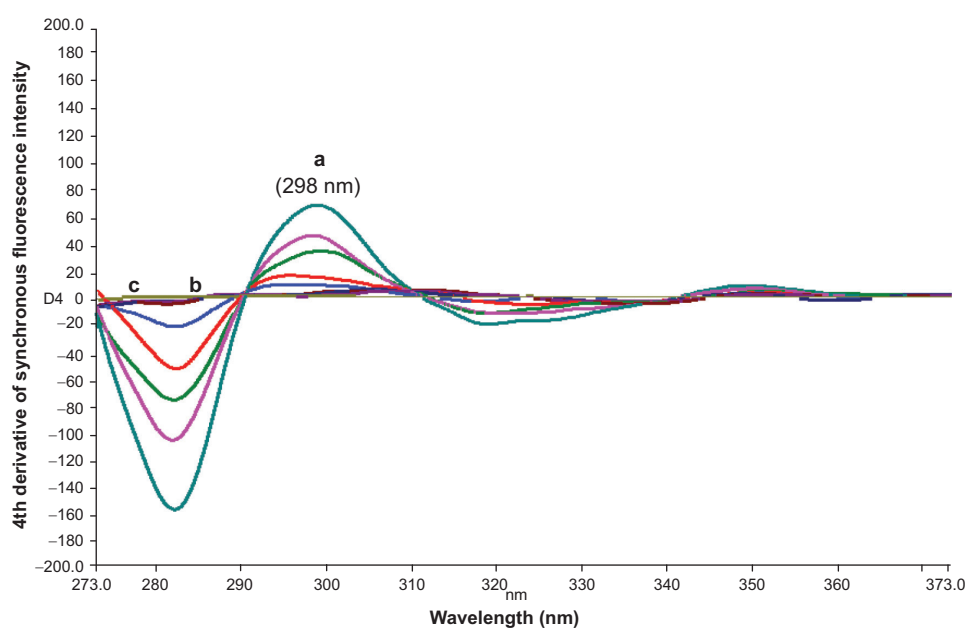
simultaneously either in their laboratory prepared mixtures or in their co-formulated tablets (Table 2). On the other hand, the spectrofluorimetric method was only able to determine AZL in presence of CLT in methanol (Table 2).

**3.3.5. Stability.** Stabilities of the standard stock solutions of AZL and CLT, stored at  $2-8^{\circ}\text{C}$ , were evaluated at various time points for more than one month. The concentrations of freshly prepared solutions and those aged for one month were calculated by the methods developed, and the difference

between them was found to be less than 2%. Also, the prepared solutions for analysis were found to be stable for at least three days at  $2-8^{\circ}\text{C}$  and for a day at room temperature. These solutions can therefore be used during this interval of time without the results being affected.

#### 4. Conclusion

Rapid, sensitive, inexpensive, and simple spectrophotometric and spectrofluorimetric methods were developed and



**Figure 10.** Fourth derivative of the synchronous fluorescence spectra for (a)  $0.01, 0.02, 0.04, 0.06, 0.08 \mu\text{g mL}^{-1}$  of AZL and (b)  $0.01, 0.02, 0.03, 0.04, 0.06 \mu\text{g mL}^{-1}$  of CLT against (c) methanol blank using the described parameters.

**Table 2.** Assay results for the determination of the studied drugs in their laboratory prepared mixtures and dosage form.

TECHNIQUE	COMPOUND	SYNTHETIC MIXTURES (CLT:AZL) (1.0:1.6)			DOSAGE FORM (EDARBCYCLOR® TABLETS)			
		AMOUNT TAKEN ( $\mu\text{g mL}^{-1}$ )	AMOUNT FOUND ( $\mu\text{g mL}^{-1}$ )	% FOUND	AMOUNT TAKEN ( $\mu\text{g mL}^{-1}$ )	AMOUNT FOUND ( $\mu\text{g mL}^{-1}$ )	% FOUND	
Spectrophotometric	CLT	6.000	6.045	100.74	6.000	6.045	100.74	
		10.000	9.839	98.39	10.000	10.286	102.86	
		18.000	17.875	99.31	18.000	17.652	98.07	
		Mean recovery%		99.48			100.56	
		$\pm$ %R.S.D		1.19			2.39	
	AZL	9.600	9.813	102.22	9.600	9.813	102.22	
		16.000	15.813	98.83	16.000	16.480	103.00	
		28.800	28.480	98.89	28.800	28.480	98.89	
			Mean recovery%		99.98			101.37
			$\pm$ %R.S.D		1.94			2.15
Spectrofluorimetric	AZL	0.0384	0.039	101.26	0.0384	0.037	97.40	
		0.0576	0.058	100.12	0.0576	0.057	99.30	
		0.0768	0.077	100.47	0.0768	0.076	98.80	
		Mean recovery%		100.62			98.50	
		$\pm$ %R.S.D		0.58			1.00	

Each result is the average of three separate determinations.

**Table 3.** Intra- and inter-day validation for the spectroscopic methods.

TECHNIQUE	MEDICATION	CONCENTRATION ( $\mu\text{g mL}^{-1}$ )	INTRA-DAY ASSAY FOUND $\pm$ % R.S.D <sup>a</sup>	INTER-DAY ASSAY FOUND $\pm$ %R.S.D <sup>a</sup>
Spectrophotometric	AZL	12.000	12.619 $\pm$ 0.63	12.124 $\pm$ 1.68
		20.000	20.124 $\pm$ 2.02	20.258 $\pm$ 1.01
		30.000	29.813 $\pm$ 1.23	29.457 $\pm$ 1.19
	CLT	4.000	4.028 $\pm$ 0.32	4.051 $\pm$ 0.64
		8.000	7.905 $\pm$ 1.63	8.054 $\pm$ 1.66
		12.000	11.923 $\pm$ 1.08	11.789 $\pm$ 1.58
Spectrofluorimetric	AZL	0.015	0.015 $\pm$ 2.09	0.015 $\pm$ 2.92
		0.030	0.030 $\pm$ 2.22	0.030 $\pm$ 2.77
		0.050	0.050 $\pm$ 1.36	0.050 $\pm$ 2.88

<sup>a</sup>Mean and % R.S.D. of three determinations.

validated for determination of AZL in its combination with CLT either in their laboratory prepared mixtures or in their combined pharmaceutical formulation. The proposed methods have many advantages regarding analysis time, sensitivity, and cost. Finally, the proposed methods can be used for the quality control of the cited drugs in ordinary laboratories without pre-separation.

### Abbreviations

AZL, azilsartan medoxomil; CLT, chlorthalidone; PBS, phosphate buffer solution; RIF, relative fluorescence intensity.

### Author Contributions

Conceived and designed the experiments: WE, EE, AE, RE, GP. Analyzed the data: WE, EE, AE, RE, GP. Wrote the first draft of the manuscript: WE, EE, AE, RE, GP. Contributed to the writing of the manuscript: WE, EE, AE, RE, GP. Agree with manuscript results and conclusions: WE, EE, AE, RE, GP. Jointly developed the structure and arguments for the paper: WE, EE, AE, RE, GP. Made critical revisions and approved final version: WE, EE, AE, RE, GP. All authors reviewed and approved of the final manuscript.



## DISCLOSURES AND ETHICS

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