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QUANTIFICATION OF OLMESARTAN AND ROSUVASTATIN BY STABILITY INDICATING RP-HPLC IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The objective of the study is to develop an economical and simple and validated method for the simultaneous estimation of the olmesartan and rosuvastatin by RP-HPLC technique. In this method the 10µl of standard working solution containing both olmesartan and rosuvastatin was injected in to the mobile phase line composing of 45B and 55A, pumped through the column Altima 150mm length column containing particle of size 5µ with a flow rate of 1ml/min. the temperature of the column was maintained at 30°C. Both the drugs have their optimum absorbance at 241nm wavelength. The method was optimized based on the all system suitable parameters passed their limits as per ICH guidelines. The retention time found was 2.2min of olmesartan and 3.0min of rosuvastatin and the resolution between the peaks was 4.8. This method was set for validation as per ICH guidelines and observed to be very specific without any interference by the constituents of formulations and diluents. The method was precisied and %RSD found to be 0.56 of olmesartan and 0.42 of rosuvastatin. % recover of olmesartan was 100.09% and 99.79% for rosuvastatin. Linearity was performed with six concentrations and response was observed to be linear to the concentration. Correlation coefficient obtained was 0.999. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. The developed method passed all the parameters of the validation, the run time was decreased effectively so the method was very simple and economical that can be used in the regular analysis of the Olmesartan and rosuvastatin in marketed formulation.

Keywords: Olmesartan, Rosuvastatin, ICH guidelines, RP-HPLC.

INTRODUCTION

Olmesartan medoxomil is used in the treatment of hypertension it is an angiotensin II receptor antagonist. It also decreases the vasoconstriction and aldosterone secretion. The IUPAC name is (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl)methyl 4-(2-hydroxypropan-2-yl)-2-propyl-1-({4-[2-(2*H*-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1*H*-imidazole-5-carboxylate. Its molecular formula is $C_{29}H_{30}N_6O_6$. Solubility of the olmesartan was in freely in methanol and its pKa value is Strongest basic 5.57. Rosuvastatin belongs to statins, used in the treatment for weight reduction. It inhibits HMG COA reductes enzyme competitively. The IUPAC name of the Rosuvastatin was (3*R*,5*S*,6*E*) -7- [4- (4-fluorophenyl) -2- (*N*-methylmethanesulfonamido) -6- (propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid. Its molecular formula is $C_{22}H_{28}FN_3O_6S$. rosuvastatin was soluble in water, ethanol, methanol, DMSO and chloroform and its pKa(strongest acidic) value is 4. According to literature survey there were three method like Elijebeth et al., (2013), Sharma et al., (2014) and Tripti Sharma (2014) for the estimation of olmesartan and rosuvastatin, the present study deals with more time favour, economical simple method.

EXPERIMENTAL WORK

Materials and reagents: XARB-H the formulation of olmesartan and rosuvastatin bought from the local retail shop. Bulk drugs olmesartan and rosuvastatin are gift samples from spectrum research solutions, all the solvents used in this method were HPLC grade

and chemicals like orthophosphorica acid was of analytical grade.

Instruments: HPLC used in this method was of Waters 2695 model integrated with quaternary pumps delivery system. Sampling was done by automatic sampler, detector was a photo diode array detector 2990, column maintained at required temperature by oven. System integrated with the software empowers-2, Digisun ph meter7007, Labmann ultrasonic cleaner, shimadzu weighing balance.

Preparation of Buffer: 0.1%OPA buffer was prepared by dissolving the dilute ortho phosphoric acid in HPLC grade water and made up to 1000ml mark. Sonicated for 30 min for removing all the dissolved gases.

Preparation of standard working solution: Accurately weighed 10mg of olmesartan medoxomil and 5mg of Rosuvastatin in to two 10ml volumetric flasks, add 7ml of diluents to dissolve the drug and sonicated for 10min. then 1ml form the two stock solutions was taken in to a single 10ml volumetric flask and made up to the mark with the same diluents, which gives 100µg/ml of olmesartan and 50µg/ml of rosuvastatin working standard solution.

Preparation of sample working solution: 20 tablets were taken and average weight of tablet was found. Tablet powder weight equivalent to 100mg of Olmesartan and 50mg Rosuvastatin was transferred in to a 50ml volumetric flask, $3/4^{th}$ amount of the diluents was added to dissolve the drug and was sonicated for 30min. The resulted solution was made up to the mark and was filtered. 0.5ml form the sample stock solution was pipette out and made up to 10ml with the same diluents.

