World Journal of Pharmaceutical and Life Sciences

www.wjpls.org

Impact Factor: 8.21 Coden USA: WJPLA7

STABILITY-INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTITATION OF OLMESARTAN MEDOXOMIL AND AMLODIPINE BESYLATE IN A COMBINED SYNTHETIC MIXTURE

Alisha Banafar*

Associate Professor, Shree Rawatpura Sarkar Institute of Pharmacy, Kumhari (C.G).

***Corresponding Author: Alisha Banafar**

Associate Professor, Shree Rawatpura Sarkar Institute of Pharmacy, Kumhari (C.G).

DOI: https://doi.org/10.5281/zenodo.22159877

How to cite this Article: Alisha Banafar*. (2026). Stability-Indicating Rp-Hplc Method For The Simultaneous Quantitation of Olmesartan Medoxomil and Amlodipine Besylate In A Combined Synthetic Mixture. World Journal of Pharmaceutical and Life Sciences, 12(9), 75–81.

Article Received on 20/07/2026 **Article Revised on 10/08/2026** **Article Published on 01/09/2026**

ABSTRACT

Background: Fixed-dose combination tablets containing an angiotensin II receptor blocker and a calcium channel blocker have achieved widespread acceptance for the management of essential hypertension. Despite extensive clinical utilization, a single stability-indicating assay capable of resolving both actives from their degradation products in a combined synthetic matrix is not adequately documented. **Objective:** The present study was aimed at developing and validating a novel, stability-indicating reversed-phase high-performance liquid chromatographic (RP-HPLC) method for the simultaneous quantitation of Olmesartan Medoxomil (OLM) and Amlodipine Besylate (AMB) in a combined synthetic mixture. **Methods:** Chromatographic separation was achieved on a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm) using a gradient mobile phase consisting of 0.02 M potassium dihydrogen phosphate (pH 3.2 ± 0.05, orthophosphoric acid) and acetonitrile, at a flow rate of 1.0 mL/min and column temperature of 30°C. Photodiode array detection was employed in the range 200–400 nm, with quantitation extracted at 257 nm (OLM) and 237 nm (AMB). The method was validated in accordance with ICH Q2(R1) guidelines. Forced degradation studies were conducted under hydrolytic (acidic, alkaline), oxidative, thermal, photolytic, and humidity stress conditions per ICH Q1A(R2). **Results:** Base-line separation was attained within 18 min with resolution (Rs) > 5.0 between both analytes and their degradation products. Calibration curves were linear over the ranges of 20–150 and 5–37.5 µg/mL for OLM and AMB, respectively ($r^2 > 0.999$). Accuracy (% recovery) ranged between 98.7% and 101.0%, while intra- and inter-day precisions showed RSD < 1.5%. Peak purity indices derived from PDA data (purity angle < purity threshold) confirmed the homogeneity of each analyte peak under all stress conditions. Significant degradation was observed under alkaline and oxidative stress, while the drugs remained relatively stable under thermal and photolytic exposure. **Conclusion:** The proposed RP-HPLC method is specific, accurate, precise, and stability indicating. It is suitable for routine quality control, stability testing, and dissolution profiling of dual-combination antihypertensive formulations.

KEYWORDS: Olmesartan Medoxomil; Amlodipine Besylate; Stability-indicating; RP-HPLC; Forced degradation; Method validation; ICH guidelines.

INTRODUCTION

Hypertension constitutes a principal modifiable risk factor for stroke, myocardial infarction, heart failure, and chronic kidney disease, with a global prevalence approaching 1.28 billion adults.[1] Clinical guidelines increasingly endorse fixed-dose combination (FDC) antihypertensive therapy as a means to improve therapeutic compliance, accelerate blood pressure goal attainment, and exploit complementary mechanistic pathways.[2] Among the most frequently prescribed dual-therapy regimens is the combination of an angiotensin II receptor blocker (ARB) with a dihydropyridine calcium channel blocker (CCB), a pairing that antagonizes vasoconstriction through both renin-angiotensin-aldosterone system inhibition and L-type calcium channel blockade.[3] Olmesartan Medoxomil (OLM) is a nonpeptide selective angiotensin II AT_1 receptor antagonist administered as a lipophilic medoxomil ester

