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METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF ALOGLIPTIN AND METFORMIN HYDROCHLORIDE BY RP-HPLC

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ABSTRACT

In the present work, RP-HPLC method has been developed and validated for simultaneous determination of Alogliptin and Metformin HCl in tablet dosage form. In this method, separation was done using Agilent C18 (150 X 4.6 mm, 5µ) and phosphate buffer of pH adjusted to 3.0 with 0.1% OPA and methanol in the ratio of 20:80 v/v as the mobile phase with a flow rate of 0.7 min/ml. The optimum detection wavelength was 242 nm. The method was validated for its linearity, accuracy, precision, specificity, robustness. The system suitability parameters were passed and linearity was observed in the range of 10-30µg/ml of Alogliptin and Metformin HCl. The accuracy was performed and % recovery was found to be 99.90% and 99.99% for Alogliptin and Metformin HCl respectively. Thus a sensitive, accurate, specific, precise method was developed for the simultaneous determination of Alogliptin and Metformin HCl in tablet dosage form by RP-HPLC Method.

KEYWORDS

Alogliptin, