



## DEVELOPMENT AND VALIDATION OF A RAPID RP-HPLC METHOD FOR THE DETERMINATION OF AMLODIPINE BESYLATE AND OLMESARTAN MEDOXOMIL IN THEIR COMBINED TABLET FORMULATION

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### ABSTRACT

Combination therapy of amlodipine besylate (AML) and olmesartan medoxomil (OLM) is used for the treatment of hypertension. In the present study a simple, precise, rapid, efficient and reproducible reversed-phase high performance liquid chromatography (RP-HPLC) method has been developed for the simultaneous estimation of amlodipine besylate (AML) and olmesartan medoxomil (OLM) present in its tablet dosage forms. Chromatographic separations were carried out isocratically at 25°C ± 0.5°C on an Microsorb C18 Column (5 µm, 250mm x 4.60mm) with a mobile phase composed of methanol- phosphate buffer (pH 4.0) in the ratio of 70:30% v/v at a flow rate of 1.2 ml/min. Detection is carried out using a UV-PDA detector at 248 nm. The retention times for AML and OLM were 5.59 ± 0.5 min and 4.26 ± 0.5 min respectively. During the method validation the linearity range and percentage recoveries for AML and OLM were found to be 10-50, 20-100 µg/ml and 98.93, 100.02% respectively. The correlation coefficients for all components were close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets were always less than 2%. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which may be useful for the routine estimation of amlodipine besylate and olmesartan medoxomil in bulk drug and in its pharmaceutical dosage form.

**Keywords:** Amlodipine besylate, Olmesartan medoxomil, RP-HPLC, Simultaneous estimation.

### INTRODUCTION

Amlodipine Besylate [(3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate] [Figure 1A] is orally administered calcium channel blocker, widely used for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents. It inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle.<sup>1,2</sup> Amlodipine besylate have an absolute bioavailability of 64 and 90% and half-life of about 30-50 hours.<sup>3</sup> Olmesartan medoxomil [2,3-dihydroxy-2-butenyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate, cyclic 2,3-carbonate.] [Figure 1B] is an angiotensin II receptor blocker (ARB). It blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle.<sup>4</sup> Olmesartan medoxomil have an

absolute bioavailability of 26% and half-life of about 13 hours.<sup>5,6</sup> Tablet dosage forms containing AML and OLM in ratio of 5mg: 20 mg of various brands are available in market. AML is official in IP,<sup>7</sup> BP.<sup>8</sup> Several analytical methods that have been reported for the estimation of amlodipine besylate in biological fluids and/or pharmaceutical formulations include spectrophotometric,<sup>9-13</sup> high-performance liquid chromatography,<sup>14,15</sup> and high-performance thin layer chromatography,<sup>16</sup> while OLM determinations have been reported by UV-Vis spectrophotometry,<sup>17</sup> HPLC,<sup>18</sup> and tandem mass spectroscopy.<sup>19</sup> Simultaneous determination of amlodipine and hydrochlorothiazide in pharmaceutical dosage forms was reported by HPTLC.<sup>20</sup> However there is no method available for the simultaneous determination of AML and OLM. Therefore, an attempt was made to develop a new, rapid and sensitive method for the simultaneous determination of AML and OLM. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is mandatory also.<sup>21,22</sup>

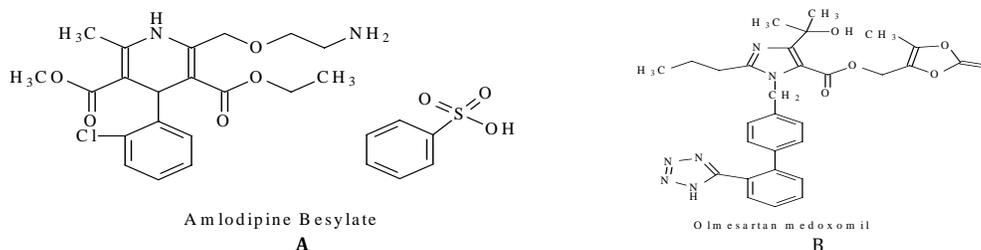


Fig. 1: Chemical structures of (A) amlodipine besylate (B) olmesartan medoxomil

### EXPERIMENTAL

#### Instrumentation

High performance Liquid chromatographic system from Younglin YL-9100 comprising of manual injector, Double parallel dual-plunger pump YL-9111 for constant flow and constant pressure delivery and Photodiode array detector YL-9160 connected to

software YL-Clarity for controlling the instrumentation as well as processing the data generated was used.

