RESEARCH ARTICLE

Stability indicating RP-HPLC method for the simultaneous estimation of Olmesartan Medoxomil and Atorvastatin calcium in bulk and tablet Dosage Form

Mohammad Yunoos*
Adinarayana Rayalla
Bandela Raju
Gonepudi Venkateswarlu

Department of Pharmaceutical Analysis, Bapatla College of Pharmacy, Bapatla, Guntur Dist., Andhra Pradesh, India.

J. Adv. Pharm. Edu. & Res.

ABSTRACT

A simple and precise stability indicating RP-HPLC method was developed and validated for the simultaneous determination of Olmesartan medoxomil (OLM) and Atorvastatin calcium (ATV) in Pharmaceutical dosage forms. Chromatography was carried out on Hypersil ODS C 18 (250mm x 4.6 mm, 5µ particle size) column using a mobile phase of phosphate buffer (adjusted to pH 2.5 with 0.1% orthophosphoric acid): acetonitrile (30:70 % v/v) at a flow rate of 1.3 ml/min. The analyte was monitored using PDA detector at 210 nm. The retention time was found to be 3.114 min and 3.828 min. for Olmesartan medoxomil and Atorvastatin calcium respectively. The proposed method was found to be having linearity in the concentration range of 5-30 µg/ml for Olmesartan medoxomil and 2.5-15 µg/ml for Atorvastatin calcium respectively. The mean % recoveries obtained were found to be 99.97-100.06% for Olmesartan medoxomil and 99.68-100.07 % for Atorvastatin calcium respectively. Stress testing which covered acid, alkali, peroxide, photolytic and thermal degradation was performed on under test to prove the specificity of the method and the degradation was achieved. The developed method has been statistically validated according to ICH guide lines and found to be simple, precise and accurate with the prescribed values. Thus the proposed method was successfully applied for the stability indicating simultaneous estimation of Olmesartan medoxomil and Atorvastatin calcium in bulk and Pharmaceutical formulations and in routine quality control analysis.

Keywords: Olmesartan medoxomil, Atorvastatin calcium, RP-HPLC, Forced degradation, Method validation.

INTRODUCTION

Olmesartan medoxomil:

Chemically it is, as shown in figure 1, (5-Methyl-2-oxo-2h-1, 3-dioxol-4-yl) methyl4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2h-1, 2, 3, 4-tetrazol-5yl) phenyl] phenyl} methyl)-1h-imidazole-5-carboxylate. Olmesartan medoxomil is an angiotensin II Type 1 receptor blocker agent 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. Olmesartan is selective for AT1 and has a 12,500 times greater affinity for AT1 than the AT2 receptor. As a result of this blockage, olmesartan reduces vasoconstriction and the secretion of aldosterone. This lowers blood pressure by producing vasodilation and decreasing peripheral resistance.

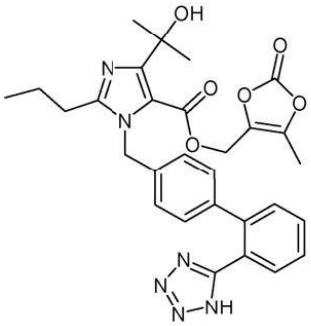

Fig.1: Structure of Olmesartan medoxomil

Atorvastatin calcium:

Chemically it is, as shown in figure 2, [R-(R*, R*)]-2-(4-fluorophenyl)-β, δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1Hpyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate. Atorvastatin is a competitive inhibitor of HMG-CoA reductase. Unlike most others, however, it is a completely synthetic compound. HMG-CoA reductase catalyzes the reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG- CoA) to

Address for correspondence

Mohammad Yunoos
Department of Pharmaceutical Analysis and Quality Assurance, Bapatla College of Pharmacy, Bapatla, Andhra Pradesh, India.
E-mail: yunoosvja@gmail.com

Access this article online
www.japer.in

mevalonate, which is the rate limiting step in hepatic cholesterol biosynthesis. Inhibition of the enzyme decreases de novo cholesterol synthesis, increasing expression of low-density lipoprotein receptors (LDL receptors) on hepatocytes. This increases LDL uptake by the hepatocytes, decreasing the amount of LDL-cholesterol in the blood. Like other statins, atorvastatin also reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol.

