IJBPAS, December, 2025, 14(12): 6217-6229

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

UV SPECTROPHOTOMETRIC QUANTIFICATION OF AZILSARTAN PHARMACEUTICALS EMPLOYING MULTIVARIATE CALIBRATION TECHNIQUE

ABDULLAH KHAN AK, SEETHARAMAN R*, MANIKANDAN K

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu - 603203, India

***Corresponding Author: Dr. Seetharaman R: E Mail:** seetharr@srmist.edu.in; seerampharm@gmail.com

Received 15th July 2024; Revised 20th Sept. 2024; Accepted 14th Nov. 2024; Available online 1st Dec. 2025

https://doi.org/10.31032/IJBPAS/2025/14.12.8350

ABSTRACT

In order to accurately determine the concentration of Azilsartan medoxomil in formulations and bulk, the current study advises developing a novel approach and evaluating it. This analytical process can be summarized as sensitive, precise, and rapid. With the aid of a UV spectrophotometer and a multivariate calibration technique, the analytical approach was carried out. The relationship between concentration and absorbance, established at five different wavelengths, including the highest peak absorbance at 250 nm, was used to establish this multivariate calibration technique with methanol as blank. Using the linear regression equations and other mathematical and statistical techniques, the strategy was verified using the ICH Q2 (R1) criterion. Accuracy, linearity, and other validation criteria listed in the instructions have all been attained. When used for periodic evaluations of drugs and formulations, this technology is sensitive, cost-effective, and yields reliable results.

Keywords: Azilsartan medoxomil, Multivariate calibration technique, UV spectrophotometric, Pharmaceutical formulations, ICH guidelines, Validation

INTRODUCTION:

Azilsartan medoxomil, A receptor inhibitor for the angiotensin II. (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]. salt of methyl in monopotassium 1H benzimidazole carboxylate. It is a white powder, crystalline solid that easily dissolves in solvents such as methanol, dimethyl formamide, dimethyl sulfoxide and but practically insoluble in water. Additionally, it is barely soluble in Tetrahydrofuran and 1-octanol and soluble in acetic acid, acetone, and acetonitrile on an intimate scale **[1]**. Since 2011, the Food and Drug Administration (FDA) states that Azilsartan may be used to treat high blood pressure. Azilsartan may be used to treat hypertension, according to the Food and Drug Administration (FDA). It is a pro-drug that instantly undergoes ester hydrolysis in the gastrointestinal tract and plasma to produce the active molecule **[2]**. Azilsartan has an empirical formula of $C_{30}H_{23}KN_4O_8$ **[3]** with a molecular weight of 606.62 g / mol. and depicted in **Figure 1**.

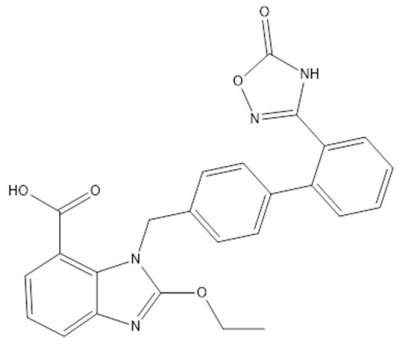

Figure 1: Structure of Azilsartan medoxomil

On February 25, 2011, the FDA approved the Edarbi® tablet for the treatment of adult hypertension **[4]**. To counteract the pressor effects of angiotensin II, azilsartan medoxomil specifically blocks angiotensin II's binding to angiotensin type 1 receptor **[5]**. The analysis of the literature reveals that a number of methodologies have been published for finding Azilsartan medoxomil in biological or pharmacological formulations. Several chromatographic methods, including HPLC **[1, 3, 5-9]**, spectrophotometric methods like UV **[10]**, HPTLC **[11]**, and LC–MS/MS **[2, 12]**, have been used to analyze the drug azilsartan medoxomil. The Multivariate calibration (MVC) technique utilizing UV spectrophotometry, which hasn't been described, was mentioned in the literature review. The present method hence focuses on the creation of the MVC method for the quantification of Azilsartan medoxomil. To reduce instrumental error and boost efficiency, the MVC method was applied. The method utilizes pharmaceutical dosage forms and is simple and affordable. MVC uses straight regression techniques with wavelengths of between 5 and 10 nm for precise results **[13]**. For choosing azilsartan medoxomil in pharmaceutical amount forms, we discussed using a UV spectrophotometric MVC technique with less mathematical content in this paper. Five different wavelengths were Consequently, it was chosen to assure the sensitivity in comparison to the traditional UV approach. The following equations **[14]** transform multivariate statistics from MVC into

univariate statistics using algorithmic approaches.

The following equations can be produced for each wavelength if the absorbed energy of a sample (x) is measured at various wavelengths (in particular, 246, 248, 250, 252, and 254nm).

