

Validation of the RP-HPLC/DAD Method for Concurrent Quantification of Various Antihypertensive Drugs in a Membrane-Moderated Matrix Monolithic Polymeric Device Type

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ABSTRACT

The goal of this study was to develop and validate the simultaneous estimate of amlodipine besylate and olmesartan medoxomil in tablet formulations. Olmesartan medoxomil and amlodipine besylate in tablet formulations can be quantified using a reversed phase high-performance liquid chromatographic (RP-HPLC) approach that uses isocratic conditions and ultraviolet detection at 280 nm. A stainless steel analytical column, the Intersil ODS C18 column (4.6 X 250 mm; 5 µm), was used to achieve successful chromatographic separation. The system was operated at 25 ± 2 °C using a mobile phase consisting of acetonitrile and HPLC grade water (pH adjusted to 4.5 with orthophosphoric acid) in a 70:30 ratio at a flow rate of 1.0 mL/minute. Olmesartan medoxomil's slope, intercept, and correlation coefficient were found to be $y = 11990x + 53124$ ($r^2 = 1$), but amlodipine besylate's were $y = 46027x - 22624$ ($r^2 = 1$). The specificity, linearity, accuracy, and precision of the suggested method were verified. The technique was found to be suitable for the concurrent quality control of amlodipine besylate and olmesartan medoxomil in both formulations and bulk medications.

Keywords: Olmesartan medoxomil, Amlodipine besylate, Isocratic separation, Method validation, RP-HPLC.

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1. INTRODUCTION

The majority of people with hypertension require many medications to achieve adequate blood pressure control. Antihypertensive medications in fixed-dose combinations, like olmesartan medoxomil/amlodipine besylate, provide advantages over monotherapy, such as increased effectiveness, less side effects, and lower costs. For those who are expected to need multiple antihypertensive drugs to achieve their blood pressure goals, amlodipine besylate and Olmesartan medoxomil tablets may be used as initial treatment ^[1].

AZOR (olmesartan medoxomil and amlodipine besylate) tablets are used to treat hypertension, either alone or in combination with other antihypertensive drugs to lower blood pressure. The risk of both fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions, is reduced when blood pressure is lowered. Controlled trials of antihypertensive drugs from a variety of pharmacologic classes, including the class to which this medication predominantly belongs, have shown these benefits. Amlodipine and Olmesartan Medoxomil

tablets have not been shown in controlled trials to reduce risk ^[2]. In 2007, the USFDA approved the combination.

The chemical formula for olmesartan medoxomil (Figure 1) is 2, 3-dihydroxy-2-butenyl-(1-hydroxy-1-methyl ethyl) -2-propyl-1-[P-(O-1H-tetrazole-5-ylphenyl) benzyl] imidazole-5-carboxylate, cyclic 2, 3-carbonate. Olmesartan medoxomil is a prodrug that releases olmesartan, its only active metabolite, when consumed. Olmesartan acts as a selective and competitive type II receptor antagonist. The abundance of esterases in the liver, plasma, and gastrointestinal tract facilitates the hydrolysis of olmesartan. For the treatment of hypertension, it is used either alone or in combination with other antihypertensive medications ^[1, 3-4].

Amlodipine besylate (Figure 2) is a monobenzene sulphonate that acts as a long-acting calcium channel blocker. Its chemical name is 3ethyl -5-methyl (±)-2-[(2-aminoethoxy) methyl] -4-(2-chlorophenyl)-1, 4-dihydro-6-methyl -3, 5-pyridine dicarboxylate ^[2-4].

Olmesartan medoxomil and amlodipine besylate can be measured using a range of HPLC techniques, both separately and in combination with other medications, as well as in stressful circumstances. Additionally, five studies have described HPLC techniques for the simultaneous measurement of valsartan and amlodipine in tablet dosage forms and in combination with other medications [5–10]. Additionally, the HPLC approach for the simultaneous measurement of hydrochlorothiazide and olmesartan medoxomil has been reported [11].