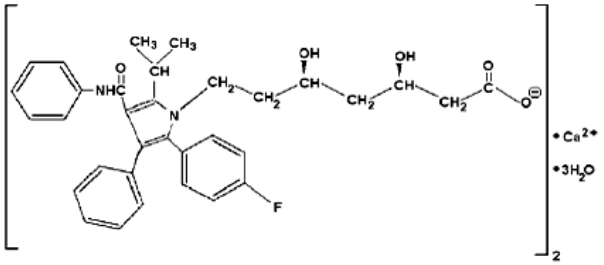

Fig.2. Structure of Atorvastatin calcium

Literature survey reveals that few analytical methods are reported like Spectrophotometric methods [1-6], RP-HPLC methods [7, 8] and HPTLC method [9] in alone or in combination with other drugs in pharmaceutical dosage forms but no simple stability indicating RP-HPLC method for the simultaneous estimation of Olmesartan medoxomil and Atorvastatin calcium in Pharmaceutical dosage forms have been reported so far. Hence author has planned to develop a simple, accurate, precise and sensitive Stability Indicating RP-HPLC method for the simultaneous estimation of Olmesartan medoxomil and Atorvastatin calcium in bulk and its Pharmaceutical dosage forms suitable for routine quality control analysis.

MATERIALS AND METHODS

2.1. Chemicals and solutions:

HPLC grade methanol and acetonitrile (Merck), HPLC grade milli-Q water, potassium dihydrogen orthophosphate and orthophos phoric acid was used for the analysis.

2.2. Instrumentation:

Quantitative HPLC was performed on Waters technologies 2695 series, PDA detector module equipped with auto injector with empower software. A reverse phase hypersil ODS C 18 (250 mm x 4.6 mm, particle size 5µm) analytical column was used. Weighing was done on shimadzu balance.

2.3. Chromatographic conditions:

Preliminary studies were conducted and trails were m ade for the method development. Separation and analysis was carried out on Hypersil ODS C 18 (250mm x 4.6mm, 5 µ particle size) column. The optimized mobile phase consisting of phosphate buffer (pH adjusted to 2.5 with 0.1% orthophosphoric acid) and Acetonitrile and in the ratio of 30:70 % v/v. Flow rate was maintained at 1.3 ml/min and run time for 7 min. Prior to sample injection, column was saturated with mobile phase for 40 min and injection volume was 10 µl injected by auto sampler. The detection response was measured at 210 nm and maintained at ambient temperature.

2.4. Preparation of standard stock solution:

Accurately Weighed and transferred 10 mg & 5 mg of Olmesartan and Atorvastatin working Standards into a 50 ml clean and dry volumetric flask, 30 ml of diluent (acetonitrile and milli-Q water in the ratio of 50:50 %v/v) was added, sonicated for 30 minutes and then made up to the final volume with diluent. From the above stock solution, 1.0 ml was pipette out in to a 10 ml volumetric flask and then make up to the final volume with diluent.

2.5. Preparation of sample stock solution:

20 tablets were weighed and average weight of each tablet was taken and then powder equivalent to 10 mg & 5 mg of Olmesartan and Atorvastatin was transferred into a 50 ml volumetric flask, 30 ml of diluent was added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution, 1.0 ml was pipette out into a 10 ml volumetric flask and made up to 10 ml with diluent.

2.6. Method validation:

2.6.1. System suitability:

System suitability was carried out by injecting standard solutions five times into the chromatographic system. The system suitability parameters were then evaluated using tailing factor, retention time and theoretical plates of standard chromatograms.

2.6.2. Accuracy:

The accuracy was performed by making three different standard concentrations at 50%, 100% and 150% levels of known amounts of studied drugs. The accuracy of an analytical method should be established across its range. The mixture was then analyzed by the proposed HPLC method at 210 nm.

2.6.3. System Precision:

The system precision was carried out by injecting standard solution preparations six times into the chromatographic system and calculate %RSD of retention time and peak area for both Olmesartan medoxomil and Atorvastatin calcium in standard preparations.