$A_{\lambda 246} = a \times C_x + k_1$ ……. (1)

$A_{\lambda 248} = b \times C_x + k_2$ ……. (2)

$A_{\lambda 250} = c \times C_x + k_3$ ……..…... (3)

$A_{\lambda 252} = d \times C_x + k_4$ ……...…. (4)

$A_{\lambda 254} = e \times C_x + k_5$ …….……...…. (5)

A_λ = Absorbance of the sample;

- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The five equations above can be rearranged in the following order:

$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T$……. (6)

Equation (6) can be re-arranged as:

$A_T = C_x (a + b + c + d + e) + K_T$ ……. (7)

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

The concentration of a sample (X) in a solution can be calculated with the equation

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+e)} \quad \text{…….. (8)}$$

MATERIALS AND METHODS:

Chemicals and solvents employed:

- Methanol
- AZTRIC TABLETS– (Label claim – 40 mg of Azilsartan Medoxomil), manufactured by INTAS PHARMACEUTICALS LTD., The authorized tablet came from the nearby market.

Solubility:

- Freely Dissolvable in methanol, and methylene chloride.

Instrumentation:

- UV-Vis double beam Spectrophotometer (Lab India UV-3092).
- Electronic balance (SHIMADZU AY-220H).
- Sonicleansonicator (model 160T, Thebarton-Australia).

Selection of solvent:

- The solvent used to solubilize the substance throughout the analysis, methanol.

Preparation of standard stock solution:

- Accurately weighing and transferring 50 mg of Azilsartan medoxomil into a 50 mL volumetric flask. The mixture was sonicated for 10 minutes with 20mL of methanol. The total volume was filled to 50ml (1000 µg mL^{-1}). After that, the solvent was employed to resulting,

yielding concentrations from 7 to 13 µg mL^{-1}

Determination of λ_{max}:

Over the wavelength between 200 and 400 nm, the Azilsartan medoxomil standardized solutions were examined with methanol as blank, which has an absorption maximum at 250 nm.

METHOD VALIDATION:

According to ICH requirements, the suggested method's linearity, accuracy, and precision were evaluated **[15]**.

Linearity:

In order to get concentrations ranging from 7 to 13 µg mL^{-1} (7, 8, 9, 10, 11,12 and 13), the stock solution was sufficiently diluted with methanol. The linearity and spectrum area of azilsartan medoxomil were then assessed at these doses. The absorbed energy of linearity mixtures at the correct wavelength was assessed for the Multivariant method.

Limit of Detection and Limit of Quantification

The below mentioned formulas were employed to predict the Limits of Detection (LOD) and Limits of Quantification (LOQ) for Azilsartan Medoxomil based on the slope of the calibration curve and the standard deviation of responses for a specific wavelength.

$$LOD = \frac{3.3 \times \text{standard deviation}}{\text{Slope}} \quad \ldots\ldots\ldots (9)$$

$$LOQ = \frac{10 \times \text{standard deviation}}{\text{Slope}} \quad \ldots\ldots (10)$$

Precision

Precision's repeatability was examined using intraday and interday precision. Accuracy levels were determined using a standard Azilsartan medoxomil solution at a concentration of 10 µg mL^{-1}. To assess repeatability, six varying wavelengths were put to the test. For the intervariation scenario, the absorbed energy of prepared mixtures was tested 3 times on the same day, at varying intervals. The absorbance was utilized for three more days to allow for intravariation every day as well as interday.

Accuracy

The Azilsartan Medoxomil methodology's precision was assessed at 80, 100, and 120 percent of the concentrations of the previously examined sample solutions, while the recovery values percentages were estimated.

Assay

Weigh and powder 10 Tablets. Weigh accurately a quantity of the tablet powder equivalent to about 50mg of azilsartan medoxomil, add 25 ml of methanol and sonicate for 10mins. Add sufficient methanol and make up to 50mL. The solution obtained above is filtered and diluted with methanol to attain 10 µg mL^{-1}concentration of Azilsartan medoxomil. By measuring the absorbance of the resulting solution at 250 nm, the

concentration of Azilsartan Medoxomil is identified.

RESULTS AND DISCUSSION:

The first scan range for Azilsartan medoxomil standard solution was 200–400 nm. Azilsartan medoxomil maximal spectrum has a wavelength of 250 nm. The typical spectrum of Azilsartan medoxomil at 10 µg mL^{-1} is shown in **Figure 2**.