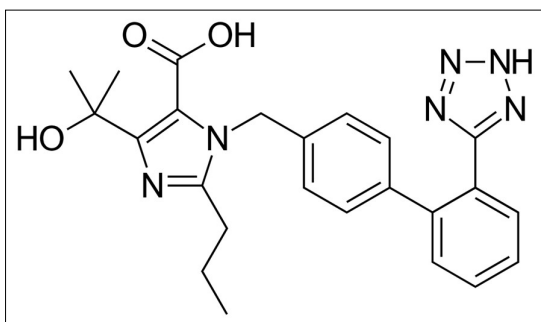


Fig.1: Chemical structure of olmesartan medoxomil

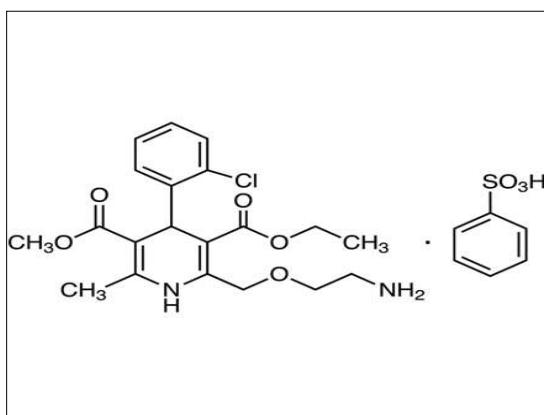


Fig. 2: Chemical structure of amlodipine besylate

According to the literature review, there is currently no technique for employing reversed-phase liquid chromatography to simultaneously measure olmesartan medoxomil and amlodipine besylate in a combination dose form. Developing a simple, accurate, and precise RP-HPLC method for the simultaneous determination of a binary drug formulation was the aim of this study [12–14]. The International Conference on Harmonisation (ICH) principles were followed in the optimisation and validation of the suggested technique [15–17].

MATERIAL AND METHOD

1. Instrumentation: A Waters Alliance 2695 separation module with a 2487 UV detector was used in this study. An Intersil ODS C18 column (4.6 X 250 mm; 5 μ m) was used in the method. An automated injector was used to administer the samples. Twenty microlitres of the sample were injected. Waters Empower 2 software was used to

track the input and output activities of the chromatographic machine. A flow rate of 1.0 mL per minute was selected. At 265 nm, the detection took place. The duration and temperature were measured at 7.0 minutes and 25 ± 2 °C, respectively. A Lab India UV 3000 spectrophotometer was used to obtain the ultraviolet spectra of the medications used in the experiment in order to calculate their λ_{max} values. Using an ultra sonicator (Power Sonic 510, Hwashin Technology), the compounds' solubility was increased.

- 2. Reagents and materials:** Surasha Pharmaceuticals, Hyderabad, Telangana, supplied the reference sample of amlodipine besylate and olmesartan medoxomil. Standard Reagents, Hyderabad, provided HPLC-grade water that was filtered using 0.45 Millipore Milli-Q. We purchased HPLC-grade acetonitrile and methanol from Merck in Mumbai. Potassium dihydrogen phosphate (Finar Chemicals, Ahmedabad) and orthophosphoric acid (Standard Reagents, Hyderabad) are the chemicals used in buffer formulation. The various solvents and solutions intended for injection into the column were filtered using membrane filters (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) with a pore size of 0.45 μ m.
- 3. Optimization of the chromatographic conditions:** The ratios of acetonitrile, methanol, and water were changed in order to execute various changes to the mobile phase. Chloroform, Tetrahydrofuran (THF), ethanol, isopropyl alcohol (IPA), n-hexane, and dichloromethane were among the modifiers used for the first separation using a column with a particle size of 5 μ m. A phosphate buffer (pH 5) mixed with acetonitrile in a 70:30 ratio produced the ideal resolution of 8.23, which is far higher than the USP resolution criterion of 2.0. Olmesartan medoxomil and amlodipine besylate have retention periods of 3.071 and 4.487 minutes, respectively.
- 4. Preparation of 0.2 % v/v ortho phosphoric acid:** In a dry, clean 1000 mL flask, 0.2 grams of orthophosphoric acid were dissolved in 900 mL of HPLC-grade water to form the orthophosphoric acid solution. The salt was fully dissolved by vigorously stirring the liquid. The pH was adjusted to 5.0 after adding an extra 100 mL of water.
- 5. Preparation of mobile phase:** In a dry, clean flask, 700 mL of HPLC-grade water (pH 5.0) and 300 mL of HPLC-grade acetonitrile were combined to create the mobile phase. For five minutes, the mixture was degassed in an ultrasonic cleaner. A 0.45 membrane filter (Advanced Micro Devices Pvt. Ltd., Chandigarh, India) was used to filter the resulting mobile phase under vacuum.
- 6. Diluent preparation:** Acetonitrile and phosphate buffer (pH 5.0) were combined in a 70:30 (v/v) ratio to create the diluent. The medication solutions used in the investigation were diluted using this solution.
- 7. Preparation of standard solution:** After precisely weighing about 20 mg of olmesartan medoxomil, it

