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Research Article

Analytical Method Validated Techniques For The Simultaneous Estimation Of Olmesartan Medoxomil And Ramipril By RP-HPLC Method

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ABSTRACT

A reverse phase high performance liquid chromatographic simultaneous estimation (HPLC) was developed and validated as per ICH 2019, US FDA 2018 guidelines for quantification of Ramipril (RAM) and Olmesartan Medoxomil. In present study, Estimatimation of Olmesartan Medoxomil (OLM) and Ramipril (RAM) in tablet formulation, accurate, and affordable approach has been established. Both OLM and RAM are angiotensin-converting enzyme inhibitors, which are antihypertensive drugs. The combination of Ramipril (RAM) and Olmesartan Medoxomil (ATV) in Telmy-R is a particularly effective pharmacological formulation since it increases the efficacy of the separate medications. The current study uses shimadzhu700 and Elico UV spectrophotometers for simultaneous estimation utilising the first derivative approach and RP-HPLC. OLM has zero crossing point at 240nm in methanol and RAM has zero crossing point at 246 nm in methanol. Both these drugs obey Beer's law in the concentration range employed for the present method. The result of analysis has been validated statistically by recovery studies. The slope and intercept for OLM were 0.0364x and 0.0078 and for RAM were 0.001 and -0.0001 respectively as determined by the method of least squares. Estimation of individual drug in the tablet formulation by Amplitude measurement method (peak to peak) was carried out at first derivative mode. The work is done within the UV range of 230nm to 280nm, where OLM has λmax at 256nm. showing the linearity range between 5-30 µg/ml. The slope and intercept for OLM were 0.4594x and 2.1693 respectively. The result of analysis has been validated statistically by recovery studies. A simple, rapid, and precise reversed-phase high-performance liquid chromatographic method has been developed for

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simultaneous determination of OLM & RAM. The two drugs are separated on Capcell pack col no. AKAD (05395(4.6 mml D X 250 mm i.d.) C18 column. The mobile phase was 0.005 M Phosphate Buffer–Acetonitrile–Methanol, 60:20:20 (v/v), pH adjusted to 4 with glacial acid, at a flow rate of 1.0 mL min−1. UV detection was performed at 210 nm. The method was validated for linearity, accuracy & precision as per ICH guidelines. The results were found satisfactory and reproducible. The method was applied successfully for the estimation of OLM and RAM simultaneously in tablet dosage form without the interference of common excipients.

INTRODUCTION

Dragon fruit (Hylocereus undatus), one of the Olmesartan is a biphenylyltetrazole. It has a function as an antihypertensive agent and an angiotensin receptor antagonist.Omlersartan medoxomil is used to treat high blood pressure (Hypertension) lowering high blood pressure helps prevent strock, heart attack and kidney problems. Olmesartan belong to class og drugs called angiotensin receptor blockers (ARBs).It work by relaxing blood vessels so that blood can flow more easily. Olmesartan medoxomil is described chemically as (5-methyl-2-oxo-1, 3-dioxolen-4-yl) methyl 4-(1-hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl)-1,1'–biphenyl-4 yl]methyl]-1H-imidazole-5-carboxylate.

Olmesartan is an angiotensin II receptor blocker with an inhibitory effect on angiotensin-converting enzyme. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Its action is, therefore, independent of the pathways for angiotensin II synthesis. Ramipril is an ACE inhibitor used for the management of hypertension and the of reduction cardiovascular mortality following myocardial infarction in hemodynamically stable patients with clinical signs of congestive heart failure. Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events.

Fig:1 Structure of Ramipril

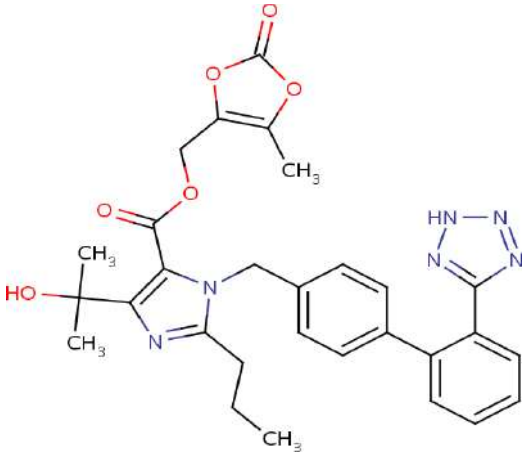

Fig:2 Structure of Olmesartan Medoxomil

MATERIAL AND METHODS

Chemicals and Materials:

