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Development and Validation of RP-HPLC Method for Simultaneous Estimation of Amlodipine Besylate and Olmesartan Medoxomil in Bulk and Its Pharmaceutical Formulations

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ABSTRACT

A new, simple, rapid, selective, precise and accurate isocratic reverse phase high performance liquid chromatography assay method has been developed for simultaneous estimation of Amlodipine Besylate and Olmesartan Medoxomil tablet formulations. The separation was achieved by using column Eclipse XDB C8, 150X4.6mm, 5µm, in mobile phase consisted of pH 3.0 phosphate buffer and Acetonitrile in the ratio of 60:40 v/v. The flow rate was 1.0 mL/min, column oven temperature 30° C, sample cooler temperature 5°C and the injection volume was 10 µL, and detection was performed at 238 nm using a photodiode array detector (PDA), Run time 10 minutes. The retention time of Amlodipine Besylate and Olmesartan Medoxomil was noted to be 2.65 minutes and 5.70 minutes respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

INTRODUCTION

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. Amlodipine decreases arterial smooth muscle contractility and subsequent vasoconstriction by inhibiting the influx of calcium ions through L-type calcium channels. Calcium ions entering the cell through these channels bind to calmodulin. Calcium-bound calmodulin then binds to and activates myosin light chain kinase (MLCK). Activated MLCK catalyzes the phosphorylation of the regulatory light chain subunit of myosin, a key step in muscle contraction.

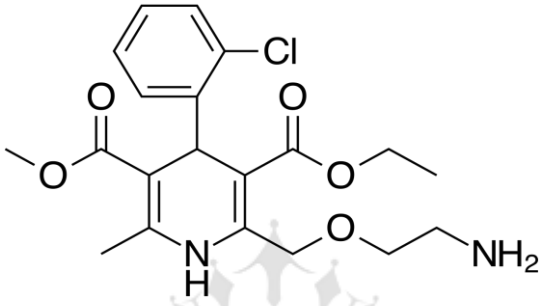

Figure No. 1: Structure of Amlodipine Besylate

Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin II receptor blockers. Olmesartan is an ARB that selectively inhibits the binding of angiotensin II to AT1, which is found in many tissues such as vascular smooth muscle and the adrenal glands. This effectively inhibits the AT1-mediated vasoconstrictive and aldosterone-secreting effects of angiotensin II and results in a decrease in vascular resistance and blood pressure.

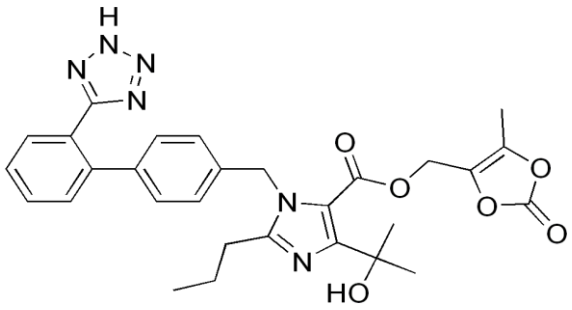

Figure No. 2: Structure of Olmesartan medoxomil

Literature survey reveals that few analytical methods have been reported for the estimation of Amlodipine Besylate and Olmesartan Medoxomil in pharmaceutical dosage form including UV-Spectroscopic method [1-4], RP-HPLC method [5-6], for combination of three drugs Amlodipine, Olmesartan and Hydrochlorothiazide using RP-HPLC method [7-9], for Amlodipine using RP HPLC method [10], very few methods have been reported so far for simultaneous estimation of both the drugs Amlodipine Besylate and Olmesartan medoxomil [11-16]. The objective of this work was to develop a new rapid, novel, and economical RP-HPLC method which can be used as a stability-indicating assay for combination drug product of Amlodipine Besylate and Olmesartan medoxomil.

