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Original Article

Stability Indicating Method Development and Validation for the Simultaneous Estimation of Azilsartan and Chlorthalidone in their Bulk and Pharmaceutical Dosage Forms by RP-UPLC

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ABSTRACT

Aim: The present study is aimed towards developing and validating novel RP-UPLC analytical method for the analysis of Azilsartan and Chlorthalidone in their pharmaceutical formulations and to carry out stability studies by forced degradation to ascertain the stability of selected drugs. The proposed method was challenging as the method used in the study is novel for the estimation of antihypertensive drugs. **Materials and Methods:** The solubility studies of Azilsartan and Chlorthalidone were carried out at 25°C using various solvents and buffers. The solubility studies revealed that the selected drugs were soluble in methanol, water and acetonitrile. Hence, a mobile phase system consisting of Acetonitrile: Water: Methanol in the ratio of 20: 40: 40 v/v/v is used. The analysis of the drugs is accomplished using Phenomenex C18 column (4.5 μm, 250 × 2.2 mm ID) at 258 nm. **Results and Discussion:** The method developed is then validated according to the guidelines of ICH. The analysis results reveal that, method developed is within the acceptance limits for all the validated parameters. The forced degradation studies revealed that 9.9% of Azilsartan degradation is observed in peroxide presence and 5.6% of Chlorthalidone degradation is observed in the presence of acid solution. **Summary and Conclusion:** From the results of the study for the selected drugs, the sample recoveries in all the formulations were within the limits, hence the proposed method can be conveniently and easily adopted to the determination of Chlorthalidone and Azilsartan in combined formulation.

Keywords: Liquid Chromatography, Azilsartan, Forced degradation, Validation, Chlorthalidone.

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INTRODUCTION

Azilsartan (Figure 1) is an antihypertensive agent is an antagonist of the receptor Angiotensin-II that decreases blood pressure.[1-3] Chlorthalidone (Figure 2) is a diuretic drug from the thiazide diuretics category which is chemically named as 2-chloro-5(2,3-dihydroxy-3-oxo-1*H*-isoindol-1-yl)benzenesulfonamide inhibits sodium and chloride ions reabsorption in the distal convoluted tubule by blocking the Na^+/ Cl^- symporter.[4-6] The present study is aimed towards the development and followed by validation of Azilsartan and Chlorthalidone in their pharmaceutical and bulk formulations using RP-UPLC method which is relatively a new technique.[7-9] The literature survey on selected drugs reveals that only HPLC (High Performance Liquid Chromatography) analysis is carried out so far hence the present work is focused towards developing a new method for the determination of Chlorthalidone and Azilsartan using UPLC. The results of the analysis were shown to be within the acceptable limits for all the validated parameters and the drugs were found to be stable when subjected to various stress conditions.

MATERIALS AND METHODS

Azilsartan is procured from Jigs Chemical Limited, Ahmedabad, India and Chlorthalidone from Jai Radhe Sales, Ahmedabad, India. Methanol, Water and Acetonitrile were purchased from E. Merck Limited (Mumbai). H_2O_2 (Hydrogen peroxide) and HCl (Hydrochloric acid) are supplied from Himedia Labs Mumbai, India and Loba Chemie, Mumbai, India respectively. UPLC of Agilent 1290 Infinity II make with DAD (Diode Array Detector), Shimadzu/AY220 Analytical balance, ultrasonicate water bath from PCI Analytics-Mumbai and pH meter of Digisun electronics/2001 were used in the study.

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Chromatographic conditions

The determination of Chlorthalidone and Azilsartan were achieved with the help of stationary phase as Phenomenex C_{18} (250 × 2.2 mm ID, 4.5 μm) column with isocratic elution using Acetonitrile:Methanol:Water (20:40:40 v/v/v) as solvent. 1.0 mL/min is the flow rate set with a run time of 10 min. The wavelength for detection is set at 258 nm with the temperature of the column is set at 25°C. 20 µL is the injection volume used for the determination.

