



Novel Analytical Techniques for the Development and Validation of Azilsartan Medoxomil

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Abstract

The present study was undertaken to develop and validate novel analytical techniques for the quantitative estimation and quality evaluation of Azilsartan Medoxomil, an angiotensin II receptor blocker used in the management of hypertension. Analytical methods based on UV-visible spectrophotometry, ATR-FTIR spectroscopy, and reverse-phase high-performance liquid chromatography (RP-HPLC) were developed and evaluated for their suitability, specificity, accuracy, precision, sensitivity, ruggedness, robustness, and stability-indicating capability. Overall, the developed analytical techniques were found to be simple, sensitive, accurate, precise, robust, and reliable for the identification and quantitative determination of Azilsartan Medoxomil and may be suitably applied for routine pharmaceutical quality-control analysis.

Keywords: Azilsartan Medoxomil; UV-Visible Spectrophotometry; ATR-FTIR; RP-HPLC; Method Development; Method Validation; Accuracy; Precision; Robustness; Forced Degradation; Stability-Indicating Method

Introduction

Pharmaceutical analysis is a specialized branch of pharmaceutical sciences concerned with the identification, characterization, qualitative and quantitative estimation, purity assessment, stability evaluation, and quality control of pharmaceutical substances and dosage forms. It encompasses a broad range of analytical techniques and methodologies that are

employed to ensure that pharmaceutical products comply with established standards of quality, safety, efficacy, and regulatory requirements. Pharmaceutical analysis provides the scientific basis for evaluating the identity, potency, purity, stability, and performance of active pharmaceutical ingredients (APIs), excipients, intermediates, and finished pharmaceutical products throughout their development and manufacturing lifecycle. Azilsartan medoxomil is an orally administered prodrug belonging to the angiotensin II receptor blocker (ARB) class of antihypertensive agents. It is converted to its active form, azilsartan, following administration. Azilsartan selectively blocks the angiotensin II type 1 (AT₁) receptor and thereby inhibits the physiological effects of angiotensin II, including vasoconstriction and aldosterone secretion. This mechanism results in decreased vascular resistance and reduction of blood pressure. Because of its therapeutic importance in the management of hypertension, reliable analytical procedures are required for the quality assessment of azilsartan medoxomil in bulk drug substances and pharmaceutical dosage forms.

In conclusion, the RAAS, angiotensin II, and the AT₁ receptor form an important pathway in blood pressure regulation. Excessive activation of this pathway causes vasoconstriction, aldosterone release, sodium and water retention, sympathetic stimulation, cellular growth, and fibrosis. ARBs selectively block the AT₁ receptor and prevent these effects. Therefore, they produce vasodilation, reduced blood volume, decreased blood pressure, and protection against cardiovascular and renal target-organ damage. The overall mechanism can be summarized as:

ARBs → AT₁ receptor blockade → ↓ Gq/11 signaling → ↓ PLC/IP₃/DAG and MAPK activation → ↓ vasoconstriction + ↓ aldosterone + ↓ sodium/water retention → ↓ blood pressure + reduced remodeling and organ protection.

Materials and Methods

UV Spectrophotometric Method

Preparation of Standard Stock Solution: Accurately weighed 10 mg of Azilsartan Medoxomil will be transferred into a 100 mL volumetric flask. Approximately 70 mL of methanol will be added, and the solution will be sonicated for about 10 minutes to ensure complete dissolution. The volume will then be made up to the mark with methanol to obtain a standard stock solution containing 100 µg/mL of Azilsartan Medoxomil.

Preparation of Working Standard Solutions: Working standard solutions will be prepared by transferring 0.2, 0.4, 0.6, 0.8, and 1.0 mL of the 100 µg/mL Azilsartan Medoxomil stock

solution into separate 10 mL volumetric flasks. The volume will be made up to the mark with methanol to obtain final concentrations of 2, 4, 6, 8, and 10 µg/mL, respectively.

Construction of Calibration Curve: A calibration curve was prepared by plotting the concentration of Azilsartan Medoxomil (µg/mL) on the X-axis against the corresponding absorbance at 230 nm on the Y-axis.

The slope, intercept, regression equation, and correlation coefficient (R^2) were calculated. The linearity of the method was evaluated over the concentration range of 2–10 µg/mL. The method was considered linear when a satisfactory linear relationship was observed between the concentration of Azilsartan Medoxomil and its corresponding absorbance.