MATERIALS AND METHODS

Chemicals and Reagents

Milli-Q Water, Acetonitrile (HPLC Grade) and Potassium dihydrogen phosphate monohydrate (AR Grade), Triethylamine, Hydrochloric acid and, sodium hydroxide (AR Grade), Hydrogen peroxide (AR Grade), orthophosphoric acid (GR Grade), were obtained from Qualigens Ltd., Mumbai. All other chemical of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

Instrumentation and Chromatographic Conditions

Instrumentation

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were use in the present assay.

Preparation of Buffer (pH 3.0)

3.40 g of potassium dihydrogen phosphate was dissolved in 1000mL of Milli-Q water and 1ml of triethylamine was added and mixed well. Then pH was adjusted to 3.00±0.05 with diluted ortho Phosphoric acid solution and then filtered through 0.45µm Nylon membrane filter.

Mobile Phase Preparation:

pH 3.0 buffer and Acetonitrile were mixed in the ratio of 60:40% v/v and sonicated to degas for 5 min.

Preparation of diluents:

Water and Acetonitrile were mixed together in the ratio of 25:75% v/v.

Preparation of Amlodipine Besylate stock solution:

35.0 mg of AML working standard was weighed and transferred into a 50 ml volumetric flask and 30 ml of diluents was added, sonicated to dissolve and then diluted to volume with diluents and mixed well.

Preparation of Olmesartan Medoxomil stock solution:

50.0 mg of OLM working standard was weighed and transferred into a 25 ml volumetric flask and 15 ml of diluents was added, sonicated to dissolve and then diluted to volume with diluents and mixed well.

Preparation of Standard Solution:

Transferred 3ml of Amlodipine Besylate stock solution and 5ml of Olmesartan medoxomil stock solution were transferred into 50 ml volumetric flask and diluted to volume with diluents.

Sample preparation:

Accurately weighed 5 tablets were directly transferred into 250 mL volumetric flask, and 200.0 mL of diluents was added and sonicated for 30 min. Then the solution was centrifuged at 4000 rpm for 10 min. The supernatant solution was collected and then diluted 5 mL of filtrate was diluted to 20 mL with diluents and then injected in to chromatograph.

Chromatographic conditions

Eclipse XDB C8, 150X4.6mm, 5µm, Column was used for analysis at 30°C column temperature and sample cooler 5°C. Mobile phase consisted of pH 3.0 phosphate buffer and Acetonitrile in the ratio of 60:40 v/v. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 10 µL. The photodiode array

detector was set to a wavelength of 238nm for the detection and chromatographic runtime was 10 minutes.

Method development

To develop a suitable and robust LC method for the determination of Amlodipine Besylate and Olmesartan medoxomil, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Zorbax XBD C8, (150X4.6 mm, 5 µm.) with the following mobile phase pH 3.0 Phosphate buffer: Acetonitrile (60:40). Detector wavelength 238 nm, column temperature 30^{o}C, Injection volume 10 µL and Flow rate 1.0 ml/min used. Peak shape was not good, due to asymmetry (tailing factor). So, another trial was made with change in flow rate.

For next trial column was changed to Eclipse XDB C8, 150X4.6mm, 5µm from Zorbax XBD C8, (150X4.6 mm, 5 µm.) remaining chromatographic conditions are same. Peak shape was satisfactory in both standard and sample preparations. Retention time of Amlodipine Besylate and Olmesartan Medoxomil were found to be 3.0 and 6.0 min acceptable. The chromatogram of Amlodipine Besylate and Olmesartan medoxomil standard using the proposed method is shown in **(Figure 3.)** System suitability results of the method are presented in **Table 1.**