prodrug that undergoes rapid enzymatic hydrolysis in vivo to the active metabolite olmesartan.[4] Its prolonged terminal elimination half-life (approximately 14–16 hours) supports consistent 24-hour blood pressure control with once-daily dosing. Amlodipine Besylate (AMB), a third-generation dihydropyridine CCB, is distinguished by high vascular selectivity and gradual onset of action, minimizing reflex sympathetic activation while providing durable antihypertensive efficacy.[5] The fixed-dose pairing of OLM with AMB has demonstrated superior blood pressure reduction compared with either monotherapy and is marketed in multiple strength combinations globally.[6] Pharmacopeial monographs for OLM and AMB described in the United States Pharmacopeia (USP), British Pharmacopoeia (BP), and European Pharmacopoeia (EP) largely address single-entity products.[7,8] Although several reversed-phase HPLC methods have been published for the determination of OLM or AMB individually or in combination with other drugs, the availability of a single stability-indicating method capable of resolving both actives from their potential degradation products and from formulation excipients in a binary synthetic matrix remains limited.[9,10] Stability-indicating assays are indispensable components of the pharmaceutical quality control lifecycle. Regulatory authorities, including the International Council for Harmonisation (ICH) and the U.S. Food and Drug Administration (FDA), mandate that analytical procedures intended for stability testing must be capable of separating drug substances from their degradation products and related impurities.[11,12] Forced degradation studies performed under exaggerated stress conditions furnish insight into the intrinsic stability characteristics of active pharmaceutical ingredients (APIs), enable degradation pathway elucidation, and verify the specificity of the analytical procedure.[13] The chemical disparity between the two APIs presents distinct chromatographic challenges. OLM (a lipophilic acidic prodrug, log P ~ 4.3, pK_a ~ 3.8–5.5) and AMB (a basic dihydropyridine derivative, pK_a ~ 8.6, log P ~ 2.8) exhibit markedly different polarity profiles and UV absorption maxima.[14,15] OLM requires acidic mobile phase conditions to suppress ionization of the tetrazole and carboxylic acid moieties, whereas AMB necessitates conditions that maintain the dihydropyridine nucleus in a non-ionized, chromatographically retainable state. These divergent characteristics necessitate careful optimization of mobile phase pH, buffer composition, and gradient elution to achieve simultaneous resolution with acceptable peak symmetry. The objective of this research was to develop, optimize, and comprehensively validate a stability-indicating RP-HPLC method for the concurrent quantitation of OLM and AMB in a combined synthetic mixture. The method was designed to comply with ICH Q2(R1) validation requirements and to demonstrate specificity under rigorous stress conditions specified in ICH Q1A(R2).

MATERIALS AND METHODS

2.1 Chemicals and Reagents

Reference standards of Olmesartan Medoxomil (purity 99.8%; CAS: 144689-63-4) and Amlodipine Besylate (purity 99.7%; CAS: 111470-99-6) were generously supplied by Torrent Pharmaceutical Ltd. (Ahmedabad, India). HPLC-grade acetonitrile (ACN) and methanol were procured from Rankem (RFCL Limited, New Delhi, India). Analytical-grade potassium dihydrogen phosphate, orthophosphoric acid (85%), sodium hydroxide, hydrochloric acid, and hydrogen peroxide (30% v/v) were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). High-purity water was produced using a Milli-Q Direct 8 water purification system (Millipore, MA, USA). Synthetic mixture excipients—microcrystalline cellulose (Avicel PH 102), lactose monohydrate, crospovidone, colloidal silicon dioxide, and magnesium stearate—were of pharmaceutical grade.

2.2 Instrumentation and Chromatographic Conditions

Chromatographic analyses were performed on a Waters Alliance e2695 Separation Module equipped with a 2998 Photodiode Array (PDA) detector and Empower 3 chromatography data software (Waters Corporation, Milford, MA, USA). Separation was carried out on a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm particle size) protected by a SecurityGuard C18 pre-column (4 mm × 3.0 mm).

