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STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF OLMESARTAN MEDOXOMIL AND ATORVASTATIN CALCIUM

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ABSTRACT

A simple, rapid, and precise, stability indicating RP-HPLC method for simultaneous analysis of olmesartan medoxomil (OLM) and Atorvastatin (ATR) in bulk has been developed and validated. The analytes were separated by using a Waters symmetry C_{18} analytical column (250mm × 4.6mm, 5 µm) with a mobile phase consisting of Acetonitrile and 0.02 M potassium dihydrogen phosphate buffer containing 0.1% heptanesulphonic acid sodium (pH 3.0, adjusted with o-phosphoric acid) in the ratio of 55:45 (v/v) at a flow rate of 1.2mL/min. The chromatographic separation was monitored at 253 nm with a run time of 10 min. A volume of 20 µL was injected into the system. Olmesartan medoxomil and Atorvastatin were eluted with approximate retention times of 4.15min and 7.36 min respectively. Calibration plots were linear over the concentration ranges 0.5 to 10µg mL^{-1} for both the drugs. The high recovery and low coefficients of variation confirms the suitability of the method for simultaneous analysis of the two drugs in formulations also.

Key Words: Simultaneous Determination, Stability Indicating Method, Validation, Olmesartan medoxomil, Atorvastatin.

INTRODUCTION

Olmesartan is an angiotensin II receptor antagonist used to treat high blood pressure. The prodrug olmesartan medoxomil is marketed worldwide. It is chemically known as 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(1H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1H-imidazole-5-carboxylic acid. Olmesartan works by blocking the binding of angiotensin II to the AT1 receptors in vascular muscle; it is therefore independent of angiotensin II synthesis pathways, unlike ACE inhibitors. By blocking binding rather than synthesis of angiotensin II, olmesartan inhibits the negative regulatory feedback on renin secretion. 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 [1-4]. Atorvastatin is a synthetic hydroxyl methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor that has been used as a lipid lowering agent [5]. Atorvastatin is not an official drug in any of the pharmacopoeia. Chemically, Atorvastatin is [R-(R*, R*)]-2-(4-flurophenyl)-B, B-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1H-pyrrole-1-heptanoic acid [6-7]. Atorvastatin is a competitive inhibitor of HMG-CoA reductase. This enzyme catalyzes the reduction of 3-hydroxy-3-methylgultaryl-coenzyme-A to mevalonate, which is the rate-determining step in hepatic cholesterol synthesis. Because cholesterol synthesis decreases, hepatic cells increase the number of LDL receptors on the surface of the cells, which in turn increase the amount of LDL uptake by the hepatic cells, and decrease the amount of LDL in the blood [8-9]. The structures of the analytes are shown in Figure 1.

Literature survey revealed that no HPLC methods were reported for the stability evaluation with

simultaneous determination of olmesartan medoxomil (OLM) and Atorvastatin (ATR) till date. Methods reported for the quantification of olmesartan medoxomil individually and with other combinations include [10-16]. Methods are available for the quantification of Atorvastatin individually and with other combinations [5, 17-23]. Present study involves development and validation of RP-HPLC method for the simultaneous determination of olmesartan medoxomil (OLM) and Atorvastatin (ATR) in bulk and applied to stress test for stability evaluation of Drug substance.

MATERIALS AND METHODS

Instrumentation and Chromatographic conditions: Waters HPLC system consisted of 2695 separation module-Alliance LC, auto injector and a 2996 Photo Diode Array detector (Waters, Milford, MA, USA). The data was analyzed and processed by using waters Millennium[32] software. Chromatographic separation was achieved using a Waters symmetry C_{18} analytical column (250mm × 4.6mm, 5 µm) with a mobile phase consisting of Acetonitrile and 0.02 M potassium dihydrogen phosphate buffer containing 0.1% heptanesulphonic acid sodium (pH 3.0, adjusted with o-phosphoric acid) in the ratio of 55:45 (v/v). The mobile phase was filtered, degassed and pumped at a flow rate of 1.2mL/min. The chromatographic separation was monitored at 253 nm with a run time of 10 min. A volume of 20 µl was injected into the system. For pH measurement Multical®, WTW, USA pH meter was used.

