

## **Determination of Azilsartan Medoximil and Chlorthalidone in tablets exposed to forced degradation by using RP-HPLC.**

**T Hemant Kumar<sup>1\*</sup>, Ayesha Begum<sup>1</sup>, D Gowri Sankar<sup>2</sup>**

<sup>1</sup>Department of Pharmaceutical Analysis and Quality Assurance, Vignan Institute of Pharmaceutical Technology, Visakhapatnam, India

<sup>2</sup>Department of Pharmaceutical Analysis, Andhra University, Visakhapatnam, India

### **Abstract**

An RP-HPLC method was developed which was simple, specific and precise accurately and was validated for the simultaneous estimation of Azilsartan Medoximil and Chlorthalidone by following forced degradation studies in pharmaceutical formulation. The method has been developed using Enable C 18G column (250 × 4.6 mm, 0.5 μm) and a mobile phase comprising of acetonitrile and 0.1% trifluoroacetic acid in water at the ratio of 40:60%v/v with a flow rate of 0.8 mL/min. UV detection was carried out at 240 nm. The retention time for Azilsartan Medoximil and Chlorthalidone were found to be 6.982 and 7.748 min respectively. The proposed method was validated for various parameters like linearity, range, accuracy, precision, robustness, LOD and LOQ. Linearity was observed over a concentration range 5-80 μg/ml for Azilsartan Medoximil ( $r^2=0.9995$ ) and 2.5-25 μg/ml for Chlorthalidone ( $r^2=0.9973$ ). The % RSD for Intraday and Interday precision was found as 0.41 and 0.41 for Azilsartan Medoximil and 0.32 and 0.47 for Chlorthalidone respectively. The LOD and LOQ were found as 0.0189 μg/ml and 0.0305 μg/ml for Azilsartan Medoximil and LOD and LOQ were found to be 0.1109 and 0.3522 μg/ml for Chlorthalidone respectively. Azilsartan Medoximil and Chlorthalidone have been subjected to stress conditions of degradation including acidic, alkaline, oxidative, and thermal and photolysis.

**Keywords:** Azilsartan Medoximil, Chlorthalidone, RP-HPLC, Forced Degradation.

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### **Introduction**

Azilsartan Medoximil (Figure 1) is an Angiotensin II receptor antagonist which has the chemical name (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 monopotassium salt. It is a white crystalline powder which is practically insoluble in water but freely soluble solvents like methanol, dimethyl sulfoxide and dimethyl formamide, soluble in acetic acid, slightly soluble in acetone and Acetonitrile and very slightly soluble in Tetra Hydro furan and 1-octanol. It is available in dosages of 40 mg and 80 mg, the recommended dosage is 80 mg per day. By hydrolysis of medoximil ester, the active moiety of Azilsartan Medoximil can be released. It is an active ARB (AT1) type which is more active in lowering blood pressure within 24 hours when compared to other ARBs. Literature survey shows various analytical methods, either alone or in combination with other drugs includes UV spectrophotometric [1,2], HPLC [3-6], LC-MS [7-10], HPTLC [11] in pure drug, pharmaceutical formulations as well as biological fluids.

Chlorthalidone (Figure 2) is chemically 2-Chloro-5-(1-hydroxy-3-oxo-2,3-dihydro-1H-isoindol-1-yl)benzenesulfonamide. Chlorthalidone is practically insoluble in water, ether and chloroform and soluble in methanol and also slightly soluble in alcohol. It is a thiazide type diuretic used to treat hypertension. It acts in a similar way like the thiazides in causing diuresis but do not possess a benzothiadiazine moiety in it. At the proximal portion of the distal convoluted tubule of the nephron, it shows its action with longest duration of action as compared to other thiazide diuretics. Based on extensive evaluation in clinical trials, the combination of Azilsartan Medoximil with Chlorthalidone has greater efficacy to other ARBs alone or in combination with hydrochlorothiazide. This advanced efficacy is not offset by a large imbalance in clinically important adverse events [12]. A number of analytical methods have been reported so far for the determination of Chlorthalidone either alone or in combination with other drugs in pure drug, pharmaceutical dosage forms and also in biological samples using spectrophotometry [13,14], HPLC [15-17], GC [18,19] and SFC [20]. The combination of Azilsartan Medoximil and Chlorthalidone proves effective in the treatment of hypertension. Various other analytical methods have been reported for simultaneous

estimation of Azilsartan Medoxomil and Chlorthalidone in pure drug, pharmaceutical formulations and biological fluids by spectrophotometric [21] and HPLC [22-26]. From the reported RP-HPLC method for the simultaneous estimation of both drugs in pharmaceutical formulation two methods was stability indicating one but these developed methods showed low linearity range and were less sensitive. As RP-HPLC [27-32] is most commonly used analytical method for estimation of drugs for specificity as well as sensitivity, therefore in the current study an attempt was made to develop a simple, precise, accurate RP-HPLC [33-36] method with forced degradation studies for the analysis of Azilsartan Medoxomil and Chlorthalidone in the pharmaceutical formulation.

