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RAPID SIMULTANEOUS SEPARATION OF FOUR SARTANS BY ISOCRATIC RP-HPLC METHOD: APPLICATION TO DETERMINATION OF OLMISARTAN IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, fast, precise, specific, highly sensitive, an accurate and reproducible isocratic reversed phase HPLC method has been developed for the separation of four sartans and validated for quantitation of Olmesartan in pure and in pharmaceutical formulation. One of the prominent aims for the method development is to achieve constant, reproducible separation. The effects of mobile phase composition, buffers, pH, and acetonitrile concentrations were investigated on the separation of sartans (Valsartan, Telmisartan, Olmesartan and Irbesartan) were assayed without any interference. The chromatographic separation was achieved on Welchrom C_{18} Column (4.6 X 250 mm, 5 µm) as stationary phase with the mobile phase comprising of phosphate buffer pH-3.3 and acetonitrile in the portion of 50:50 v/v. Isocratic elution at a flow rate of 1ml/min was employed. The detection was performed with Shimadzu SPD-20A prominence UV-Vis detector set at 232 nm. Total run time was 8 minutes and the elution window of two minutes. The validated method was successfully applied to the commercially available Olmesartan pharmaceutical dosage form yielding very good and reproducible results. During method validation parameters such as linearity, precision, specificity, robustness and ruggedness were evaluated from spiked tablet samples according to ICH guidelines, which remained within acceptable limits. The proposed method can also be extended for the determination of other three sartans or their combinations with hydrochlorothiazide. This method provides a fast simple method with good retention, excellent peak shape and high resolution.

KEY WORDS: Valsartan, Telmisartan, Olmisartan, Irbesartan, RP-HPLC.

1. INTRODUCTION

Hypertension is the major health problem worldwide. Angiotensin II receptor antagonists are the agents act by blocking the Angiotensin II receptors of subtype 1 (AT_1 receptors) which regulates the effects of angiotension II on BP, heart and sodium and water balance. ARBs are highly effective at reducing blood pressure shows renoprotective properties and have placebo like tolerability. Telmisartan is an angiotensin receptor antagonist used in the management of hypertension. The maximum daily dose of Telmisartan is 40-80 mg. Valsartan is an Angiotensin receptor antagonist used in the management of hypertension.Valsartan is used to treat of high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack. Olmesartan is indicated for the treatment of hypertension. It may be used alone or in combination with other antihypertensive agents. The U.S. Food and Drug Administration (FDA) have determined that the benefits of Benicar continue to outweigh its potential risks when used for the treatment of patients with high blood pressure according to the drug label. As with all angiotensin II receptor antagonists, Irbesartan is used for the treatment of hypertension. It may also delay progression of diabetic nephropathy and is also indicated for the reduction of renal disease progression in patients with type 2 diabetes, hypertension and microalbuminuria (>30 mg/24 hours) or proteinuria (>900 mg/24 hours).Irbesartan is also available in a combination formulation with a low-dose thiazide diuretic, invariably hydrochlorothiazide, to attain an additive antihypertensive effect.

All the four drugs are alternative to ACE inhibitors mainly used in the treatment of hypertension. These drugs are very potent and are normally prescribed either individually or in combination with hydrochlorothiazide as per the demand of the situation. These four drugs are available in the market individually or in combination with Hydrochlorothiazide dosage forms. A thorough literature survey revealed that for individual estimation of each drug, analytical methods are available and even there are few methods available for estimation of two drugs at a time. The reported methods for determination of above said drugs in tablet dosage forms were Spectrophotometry (Mehulkumar P, 2009) (Jaydeep Patel, 2011) HPLC (Wakabayashi H, 1992) (Lisiane B, 2008) (Rane VP, 2009) (Bae SK, 2009) (Czerwinska K, 2001) (Megharaj D, 2012) (Purnima D, 2013) (Ravisankar P, 2014) (Santhsh Kumar M, 2014) (Kumara Swamy G, 2014) LC-MS-MS (Vaidya VV, 2008). But, there is no analytical method has been reported yet for the simultaneous separation of all the four drugs namely Valsartan, Telmisartan, Olmisartan, and Irbesartan and estimation of Olmesartan. Infact the present research work aims to separate and develop a fast,

simple, convenient, rapid, precise, accurate, and economical and user friendly methodology with a simple and easily available mobile phase for the simultaneous separation and quantitative estimation of Olmesartan in bulk and pharmaceutical dosage forms by RP-HPLC method. The proposed method was validated as per International Conference on Harmonization (ICH Q2 (R1) 2005) guidelines. The chemical structures of four sartans are shown in the Figure1.

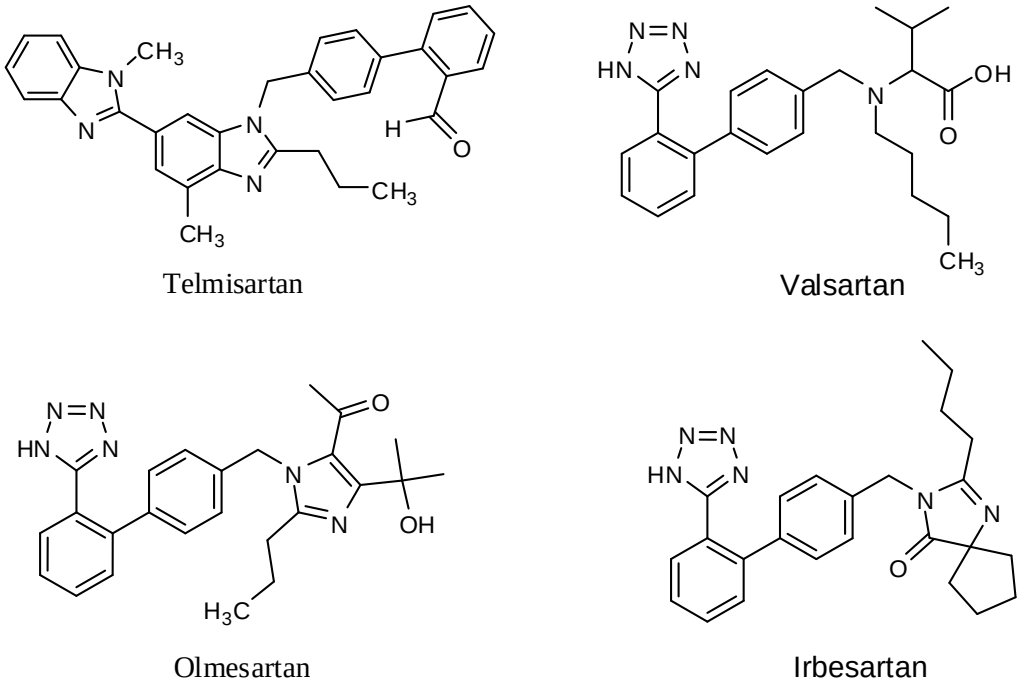

Figure 1. Chemical Structures of the drugs used in the present investigation.

2. MATERIALS AND METHODS

Quantitative HPLC was performed on a high pressure gradient high performance liquid chromatograph (Shimadzu LC-20AT prominence liquid chromatograph) with two LC-20AT VP pumps, manual injector with loop volume of 20 µl (Rheodyne), programmable variable wavelength Shimadzu SPD-20A prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm). The HPLC system was equipped with "Spincotech" software. In addition an electronic balance (Essae-Teraoka Ltd.,) digital pH meter (Systronics model 802), Ultra sonic bath sonicator (spectra lab, model UCB 40), Double beam spectrophotometer (Systronics model- 2203) were used in this study.

Standards and chemicals used: Samples of Telmisartan and Olmesartan were provided by Hetero Labs, Valsartan by Aristo Pharma, and Irbesartan by Ananth Pharmaceuticals. All chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from S.D Fine-Chem. Ltd., Mumbai, India While acetonitrile. (HPLC grade) and from Merck Pharmaceuticals Private Limited, Mumbai, India. Commercial tablets of Olmesartan were purchased from local market. Benicar-40mg tablets are manufactured by Glenmark Pharmaceuticals Ltd., Mumbai, India.

Preparation of pH 3.3 phosphate buffer: A 10 mM phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen orthophosphate in 445 ml of HPLC grade water. To this 55 ml of 0.1 M phosphoric acid was added and pH was adjusted to 3.3.

Preparation of mobile phase: The above prepared buffer and acetonitrile were mixed in the proportion of 50: 50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of standard stock solution and working standard solution of Olmesartan: About 100 mg of pure Valsartan, Telmisartan, Olmisartan, and Irbesartan was accurately weighed and dissolved in 100 ml of mobile phase in separate volumetric flasks to get 1 µg/ml standard stock solution of each drug. Working standard solution of Olmesartan was prepared with mobile phase. To a series of 10 ml volumetric flasks, standard solutions of Olmesartan in the concentration range of 2, 4, 6, 8, 10 µg/ml were transferred. The final volumes were made with the mobile phase in 10 ml volumetric flasks.

Optimization of mobile phase and method development:A series of trials were conducted for optimization of mobile phase. A series of trials were conducted in order to get proper optimized HPLC conditions. In the first instance several mobile phase trials were done such as methanol: water, acetonitrile: HPLC grade water, methanol: acetonitrile: water in different ratio and adjusting pH to obtain required separations. Finally after reviewing the results, a mobile phase consisting of phosphate buffer mixture properly adjusted to pH 3.3, acetonitrile in the proportion of 50:50 v/v which full fill all the criteria of system suitability and also obtained sharp, gaussian shape peak. This mobile phase was also selected as the diluent because the drug is freely soluble in the mobile phase. The stationary phase made up of Welchrom C_{18} column with 4.6 X 250 mm, 5 µm were observed and they are found to be utmost suitable for separation of four sartans. The ultra violet spectrum of four sartans scanned individually in the region between 200-400 nm. The UV overlain spectra of these four drugs showed that they absorbed at 232 nm. So this wavelength was selected for the detection of sartans. Figure 2 shows UV overlain spectra of four sartans. The developed method gave symmetric peak at retention time of 5.243 minutes for Olmisartan and satisfied all the peak properties as per ICH guidelines. Based on the above said optimized conditions for the developed method the retention times of Valsartan, Telmisartan, Olmisartan, and Irbesartan were found to be 3.377 min,4.463 min, 5.243 min, and 5.563 min respectively. A chromatogram shows how the peaks of these four drugs are well separated which is presented in Figure 3. Optimized chromatographic conditions are shown in Table 1.