Azilsartan standard (stock) solution preparation

Azilsartan (10.0 mg) is transferred to the volumetric flask (100 mL) containing minimum quantity of methanol and when the Azilsartan dissolved completely, the flask is filled to the mark using methanol. From this solution, dilute solution of Azilsartan is prepared by mixing 1 mL to 10 mL using methanol to get 10 µg/mL concentration.

Preparation of Chlorthalidone standard stock solution

Chlorthalidone (10.0 mg) is carefully transferred after weighing to volumetric flask (100 mL) and having minimum quantity of methanol and then the flask is filled to 100 mL using methanol. 10 µg/mL solution is prepared from the above solution by diluting 1 mL to 10 mL using methanol.

Preparation of mobile phase

20 volumes of acetonitrile, 40 volumes of methanol, and 40 volumes of water are combined to create the combination. For the purpose of degassing the mobile phase, the mobile phase has been sonicated for 10 min in order to remove gases.

Method validation

Linearity

Azilsartan (50.0 mg) and Chlorthalidone (12.5 mg) is weighed accurately and dissolved in volumetric flask of 100 mL with 70 mL of solvent and flask is filled to the mark using solvent. The concentrations of 25, 32.5, 50, 62.5, 75 µg/mL and 6.25, 9.38, 12.5, 15.625, 18.75 µg/mL were prepared for Azilsartan and Chlorthalidone respectively for linearity studies.

Accuracy

Recovery studies tend to be conducted to measure the reliability of the technique. The formulation (preanalyzed sample) involves additions of the pharmaceutical reference standards at levels that include 50%, 100%, and 150%. Following three recovery measurements, the drug's recovery percentage and % mean recovery was calculated and tallied. In order to verify the exactness of the method, recoveries studies were performed by incorporating standard drug solution to preanalyzed solutions of samples at three distinct concentrations: 50%, 100%, and 150%.

Method precision

Chlorthalidone and Azilsartan sample preparations were made in compliance with the test technique and administered 6 times to the column.

Preparation of mixed standard solution

Accurately weighed Azilsartan (50.0 mg) and Chlorthalidone (12.5 mg) in a volumetric flask of 100 mL, which were then dissolved in 70 mL of solvent and the remaining capacity was filled to the mark using mobile phase. By dilution with mobile phase of 5 mL to 50 mL of this stock, Azilsartan (50 µg/mL) and Chlorthalidone (12.5 µg/mL), respectively, are prepared.

Sample solution preparation

Five tablets, each containing Chlorthalidone (12.5 mg) and Azilsartan (50.0 mg), were weighed, and ground to a powder in the mortar, and then combined thoroughly. 12.5 mg of Chlorthalidone, and 50.0 mg of Azilsartan were dissolved in 70 mL of solvent by 30 min of ultrasonication. The sample was then centrifuged at 5000 rpm for the period of 10 min. By dilution with mobile phase of 5 mL to 50 mL of this stock solution, Chlorthalidone (12.5 µg/mL) and Azilsartan (50 µg/mL), respectively, are prepared.

System Suitability

Chlorthalidone at a concentration of 12.50 µg/mL and Azilsartan at a concentration of 50 µg/mL are injected into test subjects 6 times, and for each injection the chromatograms were recorded to ascertain that the analytical method is functioning properly and thus can produce exact and reproducible findings.

Robustness

Variations in chromatographic conditions

To show the method's robustness, solutions were injected under a variety of changing conditions, including temperatures of 20°C and 30°C and wavelengths of 235 and 245 nm. Parameters for system appropriateness and procedure precision were compared.

Forced degradation Studies

A procedure known as forced degradation involves subjecting a medicinal product to varied stresses, which causes a variety of degradation products to be produced. These studies are sometimes named to be stress test and stress degradation studies. These methods are mostly employed for determining a molecule's stability under time-sensitive conditions. In the present study, the selected compounds were subjected to stress conditions like Thermal degradation, Photolytic degradation, Acid degradation, Base degradation and Peroxide degradation.

