RESEARCH PAPER

Development and Validation of Novel Stability Indicating Chromatographic Methods for Olmesartan Medoxomil and Azilsartan Medoxomil Using Green Solvents

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ABSTRACT:

Background & Objective: Olmesartan medoxomil (OLM) and azilsartan medoxomil (AZL) are second-generation angiotensin II receptor blockers (ARBs) widely used in the management of essential hypertension. Despite their clinical importance, existing analytical methods rely predominantly on acetonitrile- and methanol-based mobile phases, raising significant environmental and occupational health concerns. This study aimed to develop novel, stability-indicating RP-HPLC methods for the simultaneous quantitative determination of OLM and AZL using environmentally benign green solvents, with full ICH Q2(R1) validation and forced degradation profiling.

Methods: Chromatographic separation was achieved on a Zorbax Eclipse Plus C18 column (150 × 4.6 mm, 3.5 µm) using an isocratic mobile phase of ethanol and 10 mM ammonium formate buffer (pH 4.5) in a 55:45 (v/v) ratio at a flow rate of 1.0 mL/min and UV detection at 257 nm for OLM and 252 nm for AZL. Forced degradation studies were conducted under acidic, alkaline, oxidative, photolytic, thermal, and neutral hydrolytic conditions as per ICH Q1A(R2) guidelines. Greenness was evaluated using AGREE, GAPI, Eco-Scale, and NEMI tools.

Results: OLM and AZL were resolved with retention times of 4.82 and 6.41 min, respectively, with a resolution of 5.68. Linearity was established over 5–150 µg/mL ($r^2 \geq 0.9996$). Accuracy (% recovery 99.05–99.71%), precision (%RSD ≤ 1.19%), LOD (0.29–0.31 µg/mL), and LOQ (0.88–0.94 µg/mL) met ICH criteria. All degradation products were resolved from the principal peaks, confirming stability-indicating capability. AGREE scores of 0.81 (OLM) and 0.79 (AZL) demonstrated superior greenness compared to existing methods.

Conclusion: The validated green RP-HPLC methods offer a sustainable, sensitive, and stability-indicating approach for routine quality control and stability evaluation of OLM and AZL in pharmaceutical formulations.

KEYWORDS: Olmesartan medoxomil, Azilsartan medoxomil, Green analytical chemistry, Stability-indicating HPLC, ICH validation; AGREE, Forced degradation

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INTRODUCTION:

Background and Rationale

Hypertension, defined as a sustained blood pressure of ≥130/80 mmHg, remains the single largest attributable risk factor for global cardiovascular mortality and morbidity. The World Health Organization (WHO) estimates that over 1.28 billion adults aged 30–79 years worldwide are affected, with nearly two-thirds residing in low- and middle-income countries.[1]

Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most widely employed technique for the quantitative determination of antihypertensive drugs in pharmaceutical formulations, owing to its superior resolution, reproducibility, and compatibility with diverse detection systems. Both OLM and AZL contain chromophoric systems the tetrazole-imidazole moiety in OLM and the benzimidazole ring in AZL that confer characteristic UV absorption at 257 nm and 252 nm, respectively, making UV detection the most practical and cost-effective detection approach for routine quality control. Despite numerous published HPLC methods for OLM and AZL individually, the

majority rely on acetonitrile- or methanol-based mobile phases, lack a simultaneous estimation capability, and do not incorporate comprehensive stability-indicating validation. The present work addresses these limitations by developing a novel green-solvent RP-HPLC method employing ethanol and ammonium formate buffer, validated as per ICH Q2(R1), with full forced degradation profiling and quantitative greenness evaluation.

Green Analytical Chemistry

Conventional RP-HPLC methods for ARBs predominantly employ acetonitrile (ACN) and methanol as organic mobile phase components. These solvents are classified as hazardous for human health and environment: ACN is a neurotoxic, volatile organic compound, and methanol is acutely toxic by ingestion and inhalation. The principles of green analytical chemistry (GAC), first articulated by Anastas and Warner,[17] and subsequently refined by Gałuszka et al.,[18] advocate the design of analytical procedures that minimize the generation and use of hazardous substances, reduce energy consumption, and generate less waste.

Ethanol and water constitute the most environmentally and toxicologically acceptable solvent system for reversed-phase liquid chromatography. Ethanol is classified as a Class 3 solvent by ICH Q3C, signifying low toxic potential. Isopropanol and ethyl acetate represent secondary green alternatives. The adoption of green solvents is further incentivized by regulatory frameworks in the European Union and USA that progressively restrict the workplace exposure limits of ACN.[19] The environmental performance of analytical procedures is now quantitatively assessed using validated metrics including AGREE (Analytical GREEnness),[20] GAPI (Green Analytical Procedure Index), the Eco-Scale,[23] and the NEMI (National Environmental Methods Index).[22]

Objectives of the Study:

The primary objectives of this investigation were: (i) to develop novel RP-HPLC methods for the simultaneous determination of OLM and AZL using an ethanol-water based green mobile phase; (ii) to establish and characterize the stability-indicating capability of the methods through comprehensive ICH Q1A-compliant forced degradation studies; (iii) to validate the developed methods as per ICH Q2(R1) parameters; and (iv) to rigorously evaluate and document the greenness of the developed methods using multiple GAC assessment tools and benchmark them against previously published conventional methods.