Linearity

According to ICH Q2 R1 specifications, the linearity findings for the developed approach for Azilsartan medoxomil were established within the concentration range of 70 to 130 percent for 10 µg mL^{-1} (7 to 13 µg mL^{-1}). **Figure 3** shows the Azilsartan medoxomil spectrum. Calculating the absorbed energy of reference mixtures at five different wavelengths led to the creation of the calibrated curve. The linearity spectrum obtained at 5 different wavelength is described in **Figure 4-8**. The absorbance obtained at different wavelength is tabulated in **Table 1** and the statistical parameters of linearity are tabulated in **Table 2**.

Limit of Detection and Limit of Quantification

The LOD and LOQ for Azilsartan medoxomil were estimated using the slope linearity, and numerous sample studies have backed up this approach. The LOD for Azilsartan medoxomil was discovered using the mean of all absorbed measurements, and it was discovered to be 0.318 µg mL^{-1}. The LOQ for Azilsartan medoxomil was calculated to be 0.963 µg mL^{-1} using the average of all absorbances **(Table 2)**.

Precision

The Azilsartan medoxomil system's precision spectra are displayed **in Figure 9**. The Azilsartan Medoxomil Interday Precision Spectra are shown in **Figure 10**. The intraday precision spectrum for Azilsartan medoxomil is shown in **Figure 11**. For medoxomil Azilsartan, the % RSD of the system's intraday and interday precision was calculated. The precision of the approach was discovered to be less than 2% **(Table 3)**. When compared to the outcomes of the currently employed accuracy methodologies, the findings from the proposed method show a high level of precision.

Accuracy

Table 3 lists the dosages of Azilsartan and medoxomil, and the obtained findings were deemed to it displays the Azilsartan medoxomil overlay spectrum. The outcomes are acceptable. At 80, 100, and 120%, the accuracy of azilsartan medoxomil was evaluated. **Figure 12** shows the overlay spectra of accuracy of Azilsartan medoxomil at 80, 100, 120%.

Assay of marketed formulations:

The amount of Azilsartan medoxomil in the tablet was calculated using the proposed spectrophotometric technique. The UV absorption spectra of a common tablet was

assessed three times. Throughout extraction and filtration, the pharmaceutical formulation's outstanding analytical recovery values remained stable. The results are shown in **Table 4**.

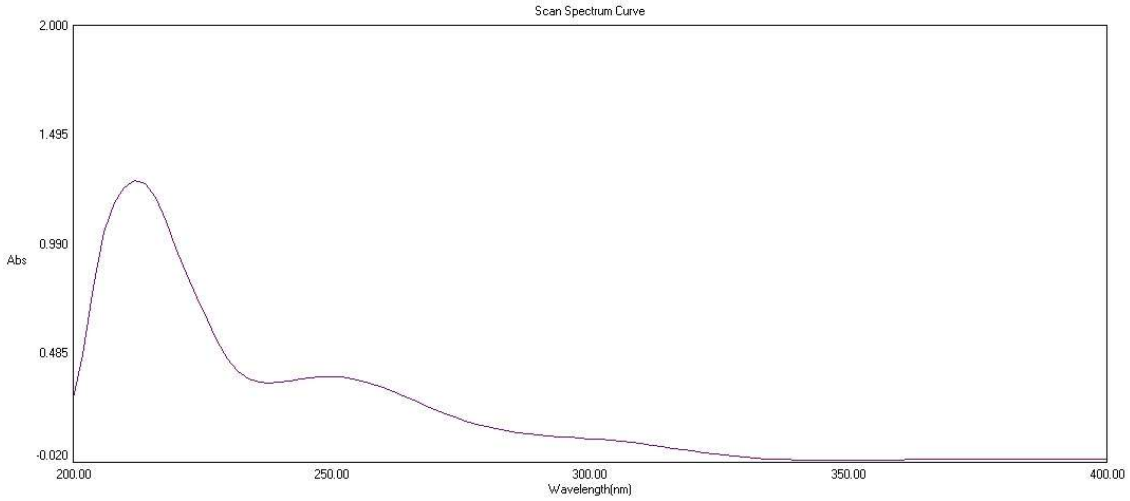

Figure 2: UV spectrum of standard azilsartan (10 µg mL^{-1}) using methanol as blank