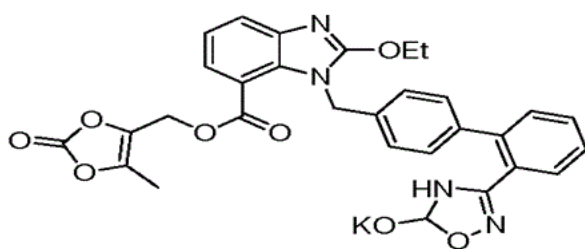


Figure 1. Structure of Azilsartan Medoxomil.

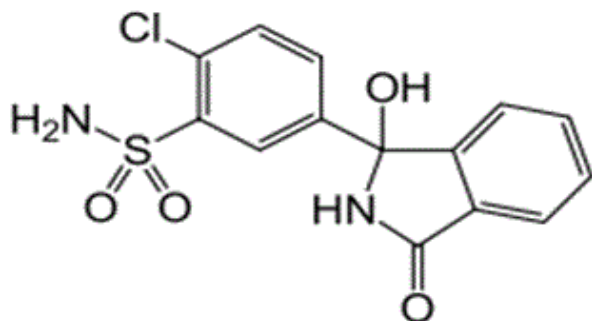


Figure 2. Structure of Chlorthalidone.

## Material and Methods

### Materials and chemicals

Azilsartan Medoxomil and Chlorthalidone standard were obtained from pharma industry, as gifted sample. Azilsartan Medoximil and Chlorthalidone tablets (Edarbychlor Tablets) containing Azilsartan Medoximil 40 mg and Chlorthalidone 12.5 mg were purchased from a pharmacy online. HPLC grade water and acetonitrile was obtained from MERCK India Ltd. HPLC grade methanol was obtained from standard reagent pvt ltd Hyderabad. Analytical grade hydrochloric acid, sodium hydroxide, hydrogen peroxide, trifluoroacetic acid, was obtained

from SD Fine chemicals Mumbai, India. Nylon membrane filters 0.2  $\mu\text{m}$  and 0.45  $\mu\text{m}$  were from PALL life sciences Mumbai, India. Ultrasonicator used was from LAB India Ltd Mumbai. pH meter was of Elico LI 120 make. UV Spectrophotometer was of Elico SL 210 model consisted of spectral treats software.

### Instrumentation

The chromatographic system which was used for the method development and validation consisted of Shimadzu HPLC comprising of LC-20AD a binary gradient pump, a SCL 20A system controller, a variable wavelength programmable SPD-20A detector. A Rheodyne injector 7725i was fitted with a 20  $\mu\text{L}$  loop which was used and the data was recorded and evaluated by using LC solutions software version 5.0.

### Chromatographic conditions

Chromatographic analysis was performed on Enable C18 G column (250  $\times$  4.6 mm i.d, 5  $\mu$ ). The mobile phase consisted of acetonitrile and 0.1% trifluoroacetic acid in water at the ratio of 40:60%v/v. The flow rate was 0.8 mL/min, injection volume was 20  $\mu\text{L}$  and detection was carried out at 240 nm using a UV detector.

### Preparations of Azilsartan Medoxomil and Chlorthalidone stock solution

Stock solution of Azilsartan Medoximil (1000  $\mu\text{g/ml}$ ) and Chlorthalidone (1000  $\mu\text{g/ml}$ ) was prepared separately by transferring accurately weighed 50 mg of Azilsartan Medoximil and 50 mg of Chlorthalidone into a 50 ml volumetric flask and to it added a 20 ml methanol. The mixture was sonicated for 5 min to dissolve the drug and the solution was diluted up to the mark with methanol. To prepare a binary mixture of Chlorthalidone and Azilsartan Medoximil appropriate volume of standard solution was transferred into a 10 ml volumetric flask and diluted with mobile phase to get a solution containing 12.5  $\mu\text{g/ml}$  of Chlorthalidone and 40  $\mu\text{g/ml}$  of Azilsartan Medoximil.

### Analysis of Azilsartan Medoxomil and Chlorthalidone in combined dosage form

Accurately weighed about twenty tablets and average weight of tablet was determined. The tablets were transferred into mortar and triturated to a fine powder form. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 100 ml volumetric flask. To it 20 ml HPLC grade methanol was added and sonicated for 5 min to dissolve the drugs. The content of the flask was kept for 10 min at laboratory temperature and diluted up to mark with HPLC[37-41] grade methanol this gives a concentration of Chlorthalidone 125  $\mu\text{g/ml}$  and Azilsartan Medoximil 400  $\mu\text{g/ml}$ . The above solution was filtered through 0.2  $\mu$  membrane filter. The 1 ml of the filtrate was transferred into a 10 ml volumetric flask and diluted with mobile phase to

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get a concentration of 12.5 µg/ml and 40 µg/ml for Chlorthalidone and Azilsartan Medoximil respectively.

### **Method Validation**

The method was validated for accuracy, precision, linearity, specificity, robustness, limit of detection, limit of quantitation.