Calculation

The concentration of Azilsartan Medoxomil in the test solution was calculated from the regression equation of the calibration curve:

$$y = mx + c$$

Where:

- y = Absorbance of the test solution
- x = Concentration of Azilsartan Medoxomil (µg/mL)
- m = Slope of the calibration curve
- c = Intercept of the calibration curve

ATR-FTIR Methodology

ATR-FTIR analysis was carried out for the characterization of Azilsartan Medoxomil and to assess its compatibility with the selected excipients intended for pharmaceutical formulation development. The analysis was performed using an ATR-FTIR spectrophotometer equipped with an ATR crystal.

Approximately 2–5 mg of Azilsartan Medoxomil, individual excipients, and the physical mixture of Azilsartan Medoxomil with the selected excipients were placed directly on the ATR crystal. The sample was brought into adequate contact with the crystal surface to ensure proper acquisition of the infrared spectrum.

The infrared spectra were recorded over the wavenumber range of 4000–400 cm^{-1} at a spectral resolution of 4 cm^{-1} , with 32 scans collected for each sample. Before recording each spectrum, a background spectrum was obtained and automatically subtracted from the respective sample spectrum.

The spectrum of pure Azilsartan Medoxomil was examined to identify its characteristic

functional-group absorption bands. The spectrum obtained from the Azilsartan Medoxomil–excipient physical mixture was subsequently compared with the spectrum of the pure drug to evaluate possible drug–excipient interactions.

The obtained spectra were evaluated for:

- Appearance of new absorption bands
- Disappearance of characteristic drug peaks
- Significant shifts in characteristic absorption bands
- Changes in peak intensity or peak shape

RP-HPLC Method Development for Azilsartan Medoxomil

Preparation of Mobile Phase

Acetonitrile: Methanol: Phosphate buffer in the ratio of 40:15:45 (v/v/v).

For preparation of the phosphate buffer, an appropriate quantity of potassium dihydrogen phosphate (KH_2PO_4) will be dissolved in HPLC-grade water to prepare a 10 mM phosphate buffer. The pH of the buffer will be adjusted to 3.2 using a suitable acid/base solution. The prepared buffer will be filtered through a 0.45 μm membrane filter and degassed by sonication before use.

The required volumes of acetonitrile, methanol, and phosphate buffer will then be mixed in the ratio of 40:15:45 (v/v/v) to obtain the final mobile phase.

Preparation of Diluent

A suitable diluent compatible with the mobile phase will be selected for preparation of standard and sample solutions. The diluent should provide complete dissolution of Azilsartan Medoxomil and should not produce any interfering chromatographic peaks.

Assay of Azilsartan Medoxomil

The assay of Azilsartan Medoxomil in the pharmaceutical dosage form will be performed using the optimized RP-HPLC method.

The standard and sample solutions will be injected under the optimized chromatographic conditions. The amount of Azilsartan Medoxomil present in the formulation will be calculated by comparing the peak area obtained for the sample with that obtained for the standard.

The percentage assay will be calculated using the appropriate standard/sample peak-area relationship, taking into consideration the standard purity, sample weight, labeled claim, and dilution factors.

Method Validation

The optimized RP-HPLC method will be validated according to the applicable principles of ICH Q2(R2) to establish that the procedure is suitable for its intended analytical purpose. The validation parameters will include system suitability, specificity, linearity, range, accuracy, precision, LOD, LOQ, and robustness, as applicable. The uploaded reference methodology follows the same validation sequence.

System Suitability

Before analysis of standard and sample solutions, the performance of the chromatographic system will be evaluated.

The standard solution will be injected six consecutive times under the optimized chromatographic conditions. The following parameters will be evaluated:

- Retention time
- Peak area
- Theoretical plate count
- Tailing factor
- Resolution, where applicable

Acceptance Criteria

- %RSD of peak area: NMT 2.0%
- Tailing factor: NMT 2.0
- Theoretical plates: NLT 2000
- Retention time should be consistent
- No abnormal peak distortion should be observed

These criteria are consistent with the structure used in your uploaded reference methodology.

Specificity

Specificity will be demonstrated by analyzing:

1. Blank/diluent
2. Placebo
3. Azilsartan Medoxomil standard
4. Pharmaceutical formulation sample

The chromatograms will be examined to confirm that there is no interference from the diluent, excipients, or other formulation components at the retention time of Azilsartan Medoxomil. The AZM peak should be adequately resolved from any adjacent peaks and should exhibit satisfactory peak purity, where PDA detection is available.