Method Validation: The developed method of analysis was validated as per the ICH for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability: Set up the chromatographic system, allow the HPLC system to stabilize for forty minutes. Inject blank preparation (single injection) and standard preparation (six replicates) and record the chromatograms to evaluate the system suitability parameters like resolution (NLT 2.0), tailing factor (NMT 1.5), theoretical plate count (NLT 3000) and % RSD for peak area of six replicate injections of Olmesartan standard (% RSD NMT 2.0). If system suitability parameters are met, then inject sample preparation in duplicate and record the chromatograms and the system suitability parameters are shown in Table 2.

Specificity: The specificity of the proposed method was established by reviewing the effect of various excipients and other additives usually present in the preparations of Olmesartan in the determinations under ideal conditions. The blank, standard, placebo, placebo spiked with analyte and test preparations were analyzed as per the method to examine the interference of blank and placebo with Olmesartan peaks. The common excipients such as lactose anhydrous, microcrystalline cellulose, purified talc and magnesium stearate have been added to the placebo solution and injected and tested. Furthermore the well-shaped peaks also indicate the specificity of the method. The results of specificity study are shown in Table 3.

Linearity: Linearity for Olmesartan was determined by standard solution aliquots of Olmisartan (100 µg/ml; 0.2-1ml) were transferred in to a 10 ml of five volumetric flasks and the volume was made up to the mark with mobile phase to get different concentrations 2-10 µg/ml. By attaining 3 replicate measurements at 5 different points of concentration the response of peak area was determined for Olmisartan. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient, regression data values. A calibration curve was designed between average peak areas against concentration of standard drug. Linearity data of Olmesartan is shown in Table 4. The linear regression data for the Olmisartan is presented in Table 5. The chromatograms of calibration standards for four sartans are shown in Figures from 4 to 7. Calibration standards for Olmisartan are shown in Figures 8 to 12. Linearity graph for Olmisartan is shown in Figure 13.

Precision: Intra-day and inter-day precision of the procedure were determined by performing six determinations at the same concentration (10 µg/ml) of Olmesartan during the same day, under the same experimental conditions and on a different day respectively. The percent relative standard deviation (% RSD) was calculated which is within the acceptable criteria of not more than 2.0. The results of precision study are presented in Tables 6 and 7.

Accuracy/Recovery: The accuracy of the method was assessed in triplicate at 3 different concentrations equivalent to 80 %, 100 % and 120 % of the active ingredient, by adding a known amount of Olmesartan standard to a sample with pre-determined samples of Olmesartan. The recovered amount of Olmesartan, % recovery, % RSD is calculated to determine the accuracy. The results of accuracy data is shown in Table 8.

Robustness: The Robustness of developed analytical method was proven by the analysis of Olmesartan under different experimental conditions such as making deliberate changes in chromatographic conditions like flow rate (± 0.2 ml/min), detection wavelength (±5 nm) and Mobile phase composition (±5 %). The results are summarized in Table 9.

LOD and LOQ: Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantitation were calculated using following formula LOD = 3.3σ/S and LOQ = 10σ/S, where SD = standard deviation of response (peak area) and S = slope of the calibration curve. The LOD and LOQ results are shown in Table 10.

Assay of marketed formulations of Olmesartan: The content of twenty tablets was transferred into a mortar and ground to a fine powder. From this tablet powder a quantity equivalent to 25 mg of Olmesartan was taken in 25 ml of volumetric flask. 25 ml of mobile phase (diluent) was added to it. The resulting solution was filtered through 0.25 µm nylon membrane filter and degassed by sonication. The volume was adjusted with diluent after the filtration of sonicated solution. 1.0 ml of this solution was poured in 10 ml volumetric flask and adjusted to the mark with diluent Olmesartan (100 µg/ml). From this solution 0.5 ml was transferred to 10 ml volumetric flask and adjusted up to the mark with mobile phase to attain the concentration of Olmesartan 5 µg/ml. The test solutions were injected into the system by filling a 20 µl fixed volume loop manual injector. The chromatographic run time of eight minutes was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 232 nm. The amount of drug present in sample was computed from the calibration graph. The result of the assay is presented in Table 11 and the sample chromatogram of Olmesartan is shown in Figure 10.

RESULTS AND DISCUSSION

The present study was designed to develop a rapid, accurate and precise HPLC method for the separation of four sartans and subsequent determination of Olmesartan in pharmaceutical dosage forms. In order to set up analysis of the component peaks under isocratic conditions, mixtures of phosphate buffer, acetonitrile in different combinations were tested as mobile phase on a C_{18} stationary phase. A combination of phosphate buffer (pH - 3.3, adjusted using o-phosphoric acid) and acetonitrile in a ratio of 50:50, v/v, was used as a mobile phase at a flow rate of 1ml/min was proved to be the most suitable of all combinations of mobile phase tried since the chromatographic peak obtained was well shape symmetrical peak. The wave length of detection was set at 232 nm from UV overlain spectra of drugs under study. The system suitability was carried out on freshly prepared Olmesartan standard solution for the evaluation of system suitability parameters such as retention time, peak area, peak tailing and number of theoretical plates. Six replicate injections for system suitability test were injected into the chromatographic system for optimized chromatographic conditions for the above said method. The retention times and peak areas of four sartans in combination were shown in Table 2. From the above experiment 3.377, 4.463, 5.243 and 5.563 minutes of retention time, 13293, 14264, 15070 and16039 of efficiency (number of theoretical plates), 1.036, 1.154, 1.192 and1.152 tailing factors were obtained for Telmisartan, Valsartan, Olmesartan and Irbesartan. The resolutions for Valsartan, Olmesartan and Irbesartan are found to be 3.615, 4.675 and 4.844 respectively. The above said results are all within the limits. These results indicated excellent peak shape, phenomenal plate count and shorter run-time. Under the said conditions described the separation of the sartans mixture was achieved with a run time of 8 minutes with an elution window of around 2 minutes for all four analytes.