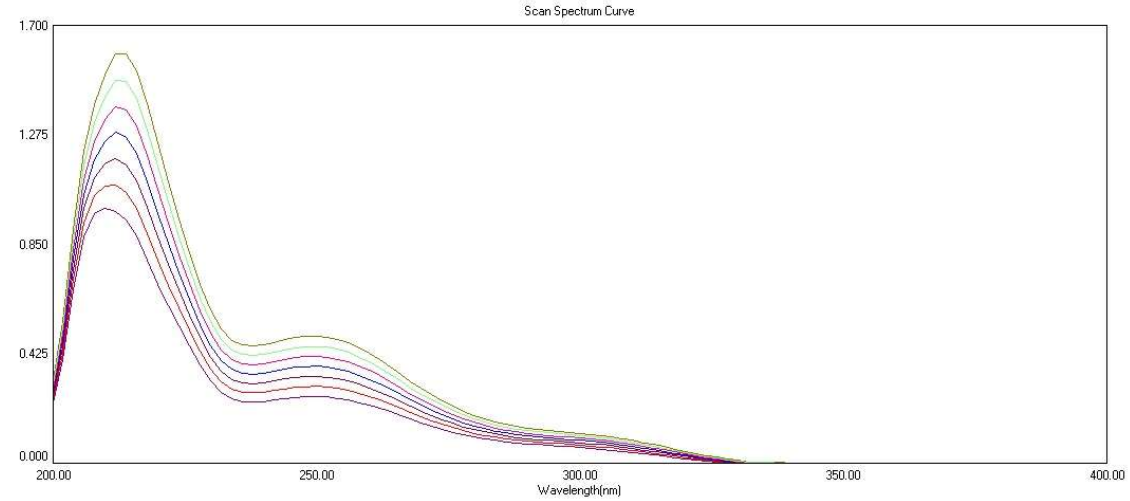

Figure 3: Linearity spectrum of azilsartan (7-13 µg mL^{-1}) using methanol as a blank

Table 1: Multivariate UV calibration data at five selected wavelengths

Concentration (µg mL^{-1})	246nm	248nm	250nm	252nm	254nm
7	0.255	0.257	0.258	0.256	0.251
8	0.291	0.296	0.297	0.294	0.289
9	0.332	0.335	0.336	0.333	0.33
10	0.371	0.374	0.375	0.372	0.365
11	0.409	0.41	0.411	0.411	0.403
12	0.448	0.452	0.453	0.445	0.441
13	0.487	0.491	0.492	0.488	0.479