“Validation of the RP-HPLC/DAD Method for Concurrent Quantification of Various Antihypertensive Drugs in a Membrane-Moderated Matrix Monolithic Polymeric Device Type”

was put into a dry, clean 50 mL volumetric flask. 30 millilitres of diluent were used to first dissolve the medication. To guarantee that the medication was completely dissolved, the solution was sonicated for fifteen minutes. The same solvent was used to prepare the final volume. A clean, dry 25 mL volumetric flask was filled with 5.0 mL of the prepared solution, which was then diluted to the appropriate level using the same diluent. Olmesartan medoxomil is included in the stock solution at a concentration of 80 µg/mL.

Similarly, 5 mg of amlodipine besylate was carefully weighed and added to a dry, clean 50 mL volumetric flask. First, 30 millilitres of diluent were used to dissolve the medication. To make sure the medication was completely dissolved, the solution was sonicated for fifteen minutes. The same solvent was used to prepare the final volume. A clean, dry 25 mL volumetric flask was filled to the brim with the same diluent after five millilitres of the prepared solution were added to it. The concentration of amlodipine besylate in this stock solution is 20 µg/mL.

8. **Preparation formulation (tablet) solution:** The commercial tablet brand AZOR, made by Torrent Pharmaceuticals Limited, was used in this investigation. Each pill included 5 mg of amlodipine besylate and 20 mg of olmesartan medoxomil. After weighing and finely powdering twenty tablets, a sample of the powdered tablets equal to 404 was precisely weighed. Using a small amount of diluent in a dry, clean 50 mL volumetric flask, a total of 30 mg was extracted. To guarantee total drug solubility, the mixture was thoroughly agitated and let to stand for 15 minutes with sporadic sonication. A 0.45µ membrane filter is used to filter the contents after they have been diluted to the required concentration. A clean, dry 25 mL volumetric flask was filled with five millilitres of the filtrate using a pipette. Olmesartan medoxomil and amlodipine besylate concentrations of approximately 80.0 and 20.0 µg/mL were obtained by adjusting the volume using the diluent. After injecting a 20 µL sample, chromatography was performed. The peak areas' mean was calculated.

9. **Method suitability:** The commercial tablet formulation of amlodipine besylate and olmesartan medoxomil, referred to as AZOR (produced by Torrent Pharmaceuticals Limited), was evaluated using the proposed method, with results presented in Table 1. The values were consistent with the indicated amounts, so validating the method's appropriateness for analyzing pharmaceuticals in pharmaceutical dose forms.

RESULT AND DISCUSSION

1. **Specificity and Selectivity:** The suggested method was used to evaluate the commercial tablet formulation of amlodipine besylate and olmesartan medoxomil, known as AZOR (produced by Torrent

Pharmaceuticals Limited). The results are shown in Table 1, and the chromatograms are shown in Figure 3 A (Sample) and 3 B (Standard). The results confirmed that the procedure is appropriate for evaluating pharmaceuticals in dosage forms because they matched the stipulated levels.