The calibration curve obtained by concentration on X-axis and peak area on Y-axis shows the linearity in the concentration range of 2-10 µg/ml of Olmesartan and the linearity graph is shown in Figure 13. The calibration data was presented in Table 4. The regression equation was found to be Y = 108.64x+0.0382 with regression coefficient of r^2 = 0.9997 which indicates this method had good linearity. The linear regression data of the calibration curve for determination of Olmesartan is shown in Table 5. The linearity data results shows that there was good linear relation between peak areas and concentration of Olmesartan. The specificity results were found that there was no interference due to excipients in the tablet formulation and also that there is good correlation between the retention times of the standard and sample.

Precision was studied to find out intra-day and inter-day variations in the test methods of Olmesartan for three times on the same day and on different days. The % RSD for intra-day and inter-day precision variations studied at 6 µg/ml obtained was 0.1987 and 0.1826 respectively which is within the acceptable criteria of NMT 2.0. This reveals that the proposed method is quite precise.Concerning the accuracy of the developed method the known amount of pure standard drug is added to the pre-analyzed samples at 50 %, 100 % and 150 % (three levels) and the recovery levels were carefully studied. The solutions of the above said drug is prepared and analyzed in triplicate and achieve the high recovery values. Generally the mean percentage recovery of Olmesartan at each level was not less than 99 % and not more than 101 %. Satisfactory recoveries ranging from 99.63 - 99.92 % were obtained by the proposed method It was also found that the % RSD were also got to be less than two percent for Olmisartan which shows that the method is absolutely accurate.

Robustness was done by deliberate changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc., deliberate changes in developed method had not much affected the peak tailing, theoretical peaks and % assay which demonstrated that the developed method was Robust in nature.The results regarding to limit of detection (LOD) and limit of quantitation (LOQ) for Olmisartan found to be 0.2376 and 0.7202 respectively. These results pellucid states that the method possesses relatively low values of LOD and LOQ. The said developed method was lastly used for estimation of marketed formulation. The mean assay values for Olmisartan is arrived at 99.875 ± 0.11 %. The results were very close to the labeled value of commercial tablets. So this method developed by the author was found to be suitable for determining the Olmesartan in commercial formulations as well as bulk drugs.

Table.1.Optimized chromatographic conditions for Olmesartan.

Parameter	**Chromatographic conditions**
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	WELCHROM C_{18} Column (4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	10mM Phosphate Buffer(pH-3.3): Acetonitrile (50:50 v/v)
Mobile phase	10mM Phosphate Buffer(pH-3.3): Acetonitrile (50 : 50 v/v)
Flow rate	1ml/min.
Detection wave length	By UV at 232 nm.
Run time	8 minutes
Temperature	Ambient temperature (25^{o}C)
Volume of injection loop	20 µl
Retention time (R_t)	5.243 min (Olmesartan)
Theoretical plates [th.pl] (Efficiency)	14264
Tailing factor (asymmetry factor)	1.154

Table.2.System suitability parameters of four sartans

Compound name	Peak ID	Retention time (min)	Asymmetry	Efficiency(theoretical plates)	Resolution
Telmisartan	1	3.773	1.036	13293	-
Valsartan	2	4.463	1.154	14264	3.615
Olmesartan	3	5.243	1.192	15070	4.675
Irbesartan	4	5.563	1.152	16039	4.844

Table.3.Specificity study for Olmesartan

Name of the solution	**Retention time, (t_R) min.**
Mobile phase	No peaks
Placebo	No peaks
Olmesartan, 10 µg/ml	5.243 min.

Table.4.Calibration data of the proposed HPLC method of Olmesartan

S.No	Concentration, µg/ml.	Retention time,(R_t) min.	Peak area, mV.s.
1.	0	-	0
2.	2	5.243	208.199
3.	4	5.243	446.726
4.	6	5.243	651.498
5.	8	5.243	870.211
6.	10	5.243	1082.801

Table.5.Linear regression data of the proposed method of Olmesartan

Parameter	Method
Detection wavelength(λ max)	By UV at 232nm
Linearity range (µg/ml)	2-10 µg/ml
Regression equation (Y=ax+b)	Y= 108.64x+0.0382
Slope (b)	108.64
Intercept (a)	0.0382
Standard deviation of slope (S_b)	0.9348
Standard deviation of intercept (S_a)	5.6610
Correlation coefficient (r^2)	0.999
Limit of detection (µg/ml)	0.2376
Limit of quantitation(µg/ml)	0.7202

Table.6.Results of Precision study (Intra-day).

Sample	Concentration (µg/ml)	Injection no.	Peak area	%RSD(acceptance criteria < 2.0)
Olmesartan	6	1	651.498	0.1987
		2	652.786	
		3	650.654	
		4	653.786	
		5	652.237	
		6	654.476	

Table.7.Results of Precision study (Inter-day).

