IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CANDESARTAN AND ALOGLIPTIN

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Abstract

A novel and new chromatographic technique has been developed for the simultaneous estimation of Candesartan and Alogliptin. Mobile phase was prepared using methanol: water (8:2) was found to resolve Candesartan and Alogliptin. The degassing of mobile phase was done by sonication for 30 min. the flow rate was set to 1 ml/min. both drug show good absorbance at 273nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature. The best results were obtained by using HiberR 250-4.6 HPLC column purosphenR STAR RP18 as compared to other different phases. Appropriate wavelength for the detection of the drug in mobile phase was determine by scanning standard solution of both drugs over the wavelength range of 200-400nm and after scanning an appropriate wavelength was selected. From overlain UV spectra of Candesartan and Alogliptin 273nm was selected as wavelength for HPLC analysis of both drugs. Beer's law obeyed the concentration range of 10 - 50 µg/ ml, and 10-50 µg / ml, Candesartan for and Alogliptin, respectively. The calibration graphs were plotted. The correlation coefficient values for the two drugs were more than 0.999. The optical parameters like the Molar absorptivity, Correlation coefficient, Slope, Intercept, LOD and LOQ, and were calculated.

Keywords: Hypertension, Diabetes, HPLC, Chromatography, Candesartan, Alogliptin

Introduction:

Hypertension, also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure typically does not cause symptoms. Long-term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral arterial disease, vision loss, chronic kidney disease and dementia. Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. For most adults, normal blood pressure at rest is within the range of 100–130 millimeters mercury (mmHg) systolic and 60–80 mmHg diastolic. For most adults, high blood pressure is present if the resting blood pressure is persistently at or above 130/80 or 140/90 mmHg. Different numbers apply to children. Ambulatory blood pressure monitoring over a 24-hour period appears more accurate than office-based blood pressure measurement.

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period of time. Symptoms often include frequent urination, increased thirst, and increased appetite. If left untreated, diabetes can cause many complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes and cognitive impairment. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body not responding properly to the insulin produced.

MATERIALS AND METHODS:

Chemical, solvent and Reagents:

The pharmaceutical grade Candesartan & Alogliptin is purchased from MSPL. The solvent use for procedure is analytical grade. The HPLC grade chemical used is Methanol and double distilled water and they were obtained from S D Fine Chem. Ltd, Mumbai Finar Ltd All the solvents used for HPLC such as water, methanol were of HPLC grade which were initially sonicated for 5 minutes and then filtered through membrane filter to remove any particulate present.

Apparatus: U.V. Visible double beam spectrophotometer having model UV-3000-M. Stock solutions of the samples were prepared in AR grade Methanol and used for analysis. The HPLC system used is the water HPLC model HPLC 3000 series. The column used was Cosmosil C18 (250mm ×4.6ID, Particle size: 5 micron) The auto sampler Cosmosil C18 (250mm ×4.6ID, Particle size: 5 micron).

Chromatographic conditions: Mobile phase was prepared using methanol: water (8:2) which was found to resolve Candesartan and Alogliptin appropriately. The degassing of mobile phase was done by sonication for 30 min. and flow rate was set to 1ml/min. both drugs show good absorbance at 273nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature.

Preparation of std stock solution : Candesartan 10 mg was weighed by using electronic balance (model). And dissolved in small amount of methanol and then completely dissolved. After this the solution was adjusted to 100 ml in volumetric flask, so that the solution of 100µg/ml was prepared.

Preparation of std solution : From the stock solution of Candesartan and Alogliptin the standard solution of Prepared in the 10-50µg /ml concentration range for Candesartan and 10-50µg/ml concentration range Alogliptin respectively.

Preparation of sample solution: A sample was prepared by taking 1ml of each Candesartan and Alogliptin from stock solution in a 10 ml volumetric flask, mix well and make volume up to mark using distilled water.