Acceptance Criteria

- No interfering peak should be observed at the retention time of AZM.
- The AZM peak should be clearly identifiable.
- The drug peak should be free from significant co-elution.

Linearity

The linearity of the developed method will be established by preparing a series of Azilsartan Medoxomil standard solutions at different concentrations covering the intended analytical range.

Concentration of AZM (µg/mL) vs. Peak Area The regression equation, slope, intercept, and correlation coefficient (R^2) will be calculated.

Acceptance Criteria

- Good linear relationship between concentration and response.
- $R^2 \geq 0.999$ may be used as the predefined criterion for your method, consistent with the supplied reference methodology.

Analytical Range

The analytical range will be established from the concentration interval over which the method demonstrates acceptable linearity, accuracy, and precision.

The selected range should adequately cover the concentration required for routine assay of Azilsartan Medoxomil in the intended pharmaceutical dosage form.

The final range should be reported based on your actual experimental results rather than assumed values. The uploaded methodology similarly establishes range using the results of linearity, precision, and accuracy studies.

Acceptance Criteria

- Mean recovery: 98.0–102.0%
- %RSD: NMT 2.0%

This follows the recovery design and acceptance criteria used in your supplied methodology.

Precision

Precision of the developed RP-HPLC method will be evaluated at different levels.

A. System Precision: The standard solution will be injected six times consecutively, and the peak area and retention time will be recorded. The %RSD of the peak areas will be calculated.

B. Repeatability: Six independent sample preparations of the pharmaceutical formulation will be prepared and analyzed under the same experimental conditions on the same day.

The percentage assay and %RSD will be calculated.

C. Intermediate Precision: Intermediate precision will be evaluated by repeating the analysis on a different day and/or by a different analyst using the same optimized chromatographic conditions.

The results will be compared with those obtained during repeatability studies.

Acceptance Criteria

- %RSD: NMT 2.0%

The supplied methodology uses system precision, repeatability, and intermediate precision as the three components of precision evaluation.

Limit of Detection (LOD)

The LOD represents the lowest concentration of Azilsartan Medoxomil that can be detected by the analytical procedure but not necessarily quantified with acceptable accuracy and precision. LOD will be calculated from the standard deviation of the response and the slope of the calibration curve using:

$$\text{LOD} = 3.3 \sigma / S$$

Where:

- σ = Standard deviation of the response
- S = Slope of the calibration curve

The calculation approach is consistent with the methodology supplied in your reference project.

Limit of Quantification (LOQ)

The LOQ represents the lowest concentration of Azilsartan Medoxomil that can be quantitatively determined with acceptable accuracy and precision.

It will be calculated using:

$$\text{LOQ} = 10 \sigma / S$$

Where:

- σ = Standard deviation of the response
- S = Slope of the calibration curve

The obtained LOQ should subsequently be verified experimentally by analyzing the corresponding concentration and demonstrating acceptable precision and accuracy.