Sample	Concentration (µg/ml)	Injection no.	Peak area	%RSD (acceptance criteria < 2.0)
Olmesartan	6	1	651.651	0.1826
		2	649.940	
		3	652.743	
		4	653.619	
		5	654.671	
		6	654.476	

Table.8.Recovery data of the proposed Olmesartan by RP-HPLC method.

Drug	Level	Amount of Sample concentration taken (µg/mL)	Amount of standard spiked (%)	Mean % recovery ± SD*	% RSD#
Olmesartan	**I**	5	50	99.63±0.23	0.24
	II	5	100	99.92±0.32	0.17
	III	5	150	99.85±0.11	0.070

*SD is standard deviation,**% RSD#** is percentage of relative standard deviation.

Table.9.Robustness results of Olmesartan.

S. No.	Parameter	Optimized Condition	Used condition	Peak area (mv.s)	Retention Time (R_t), Minutes	Plate count	Peak asymmetry
1.	Flow rate (± 0.2 mL/min)	1.0 mL	0.8	1098.923	5.375	16176	1.085
			1.0	1082.801	5.243	15956	1.056
			1.2	1072.872	5.210	15998	1.076
2.	Detection wavelength (± 5 nm)	232 nm	227	1083.491	5.243	15956	1.046
			232	1082.801	5.243	15956	1.056
			237	1083.154	5.243	15956	1.063
3.	Mobile phase composition (± 5 v/v)	50:50	55:45	1082.934	5.290	15998	1.068
			50:50	1082.801	5.243	15956	1.056
			45:55	1082.978	5.187	15895	1.079

Table.10.Limit of Detection (LOD) and Limit of Quantitation (LOQ).

Limit of Detection (LOD)	0.2376µg/ml
Limit of Quantitation (LOQ)	0.7202 µg/ml

Table.11.Assay results of Olmesartan formulations.

S.No	Formulations	Standard Peak area	Sample Peak area	Labeled amount	Amount found	% Assay ±RSD*
1	Benicar	208.199	208.112	40 mg	39.85 mg	99.875±0.11

***average of six determinations**

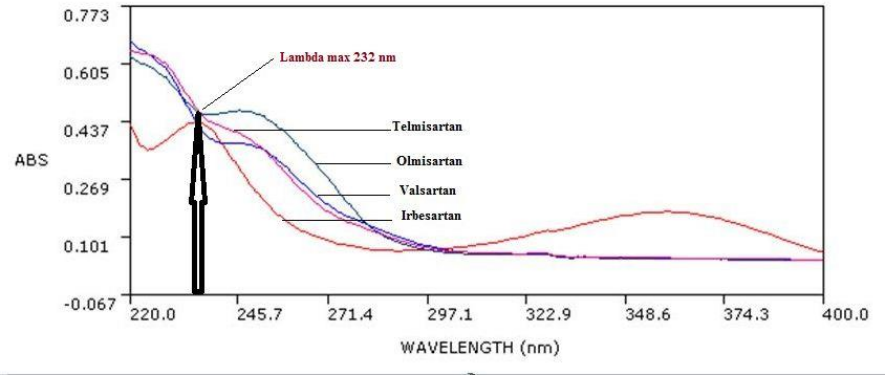

Figure.2.UV overlain spectra of four sartans

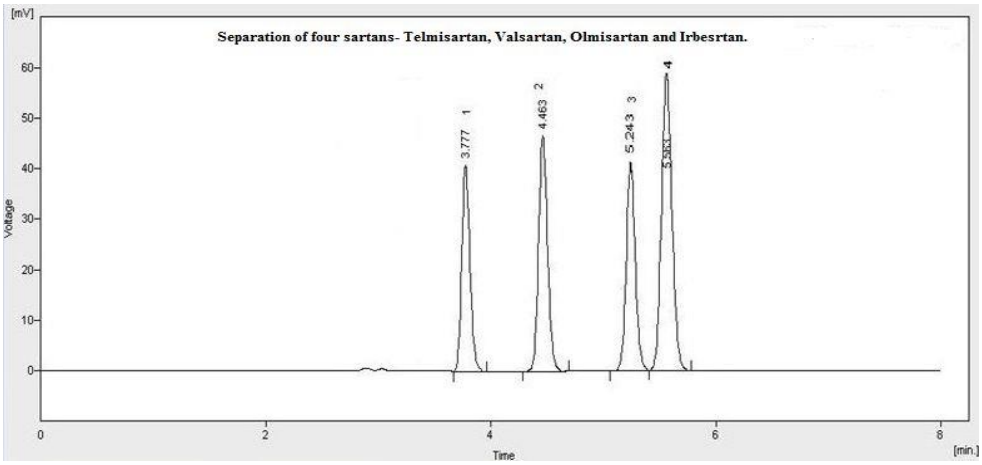

Figure.3.The separation of the four sartans. The peaks at 3.777 min, 4.463 min, 5.243 min, 5.563 min, Telmisartan, Valsartan, Olmesartan, Irbesartan respectively