#### Reagents and chemicals

Amlodipine besylate and olmesartan medoxomil were obtained as pure samples from Unichem Laboratories Ltd. Mumbai Maharashtra (India) as a gift sample. Methanol and glacial acetic acid were of HPLC grade supplied by Merck Ltd., India. Triple distilled water was

generated in house. Tablet, OLSAR A (Unichem Laboratories Ltd.) and PINOM A (Lupin Pharma Ltd.) containing AML and OLM in ratio of 5 mg: 20mg respectively was purchased from local market.

#### Chromatographic condition

The isocratic mobile phase consisted of methanol–phosphate buffer (pH 4.0) in the ratio of 70:30 v/v, flowing through the column at a constant flow rate of 1.2 ml/min. A Microsorb (C-18) Column (5 µm, 250mm x 4.60mm) was used as the stationary phase. Although the AML and OLM have different λmax viz 240 and 256nm respectively, but considering the chromatographic parameter, sensitivity and selectivity of method for two drugs, 248 nm was selected as the detection wavelength for UV-PDA detector.

#### Standard preparation

**Standard stock solution:** Standard stock solutions of 1000 µg/ml of AML and OLM were prepared in mixture of methanol: phosphate buffer pH 4.0 (70:30%v/v) respectively.

**Working standard solution:** Working standard solutions were prepared by taking dilutions ranging from 10-50, 20-100 µg/ml for AML and OLM respectively.

#### Sample preparation

Twenty tablets of OLSAR A (Unichem Laboratories Ltd.) and PINOM A (Lupin Pharma Ltd.) containing AML and OLM in ratio of 5 mg:

20mg respectively was weighed and crushed to fine powder. Powder equivalent to 5 mg AML and 20 mg of OLM was weighed and dissolved in 100 ml of diluent, sonicated for 10 min and filtered through whatmann filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

## RESULTS AND DISCUSSION

#### Chromatography

Initially reverse phase LC separation was tried to develop using methanol and water (80:20) as mobile phase, in which AML gave tailing of 2.4 although OLM responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of AML and OLM. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH 6.4 the signal to noise ratio for OLM is less and RT was also 12.5 min. Thereafter, methanol– phosphate buffer of pH 4.0 in the ratio of 70:30 v/v was selected to improve resolution and the tailing for the two peaks were reduced considerably and brought close to 1 and RT of OLM was also reduced. To analyze these two drugs various wavelengths from 230nm to 260nm were tried for detection. As λmax of AML and OLM were 240 and 256nm, therefore 248nm was found to be suitable where the two drugs could be detected simultaneously. The peak shapes of both the drugs were symmetrical and the asymmetry factor was lesser than 2.0. [Figure 2].

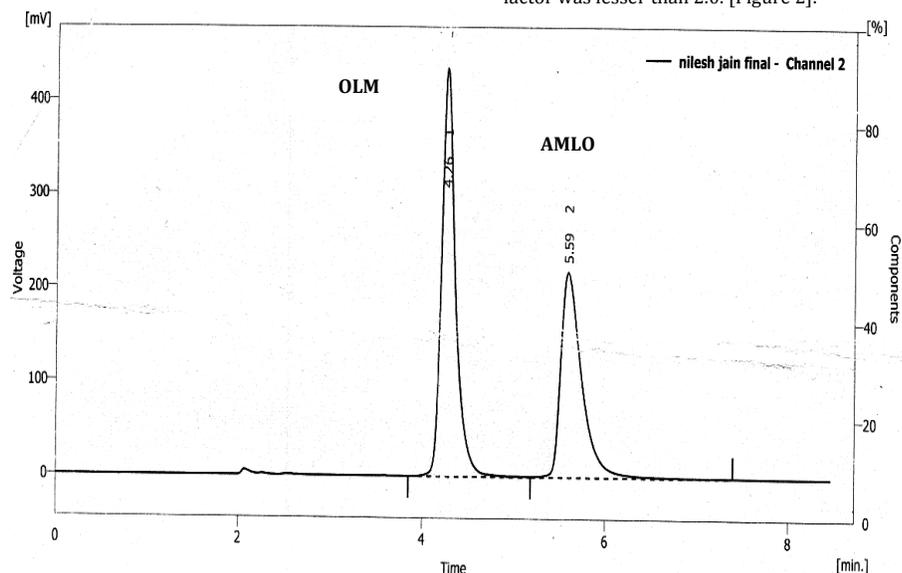


Fig. 2: Representative chromatogram of amlodipine besylate and olmesartan medoxomil