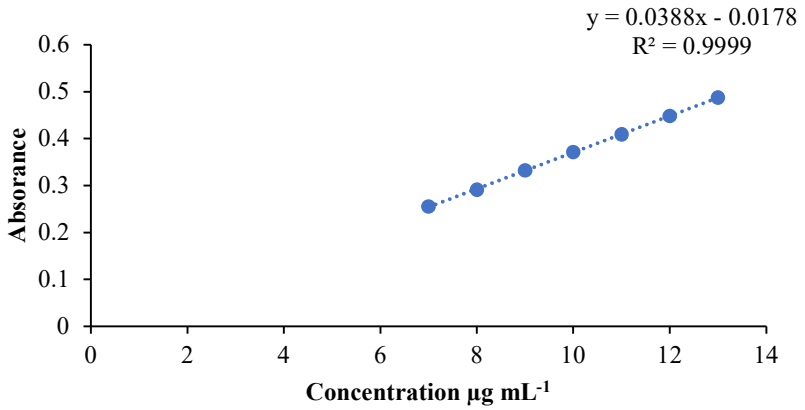

Figure 4: Calibration curve at 246nm

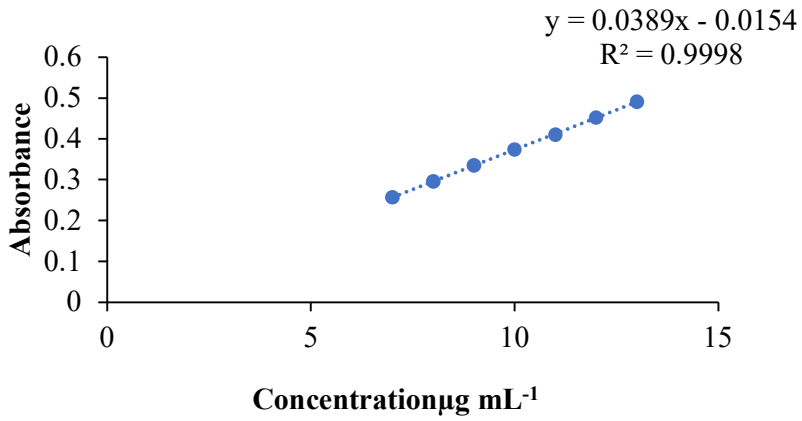

Figure 5: Calibration curve at 248nm

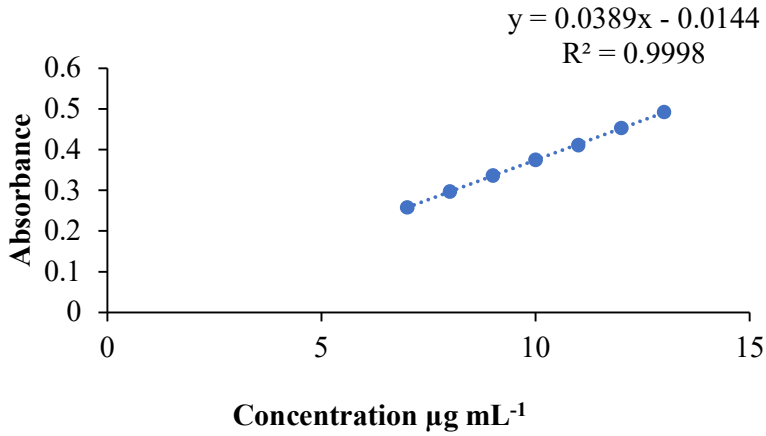

Figure 6: Calibration curve at 250nm

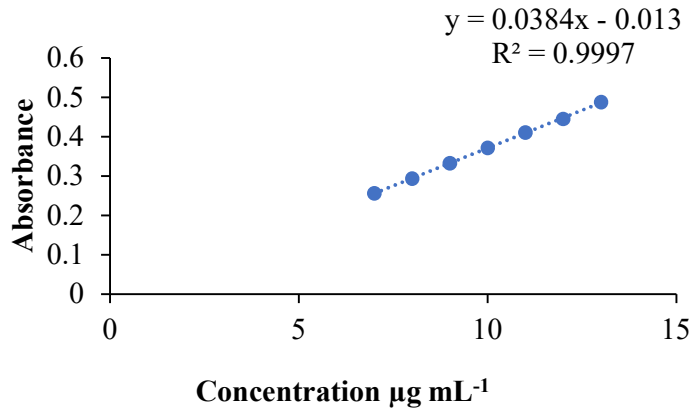

Figure 7: Calibration curve at 252nm

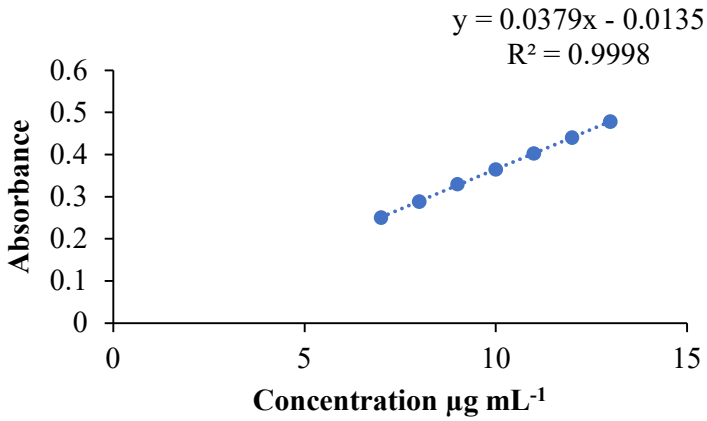

Figure 8: Calibration curve at 254nm

Table 2: Linearity data shows statistical parameters at the selected wavelengths

Wavelength(nm)	Regression equation	Slope	Intercept	R^2	LOD (µg mL^{-1})	LOQ (µg mL^{-1})
246	y = 0.0388x - 0.0178	0.0442	- 0.017	0.9999	0.0944	0.2861
248	y = 0.0389x - 0.0154	0.0446	-0.015	0.9998	0.0810	0.2456
250	y = 0.0389x - 0.0144	0.0446	- 0.014	0.9998	0.0810	0.2456
252	y = 0.0384x - 0.013	0.0176	- 0.013	0.9997	0.3169	0.9604
254	y = 0.0379x - 0.0135	0.0434	- 0.013	0.9998	0.1177	0.3569

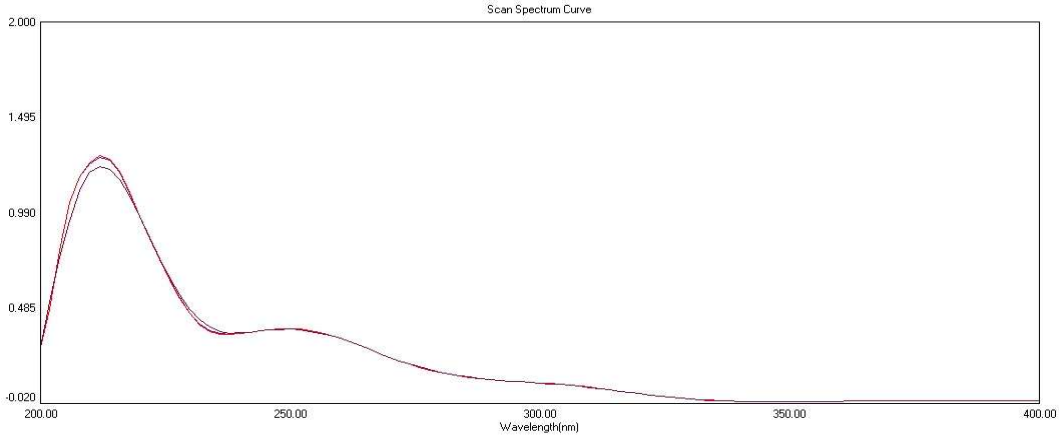

Figure 9: System precision overlay spectra of azilsartan.