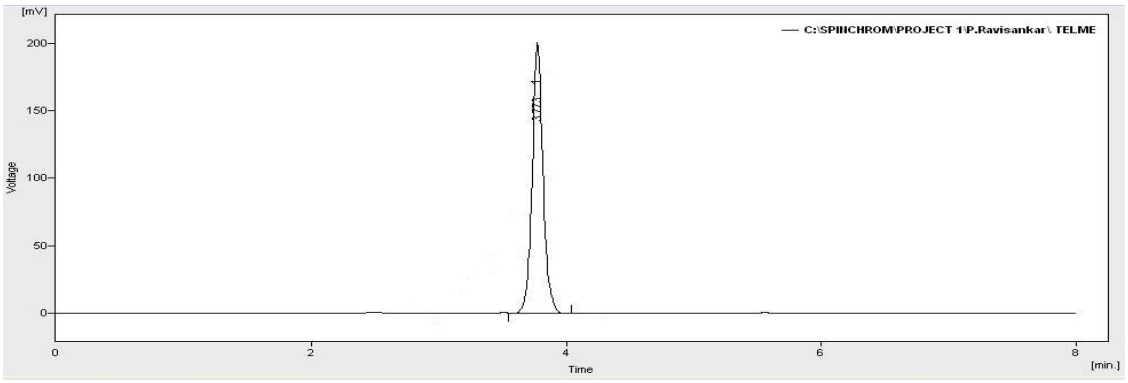

Figure.4.Standard chromatogram of Telmisartan 10 µg/ml

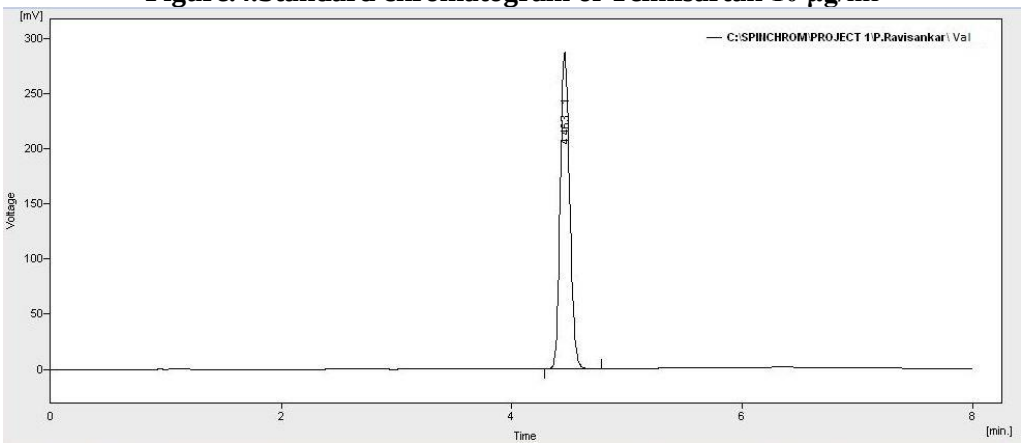

Figure.5.Standard chromatogram of Valsartan 10 µg/ml

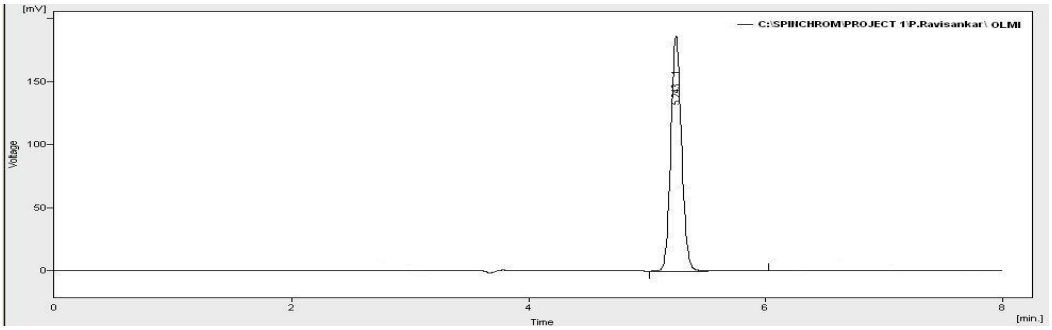

Figure 6. Standard chromatogram of Olmesartan 10 µg/ml

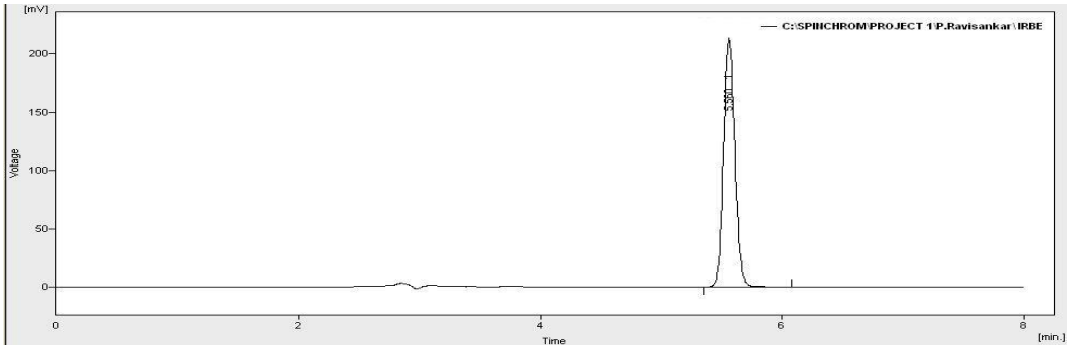

Figure 7. Standard chromatogram of Irbesartan10 µg/ml