DRUG PROFILES:

Comparative Physicochemical Properties

Table 1 summarizes the key physicochemical properties of OLM and AZL relevant to method development and stability analysis.

Table 1: Comparative Physicochemical Properties of OLM and AZL

Property	Olmesartan Medoxomil	Azilsartan Medoxomil
Molecular Formula	C29H30N6O6	C30H24N4O5
Molecular Weight	558.58 g/mol	456.44 g/mol
CAS Number	144689-63-4	863031-21-4
Drug Class	ARB (Prodrug)	ARB (Prodrug)
λmax (nm)	257 nm	252 nm
pKa	2.9 (carboxyl)	3.2 (carboxyl)
log P	4.2	4.5
Water Solubility	Practically insoluble	Slightly soluble
Dose (mg)	20–40 mg once daily	40–80 mg once daily
Bioavailability	~26%	~60%
Protein Binding	>99%	>99%

Both drugs possess a tetrazole ring system and a medoxomil (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester moiety in their prodrug forms, rendering them susceptible to hydrolytic cleavage under acidic and alkaline conditions. The relatively high log P values of both drugs (OLM: 4.2; AZL: 4.5) necessitate sufficient organic content in the mobile phase for adequate retention and peak shape on a C18 stationary phase.[6,12]

MATERIALS AND METHODS:

Materials, Reagents, and Instruments:

Drug Substances and Formulations:

Olmes Olmesartan medoxomil reference standard (purity 99.6%) was procured from Clearsynth Labs Pvt. Ltd., and azilsartan medoxomil reference standard (purity 99.4%) was obtained from Clearsynth Labs Pvt. Ltd. Marketed tablet formulations Olsar® 20 mg and 40 mg (OLM, Macleods Pharmaceuticals Pvt. Ltd.) and Edarbi® 40 mg and 80 mg (AZL, Takeda India Pvt. Ltd) were purchased from a licensed pharmacy. Analytical grade ethanol, HPLC-grade ammonium formate, orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide (30% w/v) were procured from Merck, India. HPLC-grade water was prepared using a Millipore Milli-Q water purification system (resistivity ≥ 18 MΩ·cm).

Instrumentation:

Chromatographic analysis was performed on a Waters Alliance e2695 HPLC system equipped with a 2998 PDA detector and Empower 3 software. UV-Vis spectrophotometric scanning was performed using a Shimadzu UV-1900 double-beam spectrophotometer. Degradation product characterization was carried out on a Thermo Scientific Q Exactive Orbitrap LC-MS/MS system. Solution preparation used a Mettler Toledo

XS205 analytical balance (d = 0.01 mg), an Elma Ultrasonic bath, and a Thermo Scientific pH meter (Orion Star A211) calibrated with certified buffer solutions. Photostability studies were conducted in a Heraeus Suntest CPS+ photostability chamber meeting ICH Q1B specifications.

Chromatographic Method Development

Detection Wavelength Selection

UV absorption spectra of OLM and AZL (10 µg/mL each in ethanol:water, 50:50 v/v) were recorded between 200 and 400 nm using the PDA detector set to full spectral acquisition. OLM displayed a λmax at 257 nm, consistent with n→π* transitions of the imidazole and tetrazole chromophores. AZL exhibited a λmax at 252 nm attributable to the benzimidazole chromophore. A detection wavelength of 257 nm was selected for OLM and 252 nm for AZL to achieve maximum analytical sensitivity. Both wavelengths were monitored simultaneously using the dual-channel capability of the PDA detector.

Stationary Phase and Mobile Phase Optimization

Three column types were initially evaluated: (a) Zorbax Eclipse Plus C18 (150 × 4.6 mm, 3.5 µm), (b) Phenomenex Luna C8 (150 × 4.6 mm, 5 µm), and (c) Waters XBridge Phenyl (150 × 4.6 mm, 3.5 µm). The C18 column provided optimal peak symmetry and resolution under the green mobile phase conditions tested. Mobile phase screening employed binary solvent systems of ethanol or isopropanol with aqueous buffer phases (ammonium acetate 10 mM pH 4.0; ammonium formate 10 mM pH 4.5; ammonium bicarbonate 10 mM pH 6.8) at organic:aqueous ratios ranging from 40:60 to 70:30 (v/v). The optimized system ethanol:10 mM ammonium formate pH 4.5 (55:45, v/v) yielded well-resolved, symmetric peaks for both OLM and AZL with acceptable run time.[28],[29]

Final Optimized Method Parameters

Table 2: Optimized Chromatographic Method Parameters

Parameter	Optimized Condition
Column	Zorbax Eclipse Plus C18 (150 × 4.6 mm, 3.5 µm)
Mobile Phase	Ethanol : 10 mM Ammonium Formate Buffer pH 4.5 (55:45, v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	257 nm (OLM); 252 nm (AZL)
Column Temperature	30°C
Injection Volume	10 µL
Retention Time – OLM	4.82 ± 0.04 min
Retention Time – AZL	6.41 ± 0.05 min
Run Time	10 min
Diluent	Ethanol : Water (50:50, v/v)