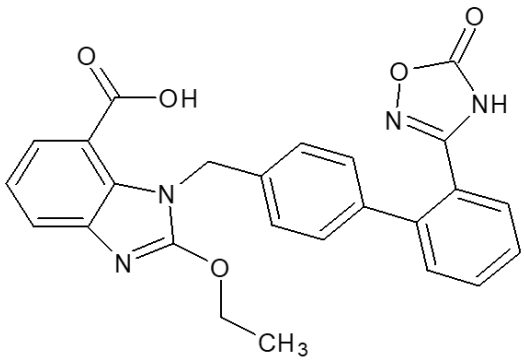

Figure 1: Azilsartan structure.

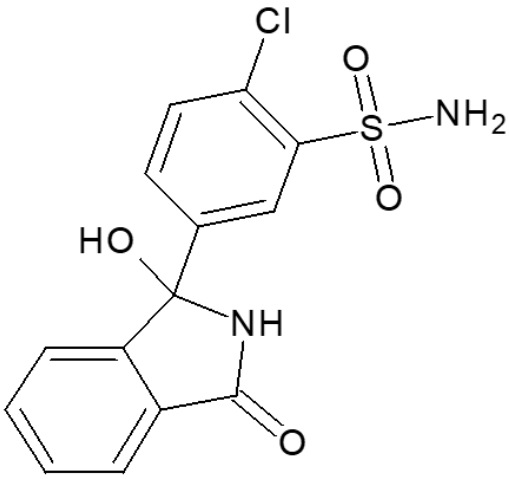

Figure 2: Chlorthalidone structure.

RESULTS

In the present work, a novel RP-UPLC method is developed for the simultaneous estimation of Azilsartan and Chlorthalidone. The method employed uses fewer organic solvents and maintain a minimal mobile phase without the addition of buffer salts or pH correction. Chlorthalidone's retention time was determined to be 5.624 min, while Azilsartan's was 2.935 min (Figure 3). The created approach was validated using ICH Q2 (R1) guidelines to see if it was accurate for analysing Azilsartan and Chlorthalidone.

Method development

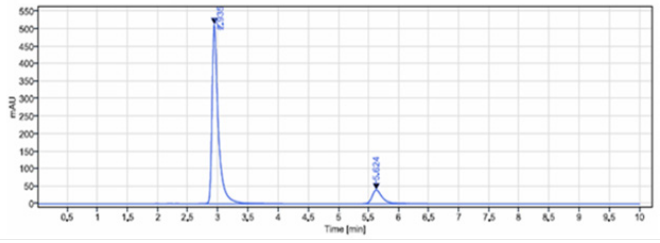

Figure 3: Chromatogram of Chlorthalidone and Azilsartan.

Method validation

Linearity

A linear relationship was exhibited in the calibration curve between the area of peak and concentration ranging from 20-100 µg/mL. Chromatograms of five preparations have been obtained as shown in Figures 4, 5, 6, 7, and 8. Linear function gave the best correlation (0.999 ± 0.002).

Accuracy

Accuracy was estimated as percentage recovery at each addition level and was calculated as the difference between the measured and theoretical value. The % average recovery was determined as 99.913% for Azilsartan (Table 1) and 100.546% for Chlorthalidone (Table 2).

Method precision

The average % assay of six injections in triplicate found to be 99.6% for Azilsartan and 99.2% for Chlorthalidone (Table 3). The %RSD is found to be less than 2.0%.

System suitability

The percentage RSD for peak area is found to be 0.05 for Azilsartan (Table 4) and 0.35 for Chlorthalidone (Table 5). The other factors such as theoretical plates and tailing factor is found to be within the limits.

Robustness

The percentage RSD for two different parameters such wavelength (235nm and 245nm) and temperature (20°C and 30°C) is found to be less than 2.0% as shown in Table 6.