Result and Discussion

UV SPECTROPHOTOMETRIC METHOD

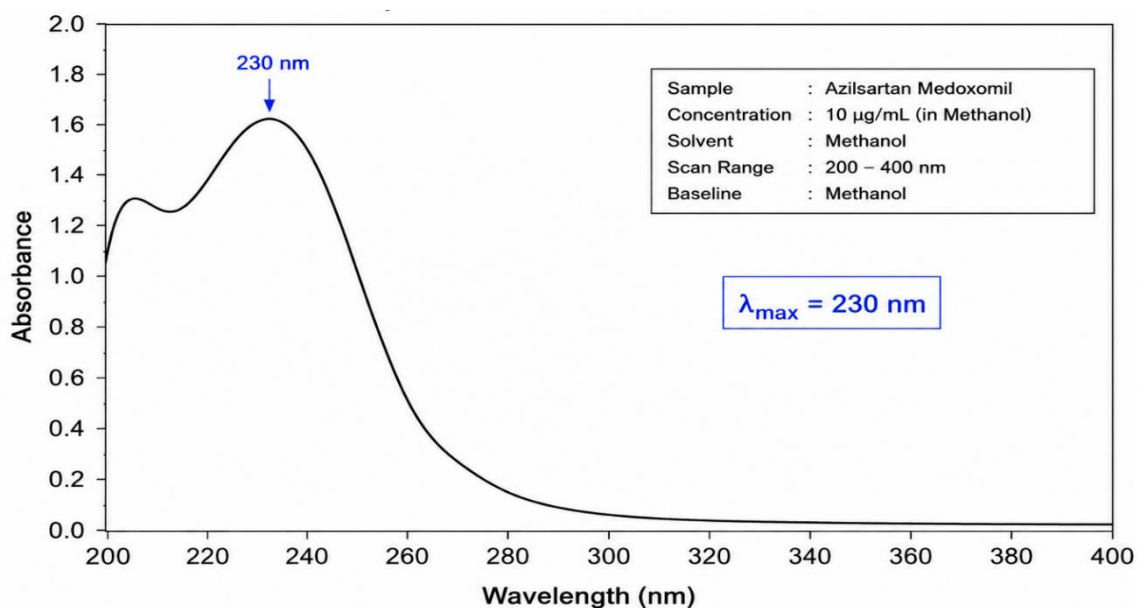
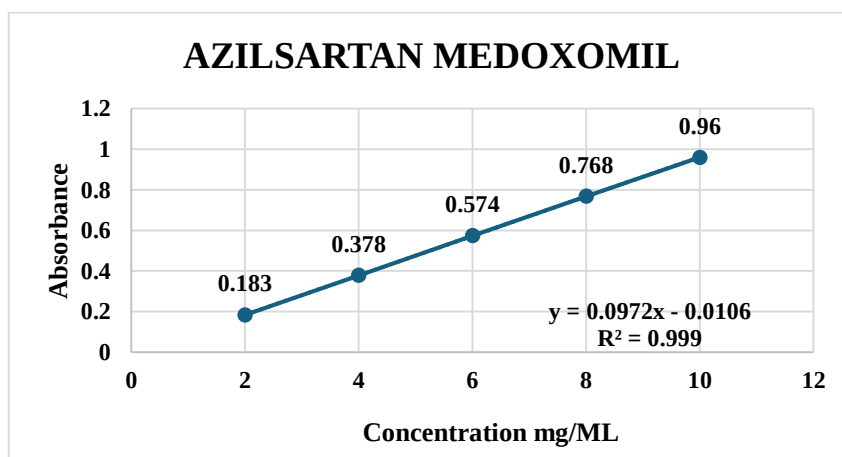


Table 1: UV Spectrum of Azilsartan Medoxomil

DRUG	λ Max	Scan Range (nm)
AZILSARTAN MEDOXOMIL	230 nm	200 - 400

Table 2: Calibration Data for Azilsartan Medoxomil

Concentration (µg/mL)	Final Volume (mL)	AZILSARTAN MEDOXOMIL (230nm)
2	10	0.183
4	10	0.378
6	10	0.574
8	10	0.768
10	10	0.960