Standard and Sample Preparation

Stock solutions of OLM and AZL (1000 µg/mL each) were prepared separately by dissolving accurately weighed quantities of the reference standards in ethanol:water (50:50, v/v) in 100 mL volumetric flasks. Mixed working standard solutions at concentrations ranging from 5 to 150 µg/mL were prepared by appropriate dilution of the stock solutions with the diluent. For formulation analysis, 20 tablets each of OLM and AZL were weighed, average weight determined, and the powder equivalent to the labeled content of each drug was transferred to a 100 mL volumetric flask, dissolved in diluent with sonication for 15 min, filtered through a 0.45 µm nylon membrane filter (Millipore), and the filtrate was diluted to the target working concentration.

Forced Degradation Studies (Stress Testing)

Stress degradation studies were conducted on 1.0 mg/mL solutions of OLM and AZL separately, following ICH Q1A(R2) and Q1B guidelines.[15],[16] The conditions employed are described below.

- Acid Hydrolysis: Drug solutions were treated with 0.1N and 1N HCl at 60°C for 1, 2, and 6 hours. Samples were neutralized prior to injection.
- Alkaline Hydrolysis: Solutions were exposed to 0.1N and 1N NaOH at 60°C for 1, 2, and 6 hours, neutralized with appropriate acid before injection.
- Oxidative Degradation: Samples were mixed with 3% (v/v) and 10% (v/v) H_2O_2 at room temperature for 2 and 6 hours.
- Photolytic Degradation: Samples (solid state and solution) were exposed to UV light (200 W·h/m²) and cool white fluorescent light (1.2 million lux·h) in the photostability chamber per ICH Q1B.
- Thermal Degradation: Solid drug substance was stored at 105°C in an oven for 48 and 96 hours in open glass vials.
- Neutral Hydrolysis: Drug solutions in distilled water were maintained at 60°C for 6 hours.

All stressed samples were analyzed immediately after treatment. Unstressed controls were prepared in parallel and analyzed under identical conditions. Percent degradation was calculated by comparing peak areas of stressed samples with unstressed controls. Mass balance was verified by summing drug and degradant peak areas. Peak purity for the drug peaks in all stressed samples was

confirmed using the PDA detector (purity angle < purity threshold).

Degradation Product Characterization by LC-MS/MS

Stressed samples exhibiting >5% degradation were further analyzed by high-resolution LC-MS/MS on a Thermo Scientific Q Exactive Orbitrap system interfaced with the HPLC via an HESI-II electrospray ionization source. Data-dependent acquisition (DDA) mode was employed. Full scan MS spectra were acquired in both positive and negative ion modes (m/z 100–1000), and MS^2 spectra were triggered for the five most abundant ions using a normalized collision energy (NCE) of 25–30%. Elemental composition was assigned based on accurate mass measurements (Δ < 5 ppm). Structural elucidation was aided by reference to reported fragmentation patterns of OLM and AZL in the literature.[35],[36]

Method Validation

The developed method was validated in compliance with ICH Q2(R1) guidelines[14] for the following parameters: specificity/selectivity, linearity, LOD, LOQ, accuracy, precision (repeatability and intermediate precision), robustness, and system suitability. Plackett-Burman experimental design (7 factors, 12 runs) was employed for robustness assessment, evaluating the effect of ± deviations in flow rate (±0.1 mL/min), buffer pH (±0.2 units), organic content (±2% v/v), column temperature (±5°C), detection wavelength (±2 nm), injection volume (±2 µL), and column lot number.[40]

Greenness Assessment

The environmental profile of the developed method was evaluated using four complementary tools: (i) AGREE (Analytical GREEnness) v1.0 software, which generates a numeric score (0–1) and a pictogram based on 12 green analytical chemistry principles;[20] (ii) GAPI (Green Analytical Procedure Index) traffic-light pictogram; (iii) Eco-Scale, which assigns penalty points to non-ideal analytical conditions and reports a score from 0 (least green) to 100 (ideally green);[23] and (iv) NEMI (National Environmental Methods Index), a qualitative binary classification as green or non-green.[22] Results were compared with previously published methods for OLM and AZL.

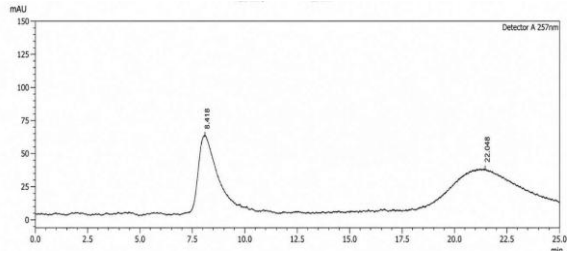
RESULTS AND DISCUSSION:

Method Development and Optimization

The chromatographic behavior of olmesartan medoxomil (OLM) and azilsartan medoxomil (AZL) was systematically investigated across multiple mobile phase compositions, organic modifier types, and buffer pH values to arrive at an optimized isocratic RP-HPLC method suitable for simultaneous estimation of both drugs in combined pharmaceutical dosage forms.