2.6.4. Method precision:

In method precision, a homogenous sample of a single batch should be analyzed six times by injecting sample solution preparations six times into the chromatographic system and calculate %RSD of retention time and peak area for both Olmesartan medoxomil and Atorvastatin calcium in sample preparations.

2.6.5. Specificity:

Specificity is the ability to assess unequivocally the analytes in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components. The specificity of the method was assessed by comparing the chromatograms obtained from standard and sample solutions. The retention times of the analytes in standard and the sample solutions were found to be same, so the method was specific and free from interference from excipients present in the tablets.

2.6.6. Linearity:

The linearity of an analytical method was carried out to check its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatograms were recorded.

2.6.7. Robustness and Ruggedness:

The robustness of the proposed method was determined by analyzing aliquots from homogenous lots by differing physical parameters like mobile phase composition, flow rate and temperature. The standard and sample solution were injected into the chromatograph at varied conditions of flow ± 0.2 ml/min, mobile phase buffer pH ± 0.2 units and temperature by ± 5 ^{0}c.

2.7. Forced degradation:

Stress testing of the drug substance can help identify the likely degradation products, which can in turn help to establish the degradation pathways and the intrinsic stability of the molecule.

2.7.1. Acid degradation studies:

To 1.0 ml of stock solution of Olmesartan and Atorvastatin, 1 ml of 2N HCL was added and refluxed for 30mins at 60^{0}c.The resultant solution was diluted to obtain 20µg/ml & 10µg/ml and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

2.7.2. Alkali degradation studies:

To 1.0 ml of stock solution of Olmesartan and Atorvastatin, 1 ml of 2N NaOH was added and refluxed for 30mins at 60^{0}c. The resultant solution was diluted to obtain 20µg/ml & 10µg/ml and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

2.7.3. Hydrolytic studies:

Stress testing under neutral conditions was studied by refluxing 1.0 ml of stock solution of Olmesartan and Atorvastatin on water bath for 6 hrs at a 60ºc. The

resultant solution was diluted to obtain 20µg/ml & 10µg/ml and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

2.7.4. Peroxide studies:

To 1.0 ml of stock solution of Olmesartan and Atorvastatin, 1 ml of 20 % H_2O_2 was added. The solution was kept for 30 min at 60^0c. The resultant solution was diluted to obtain 20µg/ml & 10µg/ml and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

2.7.5. Photolytic studies:

It is studied by exposing the 1.0 ml stock solution of Olmesartan and Atorvastatin to UV light by keeping in the UV Chamber for 7days or 200 Watt hours/m2 in photo stability chamber. The resultant solution was diluted to obtain 20 µg/ml & 10 µg/ml and 10 µl of the solution was injected into the chromatographic system and the chromatogram was recorded.

2.7.6. Thermal studies:

It is carried out by keeping 1.0 ml stock solution of Olmesartan and Atorvastatin in an oven at 105°C for 6 hrs to study dry heat degradation. The resultant solution was diluted to obtain 20µg/ml & 10µg/ml and 10µl of the solution was injected into chromatographic system and the chromatogram was recorded.

2.7.7. Solution Stability:

Standard and sample solutions of Olmesartan and Atorvastatin were prepared as per the test method and injected into the chromatographic system at initial and 24 hours by keeping solutions at room temperature. Calculated the % difference at regular intervals.

RESULTS AND DISCUSSION

From this study, it was found that a Simple, precise, accurate, sensitive and efficient Stability Indicating RP-HPLC method has been developed and validated for the estimation of Olmesartan Medoxomil and Atorvastatin Calcium in pharmaceutical dosage form. Separation was done by using mobile phase composed of phosphate buffer (adjusted to pH 2.5 with 0.1% orthophosphoric acid) and acetonitrile in the ratio (30:70 % v/v). Chromatographic separation was carried out on Hypersil ODS C 18 column (250 mm x 4.6mm 5µ particle size) at a flow rate 1.3 ml/min using PDA detection at 210 nm. The retention times of Olmesartan medoxomil and Atorvastatin calcium were found to be 3.114 and 3.828 min respectively.