Reagents and solutions: Atorvastatin calcium (ATR) was gift sample obtained from Aurobindo Pharma Ltd. (Hyderabad, India) and olmesartan medoxomil (OLM) was gift sample from Ranbaxy Laboratories Ltd. (Gurgaon, India). Deionised water was obtained from a Nanopure Diamond, Barnstead thermolyne, USA, water purification system. HPLC grade Methanol and Acetonitrile were purchased from Merck Ltd. (Mumbai, India), and O-phosphoric acid of A.R. grade was purchased from S.D fine chemicals Ltd (Hyderabad, India), Potassium dihydrogen phosphate of A.R. grade was purchased from Loba chemie Pvt.Ltd (Mumbai, India) and Lichropur® Heptane sulfonic acid was purchased from Merck Ltd. (Mumbai, India).

Preparation of standard drug solutions: An accurately weighed amount of 10 mg of each of olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) was dissolved in 10 ml of methanol to obtain a concentration of 1 mg/mL each. From 1 mg/mL solution 1 ml was taken and made to 10 ml with methanol to obtain a stock concentration of 100µg/mL each. The serial dilutions of olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) for calibration curve were prepared by suitable dilution of the stock solution with methanol.

Method validation: Method was validated accordance to ICH guidelines [24], for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification, robustness, specificity and solution stability.

System suitability: For system suitability, six replicates of standard sample were injected and studied the parameters like number of theoretical plates, tailing factor, resolution and retention time of samples. The results are presented in Table 1.

Linearity and Range: Calibration standards were prepared by spiking required volume of working standard (100µg/mL) solution into different 10 ml volumetric flasks and volume made with methanol to yield concentrations of 0.5, 1, 2.5, 5, 7.5 and 10µg/mL for each drug. A volume of 20µL was injected into HPLC. The linearity of this method was evaluated by Linear Regression Analysis and the range was 0.5-10µg/mL for both the drugs. The calibration curves for both the drugs are shown in Figure 2 and chromatogram obtained from standard preparation is shown in Figure 3.

Precision and Accuracy: Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations (0.75, 1.5 and 6µg/mL) of linearity range were prepared and injected. Peak areas of three replicated injections of each concentration were measured. Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of three consecutive days. Accuracy of the method was determined from recovery studies. The interday and intraday accuracy and precision data is shown in Table 2.

Robustness: Robustness of the method was done by changing slight variation in the parameters like mobile phase composition, flow rate and wavelength. Robustness data is shown in Table 3.

Stability: The stability of the sample solution was determined for the quality control samples by keeping them at room temperature for 24hours. Auto sampler stability was determined by storing the

samples at 5 °C for 12 hours in the auto sampler. Freeze thaw stability of QC samples was analyzed after three freeze-thaw cycles by freezing at -20 °C for 24 h and then thawing at room temperature for 24hr. The stability data is represented in Table 4.

Specificity: The specificity of the method was demonstrated through forced degradation studies conducted on the sample in acidic (1N HCl), alkaline (1N NaOH), oxidative (3% w/v H_2O_2), reductive (Zn+ 1N HCl) and neutral degradation conditions. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient. The specificity data is represented in Table 5.

RESULTS AND DISCUSSION

The method was optimized with mobile phase consisting of acetonitrile and 0.02 M potassium dihydrogen phosphate buffer containing 0.1% heptanesulphonic acid sodium (pH 3.0, adjusted with o-phosphoric acid) in the ratio of 55:45 (v/v) at a flow rate of 1.2ml/min, at 253 nm with a run time of 10min. These chromatographic conditions achieved satisfactory resolution, retention tailing for both drugs of OLM and ATR. The standard curve for both olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) were linear over a range of 0.5 to 10μg/mL with desirable correlation coefficient of more than 0.999. The recoveries were found to be between 95% -105% for olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) with less than 2.0 % RSD for both intra- and inter-day data reflecting the precision of the method. Present method did not show any significant change when the critical parameters were modified indicating robustness of the method. The recovery of both olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) in all stability studies were found to be more than 90 % with less than ± 2 % RSD. The present method effectively separated the degradation products from the pure active ingredient which indicates specificity of the method. Hence this method can be applied for quantifying the low levels of olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) in bulk.