Table 1: Result of system suitability

Serial No.	Parameters	AML	OLM
1	No. of Theoretical plates	3176	4269
2	HETP	0.079	0.059
3	Tailing factor	1.8	1.5

Table 2: Results of recovery experiments

Serial. No.	Conc. of drug in preanalyzed samples (µg/ml)		Std. drug sol. Added (µg/ml)		Recovered amount* (µg/ml)		% Recovered	
	AML	OLM	AML	OLM	AML	OLM	AML	OLM
1	10	40	10	40	9.9	40.2	99	100.5
2	20	80	20	80	19.7	80.06	98.5	100.07
3	30	120	30	120	29.8	119.4	99.3	99.5
						Mean	98.93	100.02
						S.D	0.40	0.50
						%R.S.D	0.41	0.50

\* Mean of three reading

### System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing were determined. The results obtained are shown in Table-1. The number of theoretical plates for AML and OLM were 3176 and 4269 respectively.

### Linearity

AML and OLM showed a linearity of response between 10-50 and 20-100 µg/ml respectively. The linearity was represented by a linear regression equation as follows.

$$Y (\text{AML}) = 180.68 \text{ conc.} + 70.80 \quad (r^2=0.9989)$$

$$Y (\text{OLM}) = 468.8 \text{ conc.} - 111.9 \quad (r^2=0.9998)$$

### Accuracy

Recovery studies were performed to validate the accuracy of developed method by adding a definite concentration of standard drug in to preanalyzed sample solution. These results are summarized in Table-2.

### Precision:

**Repeatability:** Five dilutions in three replicates were analyzed in same day for repeatability and results were found within acceptable limits (RSD < 2) as shown in Table-3.

**Intermediate precision:** Five dilutions in three replicates were analyzed on two different days and by two analysts for day to day and analyst to analyst variation. All Results were fall within acceptable limits (RSD < 2) as shown in Table-3.

### Robustness

As per ICH norms, small, but deliberate variations, by altering the pH or concentration of the mobile phase were made to check the method's capacity to remain unaffected. The change was made in the ratio of mobile phase, instead of Methanol: Phosphate buffer (pH 4.0) (70:30v/v), Methanol: phosphate buffer (pH 4.0) (65:35 v/v), was used as a Mobile Phase. Results of analysis were summarized in Table-4.

### Stability of sample solution

The sample solution injected after 12 hr did not show any appreciable change.

### Tablet analysis

Content of AML and OLM found in the tablets by the proposed method are shown in Table-5. The low values of R.S.D. indicate that the method is precise and accurate.

**Table 3: Results of precision**

Serial No.	Validation Parameter	% Mean*		S.D.		% R.S.D.	
		AML	OLM	AML	OLM	AML	OLM
1	Repeatability	99.2	100.1	0.42	0.51	0.42	0.50
2	Intermediate precision Day to Day	98.6	99.8	0.61	0.26	0.62	0.26
3	Intermediate precision Analyst to Analyst	99.6	100.2	0.39	0.34	0.34	0.33

\* Mean of fifteen determinations (3 replicates at 5 concentration level)

**Table 4 : Results of robustness**

Serial No.	Validation Parameter	% Mean*		S.D.		% R.S.D.	
		AML	OLM	AML	OLM	AML	OLM
1	Robustness	98.87	100.59	0.64	0.99	0.64	0.98

\* Mean of six determinations

### System suitability

System suitability parameters such as number of theoretical plates, HETP and peak tailing were determined. The results obtained are shown in Table-1. The number of theoretical plates for AML and OLM were 3176 and 4269 respectively.

### Linearity

AML and OLM showed a linearity of response between 10-50 and 20-100 µg/ml respectively. The linearity was represented by a linear regression equation as follows.

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**Table 5: Results of the HPLC analysis for tablets**

Serial No.	Parameters	OLSAR A		PINOM A	
		AML	OLM	AML	OLM
1	% Mean*	98.63	99.09	98.97	99.24
2	S.D.	0.54	0.84	0.47	0.78
3	% R.S.D	0.55	0.85	0.47	0.78
4	SEσ	0.22	0.34	0.19	0.31

\* Mean of fifteen determinations (3 replicates at 5 concentration level)

### CONCLUSION

RP-HPLC method was developed and validated for simultaneous estimation of AML and OLM in tablet dosage form. Proposed method is fast, accurate, precise and sensitive hence it can be employed for routine estimation and quality control of tablets containing these two drugs in industries.