Linearity was evaluated in the concentration range of 5-30 µg/ml for Olmesartan Medoxomil and 2.5-15 µg/ml for Atorvastatin Calcium. The calibration curves of Olmesartan medoxomil and Atorvastatin calcium were described by the equation y = 63622x + 1279.5 and y=51610x+498.71 with correlation coefficient 0.999 as shown in figure 3 and figure 4. System suitability results are shown in table no.1. The %RSD in precision, accuracy and robustness studies were found to be less than 2.0%, indicating that the method is precise, accurate and robust. Accuracy data as shown in table no.2. The validation summary parameters and assay results obtained from the marketed formulations are shown in table no.3 and table no.4.

Table 1: System suitability results

S. No.	System Suitability Parameters	Results	
		Olmesartan medoxomil	Atorvastatin calcium
1	Tailing factor (T_f)	1.12	1.11
2	Resolution (Rs)	5.00	
3	Retention time (Rt)	3.114	3.828
4	Theoretical plates (N)	7114	7322

Table 2: Accuracy study

Sample	Level	Peak area*	Mean % Recovery *± SD	% RSD
Olmesartan medoxomil	50%	536982	100.02 ±0.58	0.58
	100%	1074599	100.06±0.45	0.44
	150%	1610371	99.97± 0.32	0.31
Atorvastatin calcium	50%	273692	99.92 ±0.48	0.49
	100%	546397	100.07 ±0.33	0.33
	150%	819126	99.68 ±0.36	0.35

*Mean of three determinations

Linearity

R^2 values was found to be 0.999 and regression equation y=63622x+1279.5 for Olmesartan medoxomil and y = 59610x + 498.7 for Atorvastatin calcium.

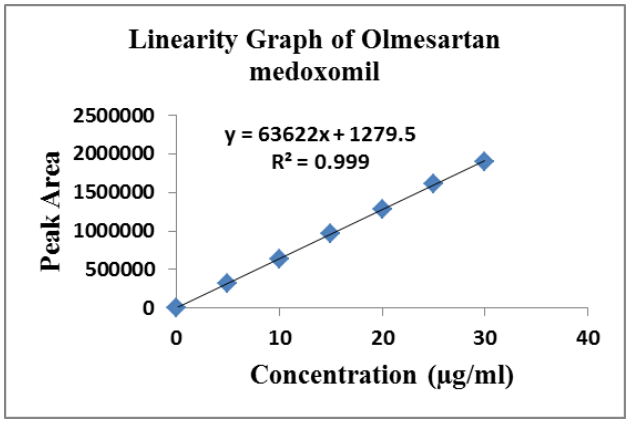

Fig.3: Linearity Graph of Olmesartan medoxomil (5-30 µg/ml)

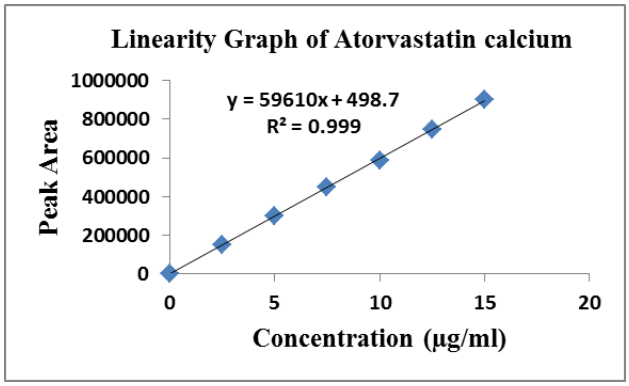

Fig.4: Linearity Graph of Atorvastatin calcium (2.5-15 µg/ml)

Specificity:

The chromatograms of standard and sample were identical to each other as shown in figure 2 and figure 3. The blank and placebo injections were also identical without any interference from the excipients.