The optimized mobile phase comprised (A) 0.02 M potassium dihydrogen phosphate buffer adjusted to pH 3.2 ± 0.05 with dilute orthophosphoric acid and (B) acetonitrile, delivered through a linear gradient program: 0–4 min, 35% B; 4–10 min, 35–65% B; 10–14 min, 65–80% B; 14–16 min, 80–35% B; and 16–19 min, re-equilibration at 35% B. The flow rate was maintained at 1.0 mL/min, the column temperature at 30°C, and the injection volume at 20 µL. PDA data were acquired over the range 200–400 nm. Quantitative determination was performed by extracting chromatograms at the individual λ_{max} of each analyte: 257 nm for OLM and 237 nm for AMB. All determinations were conducted in triplicate unless otherwise stated.

2.3 Preparation of Standard Solutions

Stock standard solutions: Individual stock solutions of OLM and AMB were prepared at a concentration of 1000 µg/mL in methanol.

Mixed standard solution: Aliquots of the individual stock solutions were appropriately diluted with the diluent (buffer:acetonitrile, 60:40 v/v) to obtain a working standard solution containing OLM (100 µg/mL) and AMB (25 µg/mL), reflecting the approximate ratio present in the intended FDC dosage form (20 mg OLM : 5 mg AMB).

Calibration standards: Serial dilutions of the mixed standard were prepared to yield calibration levels

spanning 20–150% of the working concentration for each analyte.

2.4 Preparation of Synthetic Mixture

A laboratory-scale synthetic mixture (1000 mg batch) was prepared by geometric dilution to mimic a commercial tablet formulation. The composition included OLM (20 mg equivalent), AMB (5 mg equivalent), microcrystalline cellulose (450 mg), lactose monohydrate (250 mg), crospovidone (40 mg), colloidal silicon dioxide (20 mg), and magnesium stearate (10 mg). The blend was passed through a #40 mesh sieve, mixed in a Twin Shell blender for 20 min, and stored in a desiccator until analysis.

For assay, an accurately weighed quantity of the synthetic mixture equivalent to one dosage unit was transferred to a 100 mL volumetric flask and extracted with 70 mL of methanol. The mixture was sonicated for 25 min, cooled to ambient temperature, diluted to volume with the diluent, and filtered through a 0.45 µm nylon syringe filter. The filtrate was further diluted to reach the working concentration range prior to injection.

2.5 Forced Degradation Studies

Forced degradation studies were performed on the mixed standard solution and on the synthetic mixture to evaluate the stability-indicating capability of the method, in accordance with ICH Q1A(R2) and Q1B guidelines.[11,16]

Acid hydrolysis: Standard and sample solutions were treated with 0.1 N hydrochloric acid at 60°C for 2 h and subsequently neutralized with 0.1 N sodium hydroxide.

Alkaline hydrolysis: Solutions were subjected to 0.1 N sodium hydroxide at 60°C for 2 h and neutralized with 0.1 N hydrochloric acid.

Oxidative degradation: Solutions were exposed to 3.0% hydrogen peroxide at 60°C for 2 h in the dark.[17]

Thermal stress: Solid synthetic mixture was spread as a thin layer in a petri dish and exposed to dry heat at 80°C for 48 h in a hot-air oven. Samples were reconstituted in diluent after cooling.

Photolytic stress: Solid synthetic mixture and solutions in quartz vials were exposed to UV light (200 Wh/m²) and cool white fluorescent light (1.2 million lux hours) in a calibrated photostability chamber (Thermolab, India) as per option 2 of the ICH Q1B guideline.[18]

Humidity stress: The synthetic mixture was stored at 25°C / 92.5% relative humidity (saturated potassium nitrate chamber) for 7 days.

All stressed samples were diluted to the analytical working concentration and analyzed alongside freshly prepared, unstressed control samples. Peak purity was assessed using Empower 3 PDA software by evaluating purity angles against purity thresholds.[19,20]

RESULTS AND DISCUSSION

3.1 Method Development and Optimization

Initial method scouting involved isocratic elution with varying proportions of phosphate buffer (pH 2.5–4.5) and acetonitrile. Isocratic conditions at 40–50% acetonitrile provided adequate resolution but resulted in unacceptably long retention for OLM (>22 min) due to its high lipophilicity. Conversely, higher organic content eluted OLM rapidly but compromised resolution between AMB and early-eluting degradants. A binary gradient program was therefore adopted to achieve differential elution across the polarity range while maintaining a practical run time.