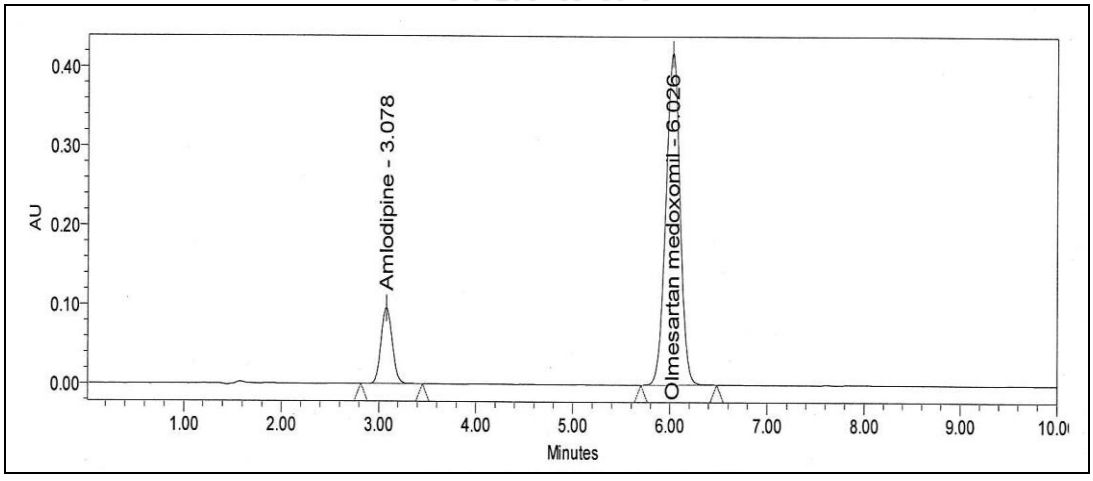

Figure No. 3: A typical HPLC Chromatogram standard

Method validation

The developed RP-LC method extensively validated for assay of Amlodipine Besylate and Olmesartan medoxomil using the following Parameters.

Specificity & System suitability

Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo was injected into the chromatograph in the defined above chromatographic conditions and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Figure 3)** showed no peaks at the retention time of Amlodipine Besylate and Olmesartan medoxomil peak. This indicates that the diluent solution used in sample preparation do not interfere in estimation of Amlodipine Besylate and Olmesartan medoxomil in tablets. Similarly, Chromatogram of Placebo solution **(Figure 4)** showed no peaks at the retention time of Amlodipine Besylate and Olmesartan medoxomil peak. This indicates that the Placebo used in sample preparation do not interfere in estimation of Amlodipine Besylate and Olmesartan medoxomil in Amlodipine Besylate and Olmesartan medoxomil tablets. The chromatogram of Amlodipine Besylate and Olmesartan medoxomil Blank using the proposed method is shown in **Figure 4**. The chromatogram of Amlodipine Besylate and Olmesartan medoxomil Placebo using the proposed method is shown in **Figure 5**.