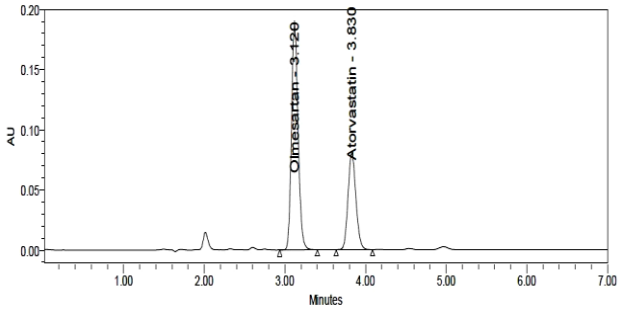

Fig.5: Chromatogram of standard solution

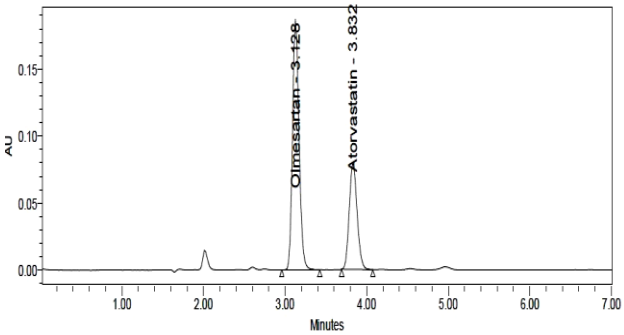

Fig.6: Chromatogram of sample solution

Limit of detection (LOD):

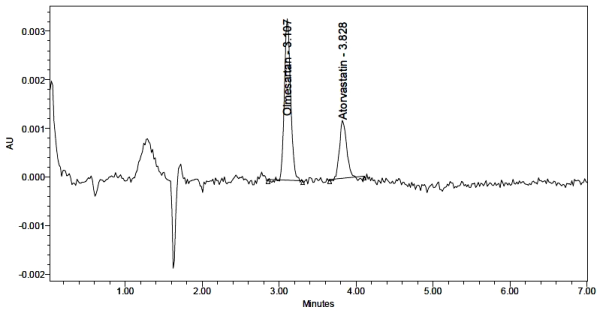

Fig.7. Chromatogram of LOD solution (0.07µg/ml of OLM & 0.03 µg/ml of ATV)

Limit of quantification(LOQ):

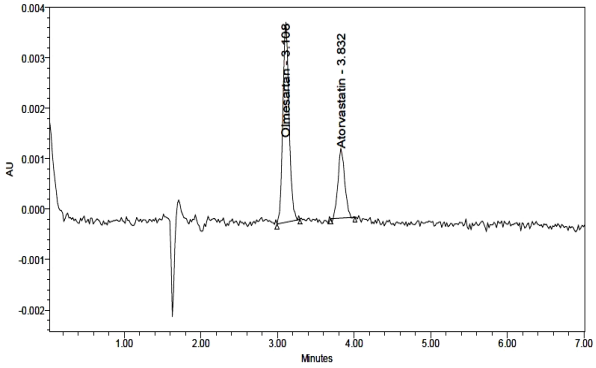

Fig.8: Chromatogram of LOQ solution (= 0.20 µg/ml of OLM & 0.08 µg/ml of ATV)

Table 3: Summary of validation parameters of the proposed RP-HPLC method

Parameter	Olmesartan medoxomil	Atorvastatin calcium
Linearity range (µg/ml)	5-30	2.5-15
Regression equation	y=63622x+1279.5	y = 59610x + 498.7
Correlation coefficient	0.999	0.999
LOD (µg/ml)	0.07	0.03
LOQ (µg/ml)	0.20	0.08
System precision (% RSD)	0.42	0.54
Method precision (% RSD)	0.51	0.44
% Assay	99.42-100.4%	99.94-100.5%

Table 4: Results of robustness study

S. No.	Parameter	Change Level	Olmesartan medoxomil			Atorvastatin calcium		
			Rt (min)	Peak area	Tailing factor	Rt (min)	Peak area	Tailing factor
1.	Flow rate (±0.1ml/min)	0.9	3.358	1153561	1.17	4.145	597630	1.14
		1.1	3.105	1065397	1.16	3.833	553368	1.12
2.	Mobile organic phase composition (±10%v/v)	55:45	3.061	1023284	1.17	3.702	530828	1.13
		35:65	3.170	1086793	1.17	3.981	589737	1.14
3.	Temperature (±5°C)	20°C	3.170 3.105	1082716 1063481	1.16 1.16	3.981 3.833	581133 549322	1.14 1.12
		30°C	3.170	1082716	1.16	3.981	581133	1.14

Forced degradation studies:

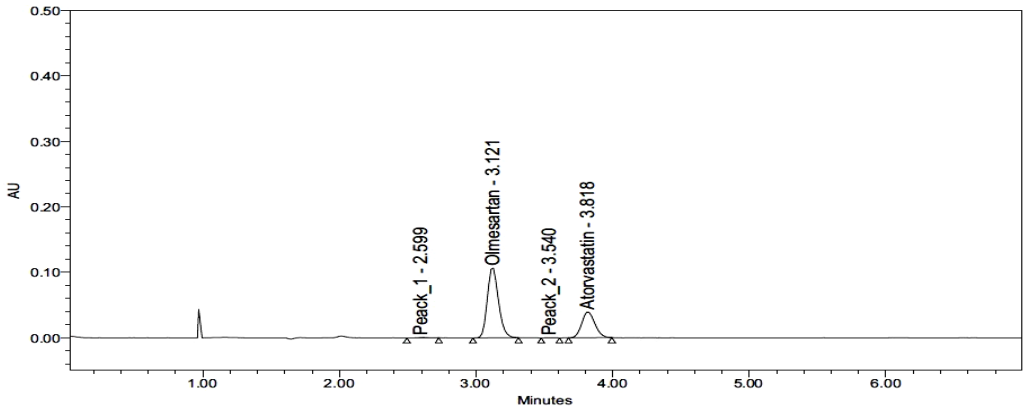

Fig.9: Chromatogram of Acid hydrolysis

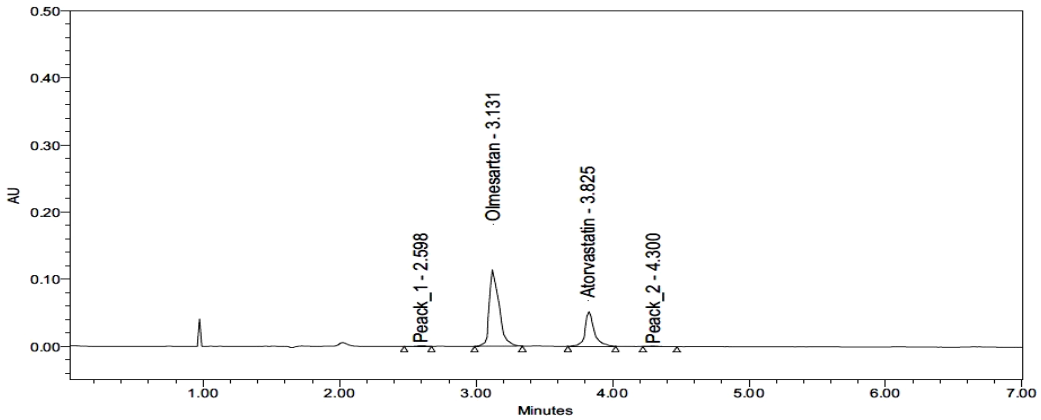

Fig.10: Chromatogram of Base Hydrolysis

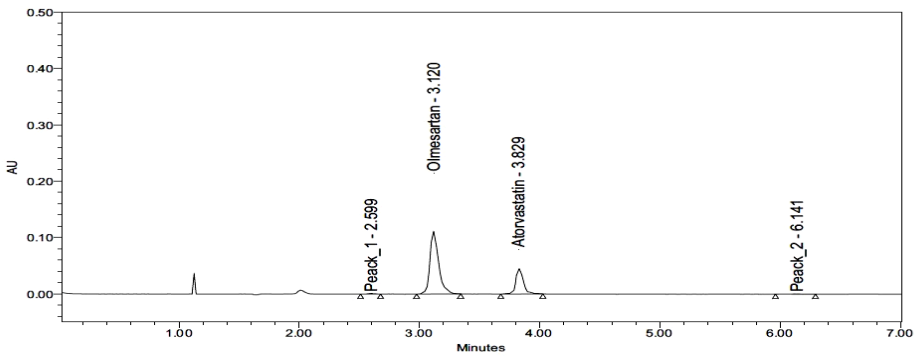

Fig.11: Chromatogram of Oxidation (peroxide)