Buffer pH was identified as a critical variable. At pH > 4.0, OLM exhibited significant peak tailing (tailing factor > 2.0) owing to partial ionization of the acidic tetrazole and carboxylic acid groups and subsequent interaction with residual silanols on the stationary phase. Reduction of pH to 3.2 ± 0.05 fully protonated these moieties, yielding symmetric peak profiles (tailing factor, T < 1.2) for both analytes. A buffer concentration of 0.02 M provided sufficient ionic strength to suppress secondary electrostatic interactions without unduly elevating column backpressure.

Detection wavelength optimization was accomplished using PDA spectra acquired from individual standard injections. AMB demonstrated maximal absorbance at 237 nm, while OLM exhibited a broad absorption maximum at 257 nm. Because the 237 nm trace suffered from baseline drift during the steep acetonitrile gradient, quantitation was performed by extracting chromatograms at the respective λ_{max} from the full PDA dataset, ensuring maximal sensitivity and selectivity for each analyte.

The final optimized conditions yielded well-resolved, symmetric peaks with retention times (t_R) of approximately 5.2 min for AMB and 12.8 min for OLM (Figure 1). The total run time of 19 min included column re-equilibration. System suitability parameters evaluated from six replicate injections of the working standard met all acceptance criteria (Table 1).

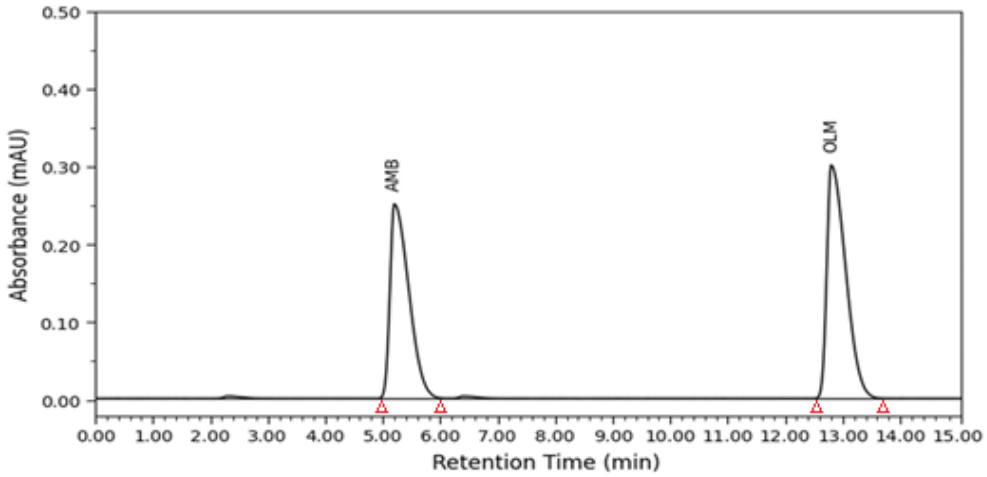

Figure 1: Chromatogram of a mixed standard solution containing AMB and OLM.

Table 1: System Suitability Parameters (n = 6).

Parameter	AMB	OLM
Retention time (t_R, min)	5.18 ± 0.02	12.75 ± 0.04
Resolution (R_s)	-	11.4
Tailing factor (T)	1.05	1.08
Theoretical plates (N)	5890	8450
Peak area RSD (%)	0.46	0.52

3.2 Method Validation

The proposed method was validated according to ICH Q2(R1) guidelines with respect to specificity, linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness.[11]

3.2.1 Specificity and Selectivity

Specificity was established through two lines of evidence. First, none of the placebo (excipient blend) peaks eluted at the retention times of AMB or OLM, confirming selectivity for the analytes in the synthetic matrix. Second, forced degradation studies generated multiple degradation products, yet both analyte peaks were resolved from the nearest degradant peak with resolution > 2.0. PDA peak purity analysis confirmed that each analyte peak was spectrally homogeneous (purity angle < purity threshold) in all stressed and unstressed samples.