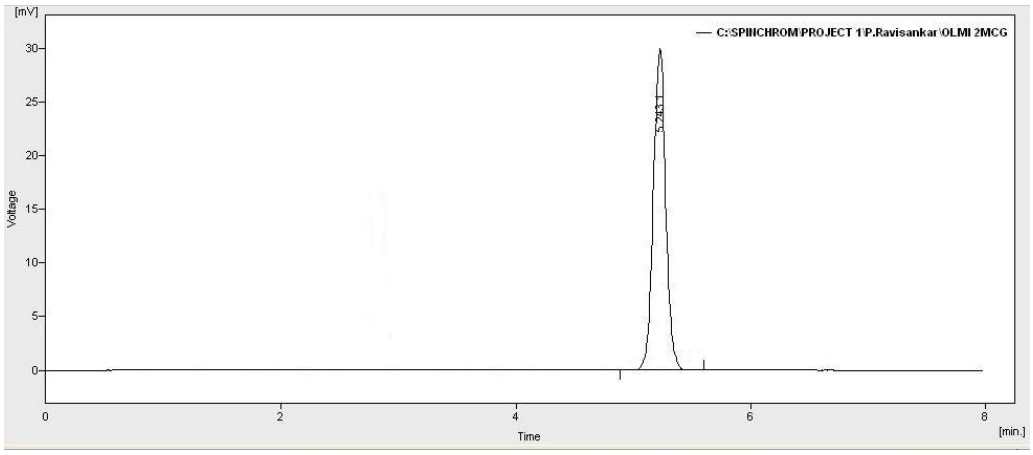

Figure.8.Standard chromatogram of Olmesartan 2 µg/ml

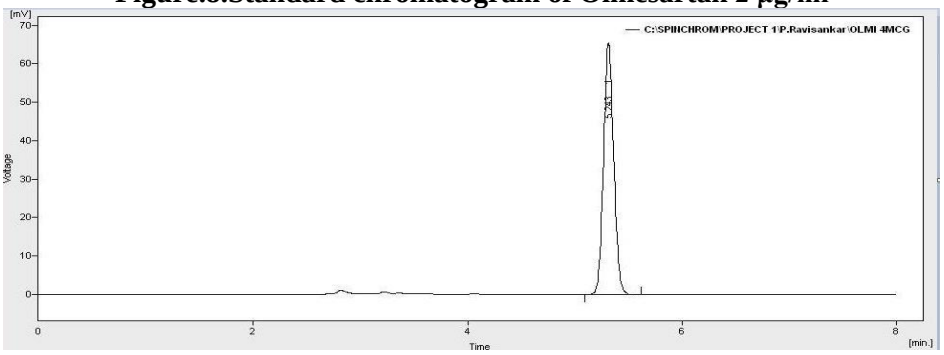

Figure 9. Standard chromatogram of Olmesartan 4 µg/ml.

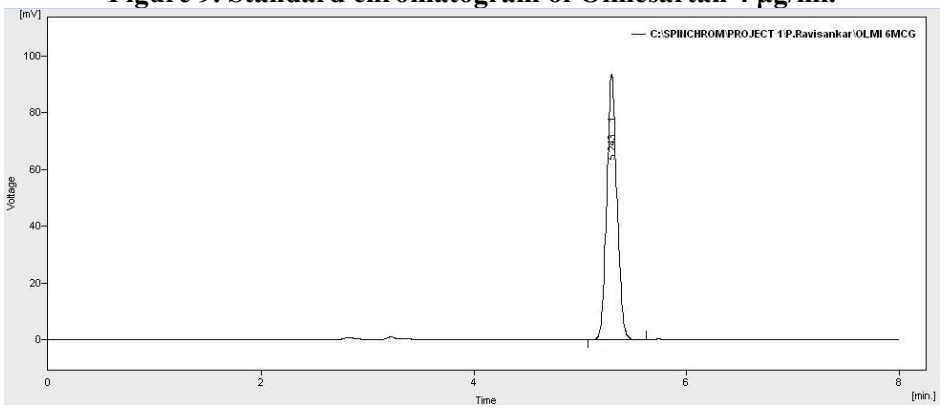

Figure 10. Standard chromatogram of Olmesartan 6 µg/ml.

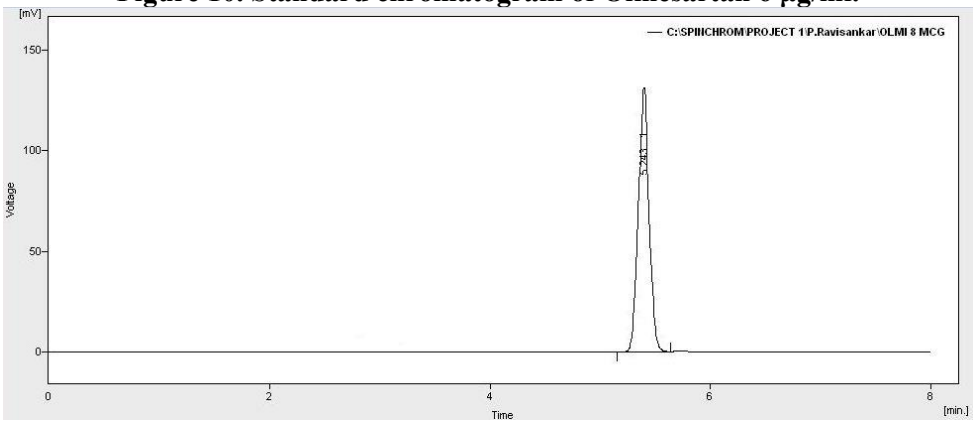

Figure 11. Standard chromatogram of Olmesartan 8 µg/ml.

