www.ijarp.com ISSN 2456-9992 Page: 01-06

International Journal Advanced Research Publications

Review Article

Volume: 02
Issue: 01

METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AZILSARTAN MEDOXOMIL AND CHLORTHALIDONE BY HPLC

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Article Received: 16 December 2025, Article Revised: 04 January 2026, Published on: 24 January 2026

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DOI: https://doi-doi.org/101555/ijarp.5810

ABSTRACT:

A simple, rapid, precise, accurate, and robust RP-HPLC method was successfully developed and validated for the simultaneous estimation of Azilsartan medoxomil and Chlorthalidone in tablet dosage form. The optimized chromatographic conditions provided good resolution. The developed method was effectively applied to the analysis of marketed tablet formulations, yielding satisfactory assay results without interference from formulation excipients. Therefore, the proposed RP-HPLC method can be reliably employed for routine quality control, stability studies, and batch release analysis of Azilsartan medoxomil and Chlorthalidone in pharmaceutical dosage forms.

KEYWORDS: Azilsartan, Medoxomil, Chlorthalidone, HPLC, Methods Development, Validation.

INTRODUCTION:

Method Development

Analytical chemistry is a branch of chemistry which deals with identification of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and another one is quantitative analysis. In qualitative analysis, there is identification of components or analyte of mixture or sample is carried out. In quantitative analysis, there is determination of amount of components or analyte of mixture or sample is carried out

(Kenkel, 2003). Analytical data is required not only in chemistry but also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space exploration and clinical diagnosis. Important areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, geological assays, fundamental and applied research (Kissinger, 2002).

Development of Analytical Methods

When there are no definitive techniques are present, new methodologies are being progressed for evaluation of the novel product. To investigate the presence of either pharmacopoeial or nonpharmacopoeial product novel techniques are developed to reduce the value besides time for higher precision and strength. These methodologies are optimized and valid through preliminary runs. Alternate ways are planned and place into practice to exchange the present procedure within the comparative laboratory information with all accessible merits and demerits.

Characterization of the standard and analyte

- All the known necessary data concerning the analyte and its structure that is to mention the physical and chemical properties such as solubility, optical isomerism, etc., are collected.
- The standard analyte is equal to 100% purity is acquired. Necessary arrangement is to becreated for the proper storage (refrigerator, desiccators, and freezer).
- In the sample matrix, when multiple parts are to be measured the amount of elements is observed duly presenting the information and the accessibility of standard are calculated.

Literature Review:

Zambre *et al.*, (2025) study aimed to establish and optimize a HPLC method for the accurate and stability-indicating quantification of Azilsartan Medoxomil Potassium (AZM) and its impurities in the active pharmaceutical ingredient (API) and formulated drug products. The validated method demonstrated high accuracy, precision, and sensitivity, with a linear response range of 10–50 µg/mL and limits of detection and quantification as low as 0.00607 and 0.01841 ng/mL, respectively. Forced degradation studies confirmed the method's selectivity and stability-indicating capabilities by identifying distinct degradation products under various stress conditions, including acidic, basic, oxidative, and photolytic environments. The validated HPLC method was successfully applied to a commercial AZM

formulation, yielding assay values within acceptable limits for quality control.

Bhasagi *et al.*, (2024) developed and validated an HPLC method for analysing azilsartan in drugs and various formulations that demonstrated stability by ICH (International Conference on Harmonization) standards. The mobile phase uses methanol: phosphate buffer (0.1% orthophosphoric acid, pH 3.2) (70:30), having the chromatographic separator is an HPLC column C18 (4.6 mm X 250 mm) with a wavelength of 249 nm. and a flow rate of 1 mL/min. The developed method showed a correlation coefficient value is 0.999 and to be linear throughout a concentration range of 2-10 µg/mL. The proposed method was precise (percent RSD 2.0%), accurate (percent recovery 99-101%), and reliable. The detection and quantification limits for azilsartan were determined to be 0.01 µg/mL and 0.04 µg/mL, respectively.

Pagare *et al.*, (2024) analytical method developed and validated of azilsartan medoxomil in bulk and pharmaceutical dosage form. An RP-HPLC technique with high sensitivity and precision has been devised to accurately determine the concentration of Azilsartan Medoxomil in its bulk formulation. The maximum wavelength (λmax) of Azilsartan Medoxomil was determined to be 248 nm in a 10 mM Ammonium acetate buffer with a Methanol solution at a ratio of 60:40 % v/v. The pH level is 3. The approach demonstrates a high level of sensitivity, with a linear range of 5 to 25µg/ml. The regression equation for this range is y = 6572.6x + 19652, with a r^2 value of 0.9994. The detection limit and quantitation limit were determined to be 0.08 µg ml^{-1} and 0.26 µg ml^{-1}, respectively.

Experimental Work and Result:

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 2-10□g/ml for AZM and 1-5□g/ml for CTD were prepared. All the solution were filtered through 0.45□m membrane filter and injected, chromatograms were recorded at 254.0 nm and it was repeat for five times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived.

Table 1: Linearity of AZM

Standard Concentration	Area under Curve (AUC)						Mean
	Rep-1	Rep-2	Rep-3	Rep-4	Rep-5	Rep-6	
2	1156.65	1150.36	1145.58	1140.36	1174.32	1160.36	1154.612
4	2215.32	2210.25	2217.85	2216.65	2218.85	2217.74	2216.116

6	3348.65	3345.69	3340.36	3374.65	3382.25	3386.65	3363.049
8	4365.85	4355.69	4245.58	4365.65	4358.78	4350.23	4340.303
10	5565.58	5560.36	5558.96	5546.69	5552.36	5574.12	5559.686
Correl Coeff (r^2)							0.9995
Slope (m)							550.03
Intercept (c)							22.13

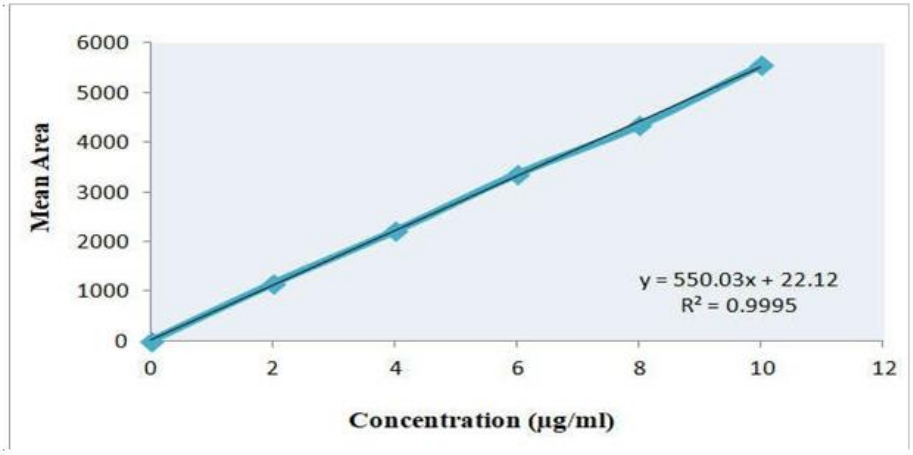

Figure 1: Calibration Curve of AZM.

Table 2: Linearity of CTD

Standard Conc. (□g/ml)	**Area under Curve (AUC)**						**Mean**
	Rep-1	**Rep-2**	**Rep-3**	**Rep-4**	**Rep-5**	**Rep-6**	
1	589.985	585.658	583.369	587.745	575.658	1160.365	680.463
2	1115.65	1120.325	1115.698	1130.369	1140.258	1140.336	1127.106
3	1589.985	1580.369	1587.745	1578.956	1570.369	1570.369	1579.632
4	2045.658	2040.369	2050.587	2040.369	2047.785	2039.987	2044.126
5	2574.658	2570.369	2565.987	2560.357	2563.321	2563.332	2566.337
Correl. Coeff (r^2)							0.9954
Slope (m)							496.43
Intercept (c)							91.858

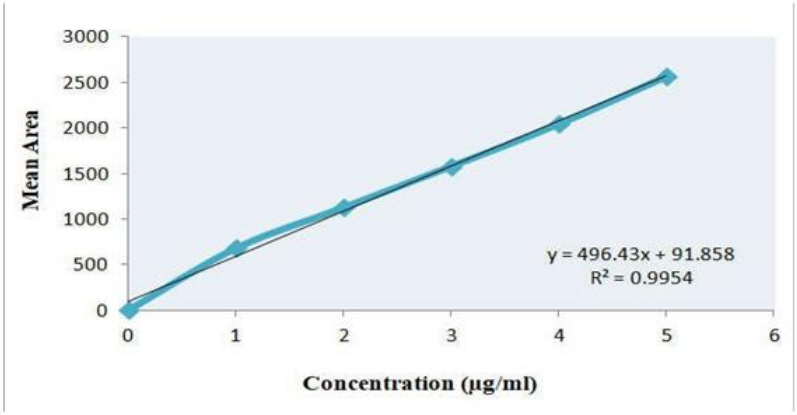

Figure 2: Calibration Curve of CTD

CONCLUSION

The present work was aimed at the development and validation of a simple, accurate, precise, and robust RP-HPLC method for the simultaneous estimation of Azilsartan medoxomil (AZM) and Chlorthalidone (CTD) in marketed tablet formulations. Initial preformulation studies such as physical characterization, melting point determination, and solubility analysis confirmed the identity and physicochemical properties of both drugs and were found to be in agreement with reported literature values.

Chromatographic separation was achieved using a Thermo C18 column (250 mm × 4.6 mm, 5 µm) with an isocratic mobile phase consisting of 20 mM potassium dihydrogen phosphate buffer and acetonitrile (20:80 v/v), adjusted to pH 3.0 with orthophosphoric acid. The flow rate was maintained at 1.0 mL/min with UV detection at 254 nm. Under optimized conditions, AZM and CTD were well resolved with retention times of 2.553 ± 0.002 min and 4.885 ± 0.001 min, respectively, without interference from excipients.

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