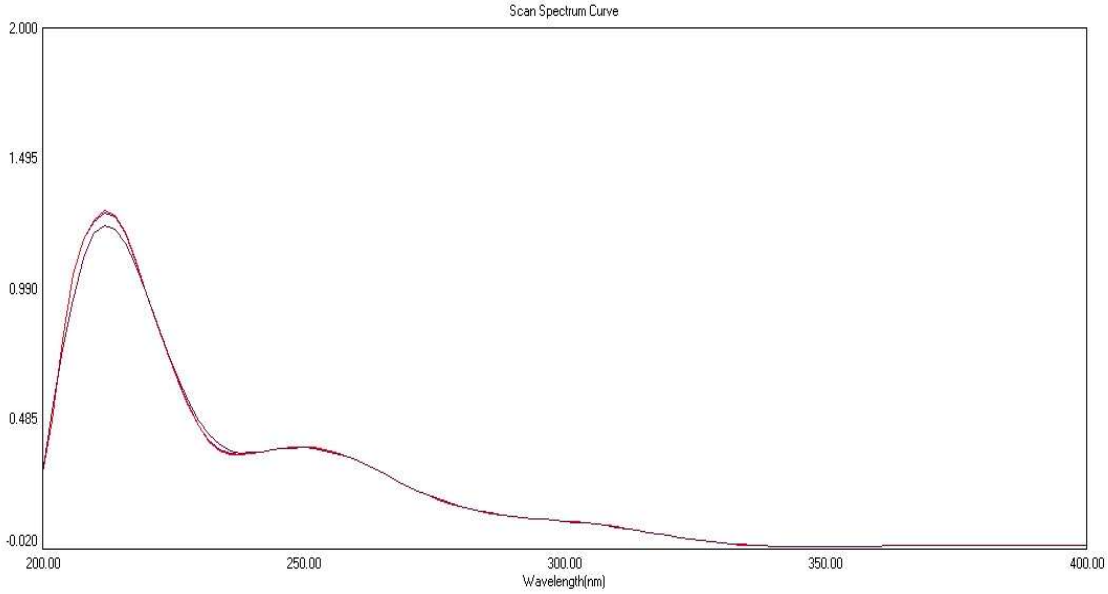

Figure 10: Interday precision overlay spectra of azilsartan

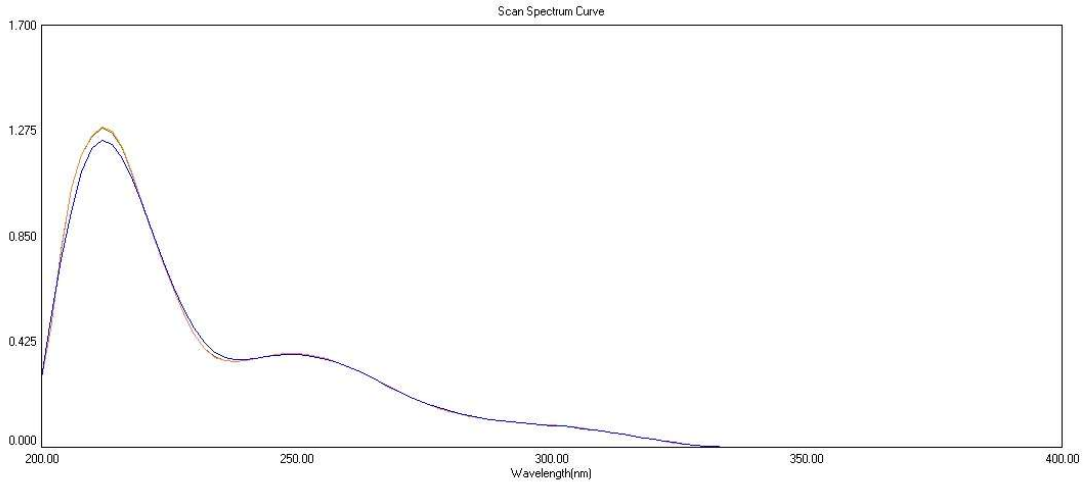

Figure 11: Intraday precision overlay spectra of azilsartan

Table 3: Results of Precision Studies

S. No.	System Precision	Intraday and Interday Precision		
	Absorbance Standard (10 µg mL^{-1})	% Recovery of sample equivalent to 10 µg mL^{-1}of sample		
		Day 1	Day 2	Day 3
1	1.857	99.64	98.99	99.47
2	1.868	98.59	98.88	99.69
3	1.851	99.21	99.48	99.27
4	1.845	99.47	99.71	99.58
5	1.852	99.78	98.55	99.45
6	1.860	98.54	98.27	99.84
Mean	1.856	99.21	99.89	99.55
SD	0.008	0.53	0.54	0.20
% RSD	0.43	0.54	0.55	0.20