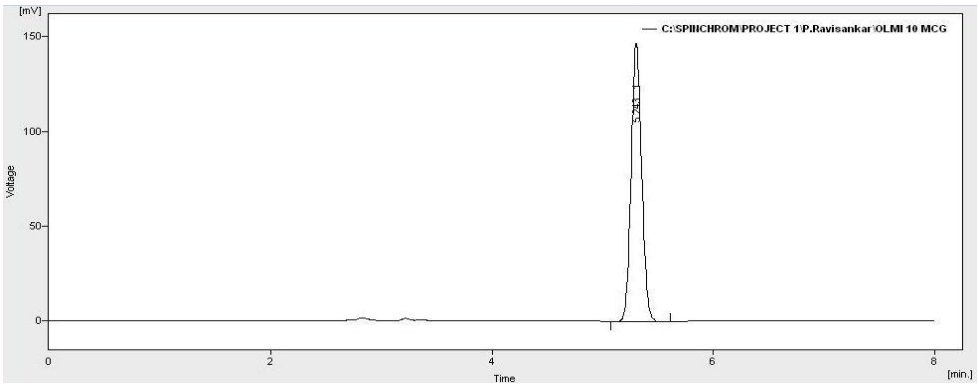

Figure 12. Standard chromatogram of Olmesartan 10 µg/ml.

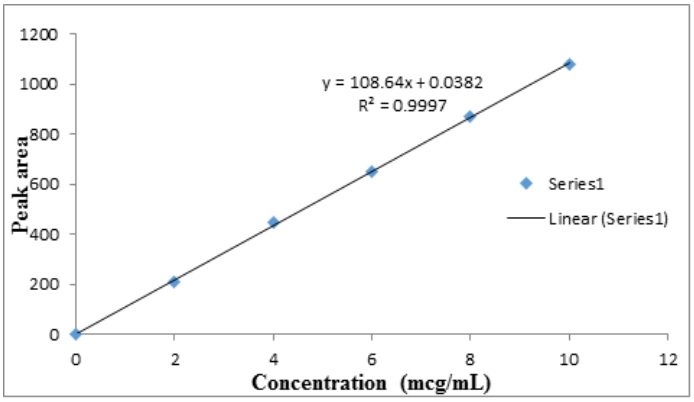

Figure 13. Calibration Plot of Olmesartan

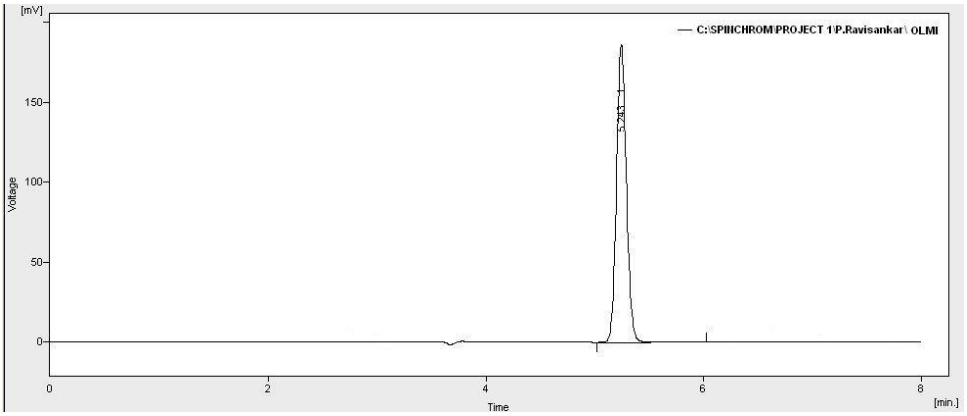

Figure 14. Sample Chromatogram of Olmesartan.

5. CONCLUSION

The ultimate goal of any separation is to achieve acceptable resolution in a reasonable time. The proposed method showed excellent resolving power and provided a very simple method for the separation of all four compounds. Because selectivity found to be lacking in respect of microbiological, as well as paper chromatographic analysis the author opted to RP-HPLC method and achieve best possible results. The mobile phase is economical and simple to prepare and used in this analysis is same for other three sartans. Detection wavelength is also same for other sartans. The run time of eight minutes and elution window of around two minutes indicates short analysis time. The analysis was performed at room temperature. The developed method provides good peak shapes and high resolution. Hence by using this method four sartans can be separated with short retention time and also this simple method gave excellent peak shape and provides excellent resolution. This highly reproducible method can be used for determination of small concentrations in short period of time. This method is extremely useful for determination of product quality of tablets. This method enables to detect cross contamination of the said sartans. The method overall provided to be simple, rapid, precise, sensitive, robust, highly reproducible, cost effective and can be conveniently feasible for the determination of Olmesartan and also other sartans in bulk and pharmaceutical dosage forms.

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