Acetonitrile (HPLC Grade, pvt. Ltd. spectrochem Mumbai, India) Methanol (HPLC Grade, SDFCL Fine-chem. Ltd. Mumbai, India) Glacial Acetic Acid (AR Grade) Disodium Hydrogen Phosphate(Hplc Grade) Potassium dihydrogen phosphate (HPLC- Grade) Ammonium Acetate (AR Grade).Sod. Hydroxide (AR Grade)Water

(HPLC Grade, spectrrochem pvt. Ltd. Mumbai India)All Chemicals used during the project work were either AR or HPLC grade. 0.2µ Millipore membrane Filter paper was used throughout the project work. Ramipril and Olmesartan were bought from PADM Laboratories Pvt. Ltd. Bangalore. Analytical grade acetonitrile was procured from standard reagent, Bangalore Milli-Q water purifying system by PADM lab (Bangalore). Analytical grade Formic acid was purchased from the Venus lab (Bangalore).

Instruments:

Jasco HPLC system consisting of Jasco PU-2089 plus HPLC pump with Jasco MD-2018 plus PDA detector and ChromNAV software was used for analysis. Separation was carried out on HiQ sil C18 (250 x 4.6 mm i.d.) column, degasser, quaternary pump, auto-injector and column oven, data acquisition by Empower-2 software. The Shodex C18, 150 mm, 4.6 mm diameter; 5 µm column. Test and standard solutions were sonicated by sonicator (Branson's).

Preparation of Standard Solution:

Accurately weighed 25mg of the Ramipril and 100mg of Olmesartan medoxomil reference standards were transferred to 50ml clean and dry volumetric flask. Then the volume was made up to the mark with the mobile phase and mixed well. This yielded a standard stock solution with concentration 250µg/ml of Ramipril and 1000µg/ml Olmesartan medoxomil.

Preparation of optimized mobile phase: Preparation of Phosphate buffer (0.05M KH2PO4): weigh 5.04gm Disodium hydrogen phosphate & 3.01gm of Potassium dihydrogen phosphate into 1000ml volumetric flask and mixed thoroughly with sufficient amount of HPLC grade water. Then add 0.05ml of Glacial Acetic Acid to adjust the PH 4.0.

Preparation of mobile phase:

The mobile phase was prepared mixing 0.005M Phosphate buffer (0.05M KH2PO4): Acetonitrile & Methanol in the ratio 60:20:20 (v/v) and pH 4.0 was adjusted with Glacial Acetic acid. The solution was than filtered through 0.2µm membrane filter and degassed.

Method Validation Parameters: The method was validated in compliance with ICH guidelines. Method validation can be defined as (ICH) "Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

Linearity:

It was evaluated by determining peak mean responses (triplicates at 5 different concentrations). The mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. Different aliquots 1, 2, 3, 4, 5 ml of standard stock were transferred into 100ml volumetric flasks and volume was adjusted to mark to obtain concentration in the ranging from 2µg/ml to 6µg/ml for Ramipril and 8µg/ml to24µg/ml for Olmesartan medoxomil were injected and peaks were recorded.

Specificity:

Accuracy: (Recovery Studies) The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Prepared solutions in at levels 80%, 100% and 120% of test concentration using Ramipril and Olmesartan medoxomil working Standard as per the test method and injected each solution in triplicate. Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. It is a measure of the

degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to a single component only i.e. that no co-elution's exist. The analytes should have no interference from other extraneous components and be well resolved from them.

Precision:

Precision is the measure of the degree of repeatability of an analytical method under normal operation and is normally expressed as the percent relative standard deviation for a statistically significant number of samples. Accurately weighed 25 mg of the Ramipril and 100mgOlmesartan medoxomil reference standards were transferred to 100ml clean and dry volumetric flask. Then the volume was made up to the mark with the mobile phase. Accurately pipette out 10ml of standard stock solution and transferred to 100ml of volumetric flask. Then volume was made up to mark with mobile phase and mixed well. Resultant solution was injected six times.

Ruggedness:

Ruggedness, according to the USP, is the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD. These conditions include different laboratories, analysts, instruments, reagents, days, etc. Standard solution was prepared containing 25µg/ml of Ramipril and 100µg/ml of Olmesartan Medoxomil and analyzed by two different analysts usingsimilar operational and chromatographic conditions. Test sample was prepared as per assay procedure. Response Factor was measured for same concentration solutions, six times and test sample in two times.

RESULTS AND DISCUSSION

By Shimadzu UV Spectrophotometer In the proposed method once the λmax value and calibration range are determined, a little time is required for analysis. Simultaneous estimation of Ramipril & Olmesartan medoxomil by RP-HPLC method was carried out.