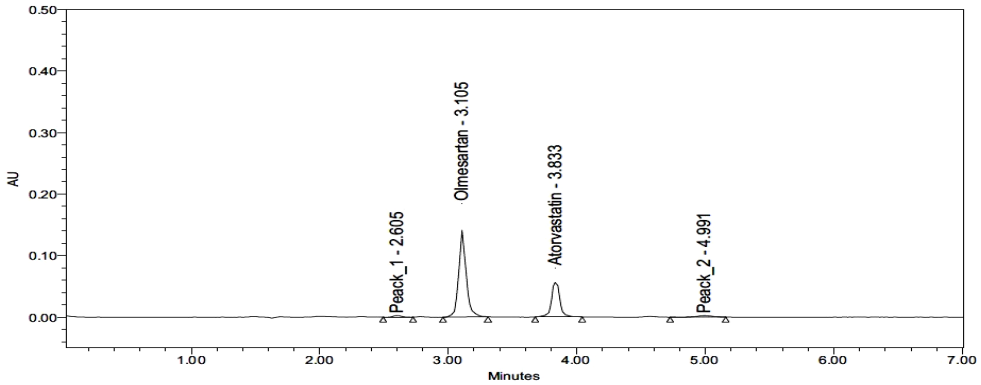

Fig.12: Chromatogram of Heat Exposure

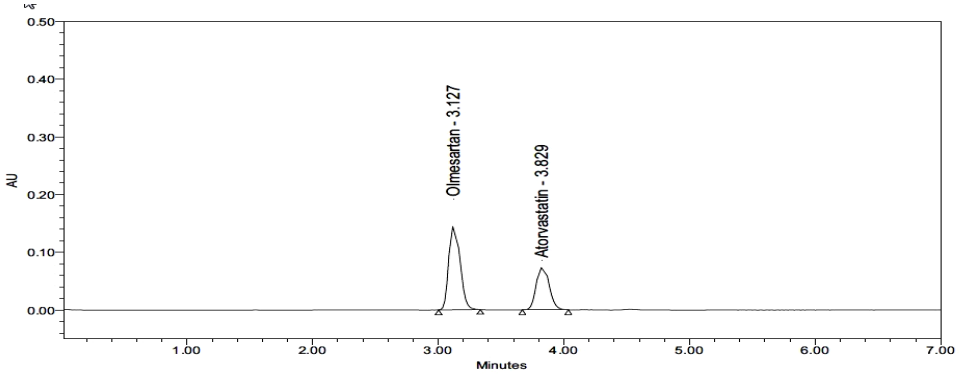

Fig.13: Chromatogram of UV Exposure

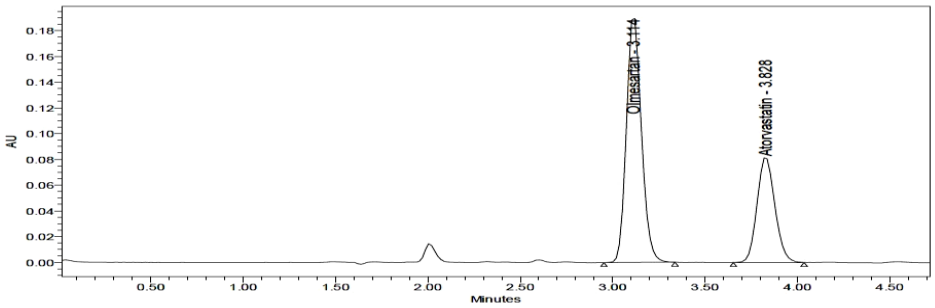

Fig.14: Chromatogram of neutral hydrolysis

Table 5: Degradation study of Olmesartan medoxomil

S. No	Name	Peak Area	Degradation % Assay	% Net Degradation
1	Acid Hydrolysis	985283	92.16	7.44
2	Base Hydrolysis	998082	93.34	6.26
3	Heat Exposure	1019786	95.37	4.23
4	Oxidation (peroxide)	1015439	94.97	4.63
5	UV Exposure	1058148	98.96	0.64
6	Neutral	1059906	99.13	0.47