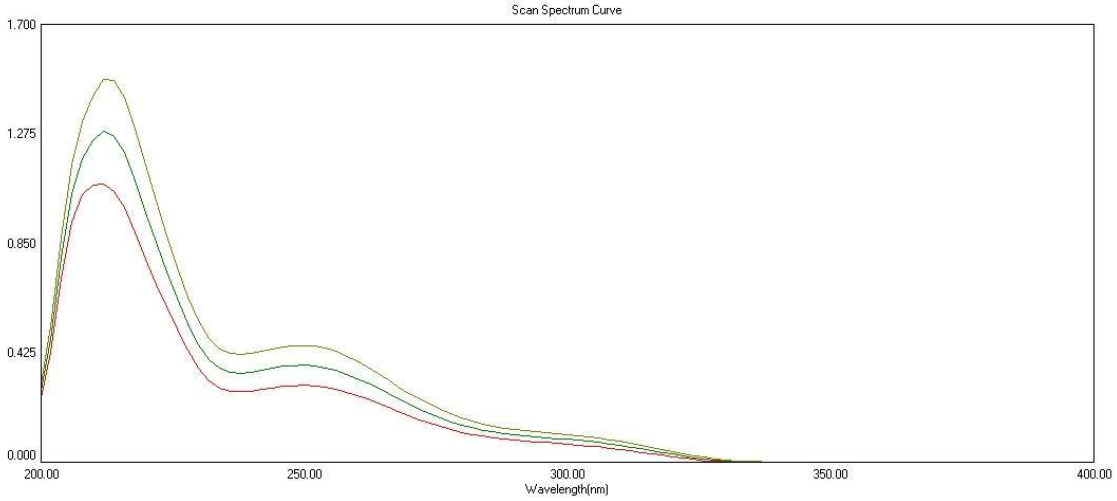

Figure 12: Overlay spectra of accuracy of Azilsartan medoxomil 80, 100, 120 % raising

Table 3: Recovery Studies

Wavelength (nm)	Amount present (µg mL^{-1})	Amount added (µg mL^{-1})	Absorbance	Amount recovered (µg mL^{-1})	% Recovery
246	4	4	0.291	4.01	100.25
		6	0.371	5.98	99.67
		8	0.448	7.98	99.75
248	4	4	0.296	3.97	99.25
		6	0.374	6.02	100.33
		8	0.452	7.97	99.63
250	4	4	0.297	3.95	98.75
		6	0.375	5.98	99.67
		8	0.453	7.96	99.50
252	4	4	0.294	3.94	98.50
		6	0.372	5.93	98.83
		8	0.445	7.96	99.50
254	4	4	0.289	4.01	100.25
		6	0.365	5.99	99.83

Table 4: Assay of Azlisartan

Label claim (mg)	Amount estimated (mg)	% Assay
40	39.98	99.95
40	40.01	100.03
40	39.95	99.88
Average	39.98	99.95
SD		0.0750
% RSD		0.0750

CONCLUSION

A number of validation parameters were examined in order to confirm the newly devised spectrophotometric approach for evaluating Azilsartan medoxomil and it was discovered that it was within acceptable ranges as per ICH standards. Azilsartan medoxomil measurement in tablet formulation was demonstrated to be sensitive, accurate, precise, and repeatable using the suggested approach. Since the suggested methodology is more accurate than existing UV spectrophotometric approaches and includes a method with

fundamental mathematical elements, we strongly advise utilizing it for a routine analysis of Azilsartan medoxomil in pharmaceutical formulations.

STATEMENT OF ETHICS

There are no animal or human participants used in this study

ACKNOWLEDGMENTS

The management of SRM Institute of Science and Technology and SRM College of harmacy at SRM Institute of Science and Technology, Kattankulathur, are to be thanked for providing the authors with a variety of reprographic sources for this study.

DISPUTE OF INTEREST

There are no financial interests that could be at odds with this content.

FUNDING SOURCES

No financing has been reported.

REFERENCE:

[1] T Hemant Kumar AB, D Gowri Sankar. Determination of Azilsartan Medoximil and Chlorthalidone in tablets exposed to forced degradation by using RP-HPLC. Biomed Res. 2019;30(5):775–85.