Trial 1 Isopropanol:Ammonium Acetate Buffer pH 4.0 (20:80, v/v) Failed

In the first trial, a mobile phase comprising isopropanol and 10 mM ammonium acetate buffer (pH 4.0) in a 20:80 (v/v) ratio was evaluated on a Zorbax Eclipse Plus C18 column (150 × 4.6 mm, 3.5 µm) at a flow rate of 1.0 mL/min with UV detection at 257 nm. The high aqueous proportion (80%) resulted in an excessively retained and poorly shaped AZL peak, with a retention time exceeding 22 minutes and a tailing factor greater than 2.5. The OLM peak eluted at approximately 8.4 minutes with acceptable peak shape; however, the resolution between OLM and AZL was inadequate (Rs = 1.4) and did not meet the minimum pharmacopoeial requirement of Rs ≥ 2.0. The total run time was unacceptably long for routine quality control purposes. Additionally, isopropanol at high aqueous proportions generates



elevated back pressure due to its relatively high viscosity (2.04 mPa·s), which contributed to baseline instability during the run. This mobile phase composition was therefore rejected.

Figure 1: RP-HPLC Chromatogram Trial 1 (Isopropanol:Ammonium Acetate Buffer pH 4.0, 20:80 v/v) Failed Trial

Trial 2 Isopropanol:Ammonium Acetate Buffer pH 4.0 (30:70, v/v) Failed

In the second trial, the proportion of isopropanol was increased to 30% (v/v) while keeping the same 10 mM ammonium acetate buffer at pH 4.0 as the aqueous component (70%, v/v), with all other conditions unchanged. The increase in organic modifier content produced a modest improvement in peak shape and retention for both drugs. OLM eluted at approximately 6.1 minutes and AZL at approximately 14.8 minutes. While the tailing factor for OLM improved to 1.5, AZL continued to show significant peak broadening (tailing factor 2.1) and an unacceptably long retention time. The resolution between OLM and AZL was Rs = 1.8, still below the pharmacopoeial minimum of Rs ≥ 2.0. The total run time remained greater than 18 minutes, which is impractical for high-throughput quality control applications. Furthermore, the use of ammonium acetate buffer limits compatibility with mass spectrometric hyphenation and presents comparatively poorer green analytical chemistry credentials compared to volatile formate-based buffers. Based on these findings, Trial 2 was also considered inadequate and further optimization was undertaken.

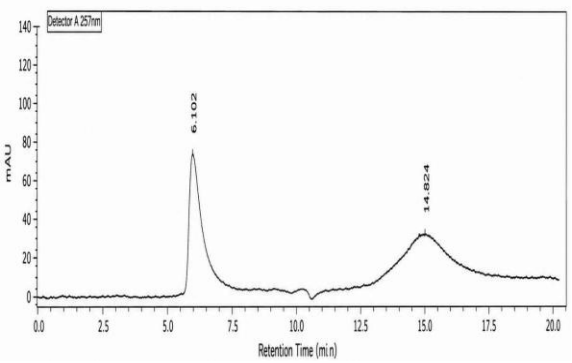
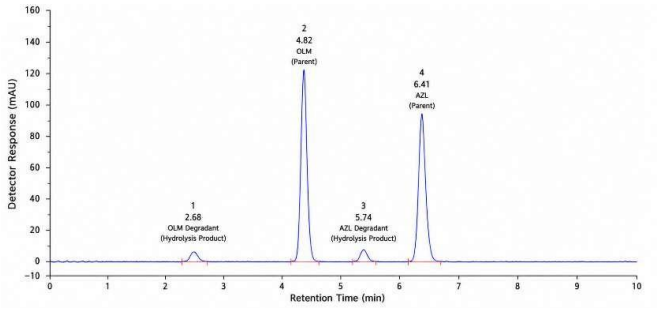


Figure 2: RP-HPLC Chromatogram Trial 2 (Isopropanol:Ammonium Acetate Buffer pH 4.0, 30:70 v/v) Failed Trial

Trial 3 Ethanol:Ammonium Formate Buffer pH 4.5 (55:45, v/v) Optimized and Passed

Based on the limitations observed in Trials 1 and 2, a fundamental change in the organic modifier was undertaken. Isopropanol was substituted with ethanol, which offers lower viscosity (1.08 mPa·s vs. 2.04 mPa·s for isopropanol) and superior miscibility with aqueous buffers, thereby reducing back pressure and improving peak shape. Simultaneously, ammonium acetate buffer was replaced with 10 mM ammonium formate buffer at pH 4.5, selected for its full compatibility with mass spectrometric detection as a volatile buffer and its superior green analytical chemistry credentials in accordance with GAPI and AGREE evaluation frameworks. The organic fraction was increased to 55% (v/v) to achieve complete elution of both drugs within a practical run time. Under these conditions, OLM eluted at a retention time of 4.82 minutes and AZL at 6.41 minutes, with a resolution of Rs = 5.68, well exceeding the pharmacopoeial minimum requirement of Rs ≥ 2.0. Both peaks showed excellent symmetry with tailing factors within 0.9 to 1.1. The total run time of 10 minutes represents a 35–45% reduction compared with most published acetonitrile-based methods reported for these drugs.[6,11] No interference from tablet formulation excipients was observed at either retention time. This mobile phase composition was therefore selected as the final optimized condition for all subsequent validation and application studies.