CONCLUSIONS

It can be seen from the results that the proposed method has good sensitivity, Specific, Precise and Robust. Hence the proposed method is suitable for simultaneous determination of olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR) in bulk, pharmaceutical formulations and as well as separation of degradants.

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Table 1: System suitability parameters

Parameter	Olmesartan Medoxomil	Atorvastatin
Calibration Range (μg mL^{-1})	0.5 - 10	0.5 - 10
Retention Time (t)	4.15	7.36
Theoretical plates (n)	8264	9083
Tailing Factor	1.21	1.33
Correlation Coefficient (r2)	0.9997	0.9995
% Recovery	97.20% -99.35%	97.69%-101.19%
System Suitability %RSD	0.87	0.45

Table 2: Interday and Intraday Accuracy and precision data (n=3)

Theoretical Concentration (μg mL^{-1})	Olmesartan Medoxomil		Atorvastatin	
	Interday	Intraday	Interday	Intraday
0.75	96.19(0.91%)	97.20(1.47%)	98.46(1.29%)	98.46(0.01%)
1.5	98.17(0.82%)	98.07(0.14%)	97.69(0.78%)	98.38(0.99%)
6	99.35(0.33%)	99.18(0.24%)	101.12(0.92%)	101.19(0.10%)

Table 3: Robustness data

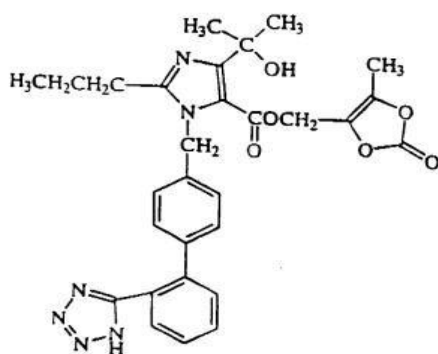
Chromatographic Conditions	Variation in parameters	System Suitability Parameters			
		Olmesartan Medoxomil		Atorvastatin	
		Theoretical Plates	Tailing Factor	Theoretical Plates	Tailing Factor
Flow Rate	+ 10 %	5912	1.25	7410	1.36
	- 10 %	4292	1.25	6275	1.33
Wavelength	- 5 nm	5398	1.21	8529	1.33
	+ 5 nm	5741	1.22	7900	1.32
% of Organic solvent in mobile phase	- 2 %	5478	1.32	7392	1.44
	+ 2 %	6212	1.22	7765	1.32

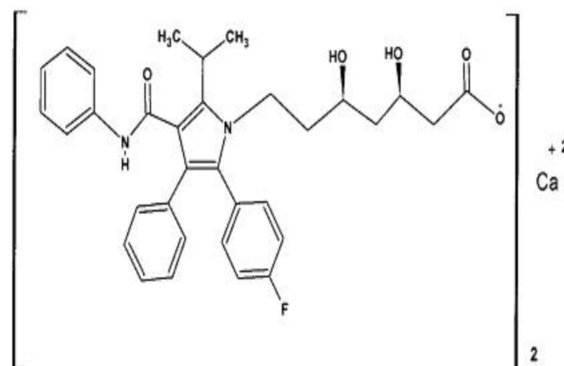
Table 4: Stability data (n=3)