Forced degradation studies

The compounds were subjected to stability indicating studies under a variety of stress conditions, including thermal (Figure 9), photolytic (Figure 10), acid (Figure 11) and alkali degradation (Figure 12). The results in all conditions show that Azilsartan degraded by 9.9% in peroxide degradation and Chlorthalidone degraded by 5.6% in acid degradation.

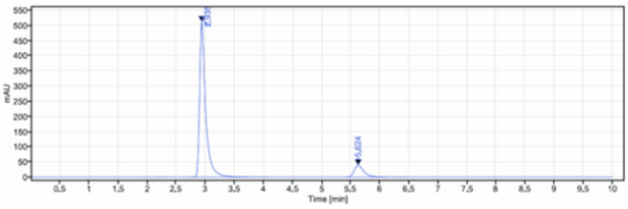

Figure 4: Chromatogram of Chlorthalidone and Azilsartan (Preparation-1).

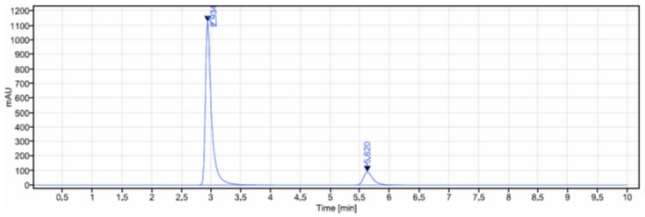

Figure 5: Chromatogram of Chlorthalidone and Azilsartan (Preparation-2).

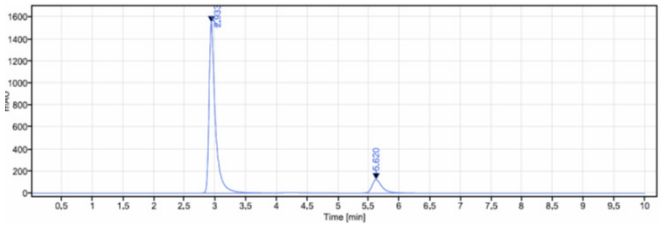

Figure 6: Chromatogram of Chlorthalidone and Azilsartan (Preparation-3).

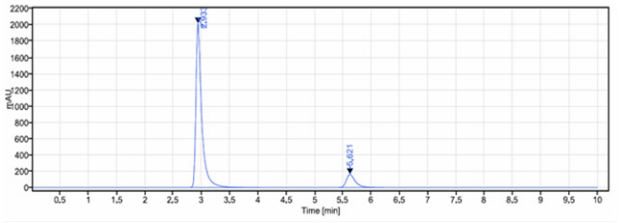

Figure 7: Chromatogram of Chlorthalidone and Azilsartan (Preparation-4).

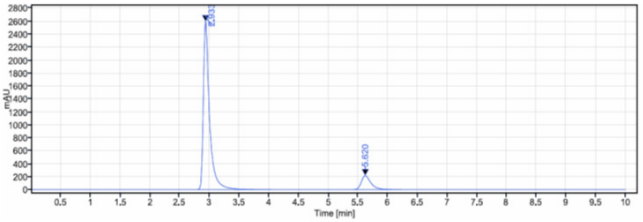

Figure 8: Chromatogram of Chlorthalidone and Azilsartan (Preparation-5).

Accuracy

Recovery results for Azilsartan

Table 1: **Azilsartan recovery results.**

Recovery Level	Accuracy of Azilsartan					Average % Recovery
	Amount taken (mcg/mL)	Area	Average area	Amount recovered (mcg/mL)	Percentage Recovery	
50%	25	5509.92	5484.24	24.65	98.60	98.13
	25	5451.81		24.39	97.56	
	25	5491.01		24.56	98.24	
100%	50	11302.35	11324.84	50.56	101.12	101.32
	50	11345.77		50.76	101.52	
	50	11325.64		50.67	101.34	
150%	75	16938.56	16869.52	75.78	100.04	100.29
	75	16825.03		75.27	100.36	
	75	16844.99		75.36	100.48	
SD						1.628015
%RSD						1.6294

Recovery results for Chlorthalidone

Table 2: Chlorthalidone recovery results.