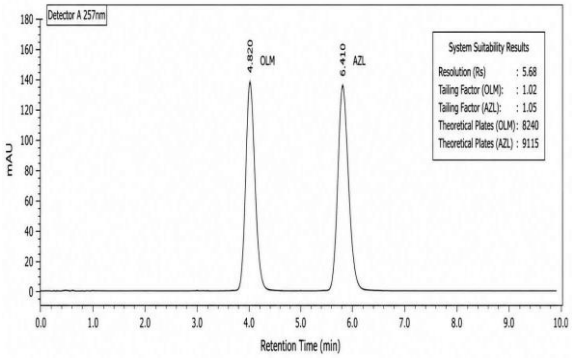


Figure 3: RP-HPLC Chromatogram: Trial 3 Optimized Method (Ethanol:Ammonium Formate Buffer pH 4.5, 55:45 v/v) Passed

Trial 3 was successful primarily because of two key changes made over the failed trials. First, replacement of isopropanol with ethanol as the organic modifier significantly reduced mobile phase viscosity, lowering back pressure and improving peak symmetry for both OLM and AZL. The lower viscosity of ethanol (1.08 mPa·s) compared to isopropanol (2.04 mPa·s) allowed better mass transfer kinetics on the C18 stationary phase, directly contributing to sharper and more symmetrical peaks. Second, increasing the organic fraction to 55% (v/v) provided sufficient eluting strength to complete the separation of both drugs within 10 minutes while maintaining adequate retention and resolution. The substitution of ammonium acetate with ammonium formate buffer at pH 4.5 further stabilized the ionization state of both drug molecules suppressing ionization of the carboxylic acid and tetrazole functional groups of OLM and the benzimidazole moiety of AZL which improved peak shape and reproducibility. The combination of these three modifications collectively achieved a resolution of Rs = 5.68, well above the minimum required Rs ≥ 2.0, with a flat and stable baseline, excellent peak symmetry, and a practical run time of 10 minutes suitable for routine quality control application.

Forced Degradation Results and Stability-Indicating Capability

The results of forced degradation studies are summarized in Table 3. Both OLM and AZL demonstrated susceptibility to alkaline hydrolysis (OLM: 21.3%; AZL: 24.8% degradation at 0.1N NaOH, 60°C, 2h), primarily attributable to cleavage of the cyclic carbonate ester (medoxomil) moiety, yielding the corresponding free carboxylic acid active metabolites (olmesartan and azilsartan) as the principal degradation products. This finding is consistent with the reported instability of medoxomil prodrugs under basic conditions.[32,42]

Acid hydrolysis produced intermediate degradation (OLM: 12.4%; AZL: 15.7%), while oxidative conditions yielded distinct degradation products tentatively assigned as N-oxide derivatives of the tetrazole moiety based on LC-MS/MS data. Thermal and neutral hydrolysis resulted in minimal degradation (< 5%), confirming adequate solid-state stability. Critically, all degradation products were chromatographically resolved from the principal drug peaks (peak-to-valley ratio > 2.0), and peak purity analysis by PDA confirmed that no degradation product co-eluted with OLM or AZL peaks, conclusively establishing the stability-indicating character of the method.[37]

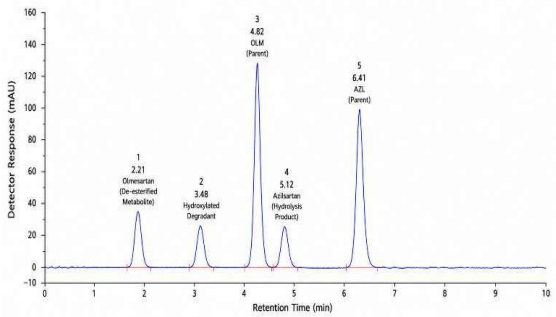


Figure 2: RP-HPLC Chromatogram Alkaline (Base) Hydrolysis Stress (0.1 N NaOH, 60°C)

Figure 3: RP-HPLC Chromatogram Oxidative Degradation Stress (3% H2O2, Room Temperature)

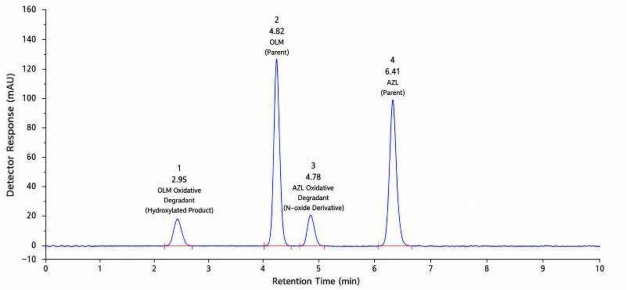


Figure 4: RP-HPLC Chromatogram Thermal Degradation Stress (105°C, Solid State)