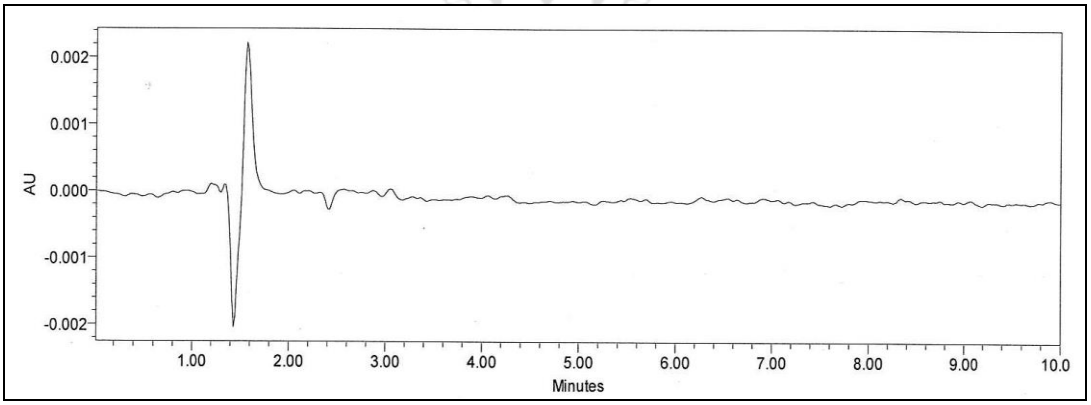

Figure No. 4: Typical Chromatogram of Blank

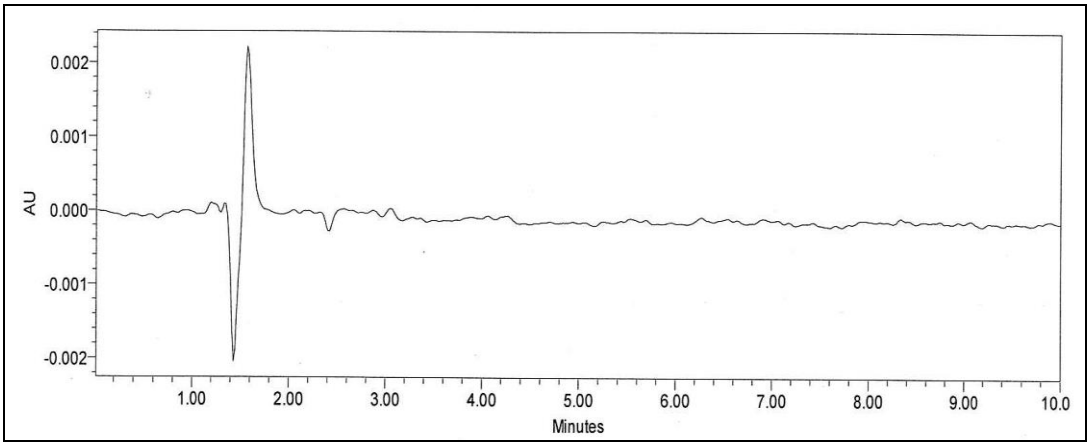

Figure No. 5: Typical Chromatogram of placebo

Table No. 1: System suitability parameters

Parameters	Amlodipine Besylate	Olmesartan medoxomil
Resolution	4.76	
Retention time (min)	3.05	6.05
No. of Theoretical plates	3323	8117
Tailing factor	1.1	1.0

Method Precision:

The precision of test method was evaluated by doing assay for six samples preparations as per test method. The content of % label claim for Amlodipine Besylate and Olmesartan medoxomil for each of the test preparation was calculated. The average content of the six preparations and % RSD for the six observations were calculated. The chromatogram was shown in **Figure 6** and data were shown in **Table 2.**

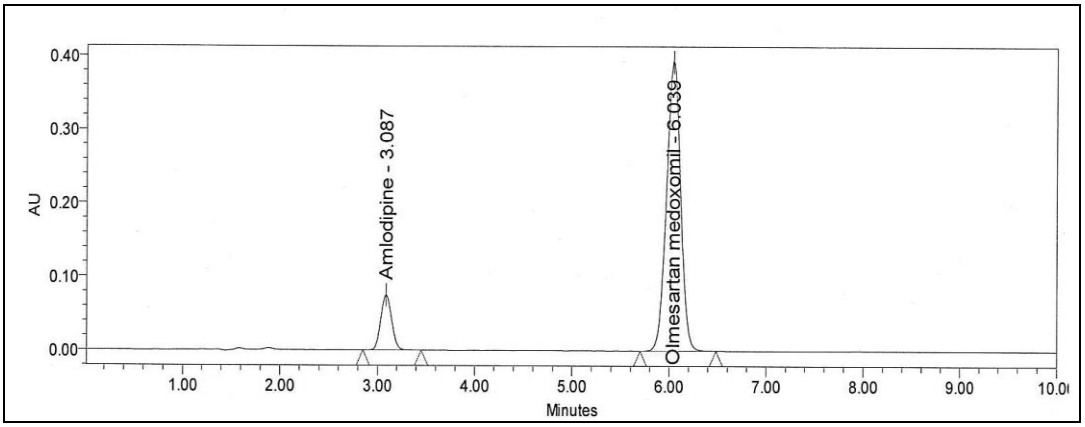

Figure No. 6: Method precision sample chromatogram

Table No. 2: Method Precision results

Preparations	Olmesartan medoxomil	Amlodipine Besylate
1	98.0	98.2
2	98.1	98.2
3	98.6	98.9
4	98.3	98.3
5	97.9	97.9
6	98.8	99.0
Mean	**98.3**	**98.4**
% RSD	**0.4**	**0.4**