Recovery Level	Accuracy of Chlorthalidone					Average % Recovery
	Amount taken (mcg/mL)	Area	Average area	Amount recovered (mcg/mL)	%Recovery	
50%	6.25	740.41	739.35	6.31	100.96	100.85
	6.25	737.96		6.29	100.64	
	6.25	739.68		6.31	100.96	
100%	12.5	1485.35	1486.77	12.67	101.36	101.44
	12.5	1487.24		12.68	101.44	
	12.5	1487.76		12.69	101.52	
150%	18.75	2185.92	2184.86	18.64	99.41	99.35
	18.75	2184.92		18.63	99.36	
	18.75	2183.75		18.62	99.30	
SD						1.077512
%RSD						1.0717

Method precision

Results for method precision

Table 3: Method precision results of Azilsartan and Chlorthalidone.

Injection	Azilsartan	Chlorthalidone
	% Assay	% Assay
1	99.2	99.6
2	100.1	100.2
3	100.2	99.6
4	99.4	98.7
5	99.6	98.7
6	99.2	98.1
Average	99.6	99.2
SD	0.4	0.8
%RSD	0.9	0.8

System suitability

Table 4: Azilsartan system suitability results.

Injection	Retention time	Peak area	Theoretical plates	Tailing factor
1	2.932	11173.05	20141	1.31
2	2.932	11185.68	20951	1.28
3	2.933	11181.99	20632	1.27
4	2.933	11175.29	20415	1.29
5	2.93	11171.9	20362	1.28
6	2.931	11172.04	20852	1.27
Mean	2.932	11176.66	-	-
SD	0.00	5.81	-	-
%RSD	0.04	0.05	-	-

Table 5: Chlorthalidone system suitability results.

Injection	Retention time	Peak area	Theoretical plates	Tailing factor
1	5.62	1469.15	25141	1.23
2	5.621	1470.2	25112	1.22
3	5.624	1455.98	25852	1.21
4	5.623	1467.12	25362	1.26
5	5.621	1467.75	25136	1.25
6	5.62	1465.52	25941	1.21
Mean	5.622	1465.95	-	-
SD	0.00	5.15	-	-
%RSD	0.03	0.35	-	-

Robustness

Table 6: Azilsartan and Chlorthalidone robustness results.

Chromatographic Changes		Theoretical Plates		Tailing factor			
		Azilsartan	Chlorthalidone	Azilsartan	Chlorthalidone	Resolution	%RSD for 5 replicate injections
Wavelength (nm)	235	20141	25614	1.31	1.33	7.54	0.31
	245	20425	24984	1.32	1.24	7.56	0.33
Temperature (°C)	20	20542	24890	1.30	1.22	7.54	0.32
	30	20515	24875	1.30	1.31	7.54	0.33