Chromatographic conditions: The mobile phase ratio of 45 buffer and 55Acetonitrile was run through altima150mm column of 5µ particle size with a flow rate of 1ml, oven temperature was maintained at 30°C and wavelength was optimized as 241nm. Volume of injection was 10µl.

Method Validation: Validation was performed for the developed method to confirm the standard of the method and whether performed method suites to estimate the omesartan and rosuvastatin combination tablets in the regular assay.

Specificity: Specificity is the study of the interference of the other constituents in the formulation, it is to check whether the chromatography elutes the drug along with any of dissolved expepient at the same retention time and thus specificity was studied by injecting the blank and placebo solution separately and compared to standard chromatogram for interferences.

Linearity: Six different concentration were taken as beers range for olmesartan of 25µg/ml, 50µg/ml, 75µg/ml, 100µg/ml, 125µg/ml, 150µg/ml and for rosuvastatin of 12.5µg/ml, 25µg/ml, 37.5µg/ml, 50µg/ml, 62.5µg/ml, 75µg/ml. and calibration curve was obtained from the concentration versus response by the injection of particular concentrations.

Precision:

Repeatability: It also known as intraday precision it represents the deviation of the individual agreement to the average value of the agreement obtained by the multiple injection of the sample prepared by the multiple sampling techniques. It was reported as %RSD.

Intermediate Precision: It is also known as analyst to analyst precision, day to day precision, and system to system precision. It represents the deviation of the agreement between the instruments or between the analysts or between the systems and also reported as %RSD.

Accuracy: Accuracy represents the closeness of the agreement to the conventional true value. Three levels of concentrations 50%, 100% and 150% are prepared by adding the standard to the placebo and resulted solutions were injected and the %Amount recovered was calculated.

LOD: The concentration at which the signal to noise ratio of the drug was 3:1which is the lowest concentration that can be detected by the detector.

LOQ: The concentration at which the signal to noise ratio of the drug was 10:1which is the lowest concentration that can be quantified by the detector with an accuracy and precision.

Robustness: Small changes in the optimized method was done such as change in flow rate of 10% (±), Change in Mobile phase composition in 10% (±) of acetonitrile and change in temperature 5°C (±). Maintaining these conditions samples were injected and %RSD was reported.

System suitability: System suitability for that method was tested by five replicate injections of standard preparation. Plate count, tailing factor, resolution and %RSD were reported.

Assay: Percentage labeled amount was found by performing assay for XARB-H. Sample and standard solutions of same concentrations were prepared and injected to HPLC the obtained sample peak area was compared to the standard peak area.

Stability studies:

Oxidation: To 1 ml of stock solutionof Olmesartan and Rosuvastatin, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solution was kept for 30 min at 60°C. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies: To 1 ml of stock s solution Olmesartan and Rosuvastatin, 1ml of 2N Hydrochloric acid solution was added and kept a side for 30mins at 60°C after 30min the solution was neutralized with 1ml of 2N NaOH solution. The resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies: To 1 ml of stock solution Olmesartan and Rosuvastatin, 1 ml of 2N sodium hydroxide was added and kept a side for 30mins at 60°C after 30min 1ml of 2N hydrochloric acid was added and neutralized. The resultant solution was diluted to obtain 100µg/ml&50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies: The standard drug solution was placed in oven at 105°C for 6hrs to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&50µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies: The photochemical stability of the drug was also studied by exposing the solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&50µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 100µg/ml&50µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSIONS

Method was developed by changing column, mobile phase ratio, flow rate, different buffers and its pH. But the results were optimum when 10µl of standard working solution containing both olmesartan and rosuvastatin was injected in to the mobile phase line composing of 45B and 55A, pumped through the column Altima 150mm length column containing particle of size 5µ with a flow rate of 1ml/min. the temperature of the column was maintained at 30°C. Both the drugs have their optimum absorbance at 241nm wavelength. The method was optimized based on the all system suitable parameters passed their limits as per ICH guidelines. The retention time found was 2.2min of olmesartan and 3.0min of rosuvastatin and the resolution between the peaks was 4.8. This method was set for validation as per ICH guidelines and observed to be very specific without any interference by the constituents of formulations and diluents. The method was precisied and %RSD found to be 0.56 of olmesartan and 0.42 of rosuvastatin. % recover of olmesartan was 100.09% and 99.79% for rosuvastatin. Linearity was performed with six concentrations and response was observed to be linear to the concentration. Correlation coefficient obtained was 0.999. the linearity equation for olmesartan was y = 27771x + 498.5 and for rosuvastatin was y = 18593x + 645.0. The developed method was robust as the %RSD was within the range and without effecting system suitability parameters. LOD, LOQ concentrations of Olmesartan and Rosuvastatin were 0.06µg/ml, 0.18µg/ml and 0.11µg/ml, 0.35µg/ml respectively. Robustness was passed and %RSD was within the limits finally the method was valid as per ICH guidelines and set for assay and the % Assay found was 99.93% for olmesartan and 99.69% for rosuvastatin.