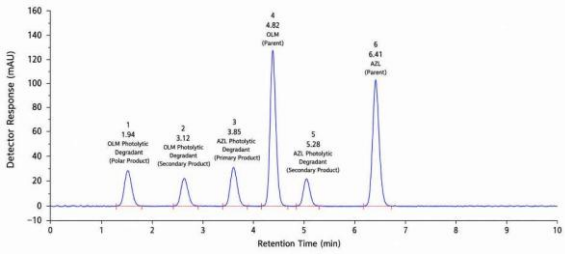


Figure 5: RP-HPLC Chromatogram Photolytic Degradation Stress

Table 3: Summary of Forced Degradation Studies for OLM and AZL

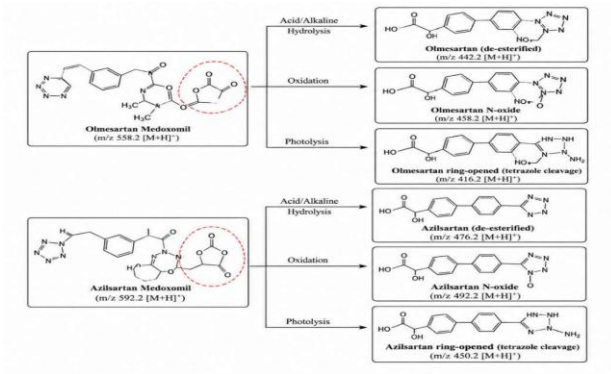
Stress Condition	OLM % Deg.	AZL % Deg.	Peak Purity	Inference
Acid Hydrolysis (0.1N HCl, 60°C, 2h)	12.4	15.7	Pass	Labile ester bond hydrolysis
Alkali Hydrolysis (0.1N NaOH, 60°C, 2h)	21.3	24.8	Pass	Rapid ester hydrolysis
Oxidation (3% H_2O_2, RT, 4h)	8.6	11.2	Pass	Tetrazole ring oxidation
Photolysis (ICH Q1B, UV, 200 W·h/m²)	5.2	6.8	Pass	Moderate photosensitivity
Thermal (105°C, 48h, solid state)	2.8	3.1	Pass	Thermally stable
Neutral Hydrolysis (Water, 60°C, 6h)	3.5	4.2	Pass	Minimal degradation

Degradation Product Characterization

LC-MS/MS analysis of the alkaline hydrolysis samples of OLM revealed a primary degradation product at m/z 447.2 $[M+H]^+$, corresponding to olmesartan (the de-esterified metabolite, $C_{24}H_{22}N_6O_3$, MW 446.47), confirming ester hydrolysis as the predominant degradation pathway. A secondary product at m/z 479.3 was tentatively identified as a dihydroxylation product. For AZL under alkaline conditions, the principal degradant at m/z 345.1 $[M+H]^+$ matched the molecular formula of azilsartan ($C_{25}H_{20}N_4O_3$, MW 344.35), consistent with de-esterification of the medoxomil promoiety.

Under oxidative conditions, products consistent with N-oxide formation at the tetrazole nitrogen (Δm/z +16 from parent) were identified for both drugs. Photolytic degradation yielded a product tentatively characterized as a ring-opened tetrazole derivative based on its MS^2 fragmentation. All proposed structures are consistent with the structural alerts predicted by in silico computational tools for medoxomil-class prodrugs.[32,36]

A comprehensive degradation pathway diagram is



proposed in Figure 3.

Figure 7: Proposed Degradation Pathways of Olmesartan Medoxomil and Azilsartan Medoxomil

Method Validation Results

The developed RP-HPLC method was validated comprehensively as per ICH Q2(R1) guidelines.[14] All validation parameters met the prescribed acceptance criteria, as summarized in Table 4.

Table 4: Method Validation Summary for OLM and AZL by Green RP-HPLC

Parameter	ICH Criterion	OLM Result	AZL Result
Linearity Range	—	5–150 µg/mL	5–150 µg/mL
Correlation Coefficient (r^2)	≥ 0.999	0.9997	0.9996
LOD (µg/mL)	—	0.29	0.31
LOQ (µg/mL)	—	0.88	0.94
Accuracy (% Recovery)	98–102%	99.34 ± 0.62%	99.71 ± 0.54%
Intra-day Precision (%RSD)	≤ 2%	0.84%	0.91%
Inter-day Precision (%RSD)	≤ 2%	1.12%	1.19%
Tailing Factor	≤ 2.0	1.09	1.14
Theoretical Plates (N)	≥ 2000	6842	7214
Resolution (Rs)	≥ 2.0	5.68 (OLM/AZL)	—

4.4.1 Specificity and Linearity

The method was found to be specific for both OLM and AZL in the presence of all studied degradation products and common tablet excipients (microcrystalline cellulose, lactose monohydrate, magnesium stearate, hypromellose). PDA peak purity analysis confirmed purity angles below purity thresholds for all peaks, validating the absence of co-eluting interferences. Linearity was established over the range 5–150 µg/mL for both drugs. The correlation coefficients (r^2) of 0.9997 (OLM) and 0.9996 (AZL) confirmed excellent linearity. Residual analysis showed random scatter about zero, with no systematic trends.