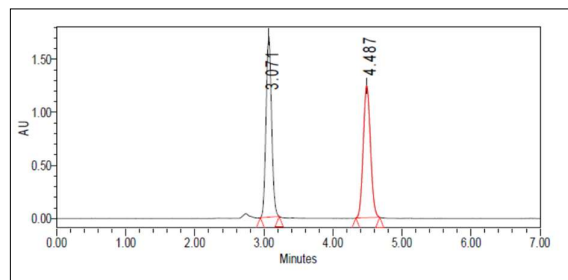


Figure 3 A: A typical chromatogram of olmesartan medoxomil and amlodipine besylate (Sample)

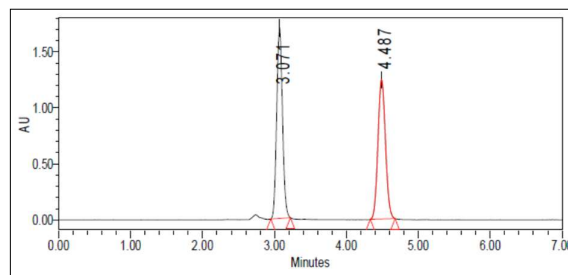


Figure 3 B: A typical chromatogram of olmesartan medoxomil and amlodipine besylate (Standard)

2. **Linearity:** The peak areas of the analyte were plotted against their concentrations to create curves that showed the linearity range of the suggested HPLC procedure. The concentrations of amlodipine besylate and olmesartan medoxomil and their corresponding peak regions showed a significant linear connection ($r^2 = 0.999$). The relevant regression equations are $y = 46027x - 22624$ ($r^2 = 1$) for amlodipine besylate and $y = 11990x + 53124$ ($r^2 = 1$) for olmesartan medoxomil, where x stands for the concentrations of amlodipine besylate (µg/mL) and y is the peak area. Table 2 represent the data, while Figures 4 and 5 show the calibration curves.

Table 2: Regression Characteristics and Validation Parameters

Sl. No.	Parameters	Olmesartan medoxomil	Amlodipine besylate
1.	λ_{max} (nm)	280	280
2.	Linearity range (µg/ml)	40 to 120	10 to 30
3.	Correlation coefficient (r^2)	1	1
4.	Regression equation	$y = 46027x - 22624$	$y = 11990x + 53124$
5.	Intercept	22624	53124
6.	Slope	460278	11990
7.	Limit of detection (LOD µg/ml)	0.25	0.08

“Validation of the RP-HPLC/DAD Method for Concurrent Quantification of Various Antihypertensive Drugs in a Membrane-Moderated Matrix Monolithic Polymeric Device Type”

8.	Limit of quantification (LOQ µg/ml)	0.82	0.28
9.	Accuracy (%) (n=3)	99.98	100.35
10.	Repeatability (RSD, %, n=6)	0.0334	0.0370
11.	Precision (RSD %), Interday (n=6)	0.032	0.033

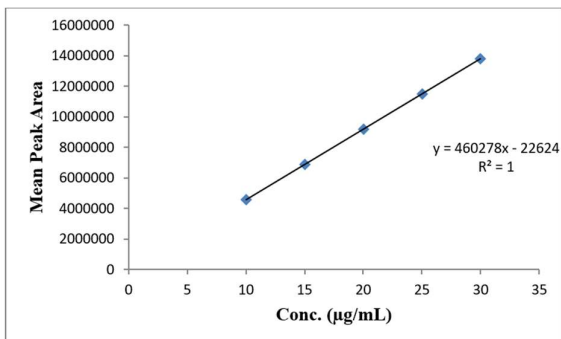


Figure 4: Calibration curve for olmesartan medoxomil

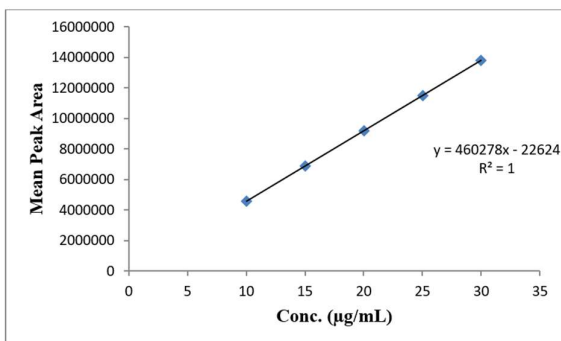


Figure 5: Calibration curve for amlodipine besylate

- Precision:** The degree of consistency between results from samples evaluated over three days (inter-day) and those analysed on the same day (intra-day) is referred to as precision. The suggested HPLC method was used to analyse solutions containing 80.0 and 20.0 µg/mL of olmesartan medoxomil and amlodipine besylate, respectively, in order to assess the method's intra- and inter-day variability. The outcomes were meticulously documented. Using percent RSD, the accuracy of the suggested method more especially, the intra- and inter-day changes in the drug solutions' peak areas was evaluated. The results are shown in table 2. According to an analysis, the drug's relative standard deviation for six injections at the linearity level was less than 2.0.
- Accuracy:** The degree of similarity between the results derived from standard samples and the actual known amounts is referred to as accuracy. Recovery studies were carried out by examining the recovery levels (50.0, 100.0, and 120.0 µg) of pure olmesartan medoxomil and amlodipine besylate in order to evaluate the correctness of the suggested