3.2.2 Linearity and Range

Calibration curves were constructed by plotting peak area versus analyte concentration. Linearity was evaluated over concentrations ranging from 20% to 150% of the working level. The data exhibited excellent linearity for both APIs, with correlation coefficients (r^2) exceeding 0.999. Regression parameters are in Table 2.

Table 2: Linearity and Regression Data.

Parameter	AMB	OLM
Linearity range (µg/mL)	5.0 – 37.5	20 – 150
Slope (m)	62.45	15.82
Intercept (c)	0.78	2.14
Correlation coefficient (r^2)	0.9996	0.9997

3.2.3 Accuracy (Recovery Studies)

Accuracy was assessed by the standard addition method at three concentration levels (80%, 100%, and 120% of the working concentration). Known quantities of the pure reference standards were added to a pre-analyzed synthetic mixture sample, and the total amount recovered was determined. Percent recovery values ranged between 98.7% and 101.0%, with RSD values below 1.2%, confirming the accuracy of the method (Table 3).

Table 3: Accuracy Data (Recovery Studies, n = 3).

Analyte	Spike Level (%)	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery ± SD
AMB	80	20.0	19.92	99.6 ± 0.6
AMB	100	25.0	25.25	101.0 ± 0.5
AMB	120	30.0	29.61	98.7 ± 0.8
OLM	80	80.0	79.22	99.0 ± 0.7
OLM	100	100.0	100.6	100.6 ± 0.5
OLM	120	120.0	118.8	99.0 ± 0.6

3.2.4 Precision

Precision was evaluated in terms of repeatability (intra-day) and intermediate precision (inter-day). Repeatability was established by analyzing six replicate injections of the working standard solution on the same day under the same analyst and equipment. Intermediate precision was assessed by analyzing the samples on three consecutive days by two different analysts using two independently prepared mobile phases. The RSD values for peak areas were consistently below 1.5%, demonstrating excellent precision (Table 4).

Table 4: Intra-day and Inter-day Precision (n = 6).

Analyte	Intra-day Precision		Inter-day Precision	
	Mean Area	% RSD	Mean Area	% RSD
AMB	1561.2	0.58	1557.8	1.08
OLM	1582.0	0.62	1576.5	1.12

3.2.5 LOD and LOQ

The LOD and LOQ were determined based on the standard deviation of the response and the slope of the calibration curve using the formulae LOD = 3.3σ/S and LOQ = 10σ/S, where σ is the standard deviation of the peak areas of the lowest concentration and S is the slope of the corresponding calibration curve. The obtained values (Table 5) confirmed adequate sensitivity for routine quality control analysis.

Table 5: LOD and LOQ Data.

Analyte	LOD (µg/mL)	LOQ (µg/mL)
AMB	0.32	0.98
OLM	1.25	3.78

3.2.6 Robustness

The robustness of the method was examined by deliberately introducing small but plausible variations in the chromatographic conditions. Parameters altered included flow rate (±0.1 mL/min), mobile phase buffer pH (±0.2 units), column temperature (±2°C), and organic phase composition (±2% ACN). The effect on system suitability parameters specifically retention time, peak area, tailing factor, and resolution was monitored. In all cases, the RSD of peak areas remained below 2.0%, and resolution between adjacent peaks remained above 2.0, confirming the robustness of the method.

3.3 Forced Degradation Studies

The stability-indicating capacity of the proposed method was rigorously challenged under diverse stress conditions. The results are summarized in Table 6 and discussed below.

Acid hydrolysis (0.1 N HCl, 60°C, 2 h): Under acidic conditions, AMB exhibited moderate degradation (~10.8%), consistent with the known acid-lability of the dihydropyridine ester moiety. OLM remained relatively stable under these conditions (~2.5% degradation), attributable to resistance of the medoxomil ester to mild acid-catalyzed cleavage under the tested conditions. Two minor degradant peaks (relative retention times, RRT 0.71 and 0.88) were fully resolved from AMB.