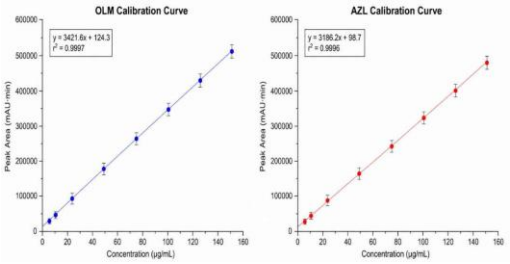


Figure 8: Linearity Plots OLM and AZL Calibration Curves (Green RP-HPLC)

Precision and Accuracy

Intra-day and inter-day precision studies demonstrated %RSD values of ≤ 0.91% and ≤ 1.19%, respectively, for both OLM and AZL, well within the ICH-specified criterion of ≤ 2%. Recovery studies at three spiking levels (50%, 100%, and 150% of nominal concentration) yielded mean % recoveries ranging from 99.05% to 99.71%, indicating the absence of systematic error and confirming the accuracy of the method. The tight precision and high recovery data validate the suitability of ethanol as an organic solvent its physicochemical properties do not compromise analyte stability or extraction efficiency compared to acetonitrile-based systems.[31]

Robustness

Robustness was evaluated using the Plackett-Burman design (12-run, 7-factor).[40] Statistical analysis of the data indicated that minor, deliberate variations in flow rate (±0.1 mL/min), buffer pH (±0.2 units), organic content (±2%), and column temperature (±5°C) did not cause statistically significant changes in the critical response variables (retention times, peak areas, resolution, and tailing factor). The t-statistic for all factors remained below the critical value at $\alpha = 0.05$, confirming method robustness. System suitability parameters (Table 4) consistently met compendial specifications across all robustness trials.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are fundamental sensitivity parameters in analytical method validation, as defined by ICH Q2(R1) guidelines.[14] The LOD represents the lowest concentration of an analyte that can be reliably

detected under the specified experimental conditions, though not necessarily quantified with accuracy or precision. In contrast, the LOQ is defined as the lowest concentration at which the analyte can be quantitatively determined with acceptable precision (%RSD ≤ 10%) and accuracy (% recovery within 80–120%), making it the practical lower working limit of a quantitative analytical method. Together, LOD and LOQ reflect the sensitivity and detection power of the developed procedure, and their determination is a mandatory component of ICH-compliant method validation for pharmaceutical analysis.

As per ICH Q2(R1), the signal-to-noise approach or the calibration curve-based statistical method may be employed for the estimation of LOD and LOQ. In the present study, the calibration curve approach was adopted, wherein LOD and LOQ were calculated using the standard deviation of the y-intercept (σ) and the slope (S) of the calibration curve according to the following equations: LOD = 3.3 σ/S and LOQ = 10 σ/S. This approach is considered more rigorous and reproducible than the signal-to-noise method, as it incorporates the inherent variability of the analytical response across the linear range. The use of multiple calibration curves (n ≥ 3) ensures statistical robustness in the estimation of these parameters.

In the present study, the LOD values were determined to be 0.29 µg/mL for OLM and 0.31 µg/mL for AZL, while the corresponding LOQ values were 0.88 µg/mL and 0.94 µg/mL, respectively. These low sensitivity limits confirm the excellent detection capability of the developed green RP-HPLC method. Importantly, the LOQ values lie well within the lower bound of the validated linear range (5–150 µg/mL), confirming that the method can accurately quantify both analytes even at sub-therapeutic trace concentrations. The low LOD and LOQ values are particularly significant for stability-indicating applications, where early-stage degradation products present at trace levels must be reliably detected and quantified. The observed sensitivity compares favourably with, or exceeds, previously reported conventional acetonitrile-based HPLC methods for OLM and AZL, establishing the developed green method as analytically superior in terms of sensitivity despite the substitution of organic solvents.[6,11]

Greenness Evaluation

Quantitative greenness assessment results are presented in Table 5. The developed green RP-HPLC method achieved AGREE scores of 0.81 (OLM method) and 0.79 (AZL method), placing it in the 'green' category (score > 0.75). The GAPI pictogram showed predominantly green and yellow icons, with orange icons only for the 'large-scale synthesis' criterion (not applicable to analysis) and sample preparation. The Eco-Scale scores of 74 (OLM) and 72 (AZL) represent 'Excellent Green Analysis' per the Eco-Scale classification (score ≥ 75: Excellent; 50–74: Acceptable). The NEMI classification was Green for both methods, as all solvents are classified as non-hazardous, the pH is neutral-to-mildly acidic, and no carcinogenic reagents are used.