Table 6: Degradation study of Atorvastatin calcium

S. No	Name	Peak Area	Degradation % Assay	% Net Degradation
1	Acid Hydrolysis	501379	92.30	7.91
2	Base Hydrolysis	508085	93.53	6.68
3	Heat Exposure	517014	95.18	5.03
4	Oxidation (peroxide)	512089	94.27	5.94
5	UV Exposure	533047	98.13	2.08
6	Neutral	538967	99.22	0.99

CONCLUSION

From this study it is concluded that the proposed Stability Indicating RP-HPLC method was found to be simple, accurate, precise, rapid and useful for routine analysis of Olmesartan medoxomil and Atorvastatin calcium in bulk & its Pharmaceutical dosage form. The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory as per ICH guidelines.

ACKNOWLEDGEMENT

The authors are grateful to Bapatla College of Pharmacy, Guntur dist., Andhra Pradesh, India for providing research facilities.

REFERENCES

1. D. Nagavalli, Venkata Suresh Babu Aluri, Development Of Uv Spectrophotometric Method for the Simultaneous Estimation of Olmesartan Medoxomil and Atorvastatin Calcium in Tablet by Simultaneous Equation and First Order Derivative Method, Journal of Pharmacy Research, 2011, 4(6), 1711-1712.
2. Nikita N. Patel, Parag R. Patel, Falguni A. Tandel, Charmy S. Kothari, Shailesh A. Shah, Ratio Derivative Spectrophotometric Method for Simultaneous Estimation of Olmesartan Medoxomil and Atorvastatin Calcium in their Combined Tablet Dosage, International Journal of Pharmacy and Pharmaceutical Sciences, 2012,4(5), 222-226.
3. Disha D Patel, Mehul M Patel, Simultaneous Estimation of Metoprolol Succinate and Olmesartan Medoxomil in Pharmaceutical Dosage Form by Uv Spectroscopy, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2012, 3(2), 935-939.
4. M.Vijayakumari, K. Prasadbabu, K. Lakshmi Prasanna, P.Sowjanya, M.Prathap, A New Method Development and Validation for Quantitative Estimation of Olmesartan Medoxomil in Pharmaceutical Dosage Form by Uv Spectrophotometry, Scholars Academic Journal Of Pharmacy, 2014, 3(3), 317-320.
5. Revanth Reddy B, Aravind G, Simultaneous Estimation of Olmesartan and Atorvastatin in Bulk and Formulation by using Uv Spectroscopy and RP-HPLC, Indian Journal Of Research in Pharmacy and Biotechnology, 2013, 1(2), 244-254.
6. Bharat G. Chaudari., Ashok B. Patel, Simultaneous Spectrophotometric Estimation of Atorvastatin calcium and Amodipine Besylate in Tablet Dosage Form, International Journal of Chem.Tech. Research, 2010, 2(1), 633 – 639.
7. Prabhat Jain, Anurekha Jain, Deepika Maliwal, and Vaibhav Jain, Development and Validation of Spectrophotometric and RP-HPLC Method for Estimation of Olmesartan Medoxomil in Tablet Dosage Form, International Journal of Pharma. and Bio Sciences, 2010, 1(2), 1-7.
8. R.A. Mhaske, S. Sahasrabudhe , A.A. Mhaske And D. J. Garole, Simultataneous Determination of Atorvastatin Calcium, Olmesartan Medoxomil, Candesartan, Hydrochlorothiazide and Chlorthalidone – Application to Commercially Available Drug Products by RP-HPLC Method , ijpsr, 2012, 3(3), 793-801.
9. Napa Delhi Raj, Sockalingam Anbazhagan, Validated HPTLC Method for the Estimation of Antihypertensive Drugs in Pharmaceutical Combined Dosage Forms, International Journal of Research And Reviews In Pharmacy And Applied Science, 2012, 2(5), 959-964.

How to cite this article: Mohammad Yunoos*, Adinarayana Rayalla, Bandela Raju, Gonepudi Venkateswarlu; Stability indicating RP-HPLC method for the simultaneous estimation of Olmesartan Medoxomil and Atorvastatin calcium in bulk and tablet Dosage Form; J. Adv. Pharm. Edu. & Res. 2014: 4(3): 372-379.

Source of Support: Nil, **Conflict of Interest:** Nil