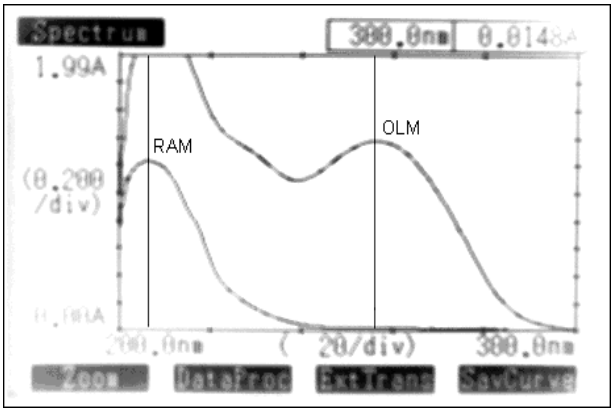

Fig:3.Overlain spectra of OLM and RAM

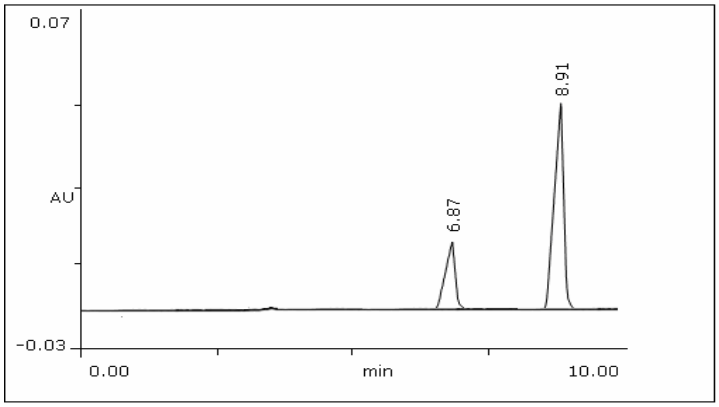

Fig:4 chromatogram of RAM &OLM

Chromatographic conditions:

Table no.1 Chromatographic conditions

Parameters	Method
Stationary phase (column)	The Shodex C18, 150 mm, 4.6 mm diameter; 5 µm column
Mobile phase	0.005M Phosphate buffer (0.05M KH2PO4): Acetonitrile & Methanol in the ratio 60:20:20 (v/v) and pH 3.5 was adjusted with orthophosphoric acid.
Flow rate (ml / min)	1.0
Column temperature (0C)	Ambient
Volume of injection (µl)	20

Detection wavelength (nm)	210
Retention Time (min.)	**RAM-** 6.87 **OLM-** 8.91

Linearity:

Linearity range for Ramipril and Olmersartan 2-6 µg/ml at respective selected wavelength. The coefficient of correlation for Ramipril 240nm and Olmersartan at 246nm.

Table no. 2 Concentrations to peak response Ramipril.

Concentration (µg/ml)	INJ-1 Area	INJ-2 Area	INJ-3 Area	Mean peak Response	Statistical Analysis
2	409893	409945	409876	409904.67	Y=156915x+88380 R^2 = 0.9994
3	549850	549895	549925	549889.33	
4	714383	714295	714356	714344.67	
5	873267	873321	873234	873274.0	
6	1032796	10327221	1032843	1032786.7	