#### **Linearity**

Linearity was performed by preparing standard solutions of Chlorthalidone and Azilsartan Medoximil at different concentration levels. Chlorthalidone was prepared in the concentration range of 2.5-25 µg/mL and 5-80 µg/mL for Azilsartan Medoximil. Twenty micro litres of each concentration from both drug solutions was injected in duplicate into the HPLC system. The response was carried out at 240 nm and the corresponding chromatograms were recorded from these mean peak areas were calculated. The calibration curve was plotted by taking concentration on x-axis and peak areas on y-axis for both the drugs.

#### **Accuracy**

The accuracy of the method evaluated by standard addition method in which a known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery of Chlorthalidone and Azilsartan Medoximil was calculated at three concentration levels of 80%, 100% and 120%. The solutions were analysed in triplicate at each level. The percent recovery and %RSD at each level was calculated.

#### **Precision**

Precision of the method was evaluated as system precision and method precision. To study the system precision, six replicate standard solutions of Chlorthalidone and Azilsartan Medoximil were analysed. The percent relative standard deviation (% RSD) was calculated for both Chlorthalidone and Azilsartan Medoximil.

Method precision of the analytical method was carried out on six preparations from the tablet formulation and percentage amount of Chlorthalidone and Azilsartan Medoximil in the tablet formulation was calculated. The intraday and interday precision study was conducted for both Chlorthalidone and Azilsartan Medoximil. The mean % assay value, standard deviation and percent relative standard deviation was calculated.

#### **Limit of detection (LOD) and Limit of quantitation (LOQ)**

LOD was measured by serially diluting the standard solutions of Chlorthalidone and Azilsartan Medoximil and determining the concentration was response of sample peaks are three times the noise peak. LOQ was measured by serially diluting the standard solutions of Chlorthalidone and Azilsartan Medoximil and determining the concentration was response of sample peaks are ten times the noise peak.

**Robustness:** Robustness of the method was determined by making slight changes in composition of organic phase  $\pm 5\%$ , flow rate by  $\pm 0.1$  ml/min and detection wavelength by  $\pm 2$  nm.

#### **Specificity**

The specificity of the proposed method was determined against blank and placebo applications. Here mobile phase was used as blank and excipients like starch, lactose, magnesium stearate were used as placebo.

#### **Forced degradation studies**

Different stress conditions were used for the forced degradation studies of formulation. These were also used to evaluate the specificity of the method. All the samples were diluted with mobile phase and filtered through 0.2 µ membrane filter.

#### **Acidic conditions**

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 100 ml volumetric flask. To this added a 40 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 5N HCl was added to it, refluxed for 6 h at 60°C, cooled to room temperature, neutralized with 5N NaOH and diluted up to the mark with the diluent. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 1 ml of the above filtered sample solution into a 10 ml volumetric flask and volume made up to the mark with diluent.

#### **Alkaline conditions**

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 100 ml volumetric flask. To this added a 40 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 10 ml of 5N NaOH was added to it, refluxed for 6 h at 60°C, cooled to room temperature, neutralized with 5N HCl and diluted up to the mark with the diluent. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 1 ml of the above filtered sample solution into a 10 ml volumetric flask and volume made up to the mark with diluent.

#### **Oxidative degradation**

Weighed accurately about twenty tablets and triturated it to a fine powder form. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 100 ml volumetric flask. To this added a 40 ml of diluent and sonicated for 10 min to dissolve the drug completely. Then 5 ml of 30% hydrogen peroxide was added, refluxed for 2 h at 60°C, then cooled to room temperature and diluted up to the mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter.

Pipetted 1 ml of the above filtered sample solution into a 10 ml volumetric flask and volume made up to the mark with diluent.

### ***Thermal degradation***

Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to thermal stress at 105°C for about 2 days. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 100 ml volumetric flask. To this added a 20 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 1 ml of the above filtered sample solution into a 10 ml volumetric flask and volume made up to the mark with diluent.

### ***Photolytic degradation***

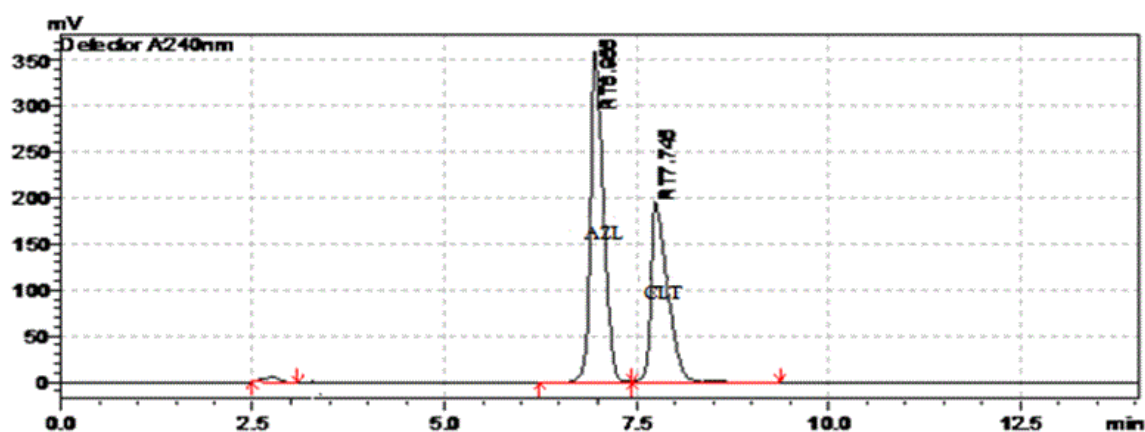
Weighed accurately about twenty tablets and triturated it to a fine powder form. The powder sample was subjected to UV light in a photo stability chamber for about 10 days. An aliquate of the powder equivalent to 12.5 mg of Chlorthalidone and 40 mg of Azilsartan Medoximil was transferred into a 50 ml volumetric flask. To this added a 40 ml of diluent and sonicated for 10 min to dissolve the drug completely the diluted up to mark with diluents. The above sample solution was filtered through 0.2 µ nylon membrane filter. Pipetted 1 ml of the above filtered sample solution into a 10 ml volumetric flask and volume made up to the mark with diluent