Forced degradation studies

Thermal degradation

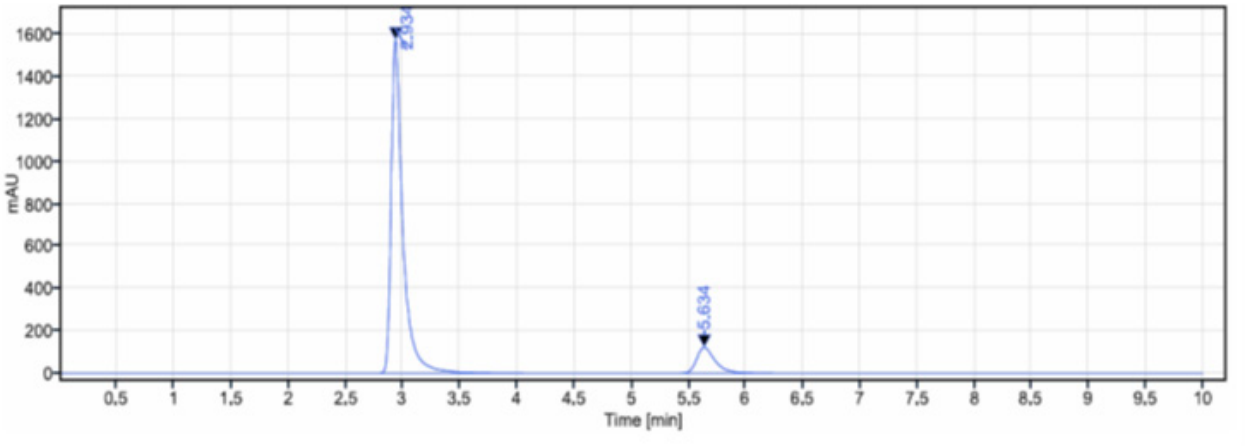

Figure 9: Chromatogram of thermal sample (105°C/48Hrs).

Photolytic degradation

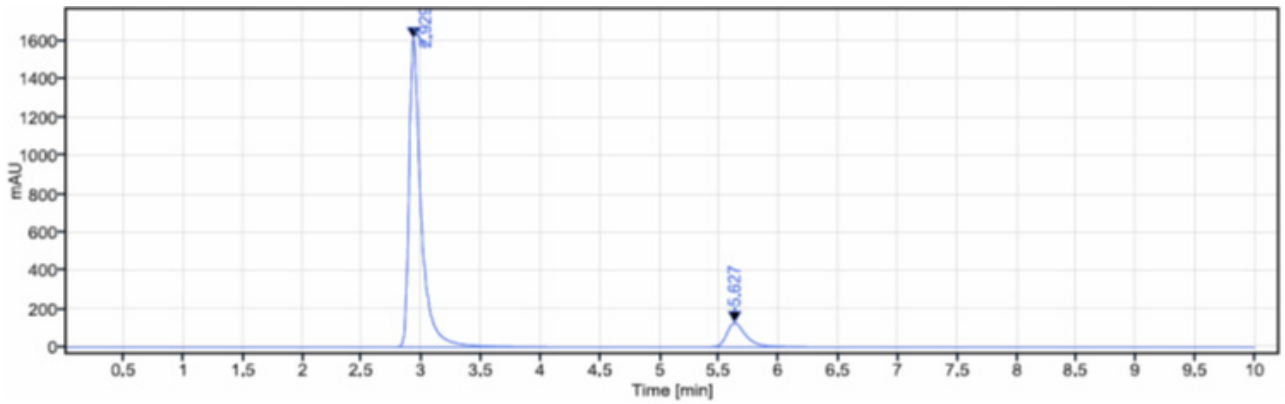

Figure 10: Chromatogram of photolytic sample (1.2mil LUX hrs).