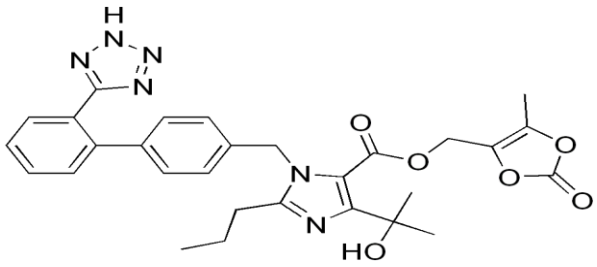

Fig.1: Olmesartan Medoxomil

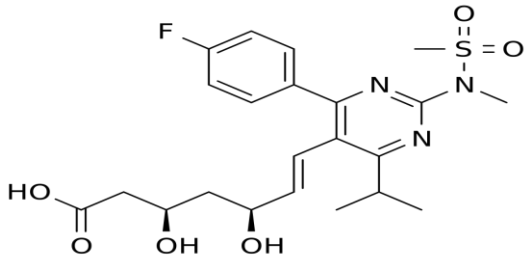

Fig.2: Rosuvastatin

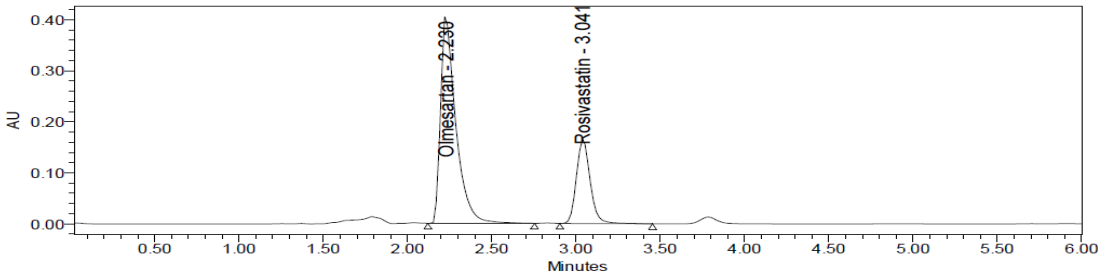

Fig.3: Chromatogram of Standard working solution

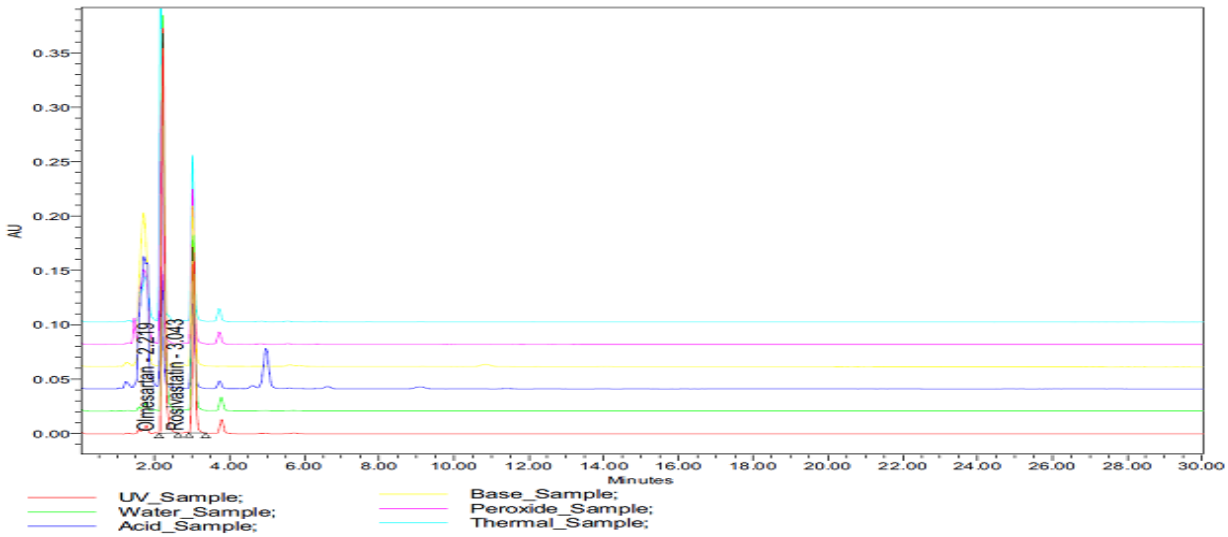

Fig.4: Degradation overlay Chromatogram of Sample working solution

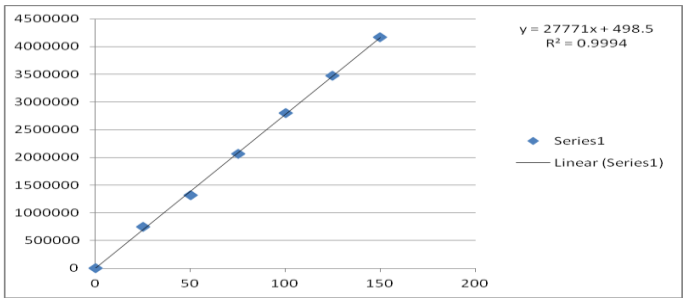

Fig.5: Calibration curve of Olmesartan

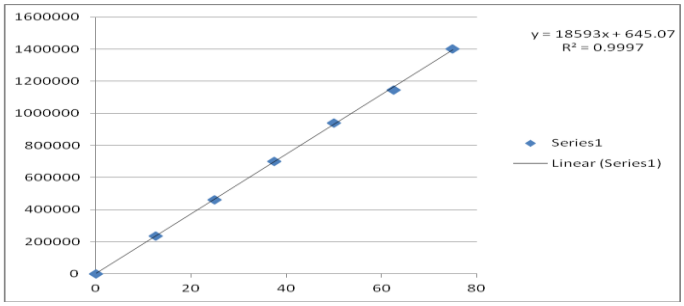

Fig.6: Calibration curve of Rosuvastatin

Table.1: Validation parameters

Parameters	Olmesartan	Rosuvastatin
Recovery	100.09%	99.79%
Intraday precision	0.56	0.34
Inter day precision	0.42	0.67
LOD	0.06µg/ml	0.11µg/ml
LOQ	0.18µg/ml	0.35µg/ml
Specificity	Specific	Specific
Robustness	1.75	0.93
Solvent stability	Stable for 24 hrs	Stable for 24 hrs

Table.2: Calibration Data

Parameters	Olmesartan	Rosuvastatin
Optimized Wavelength	241nm	241nm
Linearity range	25ppm-150ppm	12.5ppm-75ppm
Intercept	498.5	645.0
Slope	27771	18593
Correlation Coefficient	0.999	0.999
Linearity Equation	y = 27771x + 498.5	y = 18593x + 645.0

Table.3: Robustness Data

Parameters	Olmesartan	Rosuvastatin
Flow minus	0.46	0.75
Flow Plus	0.24	0.49
Mobile phase minus	1.34	0.52