## **Results and Discussion**

### ***Optimization of chromatographic conditions***

In the present work an analytical method based on RP-HPLC using UV detector was developed and validated for

simultaneous estimation of Chlorthalidone and Azilsartan Medoximil in pharmaceutical formulation. The selection of analytical conditions was based on the chemical nature of Chlorthalidone and Azilsartan Medoximil. A systematic study of various factors were undertaken by varying one parameter at a time and keeping all other conditions constant for development of analytical method. Both Chlorthalidone and Azilsartan Medoximil were soluble in polar solvents therefore RP-HPLC was chosen. The selection of stationary phase has been done on the basis of back pressure, resolution, peak shape, theoretical plates and day to day reproducibility in retention time resolution between Chlorthalidone and Azilsartan Medoximil peaks. After evaluating all these factors Enable C18 G column (250 × 4.6 mm id, 5 µ) was chosen for the analysis. For optimization of mobile phase preliminary trials were conducted under isocratic conditions using mobile phases composed of mixture of solvents like water, methanol, acetonitrile and 0.1% trifluoroacetic acid in water in different combination. A mixture of acetonitrile and 0.1% trifluoroacetic acid at a ratio of 40:60% v/v was found to be most suitable of all the combinations since the chromatographic peaks obtained were have good system suitability parameters. The Flow rate of mobile phase was optimized based on resolution between chromatographic peaks and minimal solvent consumption. The flow rate of mobile phase was changed from 0.5-2 ml/min. It was found from trials that 0.8 ml/min flow rate was ideal for successful elution of both drugs. For selection of analytical wavelength standard solutions of both drugs were scanned in wavelength range of 200-350 nm. A detection wavelength of 240 nm was selected. The chromatogram of sample was shown in Figure 3.



**Figure 3.** Chromatogram of Azilsartan Medoxomil and Chlorothalidone.

**Method validation**

**Linearity:** Linearity was studied by preparing standard solutions at different concentration levels. The linearity ranges for Azilsartan Medoxomil and Chlorthalidone were found to be 5-80 µg/mL and 2.5-25 µg/mL respectively. The linear regression equation for Azilsartan Medoxomil was found to be  $167497x+69323$  with correlation coefficient 0.9995. The linear regression equation for Chlorthalidone was found to be  $234315x-5210.6$  with correlation coefficient 0.9973. The calibration table for Azilsartan Medoxomil and Chlorthalidone was shown in Tables 1 and 2 respectively. The calibration curve of Azilsartan Medoxomil and Chlorthalidone were shown in Figures 4 and 5 respectively.

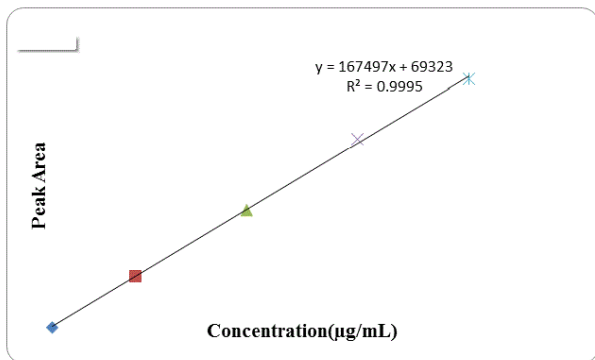


Figure 4. Linearity plot of Azilsartan Medoxomil.

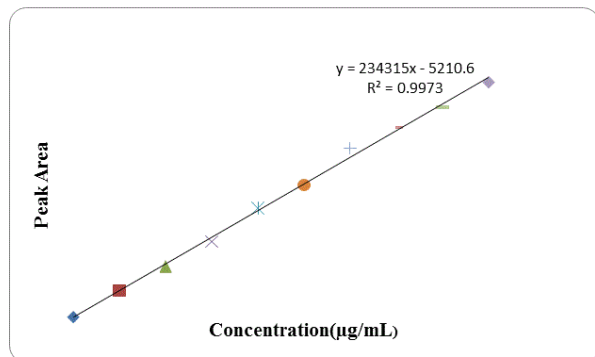


Figure 5. Linearity plot of Chlorthalidone.