### REFERENCES

- Sweetmann SC. Eds. Martindal: the complete drug reference. 32<sup>nd</sup> ed. London: The Pharmaceutical Press; 1999. p. 822.
- Budavari S. Edn., In; The Merck Index. 13<sup>th</sup> ed. NJ: Merck & Co., Inc., Whitehouse Station; 1996. p. 491.

3. Tripathi KD. Essentials of medical pharmacology. 6<sup>th</sup> ed. New Delhi: Jaypee Brother's Medical Publishers; 1999. p.531.
4. Budavari, S, Edn. In; The Merck Index. 13<sup>th</sup> ed. NJ: Merck & Co., Inc., Whitehouse Station; 1996. p.6909.
5. Scott LJ, McCormack PL. Olmesartan medoxomil: a review of its use in the management of hypertension. *Drugs*. 2008; 68(9):1239-72.
6. Chrysant SG, Oparil S, Melino M, Karki S, Lee J, Heyrman R. Efficacy and safety of long-term treatment with the combination of amlodipine besylate and olmesartan medoxomil in patients with hypertension. *J Clin Hypertens (Greenwich)*. 2009; 11(9):475-82.
7. The Indian Pharmacopoeia, The Controller of Publications, New Delhi, 1996, 72.
8. British Pharmacopoeia, CD-ROM, British Pharmacopoeia, HMSO, London, 2001, 72.
9. Gohil K, Trivedi P, Molvi KI. Spectrophotometric method for amlodipine besylate in bulk and in tablet dosage forms. *Indian J Pharm Sci*. 2005; 67:376.
10. Khopade SA, Jain NK. Difference spectrophotometric estimation of amlodipine besylate. *Indian Drugs*. 2000; 37(7):351-353.
11. Kasture AV, Ramteke M. Simultaneous UV-spectrophotometric method for the estimation of atenolol and amlodipine besylate in combined dosage form. *Indian J Pharm Sci*. 2006; 68:394-6.
12. Topale PR, Gaikwad NJ, Tajane MR. Simultaneous UV-spectrophotometric estimation of losartan potassium and amlodipine in tablet. *Indian Drugs*. 2003; 40(2):119-121.
13. Jain HK, Agrawal RK. Spectrophotometric methods for simultaneous estimation of amlodipine besylate and lisinopril in tablets. *Indian Drugs*. 2000; 37(4):196-199.
14. Rao JR, Kadam SS, Mahadik KR. RP-HPLC determination of amlodipine and benazepril hydrochloride in tablets. *Indian Drugs*. 2002; 39 (7):378-381.
15. Rajeswari K, Sankar GG, Rao AL, Seshagirirao JVLN. RP-HPLC method for the simultaneous determination of atorvastatin and amlodipine in tablet dosage form *Indian Journal of Pharmaceutical Sciences*. 2006; 68 (2), 275-277.
16. Argekar AP, Powar SG. Simultaneous determination of atenolol and amlodipine in tablets by high-performance thin-layer chromatography *J. Pharm. Biomed. Ana*. 2000; 21(6):1137-1142.
17. Celebier M, Altinoz S. Determination of olmesartan medoxomil in tablets by UV-Vis spectrophotometry. *Pharmazie*. 2007; 62(6):419-22.
18. Murakami T, Konno H, Fukutsu N, Onodera M, Kawasaki T, Kusu F. Identification of a degradation product in stressed tablets of olmesartan medoxomil by the complementary use of HPLC hyphenated techniques. *J Pharm Biomed Anal*. 2008; 47(3):553-9.
19. Liu D, Hu P, Matsushima N, Li X, Li L, Jiang J. Quantitative determination of olmesartan in human plasma and urine by liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr B*. 2007; 856(1-2):190-7.
20. Shah NJ, Suhagia BN, Shah RR, Patel NM. Development and validation of a simultaneous HPTLC method for the estimation of olmesartan medoxomil and hydrochlorothiazide in tablet dosage form, *Indian Journal of Pharmaceutical Sciences*. 2007; 69( 6):834-836
21. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.
22. Code Q2B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline, 1994, ICH Harmonised Tripartite Guideline.