approach. These were assessed at linearity levels of 80.0 and 20.0 µg/mL of amlodipine besylate and olmesartan medoxomil, respectively. There were three injections of each dilution (n=3). The medications' % recoveries were computed. Table 2 show the outcomes.

- Limit of detection (LOD) and Limit of quantitation (LOQ):** The lowest analyte concentration that yields a discernible reaction is known as LOD. The signal-to-noise ratio, which is frequently used in HPLC techniques, determines LOD. The analyte was examined in six duplicates. The lowest concentration that can be precisely and accurately measured is known as the LOQ. The lowest concentration is defined as the point where both the accuracy (expressed by the relative difference between the measured and true values) and precision (represented by the relative standard deviation, or RSD) are less than 2%.
The noise response is ten times smaller than the analyte response. The analyte was analysed and quantified using six replicates. The suggested approach yielded detection limits of 0.25 and 0.08 µg/ml for olmesartan medoxomil and amlodipine besylate, respectively. The suggested method yielded quantitative limits of 0.82 and 0.28 µg/mL for olmesartan medoxomil and amlodipine besylate, respectively.

- Robustness:** To evaluate the robustness of the approach, the HPLC conditions were changed. The mobile phase ratio and flow rate were slightly modified. The findings showed that small changes in these values had no effect on the chosen parameters. Furthermore, the technique showed resilience in the face of variations in the mobile phase of ± 5%, flow rate of ± 0.1 mL/min, and detecting wavelength of ± 5 nm. Tables 3 and 4 show the results.

Table 3: Results of the robustness study for olmesartan medoxomil

Sr. No.	Parameters	Olmesartan medoxomil		
		Retention time (min.)	Peak area	Tailing factor
1.	Standard	3.071	9668018	1.39
2.	Flow rate (0.9 mL/min.)	2.949	9658018	1.49
3.	Flow rate (1.1 mL/min.)	3.020	9666018	1.38
4.	Mobile Phase (65:35 % v/v)	2.967	9668118	1.27
5.	Mobile Phase (75:25 % v/v)	3.012	9668413	1.44
6.	Wavelength (275 nm)	2.959	9658708	1.50
7.	Wavelength (285 nm)	2.935	9667018	1.41

Table 4: Results of the robustness study for amlodipine besylate

Sr. No	Parameters	Amlodipine besylate		
		Retention time (min.)	Peak area	Tailing factor
1.	Standard	4.487	9166434	1.39
2.	Flow rate (0.9 mL/min.)	4.087	9186434	1.38
3.	Flow rate (1.1 mL/min.)	3.487	9168434	1.49
4.	Mobile Phase (65:35 % v/v)	4.769	9165534	1.50
5.	Mobile Phase (75:25 % v/v)	4.540	9167734	1.48
6.	Wavelength (275 nm)	4.356	9166234	1.27
7.	Wavelength (285 nm)	4.512	9156934	1.49

CONCLUSION

The simplicity, selectivity, sensitivity, accuracy, precision, and speed of the suggested RP-HPLC method for the quantitative measurement of olmesartan medoxomil and amlodipine besylate in bulk samples and formulations are its defining characteristics. Compared to most described methods, the method proved to be superior. Mobile phase preparation is simple and economical. The formulation excipients did not affect the estimation, as evidenced by the sample recoveries in the formulation that closely matched their corresponding label claims. Depending on the availability of chemicals and the properties of other components in the sample, this method can be used as an alternative for the regular determination of olmesartan medoxomil and amlodipine besylate. This approach can be used for pharmacokinetic, biological, and clinical research on amlodipine besylate and olmesartan medoxomil.

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