Table 1. Linearity data for Azilsartan Medoxomil.

Level	Concentration of Azilsartan Medoximil (µg/mL)	Mean peak area
Level-1	5	859346
Level-2	20	3438369
Level-3	40	6739865
Level-4	60	10298508
Level-5	80	13347435
Slope		167497
Intercept		69323
Correlation Coefficient		0.9995

Table 2. Linearity data for Chlorthalidone.

Level	Concentration of Chlorthalidone(µg/mL)	Mean peak area
Level-1	2.5	586342
Level-2	5	1162184
Level-3	7.5	1697926
Level-4	10	2245369
Level-5	12.5	2982436
Level-6	15	3496033
Level-7	17.5	4295361
Level-8	20	4753826
Level-9	22.5	5198043
Level-10	25	5748645
Slope		234315
Intercept		-5210.6
Correlation Coefficient		0.9973

Table 3. Accuracy results of Chlorothalidone.

Accurac y level	Amount taken(µg/mL)	Amount found(µg/mL)	% Recovery	Mean recovery	% RSD
80	10	9.92	99.2	100.06	1.32
	10	10.16	101.6		
	10	9.94	99.4		
100	12.5	12.42	99.36	99.41	0.25
	12.5	12.4	99.2		
	12.5	12.46	99.68		
120	15	15.19	101.2	100.04	1.02
	15	14.93	99.53		
	15	14.91	99.4		

**Accuracy:** The percent recovery of Chlorthalidone and Azilsartan Medoxomil were found to be 99.41-100.06% and 99.69-100.1%. This indicates the accuracy of the method. The results are shown in Tables 3 and 4.

**Precision**

**System precision:** The %RSD for Chlorthalidone was found to be 0.91 and for Azilsartan Medoxomil was found to be 1.15 which is within the acceptance criteria of not more than 2.0 indicates the precision of the method (Table 5).

**Table 4.** Accuracy results of Azilsartan Medoxomil.

Accuracy level (%)	Amount taken( $\mu\text{g/mL}$ )	Amount found( $\mu\text{g/mL}$ )	% Recovery	Mean Recovery	% RSD
80	32	32.12	100.3	99.9	0.35
	32	31.89	99.65		
	32	31.92	99.75		
100	40	39.99	99.97	100.1	1.33
	40	39.54	98.85		
	40	40.45	101.5		
120	48	47.89	99.77	99.69	0.21
	48	47.74	99.45		
	48	47.93	99.85		

**Table 5.** System precision results for Chlorothalidone and Azilsartan Medoxomil.

Injection No.	Peak Area of Chlorothalidone	Peak Area of Azilsartan Medoximil
1	2982436	6739865
2	2972874	6639284
3	2936438	6573948
4	2983931	6793842
5	2994725	6711174
6	2929373	6691059
Mean	2966630	6691529
SD	27118	77130
% RSD	0.91	1.15

**Method precision:** The % RSD for Intraday and Interday precision assay results of six preparations for Chlorothalidone were found to be 0.32 and 0.47 respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of method.

The % RSD for Intraday and Interday precision assay results of six preparations for Azilsartan Medoxomil were found to be 0.41 and 0.41 respectively which are within the acceptance criteria of not more than 2.0 indicates the precision of the method (Table 6).

**Limit of detection and Limit of quantitation:** The LOD and LOQ were found to be 0.0189  $\mu\text{g/mL}$  and 0.0305  $\mu\text{g/mL}$  for Azilsartan Medoxomil and the LOD and LOQ for Chlorothalidone were 0.1109  $\mu\text{g/mL}$  and 0.3522  $\mu\text{g/mL}$  respectively.

**Robustness:** To evaluate the robustness of the developed method, small deliberate variations in optimized method parameters were made.

The effect of change in flow rate, change in composition of mobile phase and detection wavelength on retention time, tailing factor and theoretical plates were studied.

The method was found to be unaffected by small changes in flow rate, change in pH, change in composition of mobile phase and detection wavelength as shown in Tables 7 and 8.

**Table 6.** Method precision results for Chlorothalidone and Azilsartan Medoxomil.

Set	Chlorothalidone (% Assay)		Azilsartan Medoximil (% Assay)	
	Intraday (n=6)	Interday (n=6)	Intraday (n=6)	Interday (n=6)
1	100.13	100.51	99.48	100.03
2	100.08	99.59	99.39	99.54
3	99.85	99.86	99.34	99.61
4	100.18	99.38	99.81	99.83
5	99.34	100.49	100.07	99.37
6	100.04	100.16	100.32	100.48
Mean	99.93	99.99	99.73	99.81
SD	0.3134	0.4685	0.4	0.4
% RSD	0.32	0.47	0.41	0.41

**Table 7.** Robustness results for Azilsartan Medoxomil.

Conditions	% Assay	System suitability parameters	
		Theoretical plates	Tailing factor
Flow Rate 0.6 mL/min	100.54	6996	1.08
Flow Rate 1 mL/min	99.48	5912	1.12
Mobile Phase-ACN(45): 0.1 % TFA(55)	99.79	5363	1.14
Mobile Phase-ACN(35): 0.1%TFA(65)	99.34	5261	1.12
Wavelength 242 nm	99.59	6980	1.11
Wavelength 238 nm	99.56	6978	1.11

**Specificity:** Specificity is the ability to unequivocally assess the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degradants or matrix.

Specificity of an analytical method is its ability to accurately and specifically measure the analyte of interest without interference from blank or placebo.

The peak purities of Azilsartan Medoxomil and Chlorothalidone were assessed by comparing the retention times of standard Azilsartan Medoxomil and Chlorothalidone and the sample, and good correlation was obtained between the retention time of the standard and sample.

Placebo and blank were injected and there were no peaks. There is no interference of degradation peaks on drug peaks.

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hence, the method is specific. The specificity results are shown in Table 9.

**Table 8.** Robustness results for Chlorothalidone.

Conditions	% Assay	System suitability parameters	
		Theoretical plates	Tailing factor
Flow Rate 0.6 mL/min	99.56	4312	1.09
Flow Rate 1 mL/min	99.79	4144	1.12
Mobile Phase-ACN(45): 0.1 %TFA(55)	99.77	4352	1.12
Mobile Phase-ACN(35): 0.1%TFA(65)	99.84	4098	1.11
Wavelength 242 nm	99.87	4107	1.12
Wavelength 238 nm	99.69	4116	1.12

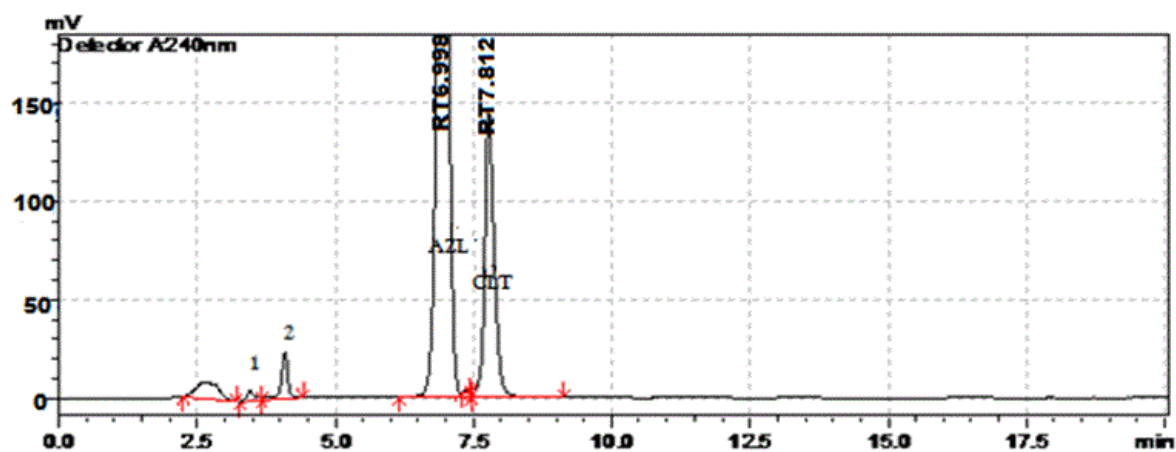
**Table 9.** Specificity results of the method.

Name of solution	Retention time
Blank	No peaks
Placebo	2.743 min
Chlorthalidone	7.748 min
Azilsartan Medoximil	6.982 min

**Analysis of commercial formulation:** The proposed method was applied for the determination of Azilsartan Medoximil

and Chlorthalidone in marketed formulations available (Edarbychlor Tablets). The % recovery was found to be  $99.65 \pm 0.86$  and  $99.76 \pm 0.67$  for Azilsartan Medoximil and Chlorthalidone respectively (Tables 10 and 11).

**Results of forced degradation studies:** Under acidic conditions Azilsartan Medoximil degraded to 1.64% and Chlorthalidone degraded to 0.45%. In these stress conditions there is an appearance of two degradant peaks at retention times of 3.512 and 4.155 min. In basic conditions Azilsartan Medoximil degraded to 16.43% and Chlorthalidone degraded to 0.46%. Under these conditions there is an appearance of two major peaks and many minor peaks degradation peaks on chromatogram. The retention times of major peaks are at 2.012 min and 3.998 min. In oxidative conditions Azilsartan Medoximil degraded to 2.52% and Chlorthalidone to 0.31%. There is an appearance of degradant peaks on chromatogram at the retention time of 4.012 min. In thermal conditions Azilsartan Medoximil degraded to 0.17% and Chlorthalidone degraded to 0.06%. There is no appearance of degradant peaks on chromatogram. In photolytic conditions Azilsartan Medoximil degraded to 1.08% and Chlorthalidone degraded to 0.82%. There is an appearance of two degradation peaks at the retention time of 3.532 min and 4.145 min on chromatogram. From the degradation studies it was concluded that both Chlorthalidone shown stability in all conditions but degraded some extent in photolytic conditions. Azilsartan medoximil shown stability in thermal conditions but in remaining conditions it degraded significantly especially in alkaline conditions (Figures 6-10).