Theor. Conc. (µg/mL)	Auto-sampler stability				Short-term stability				Freeze-Thaw stability			
	OLM		ATR		OLM		ATR		OLM		ATR	
	Pract. Conc. (µg/mL)	RSD (%)	Pract. Conc. (µg/mL)	RSD (%)	Pract. Conc. (µg/mL)	RSD (%)	Pract. Conc. (µg/mL)	RSD (%)	Pract. Conc. (µg/mL)	RSD (%)	Pract. Conc. (µg/mL)	RSD (%)
0.75	0.73	1.03	0.73	0.81	0.75	1.58	0.74	0.38	0.74	0.86	0.75	0.53
1.5	1.49	0.17	1.50	0.23	1.49	0.67	1.51	0.58	1.50	0.26	1.49	0.47
6	5.98	0.11	6.01	0.24	5.95	0.94	5.98	0.72	6.02	1.18	6.07	0.60

Table 5: Specificity data

Stress conditions	Theoretical concentration (µg/mL)	Practical concentration (µg/mL) (mean ± SD)	
		OLM	ATR
Standard	10	10.04±0.064	10.05±0.056
Oxidation	10	9.33±0.016	9.61±0.018
Reduction	10	5.21±0.016	5.32±0.018
Alkaline	10	0.04±0.001	10.07±0.081
Acid	10	9.46±0.103	1.69±0.008
Photolytic	10	7.74±0.025	9.95±0.033
Neutral	10	10.06±0.099	8.71±0.015





Olmesartan medoxomil **Atorvastatin calcium**

Figure 1: Structures of Olmesartan medoxomil (OLM) and Atorvastatin calcium (ATR).

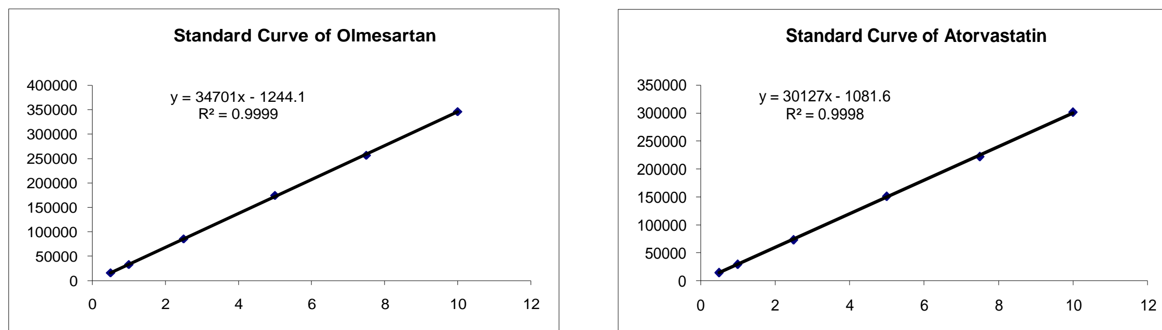


Figure 2: Calibration curve of Olmesartan medoxomil and Atorvastatin calcium.

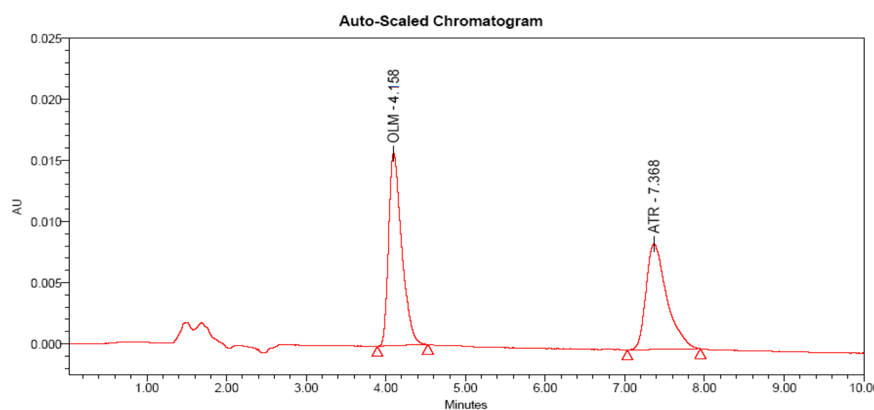


Figure 3: Standard Chromatogram of Olmesartan medoxomil and Atorvastatin calcium

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