Analytical method development :

Analytical method was developed by considering various parameters such as, mobile phase ratio, flow rate, pH, etc. Various mobile phase combinations and ratios were tried to check the maximum response. Suitable method development is essential when talking about cost, time, productivity, and effectiveness of drug

product. Analytical method development is essential for drug degradation studies, analyzing and evaluating properties of API, and to study impurities in the drug.

Method validation:

Method validation can be defined as the process of proving that a particular development analytical method is acceptable for its intended use. Validation is an important requirement in the practice of an analytical process. Method validation can be interpreted as the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with that the application requires. Validation parameters may include Specificity, Linearity, Accuracy, Precision, Robustness, Limit Of Detection (LOD), And Limit Of Quantification (LOQ).

Assay:

Standard stock solution containing Candesartan and Alogliptin were prepared individually by dissolving 1mg drug in 10ml volumetric flask by using mobile phase and volume is making up to the mark. This will get the 100µg/ml of solution from this 10ml of solution was pipette out In a 10ml volumetric flask, dilute the portable stage mark to 50µg/ml solution respectively for both the drug and used for sample injection. Mobile phase was prepared using methanol: water (8:2) which was found to resolve Candesartan and Alogliptin appropriately. The degassing of mobile phase was done by sonication for 30 min. and flow rate was set to 1ml/min. both drugs show good absorbance at 273nm, which was selected as wavelength for further analysis. The column temperature was maintained at room temperature.

Limit of detection (LOD) and Limit of quantification (LOQ):

The term LOD is the lowest concentration which can be detected. It is calculated by using the equation, LOD = 3.3 × sy/S. The term LOQ is the lowest concentration which can be quantified. It is calculated by using the equation, LOQ = 10 × sy/S. where “sy “represents the residual standard deviation of the regression line and “S” represent the slope of the calibration curve.

Precision:

Intra-day precision: To study intra-day precision, three replicate standard solutions were prepared and injected in HPLC. The results were recorded in a single day. Inter-day precision: To study inter-day precision, three replicate standard solutions (same which were used for intra-day precision) were taken and injected into HPLC. The results were recorded for 3 consecutive days. Peak area was determined and %RSD was determined.

Accuracy:

Accuracy was conducted by analyzing sample solution spiked with known amounts of the bulk drug or standard at three kinds of concentration levels of 50%, 100% and 150% of each at a specified limit. % recovery test was performed at all the three levels.

Result and Discussion:

The developed HPLC method was specific, simple, sensitive, precise, accurate and robust for the detection of Candesartan and Alogliptin in pure form and the method was validated as per ICH guidelines. The proposed method is quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The method has a wider linear dynamic range with good accuracy and precision. Hence, the method holds good for the routine analysis of Candesartan and Alogliptin in various pharmaceutical industries as well as in academics. The present study may suggest that during simultaneous treatment of antihypertension and diabetes with Candesartan and Alogliptin the dose and frequency of administration of Alogliptin are to be readjusted accordingly in order to avoid severe hypoglycemia. Along with this it is necessary to consider individual blood glucose levels and other drug therapy.