**Figure 6.** Acid degradation chromatogram.

**Table 10.** Analysis of Azilsartan Medoximil and Chlorthalidone in commercial formulation.

Formulation	Labelled claim (mg)		Amount found* (mg)		% Recovery* $\pm$ % RSD	
	Azilsartan Medoximil	Chlorthalidone	Azilsartan Medoximil	Chlorthalidone	Azilsartan Medoximil	Chlorthalidone
Edarbychlor tablets	40	12.5	39.86	12.47	$99.65 \pm 0.86$	$99.76 \pm 0.67$

\*Average of three determinations

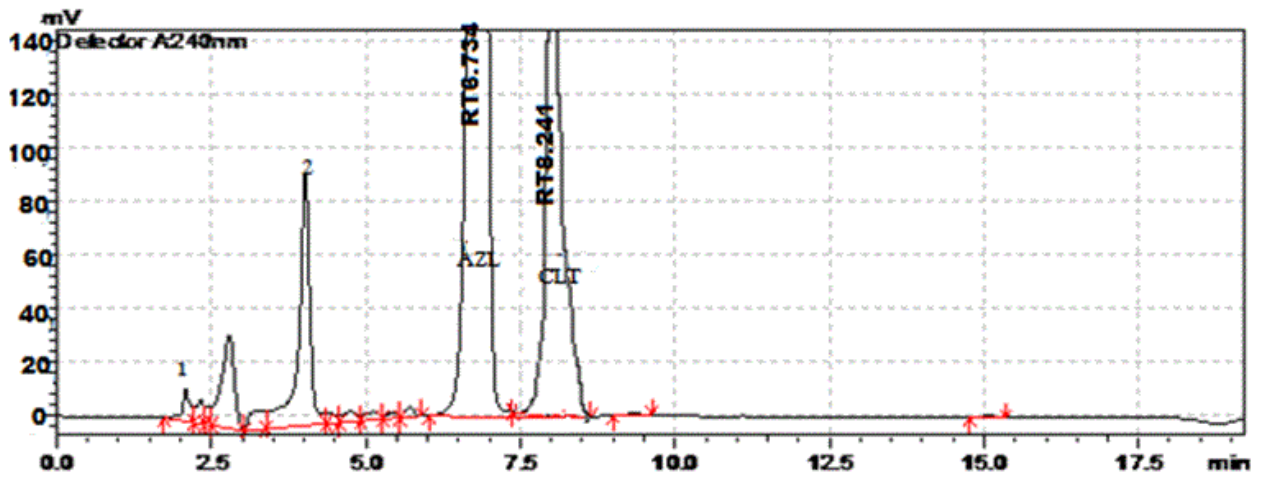


Figure 7. Base degradation chromatogram

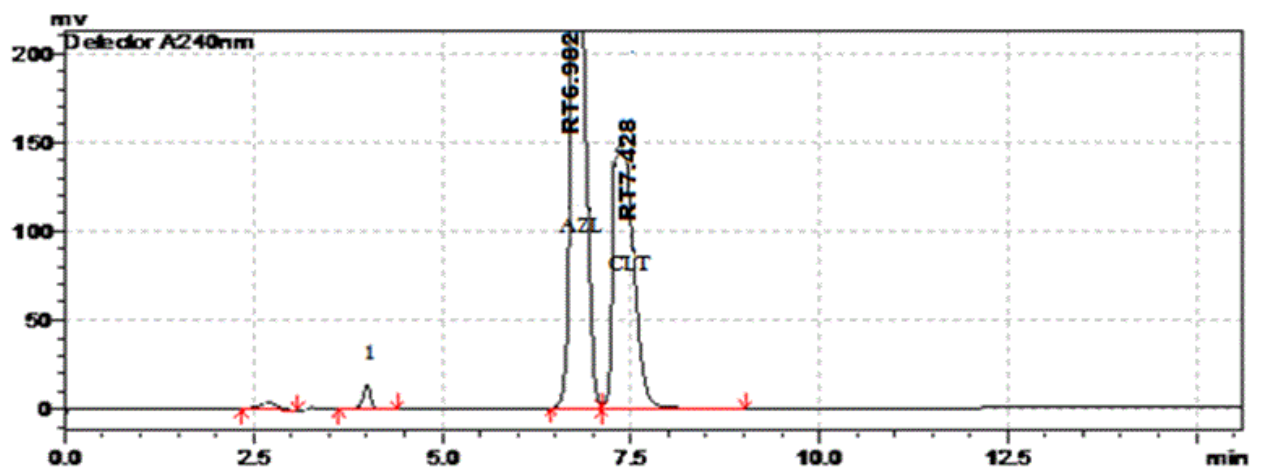


Figure 8. Oxidative degradation chromatogram.