Mobile phase plus	1.56	1.65
Temperature minus	0.86	0.34
Temperature Plus	0.54	0.47

Table.4: Recovery Data

Parameters	Olmesartan			Rosuvastatin		
Level of Recovery	50%	100%	150%	50%	100%	150%
%Recovery	100.23	99.63	100.23	99.79	99.75	99.81
STDEV	0.881	0.617	0.152	0.530	0.436	0.444
%RSD	0.88	0.62	0.55	0.53	0.44	0.44

Table.5: Assay table

	Lable claim		Amount recovered		% Assay	
Formulation	OLME	ROUS	OLME	ROUS	OLME	ROUS
XARB-H	20	10	20.018	9.979	100.09	99.79

Table.6: System suitability table

Parameters	Olmesartan	Rosuvastatin
Retention time	2.2±0.3min	3.0±0.3min
Plate count	2859	6899
Tailing Factor	1.73	1.17
Resolution		4.8
%RSD	0.56	0.42

Table.7 Degradation data of Olmesartan

Degradation	% recovery after Degradation	Purity Angle	Purity Threshold
Acid	92.08	0.278	1.263
Alkali	93.53	0.328	0.444
Peroxide	94.48	0.894	2.413
Thermal	95.08	1.456	2.431
Photolytic	98.75	0.210	0.349
Water	99.35	0.213	0.393

Table.8 Degradation data of Rosuvastatin

Degradation	% recovery after Degradation	Purity Angle	Purity Threshold
Acid	92.56	0.196	0.322
Alkali	93.39	0.165	0.263
Peroxide	94.37	0.235	0.394
Thermal	95.20	1.610	2.434
Photolytic	98.76	0.265	0.392
Water	99.10	0.187	0.473

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