Intermediate Precision:

The intermediate precision of test method was demonstrated by carrying out method precision study in six samples, representing a single batch by two different analysts on two different days, different column, different HPLC system and by different analyst. These samples were prepared as per the test method. The % assay was calculated for each of these samples. The precision of the method was evaluated by computing the % Relative standard deviation of % assay of Amlodipine Besylate and Olmesartan medoxomil. The chromatogram was shown in **Figure 7** and data were shown in **Table 3.**

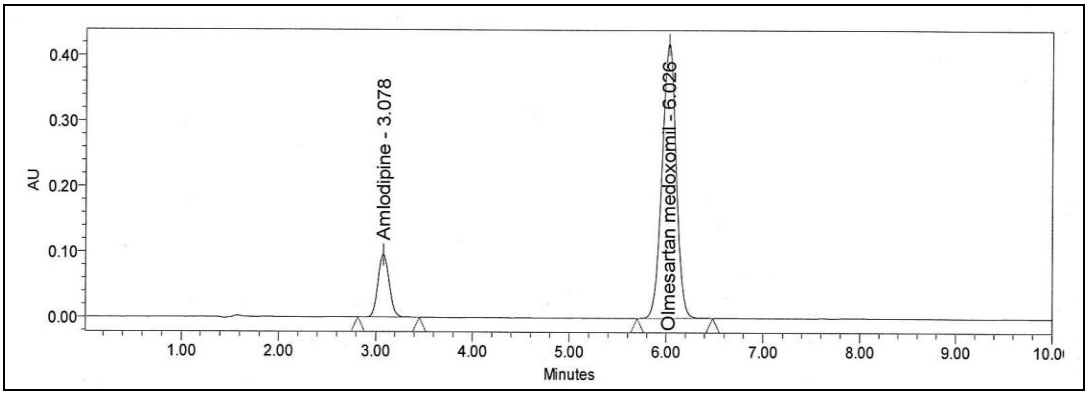

Figure No. 7: Intermediate precision sample chromatogram

Table No. 3: Intermediate Precision results

Preparations	Olmesartan medoxomil	Amlodipine Besylate
1	97.2	97.2
2	97.1	97.1
3	97.7	97.7
4	97.7	97.6
5	97.8	97.5
6	99.5	99.1
Mean	**97.8**	**97.7**
% RSD	**0.9**	**0.7**

- Overall and individual % of Assay are complies as per test method specification.
- The relative standard deviations of six assay preparations are 0.4.
- The overall relative standard deviation of six assay preparations of precision study and six assay preparations of intermediate precision study is 0.7.

Accuracy:

The accuracy of the test method was demonstrated by preparing recovery samples of Amlodipine Besylate and Olmesartan medoxomil at 10%, 50%, 75%, 100% and 150% of the target concentration level. The recovery samples were prepared in triplicate for each concentration level except 10% and 150 % (10% and 150% are six preparations). The percentage recoveries with found in the range of 99.4 to 101.3 for Amlodipine Besylate and The percentage recoveries with found in the range of 99.5 to 101.4 for Olmesartan medoxomil. From the data obtained which given in **Table 4 and Table 5** the method was found to be accurate.