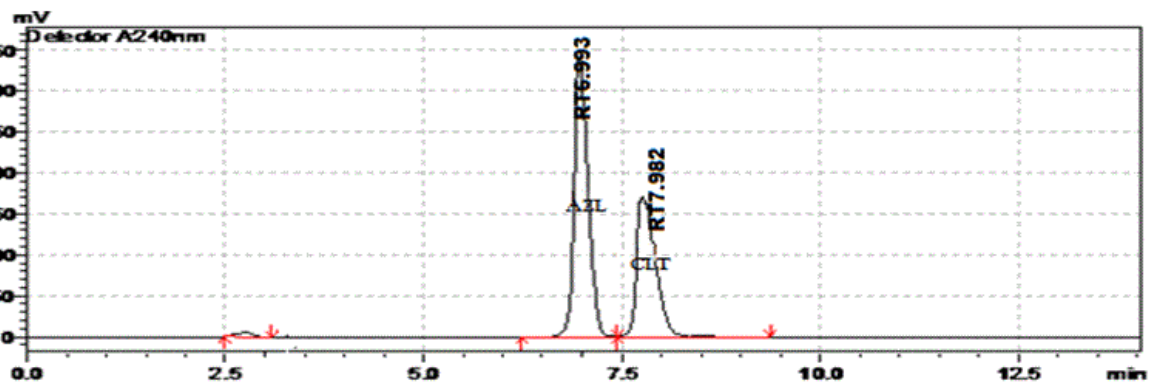


Figure 9. Thermal degradation chromatogram.



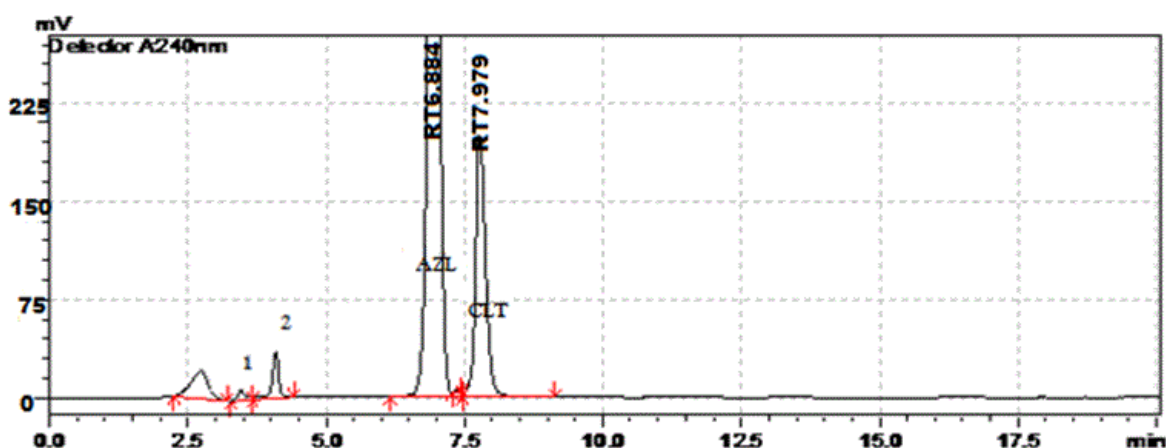


Figure 10. Photolytic degradation chromatogram.

Table 11. Forced degradation studies of Chlorthalidone and Azilsartan Medoximil.

Stress Conditions	Duration	% Drug recovered	% Drug decomposed	Retention time (min)	Theoretical plates	Tailing factor
Chlorthalidone						
Control Sample	---	99.76	---	7.748	4287	1.1
Acid Degradation	6 h	99.26	0.45	7.812	4596	1.15
Alkaline Degradation	6 h	99.29	0.46	8.241	3990	1.05
Oxidative Degradation	2 h	99.39	0.31	7.428	4261	1.42
Thermal Degradation	2 days	99.67	0.06	7.982	3986	1.34
Photolytic Degradation	10 days	98.92	0.82	7.979	4984	1.11
Azilsartan Medoximil						
Control Sample	---	99.65	---	6.982	6899	1.08
Acid Degradation	6 h	98.01	1.64	6.998	6483	1.12
Alkaline Degradation	6 h	82.79	16.43	6.734	6244	1.13
Oxidative Degradation	2 h	97.08	2.52	6.982	6534	1.11
Thermal Degradation	2 days	99.26	0.17	6.993	6623	1.09
Photolytic Degradation	10 days	98.56	1.08	6.884	6834	1.11

## Conclusion

The proposed method for the simultaneous estimation of Azilsartan Medoximil and Chlorthalidone validated as per the ICH guidelines and it is simple, specific and reliable. The data generated from the forced degradation studies enabled the evaluation of Azilsartan Medoximil and Chlorthalidone stability under a variety of ICH recommended conditions. These data are valuable for the safety and potency assessment of a drug product. Furthermore, this simple and rapid RP-HPLC method can also be used successfully for the determination of Azilsartan Medoximil and Chlorthalidone in

pharmaceutical formulations without any interference from the excipient.

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**\*Correspondence to**

T. Hemant Kumar

Department of Pharmaceutical Analysis and Quality Assurance

Vignan Institute of Pharmaceutical Technology

Visakhapatnam

India