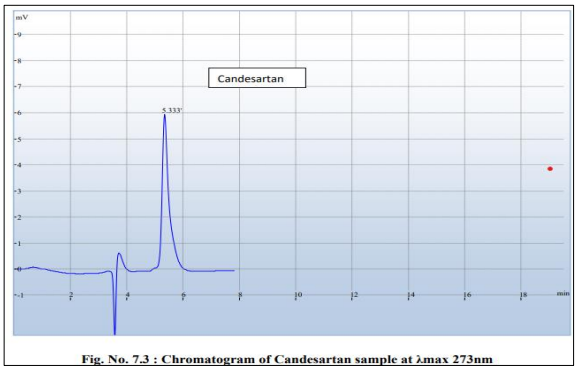

Fig. No. 7.3 : Chromatogram of Candesartan sample at λmax 273nm

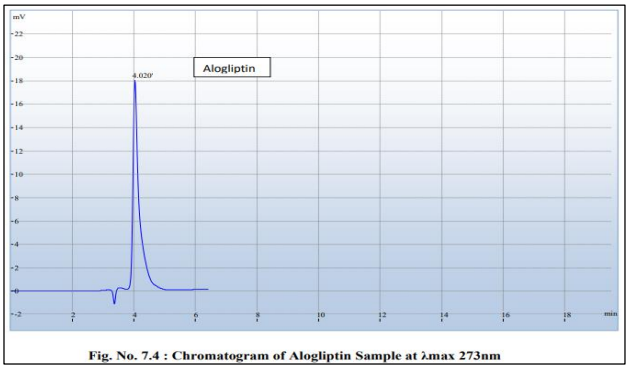

Fig. No. 7.4 : Chromatogram of Alogliptin Sample at λmax 273nm

Method Validation :

Linearity:

The calibration curves were plotted for both Candesartan and Alogliptin at 256nm and 277nm. Both drugs show linearity and obey the beer's law in the concentration range for Candesartan 10- 50µg/ml and for Alogliptin 10-50µg/ml. the correlation coefficients of calibration curve were found to be 0.999 and 0.995. Where, Y =area of chromatogram;

X = Concentration

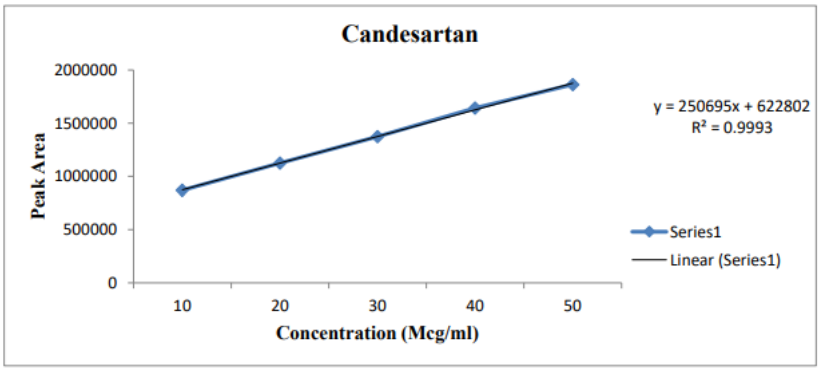

Fig no 1 : Standard calibration curve of Candesartan in Methanol (8:2)

Table no 1 : Linearity of Candesartan

Level	Conc. in µg /ml	Area
I	10	906581
II	20	1175466
III	30	1404550
IV	40	1655678
V	50	1879106

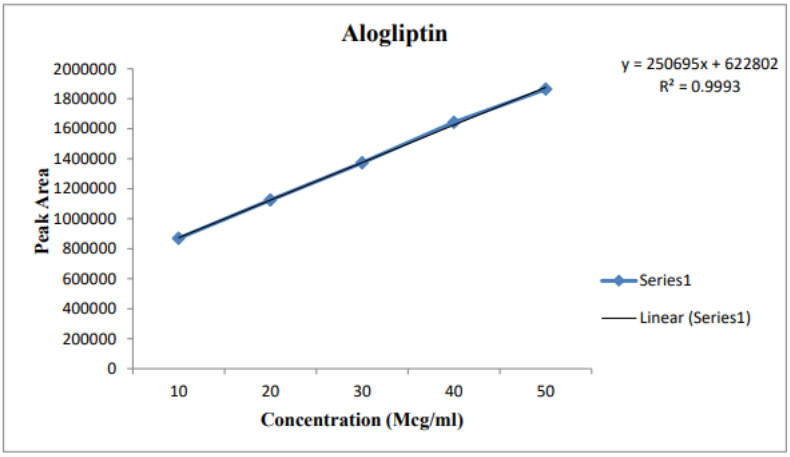

Fig.No 2 :- Standard Calibration Curve of Alogliptin in Methanol and Water (8:2)

Table No2 : Linearity of Alogliptin

Level	Conc. in µg /ml	Area
I	10	869714
II	20	1124558
III	30	1373453
IV	40	1642491
V	50	1864224

Specificity and selectivity-

Specificity is the ability of Process for the unambiguous assessment of the analyt in the presence of elements that may be anticipated to be present. These may typically include impurities, degredants, matrix and other unwanted material etc. This definition has the following consequences: identification: ensuring an analysts identity. Purity tests: ensuring that all analytical procedures conducted allow a precise declaration of an analyst's impurity content, i.e. other substance tests, heavy metals, content of remaining solvents, etc. Assay (substance or power): Specificity is very important to obtain an accurate result.