[2] Kaushik D, Kaur J, Paul Kaur V, Saini B, Bansal Y, Bansal G. Forced degradation, LC–UV, MSn and LC–MS–TOF studies on azilsartan: Identification of a known and three new degradation impurities. J Pharm Biomed Anal [Internet]. 2016 Feb;120:202–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0731708515302934

[3] Mantena BPV, Rao S V., Appa Rao KMC, Ramakrishna K, Srikanth Reddy R. Method Development and Validation for the Determination of Potential Impurities Present in Azilsartan medoxomil Tablets by Reverse Phase-Ultra Performance Liquid Chromatography. Anal Chem Lett [Internet]. 2014 Nov 2;4(5–6):287–301. Available from: http://www.tandfonline.com/doi/abs/10.1080/22297928.2014.1000966

[4] Darwish HW, Bakheit AH, Abdelhameed AS, Mustafa B. A novel method to determine new potent angiotensin inhibitor, azilsartan, in human plasma via micelle-enhanced spectrofluorimetry using cremophor RH 40. Trop J Pharm Res [Internet]. 2016 May 30;15(5):1003. Available from: http://www.ajol.info/index.php/tjpr/article/view/136344

[5] Kayesh R, Jahan MS, Sultan MZ. Development Using Response Surface Methodology and Validation of a Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Azilsartan Medoxomil and Chlorthalidone in Solid Dosage Form. Chromatographia [Internet]. 2017 Apr 3;80(4):593–603. Available from: http://link.springer.com/10.1007/s1033

7-017-3276-4

[6] Gong C, Wang J, Sun Y, Ding D, Zhong L, Zhu M, et al. UPLC–MS/MS for the determination of azilsartan in beagle dog plasma and its application in a pharmacokinetics study. Asian J Pharm Sci [Internet]. 2015 Jun;10(3):247–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S181808761400097X

[7] Vekariya PP, Joshi HS. Development and Validation of RP-HPLC Method for Azilsartan Medoxomil Potassium Quantitation in Human Plasma by Solid Phase Extraction Procedure. ISRN Spectrosc [Internet]. 2013 Sep 14;2013:1–6. Available from: https://www.hindawi.com/journals/isrn/2013/572170/

[8] Prasad Minnath Gosavi, Mahesh Mukund Deshpande MJC. stability indicating RP-HPLC and uv spectrophotometric method for the estimation of azilsartan medoxomil in bulk and pharmaceutical formulations. Int J Res Anal Rev. 2020;7(3):500–23.

[9] Sreenivasulu J, Venkata Ramana P, Sampath Kumar Reddy G, Nagaraju CVS, Thirumalai Rajan S, Eswaraiah S. A Rapid Novel HPLC Method for Estimation of Eight Related Compounds in Azilsartan Kamedoxomil and Identification of Degradation Compounds by Using LC–MS. J Chromatogr Sci [Internet]. 2015 Oct;53(9):1463–74. Available from: https://academic.oup.com/chromsci/article-lookup/doi/10.1093/chromsci/bmv039

[10] Kunal Sharad Surwade RBS. UV Spectrophotometric method for the estimation of azilsartan medoxomil in bulk and pharmaceutical formulations. World J Pharm Res. 2014;4(1):1667–72.

[11] Khan ZG, Bari SB, Gujarathi SN, Gujarathi SB, Patil PB. Azilsartan: A Review of Analytical Methods for estimation in Pharmaceutical Formulation. Asian J Pharm Anal [Internet]. 2018;8(4):227. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:ajpa&volume=8&issue=4&article=009

[12] Samanthula G. Rapid LC-MS Compatible Stability Indicating Assay Method for Azilsartan Medoxomil Potassium. J Anal Bioanal Tech [Internet]. 2015;6(4). Available from: https://www.omicsonline.org/open-access/rapid-lcms-compatible-stability-indicating-assay-method-for-azilsartanmedoxomil-potassium-2155-9872-1000254.php?aid=58211

[13] Nayabaniya A, Seetharaman R, Lakshmi K. Spectrophotometric Determination of Carvedilol in Bulk

drug and its Formulation by Multivariate Calibration Technique. Res J Pharm Technol [Internet]. 2020;13(2):915. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=13&issue=2&article=077

[14] Rani DN, Kokilambigai KS, Lakshmi KS. Multivariate Calibration Technique for the Spectrophotometric Quantification of Rasagiline in Bulk drug and Pharmaceutical Formulations. Res J Pharm Technol [Internet]. 2020;13(2):843. Available from: http://www.indianjournals.com/ijor.aspx?target=ijor:rjpt&volume=13&issue=2&article=063

[15] Borman P, Elder D. Q2(R1) Validation of Analytical Procedures. In: ICH Quality Guidelines [Internet]. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2017. p. 127–66. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9781118971147.ch5