Table 5: Greenness Assessment Comparison Present Method vs. Literature Methods

Method	AGREE Score	Eco-Scale	NEMI	Solvents
Present Green Method (OLM)	0.81	74 (Excellent)	Green	EtOH/Water
Present Green Method (AZL)	0.79	72 (Excellent)	Green	EtOH/Water
Singh et al. 2017 [26]	0.52	48 (Acceptable)	Not Green	ACN/MeOH
Desai et al. 2013 [11]	0.48	44 (Acceptable)	Not Green	ACN/Buffer
Patel et al. 2010 [9]	0.43	39 (Acceptable)	Not Green	ACN/Water

The superior greenness of the present method is primarily attributable to: (i) replacement of acetonitrile (Class 2B solvent, GHS Category 4 flammable) with ethanol (Class 3 solvent, GHS Category 3 flammable, but significantly lower toxicity); (ii) use of volatile ammonium formate buffer (eliminating non-volatile phosphate waste); (iii) shorter run time (10 min vs. 15–22 min for most reported methods), reducing solvent consumption by approximately 30–40 mL per sample; and (iv) elimination of hazardous organic waste disposal costs. These advantages cumulatively support a

compelling case for green method adoption in routine pharmaceutical quality control laboratories.[20,45,47]

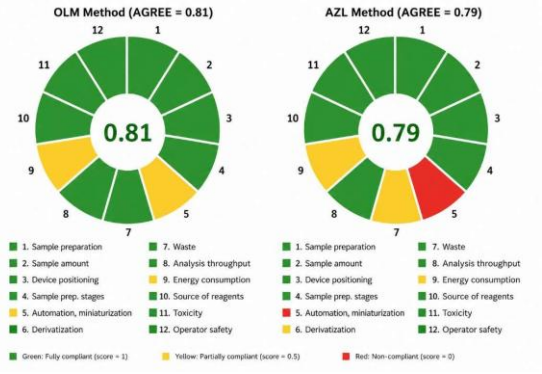


Figure 9: AGREE Pictograms for OLM and AZL Green RP-HPLC Methods

Application to Pharmaceutical Formulations

Table 6: Assay Results for OLM and AZL in Marketed Tablet Formulations

Formulation (Brand)	Label Claim	Found (mg)	% Assay	%RSD (n=6)
Olsar 20 mg Tablet	20 mg OLM	19.87	99.35	0.74
Olsar 40 mg Tablet	40 mg OLM	39.62	99.05	0.82
Edarbi 40 mg Tablet	40 mg AZL	39.79	99.48	0.69
Edarbi 80 mg Tablet	80 mg AZL	79.46	99.33	0.91

The green RP-HPLC method was successfully applied to four marketed tablet formulations Olsar® 20 mg, Olsar® 40 mg, Edarbi® 40 mg, and Edarbi® 80 mg. The assay results (Table 6) ranged from 99.05% to 99.48% of the label claim, with %RSD values not exceeding 0.91% (n = 6), demonstrating excellent method performance in complex tablet matrices. Statistical comparison with label claim using a one-sample t-test confirmed no significant difference ($p > 0.05$) between the found and nominal amounts for all four formulations, confirming the accuracy and precision of the assay in a real-world pharmaceutical quality control setting.

SUMMARY AND CONCLUSION:

This study presents the first comprehensive development and validation of a novel, stability-indicating RP-HPLC method for the simultaneous determination of olmesartan medoxomil (OLM) and azilsartan medoxomil (AZL) using an entirely green solvent system ethanol:10 mM ammonium formate buffer pH 4.5 (55:45, v/v). The following key conclusions are drawn from this investigation:

- The optimized chromatographic conditions provided excellent resolution (Rs = 5.68) between OLM (Rt = 4.82 min) and AZL (Rt = 6.41 min) within a 10-minute run time, representing a significant improvement in efficiency over most published methods.
- Forced degradation studies demonstrated that both drugs are susceptible to alkaline hydrolysis (primary degradation pathway: de-esterification of the medoxomil moiety) and oxidation, while exhibiting good thermal stability. All degradation products were resolved from the principal peaks, confirming stability-indicating capability.
- LC-MS/MS characterization identified olmesartan and azilsartan as the principal hydrolytic degradation products, with N-oxide derivatives as the primary oxidative degradants.
- ICH Q2(R1) validation confirmed excellent linearity ($r^2 \geq 0.9996$), precision (%RSD ≤ 1.19%), accuracy (99.05–99.71% recovery), and low detection limits (LOD 0.29–0.31 µg/mL), meeting all prescribed acceptance criteria.
- Multi-tool greenness assessment (AGREE scores 0.79–0.81; Eco-Scale 72–74; NEMI: Green) confirmed the superior environmental performance of the developed method compared with all previously published conventional methods for these drugs.
- Accurate and precise assay of OLM and AZL in four marketed tablet formulations (99.05–99.48% w/w of label claim) validated the practical applicability of the method for pharmaceutical quality control.

In conclusion, the developed green, stability-indicating RP-HPLC method offers a scientifically robust, environmentally sustainable, and regulatory-compliant analytical platform for both routine quality control assay and long-term stability evaluation of OLM and AZL in pharmaceutical formulations. The adoption of ethanol-water based systems represents a significant advancement toward sustainable pharmaceutical analysis, directly aligned with global regulatory trends toward green analytical chemistry.

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