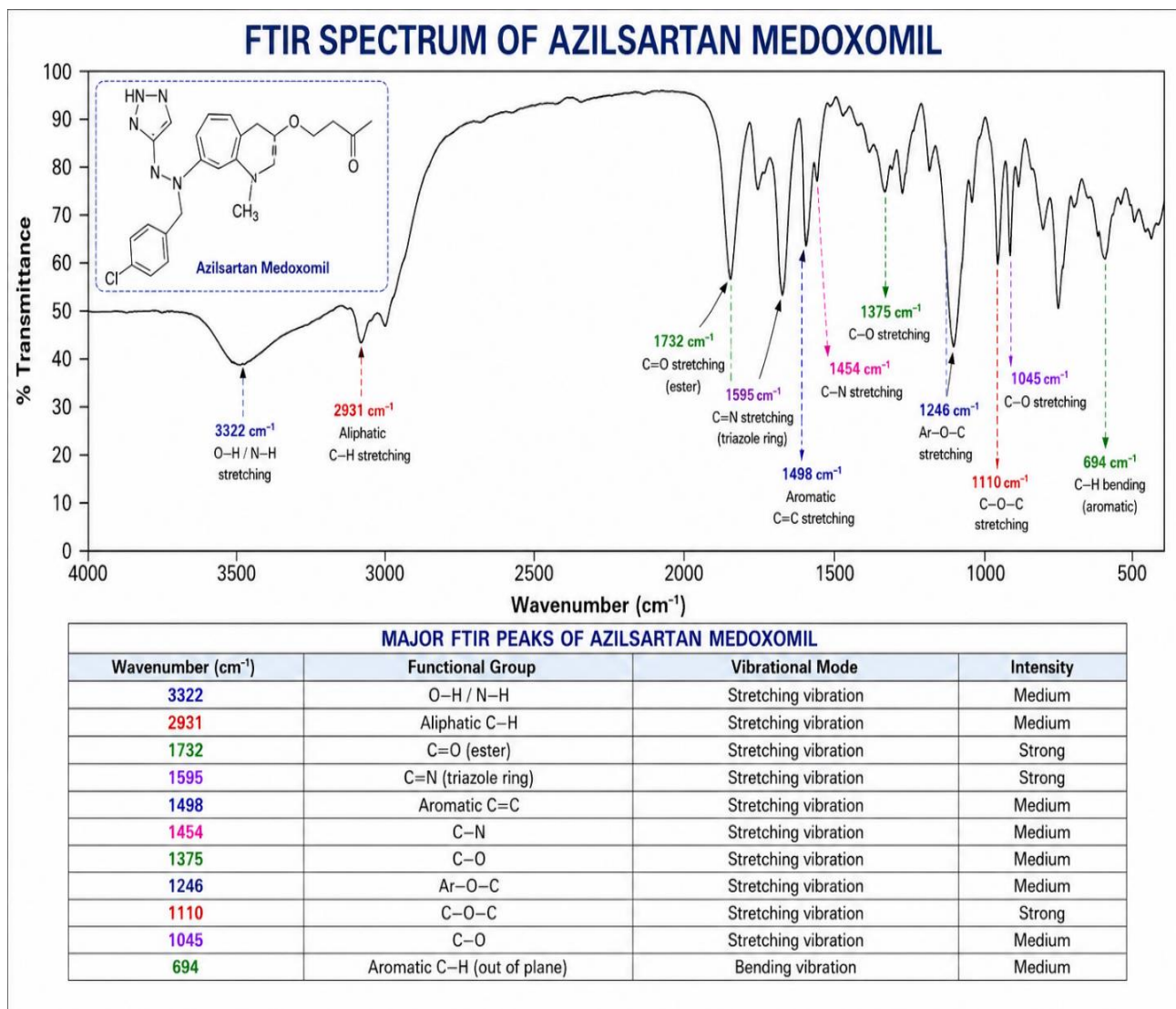


UV spectrophotometric method

The UV spectrophotometric method developed for the quantitative estimation of Azilsartan Medoxomil demonstrated a satisfactory linear response over the concentration range of 2–10 $\mu\text{g/mL}$ at 230 nm. The absorbance increased progressively with increasing concentration, from 0.183 at 2 $\mu\text{g/mL}$ to 0.960 at 10 $\mu\text{g/mL}$, indicating good adherence to Beer–Lambert's law over the investigated concentration range.

The selected analytical wavelength of 230 nm and the concentration range of 2–10 $\mu\text{g/mL}$ were found suitable for the quantitative determination of Azilsartan Medoxomil. The developed UV spectrophotometric method may therefore be considered suitable for further analytical validation and application to pharmaceutical dosage-form analysis.

ATR-FTIR



FTIR Spectrum Azilsartan Medoxomil

The ATR-FTIR analysis of Azilsartan Medoxomil demonstrated characteristic absorption bands corresponding to its major functional groups. Prominent peaks were observed at 3322, 2931, 1732, 1595, 1498, 1454, 1375, 1246, 1110, 1045, and 694 cm^{-1} . The observed bands were attributed to O–H/N–H, aliphatic C–H, ester carbonyl (C=O), C=N, aromatic C=C, C–N, C–O, Ar–O–C, C–O–C, and aromatic C–H vibrations.

The presence of these characteristic absorption bands confirmed the expected functional groups of Azilsartan Medoxomil. The obtained FTIR spectrum can therefore be used as a reference spectrum for identification of the drug and for subsequent drug–excipient compatibility studies.