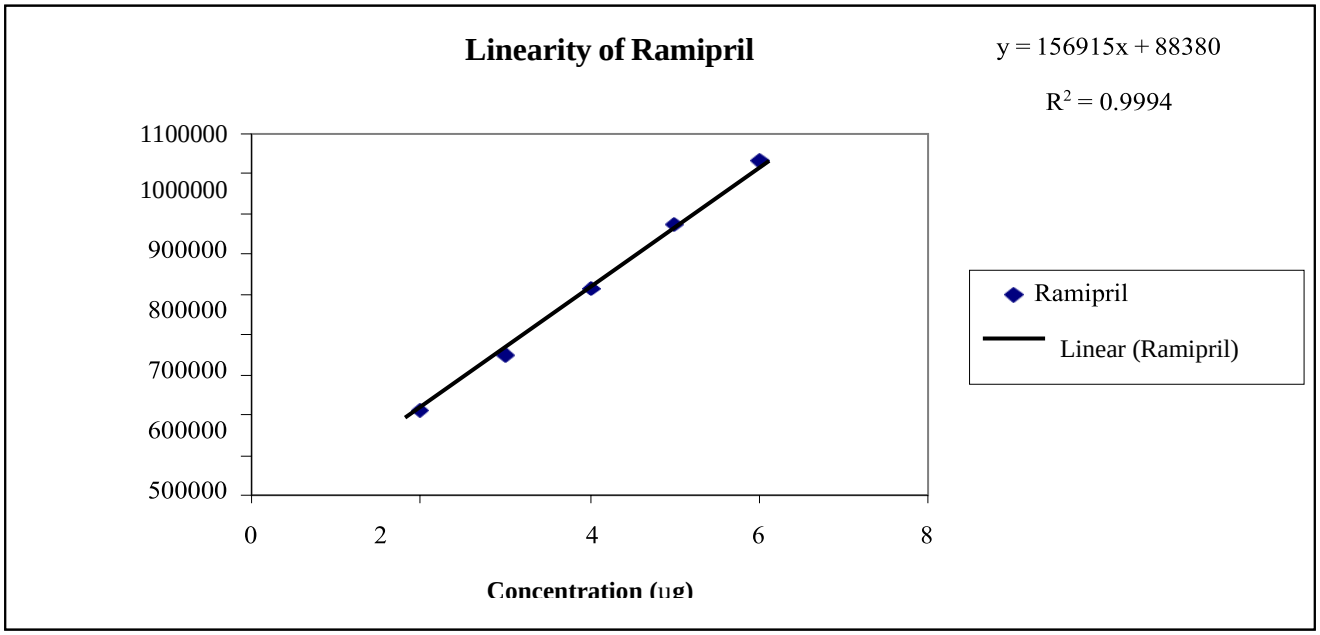

Fig no:5 Calibration Curve of Ramipril

Table no:3. Concentration to peak response Olmesartan medoxomil.

Concentration (µg/ml)	INJ-1 Area	INJ-2 Area	INJ-3 Area	Mean peak Response	Statistical Analysis
8	1461260	1461315	1461235	1461270	Y=493245x-3E+06 R^2 = 0.9992
12	3451892	3451820	3451772	3451828	
16	5228790	5228843	5228735	5228789.3	
20	7410888	7410810	7410943	7410880.3	
24	9323624	9392715	9323580	9346639.7	