Table No. 4: Recovery studies for Amlodipine Besylate by proposed method

S. No.	% spike level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Mean recovery	% RSD
1	10%	2.52	2.51	99.6	99.4	0.3
2		2.52	2.50	99.1		
3		2.52	2.51	99.5		
4		2.52	2.50	99.1		
5		2.52	2.51	99.5		
6		2.52	2.52	99.8		
1	50%	12.4	12.51	100.9	101.2	0.6
2		12.33	12.57	101.9		
3		12.4	12.50	100.8		
1	75%	18.75	18.92	101	100.9	0.1
2		18.75	18.92	100.9		
3		18.82	18.99	100.9		
1	100%	25.09	25.35	101	100.8	0.3
2		25.09	25.22	100.5		
3		25.02	25.25	100.9		
1	150%	37.71	38.02	100.8	101.3	0.4
2		37.49	38.05	101.5		
3		37.64	38.00	101		
4		37.42	37.96	101.4		
5		37.56	38.24	101.8		
6		37.49	38.01	101.4		

Table No. 5: Recovery studies for Olmesartan medoxomil by proposed method

S. No.	% spike level	Amount added(mg)	Amount recovered(mg)	% Recovery	%Mean recovery	% RSD
1	10%	20.26	20.15	99.5	99.7	0.2
2		20.26	20.21	99.8		
3		20.16	20.14	99.9		
4		20.26	20.13	99.4		
5		20.16	20.11	99.8		
6		20.26	20.16	99.5		
1	50%	99.8	99.43	99.6	99.5	0.1
2		100.2	99.68	99.5		
3		100.1	99.49	99.4		
1	75%	150	151.25	100.8	100.9	0.2
2		150.2	151.2	100.7		
3		150	151.7	101.1		
1	100%	199.8	203.37	101.8	101.4	0.4
2		200	202.58	101.3		
3		200	202.29	101.1		
1	150%	300	303.35	101.1	101.3	0.3
2		299.7	303.58	101.3		
3		300.1	303.12	101		
4		299.8	303.11	101.1		
5		299.8	305.36	101.9		
6		300	303.55	101.2		

Linearity of detector response:

The standard curve was obtained in the concentration range of 300-600 µg/ml for Amlodipine Besylate and 10-30 µg/ml for Olmesartan medoxomil. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r2] of standard curve were calculated and given in **Figure 8** for Amlodipine Besylate and **Figure 9** for Olmesartan medoxomil to demonstrate the linearity of the proposed method. From the

data obtained which given in **Table 6** For Amlodipine Besylate and **Table 6** for Olmesartan medoxomil the method was found to be linear within the proposed range.

Table No. 6: Linearity studies for Amlodipine Besylate

S. No	Linearity Level	Concentration ppm	Average area
1	10	5.083	134874
2	50	25.415	667610
3	80	40.664	1074944
4	100	50.831	1347962
5	120	60.997	1640413
6	150	76.246	2049354
correlation coefficient			0.9999
Slope			26959.983
Intercept			-12306.878
%Y-intercept			-0.91

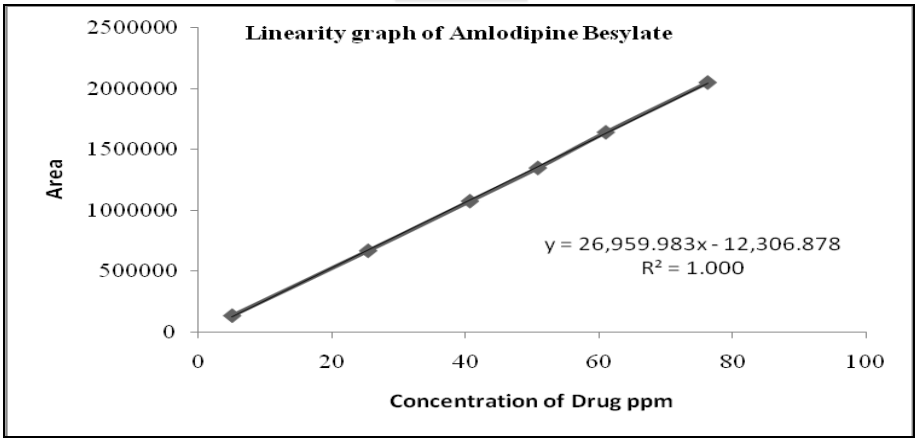

Figure No. 8: Calibration curve for Amlodipine Besylate

Table No. 7: Linearity studies for Olmesartan medoxomil

S. No	Linearity Level	Concentration ppm	Average area
1	10	19.919	444182
2	50	99.597	2208873
3	80	159.355	3533658
4	100	199.194	4457682
5	120	239.032	5409409
6	150	298.79	6804609
correlation coefficient			0.9998
Slope			22809.173
Intercept			-52188.187
%Y-intercept			-1.17