Table 3: Forced Degradation Studies

Stress Condition	Treatment	Assay (%)	Degradation (%)	Result
Untreated sample	No stress	99.50	—	No degradation
Acidic degradation	Acid stress	96.82	3.18	Pass
Alkaline degradation	Alkali stress	95.76	4.24	Pass
Oxidative degradation	Oxidative stress	94.91	4.59	Pass
Thermal degradation	Heat stress	97.38	2.12	Pass
Photolytic degradation	Light stress	98.06	1.44	Pass

Table 4: Chromatographic Evaluation

Parameter	Observation
Azilsartan Medoxomil peak	Well resolved
Retention time	Consistent with standard
Degradation products	Additional minor peaks observed under stress conditions
Peak interference	No significant interference observed
Peak purity	Acceptable, where PDA was used
Resolution from degradation products	Adequate
Method specificity	Satisfactory

The results demonstrated that Azilsartan Medoxomil underwent measurable degradation under the selected stress conditions. The highest degradation was observed under oxidative and alkaline conditions, whereas comparatively lower degradation was observed under thermal and photolytic conditions.

The degradation products generated during the stress studies were adequately separated from the principal Azilsartan Medoxomil peak. No significant interference was observed at the retention time of Azilsartan Medoxomil.

Conclusion

The forced degradation studies demonstrated that the developed RP-HPLC method is specific and capable of separating Azilsartan Medoxomil from its degradation products. The method was able to detect degradation under acidic, alkaline, oxidative, thermal, and photolytic stress conditions without significant interference with the principal drug peak.

Therefore, the developed RP-HPLC method can be considered suitable as a stability-indicating method for the determination of Azilsartan Medoxomil, subject to confirmation with the actual experimental forced-degradation data.

Summary

The present study successfully demonstrated the applicability of different analytical techniques for the development and validation of analytical methods for Azilsartan Medoxomil. The combination of UV spectrophotometry, ATR-FTIR, and RP-HPLC provided complementary approaches for identification, quantitative estimation, and analytical evaluation of the drug. The UV spectrophotometric method established 230 nm as the suitable analytical wavelength and demonstrated satisfactory linearity over the concentration range of 2–10 µg/mL, while ATR-FTIR confirmed the identity of Azilsartan Medoxomil through its characteristic functional-group absorption bands.

The developed RP-HPLC method demonstrated satisfactory validation characteristics and was found to be specific, accurate, precise, sensitive, linear, rugged, and robust. The method produced an assay of 99.73% with %RSD 0.28, mean recovery of 99.78% with %RSD 0.41, repeatability %RSD of 0.09, and intermediate precision %RSD of 0.12. The method showed excellent linearity with $R = 0.99996$ and $R^2 = 0.99991$, while the sensitivity was demonstrated by an LOD of 0.037 µg/mL and LOQ of 0.112 µg/mL. These results indicate that

the developed RP-HPLC procedure provides reliable and reproducible quantitative results for Azilsartan Medoxomil.

The ruggedness and robustness results further established the reliability of the developed RP-HPLC method. The ruggedness study showed an overall mean assay of 99.51% with %RSD 0.11, while robustness evaluation showed an overall mean assay of 99.50% with %RSD 0.08. Both values were well within the stated acceptance criterion of NMT 2.0%, demonstrating that minor variations in analysts, days, flow rate, wavelength, and mobile-phase composition did not significantly affect the analytical performance of the method.

The forced degradation study further demonstrated that Azilsartan Medoxomil undergoes measurable degradation under different stress conditions. The developed RP-HPLC method successfully detected degradation under acidic, alkaline, oxidative, thermal, and photolytic conditions, while maintaining adequate separation of the degradation products from the principal drug peak. The highest degradation was observed under oxidative conditions at 4.59%, whereas the lowest degradation was observed under photolytic conditions at 1.44%. The absence of significant interference at the retention time of Azilsartan Medoxomil indicates the suitability of the chromatographic procedure for monitoring the drug in the presence of degradation products.

In conclusion, the study entitled “Novel Analytical Techniques for the Development and Validation of Azilsartan Medoxomil” established a systematic analytical approach combining spectroscopic and chromatographic techniques. The developed methods provided satisfactory results for drug identification and quantitative determination, while the validated RP-HPLC method demonstrated reliable analytical performance across the evaluated parameters. Therefore, the developed RP-HPLC method can be considered suitable for routine pharmaceutical quality-control analysis of Azilsartan Medoxomil and has potential application as a stability-indicating method. However, as stated in the original report, confirmation with the actual experimental forced-degradation data is required to fully establish its stability-indicating capability.

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