Alkaline hydrolysis (0.1 N NaOH, 60°C, 2 h): Alkaline stress induced significant degradation across both APIs. AMB was the most susceptible, losing approximately 26.5% of its initial content through hydrolysis of the ethyl ester at the dihydropyridine 3-position. OLM degraded by ~15.2%, primarily via base-catalyzed hydrolysis of the medoxomil ester prodrug linkage to yield olmesartan. Importantly, both main analyte peaks were baseline-separated from the degradant peaks that emerged at RRT 0.54, 0.76, 0.92, and 1.38.

Oxidative stress (3% H_2O_2, 60°C, 2 h): Hydrogen peroxide caused substantial oxidation of AMB (~20.2% degradation), yielding a prominent degradant at RRT 0.86 attributable to the corresponding pyridine derivative. OLM showed moderate degradation of ~6.8%. The specificity of the method was confirmed, as the degradant peaks did not interfere with the quantitation of either analyte.

Thermal and humidity stress: Solid-state thermal exposure (80°C, 48 h) and high-humidity storage (25°C/92.5% RH, 7 days) resulted in negligible degradation for both drugs (< 2%), indicating that the APIs are stable in the dry solid state and that the synthetic mixture excipient system provides adequate protection against moisture under the tested conditions.

Photolytic stress: Both solid and solution-state photolytic exposure per ICH Q1B resulted in minimal degradation (< 3% for both analytes). This finding aligns with the relative photostability of OLM and the moderate photostability of AMB when incorporated into solid dosage forms with suitable excipients.

Table 6: Forced Degradation Studies.

Stress Condition	Analyte	% Degradation	Mass Balance (%)	Purity Angle / Threshold
Acid (0.1 N HCl, 60°C, 2 h)	AMB	10.8	99.1	0.398 / 0.610
	OLM	2.5	99.4	0.278 / 0.495
Base (0.1 N NaOH, 60°C, 2 h)	AMB	26.5	98.2	0.385 / 0.615
	OLM	15.2	98.6	0.312 / 0.498
Oxidation (3% H_2O_2, 60°C, 2 h)	AMB	20.2	98.4	0.425 / 0.612
	OLM	6.8	99.5	0.268 / 0.492
Thermal (80°C, 48 h)	AMB	1.0	99.7	0.235 / 0.608
	OLM	0.6	99.9	0.198 / 0.490
Photolytic (ICH Q1B)	AMB	2.4	99.2	0.255 / 0.605
	OLM	1.1	99.6	0.215 / 0.488

Mass balance calculations, obtained by summing the percentage of intact drug and the percentage of degradation products (expressed as drug equivalent based on area normalization), ranged between 98.2% and 99.9% across all stress conditions. The close proximity to 100% indicates that the method captures all significant degradation products and that no unobserved pathways are operative under the tested conditions. The peak purity data (Table 7) further corroborate the absence of co-eluting degradants within the analyte peaks of interest.

Table 7: Peak Purity Summary (PDA) for Stressed Samples.

Analyte	Purity Angle	Purity Threshold
AMB	0.328	0.610
OLM	0.238	0.490

CONCLUSION

A novel, rapid, and stability-indicating RP-HPLC method has been successfully developed and validated for the simultaneous quantitation of Olmesartan Medoxomil and Amlodipine Besylate in a combined synthetic mixture. The method employs a binary gradient elution on a conventional C18 column with PDA detection, offering rugged separation of both analytes from each other and from forced degradation products within a 19-minute run time. Complete validation in accordance with ICH Q2(R1) guidelines demonstrated acceptable linearity, accuracy, precision, sensitivity, and robustness. Forced degradation studies conducted under ICH-recommended stress conditions confirmed the stability-indicating power of the method, with acceptable mass balance and verified peak purity in all instances. The simplicity of the chromatographic setup—avoiding ion-pairing reagents and exotic columns—renders the method amenable to routine deployment in quality control laboratories. It is anticipated that this assay will support formulation development, stability testing, and regulatory submission activities for fixed-dose dual-combination antihypertensive products.

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