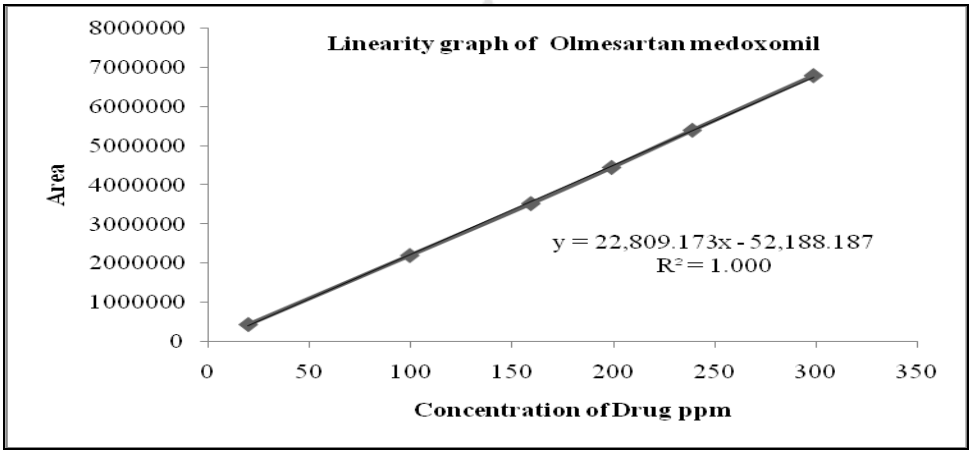

Figure No. 9: Calibration curve for Olmesartan medoxomil

RESULTS AND DISCUSSION

An RP-HPLC method for simultaneous estimation of Amlodipine Besylate and Olmesartan medoxomil was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. As there is no interference of blank and placebo at the retention time of Amlodipine Besylate and Olmesartan medoxomil hence method was specific. Linearity was observed over a concentration range of 5.083-76.246µg/ml for Amlodipine Besylate and 19.919-298.79µg/mL

for Olmesartan medoxomil. The correlation coefficient Amlodipine Besylate was found to be 0.9999 and 0.9998 for Olmesartan medoxomil.

Relative standard deviation for method precision was found to be 0.4% for Amlodipine Besylate and 0.4% for Olmesartan medoxomil. Intermediate precision was found to be 0.9% for Amlodipine Besylate and 0.7% for Olmesartan medoxomil.

The accuracy studies were shown as % recovery at 10 to 150% level for Amlodipine Besylate. The limit of % recovered shown is in the range of 99.5% to 101.4% and the results obtained were found to be within the limits. The relative standard deviation values of recoveries 0.1% to 0.4%. Hence the method was found to be accurate. The accuracy studies were shown as % recovery at 10 to 150% level for Olmesartan medoxomil. The limit of % recovered shown is in the range of 99.4% to 101.3% and the results obtained were found to be within the limits. The relative standard deviation values of recoveries 0.1% to 0.6%. Hence the method was found to be accurate.

CONCLUSION

The developed method was validated as per ICH Q2A (R1) guideline. The developed method was found to be specific because there was no interference from the placebo, matrix, and degradation products at the retention time of analyte. In conclusion, the developed method can be used for routine quality control analysis and stability studies of Olmesartan Medoxomil and Amlodipine Besylate in combination products.

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CONFLICT OF INTERESTS

The authors claim that there is no conflict of interest.

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