Acid degradation

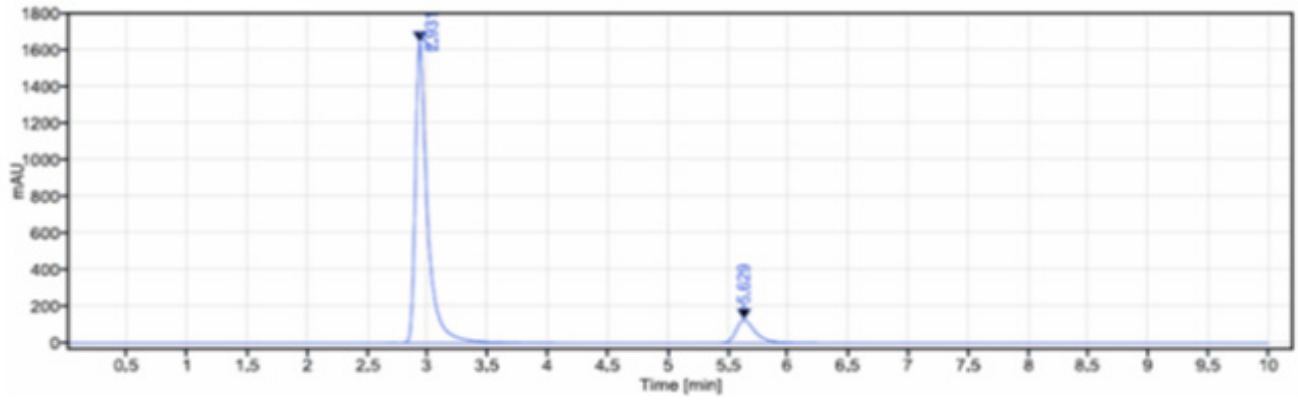

Figure 11: Chromatogram of acid sample preparation (5N HCl/4Hrs/60°C).

Alkali degradation

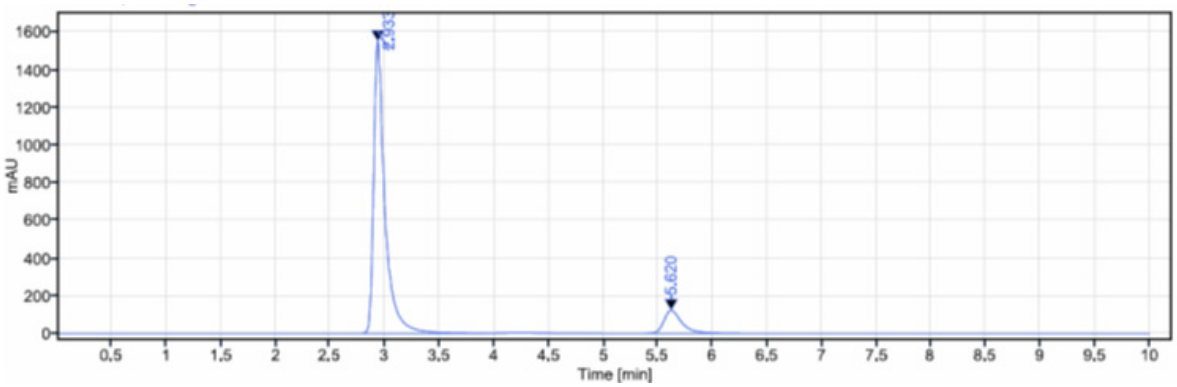

Figure 12: Chromatogram of base sample preparation (5N NaOH/4Hrs/60°C).

DISCUSSION

For conventional formulations of Chlorthalidone and Azilsartan, the correlation coefficient for the linearity produced between concentration versus area is 0.9981 and 0.9988, respectively. It was discovered that the correlation coefficient is within acceptable bounds and that the relationship between the concentration of Azilsartan and Chlorthalidone and the area of Azilsartan and Chlorthalidone is linear throughout the range investigated. Azilsartan and chlorthalidone have mean percentage recovery rates of 100.0% and 100.6%, respectively. Azilsartan and chlorthalidone recovery data demonstrate that the assay results' %RSD is within acceptable bounds. Azilsartan and chlorthalidone percentage assays ranged from 90.0 to 110.0%. The procedure was tough, as evidenced by the ruggedness results, which reveal that the %RSD between two analysts' measurements is not larger than 2.0%. The chosen compounds were subjected to stability indicating studies under a variety of stress conditions, including thermal, peroxide, acid, alkali, and peroxide degradation. The results in all conditions show that Azilsartan degraded by 9.9% in peroxide degradation and chlorthalidone degraded by 5.6% in acid degradation. The main analyte's peak purity was also passed, demonstrating the effectiveness of this method as a stability indicating method.

SUMMARY AND CONCLUSION

As per the findings of the study for the chosen combination, sample recoveries in all formulations were in line with the claims made on each drug's label, and the suggested method was found to be accurate, sensitive, quick, and affordable for the detection of Azilsartan and Chlorthalidone in combined pharmaceutical formulations. Satisfactory findings were obtained after validating the suggested approach in accordance with guidelines of ICH and comparing the acquired values with the standard values. As a result, the technique can be used to determine the combination dosage form of Chlorthalidone and Azilsartan.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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