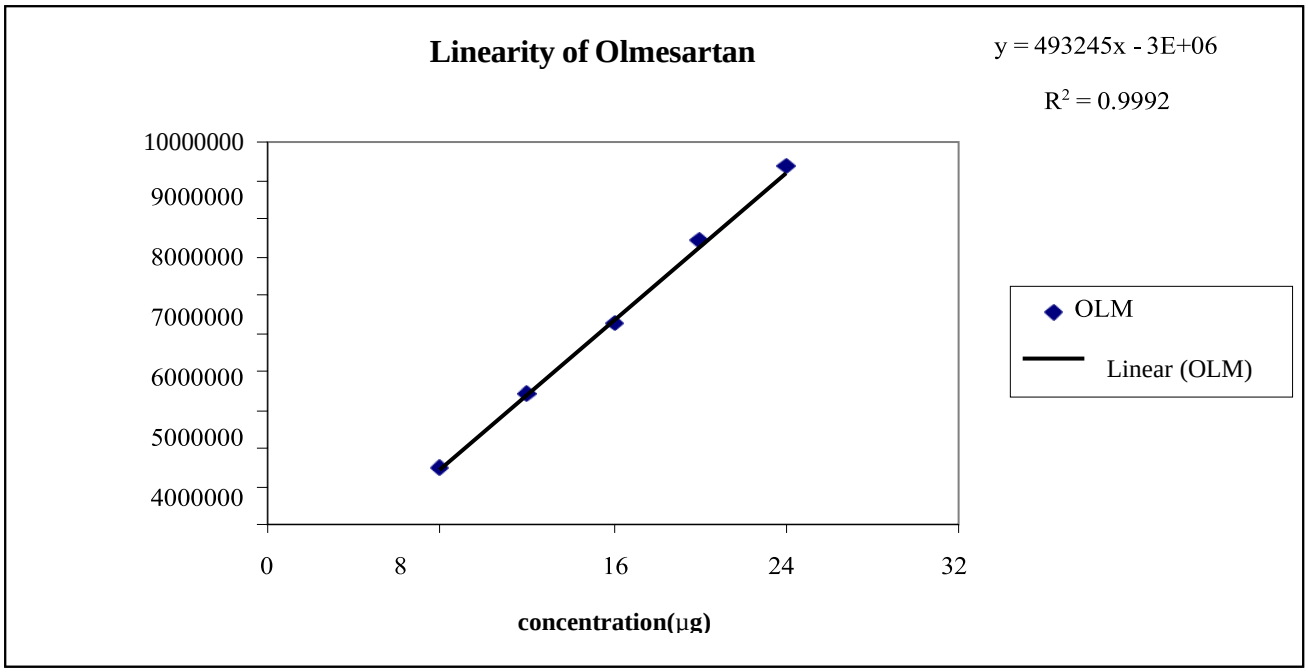

Fig. no.6 Calibration Curve of Olmersartan

Accuracy (Recovery Studies) % recovery was found within limit i.e. the recovery of Ramipril ranges from 99.05-100.07% and recovery of Olmesartan ranges from 98.87-100.67%, which shows the accuracy of proposed method.

Table no. 4 Results of Accuracy parameter for Ramipril

Conc.	INJ-1	INJ-2	INJ-3	Mean	SD	%RSD	%Recovery
80%	713742	714383	713578	713914	418.34	0.0586	99.05
100%	874352	873267	874157	873958	578.6	0.0662	99.35
120%	1032573	1032796	1033912	1033093	897.75	0.0869	100.07

Table no. 5 Results of Accuracy parameter for Olmesartan medoxomil

Conc.	INJ-1	INJ-2	INJ-3	Mean	SD	%RSD	%Recovery
80%	5228735	5228643	5228970	5228782	168.63	0.00323	98.67
100%	7410943	7411542	7410356	7410947	593.01	0.008	99.42
120%	9323580	9323196	9324113	9323629	460.51	0.00494	100.10

Specificity:

The method is quite selective. There was no other interfering peak around the retention time of Ramipril and Olmesartan medoxomil; also, the base line did not show any significant noise

Table no.6: Results of Repeatability

Sr. No	Ramipril		Olmesartan Medoxomil	
	Area	RT	Area	RT
1	863372	6.87	7521875	8.91
2	867453	6.788	7532758	8.734
3	874357	6.814	7613249	8.773
4	869723	6.773	7517345	8.693
5	872247	6.745	7624356	8.687
6	856264	6.805	7524985	8.793
Mean	**867236**	**6.786**	**755576**	**8.738**
SD	**4870.67**	**0.0246**	**15494126**	**0.0425**
%RSD	**0.56163**	**0.36306**	**0.020506**	**0.48656**

Precision:

The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (Coefficient of variation) of a series of measurement.

Table no. 7.Results of Method precision for Ramipril

Sr. No	Ramipril		
	INJ 1	INJ 2	Mean
1	862537	863277	862907
2	875463	876653	876058
3	865528	865342	865435
4	859725	859963	859844
5	876532	874675	875604
6	865563	864451	865007
Mean	**867558**	**867393**	**867475**
S.D.	**4690.2**	**6690.04**	**2137.75**
%R.S.D	**0.54062**	**0.77128**	**0.24643**

Table no. 8.Results of Method precision for Olmesartan Medoxomil

Sr. No	Olmesartan Medoxomil		
	INJ 1	INJ 2	Mean
1	7521875	7532526	7527200
2	7517345	7526355	7521850
3	7524372	7535462	7529917
4	7514682	7512543	7513612
5	7536235	7536546	7536390
6	7515427	7531352	7521089
Mean	**7521656**	**7529297**	**7525393**
S.D.	**8074.59**	**8883.20**	**7746.31**
%R.S.D	**0.10735**	**0.11798**	**0.10294**

Ruggedness:

These conditions include different laboratories, analysts, instruments, reagents, days, etc. In the guideline on definitions and terminology, the ICH did not address ruggedness specifically.

Table no. 9 Result of Ruggedness of Ramipril and Olmersartan Medoxomil

Sr. No	Drug	Label Claim (in mg)	Recovery (in mg)	Amount Found (%)
Analyst I	Ramipril	5	4.97	99.4
	Olmesartan Medoxomil	20	19.93	99.65
Analyst II	Ramipril	5	4.96	99.2
	Olmesartan Medoxomil	20	19.89	99.45

CONCLUSIONS

The described methods give accurate and precise results for determination of RAM and OLM mixtures in tablets without prior separation and are easily applied for routine analysis. From the experimental studies it can be conclude that HPLC method developed for the Simultaneous estimation of Ramipril and Olmesartan Medoxomil for their dosage form. The Proposed methods for the selected drugs were found to be accurate and precise. However, this method is more reproducible. It is also more sensitive and specific method. Result of validation parameter demonstrate that the analytical procedure is suitable for its intented purpose and meets the criteria defined in ICH Q2A/B.

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