Limit of detection (LOD) and Limit of quantification (LOQ):

Limit of detection(LOD) -Limit of detection calculated by

$LOD=3.3\sigma/S$

Where, σ - Standard Deviation,

S- Slop of calibration curve.

LOD was found to be 0.699 and 0.084 at wavelength 256nm and 277nm, for Candesartan and Alogliptin respectively.

Limit of quantitation (LOQ) -Limit of Quantitation calculated by

$LOQ=10\sigma/S$

Where, σ - Standard Deviation,

S- Slop of calibration curve.

LOQ was found to be 2.330 and 0.254 at wavelength 256nm and 277nm for Candesartan and Alogliptin respectively.

Precision :

The precision of an analytical procedure expresses the close agreement (degree of scatter) between a sequences of measurements acquired under the prescribed conditions from various sampling of the same homogeneous sample. Precision is usually considered at three levels: Repeatability, accuracy and reproducibility intermediate. Precise use of homogeneous, authentic samples should be investigated. However, if it is not possible to obtain a homogeneous sample it may be searched using artificially prepared samples or a sample solution

Day	Conc. Of drug	Absorbance		SD		%RSD	
		Candesartan	Alogliptin	Candesartan	Alogliptin	Candesartan	Alogliptin
1	10 (n=3)	1404550	1373453	1453.34	2336.05	0.103	0.170
		1407628	1367790				
		1404540	1371332				
2	20 (n=3)	1408270	1376685	4529.92	2567.28	0.322	0.186
		1397177	1374092				
		1402500	1370427				
3	30 (n=3)	1407386	1375086	2032.05	268.80	0.144	0.019
		1402596	1374592				
		1403819	1375216				

Limit: %RSD for Area NMT 2.0%

Table No 3:-Interday Precision Studies

Accuracy:

Accuracy is very important because it shows a comparison between the value accepted as either a true value or an accepted reference value and the value discovered as precision. Accuracy should be displayed across the analytical procedure specified range.

Conc.	Absorbance		SD		%RSD	
	Candesartan	Alogliptin	Candesartan	Alogliptin	Candesartan	Alogliptin
1.	906581	869714	5048.89	471.28	0.55	0.05
	915850	868852				
	904125	868618				
2.	1404550	1373453	1453.34	2336.04	0.10	0.17
	1407628	1367790				
	1404540	1371332				
3.	1879106	1864224	6001.44	12945.41	0.32	0.69
	1875247	1844864				
	1864892	1876293				

Table No 4: Accuracy Study

Conclusion:

The developed HPLC method was specific, simple, sensitive, precise, accurate and robust for the detection of Candesartan and Alogliptin in pure form and the method was validated as per ICH guidelines. The proposed method is quite simple and do not require any pretreatment of the drug and tedious extraction procedure. The method has a wider linear dynamic range with good accuracy and precision. Hence, the method holds good for the routine analysis of Candesartan and Alogliptin in various pharmaceutical industries as well as in academics. The present study may suggest that during simultaneous treatment of antihypertension and diabetes with Candesartan and Alogliptin the dose and frequency of administration of Alogliptin are to be readjusted accordingly in order to avoid severe hypoglycemia. Along with this it is necessary to consider individual blood glucose levels and other drug therapy.

Acknowledgements:

It is great pleasure for me to acknowledge my Parents, Project guide, Management of Aditya Pharmacy College Beed for providing necessary facilities.

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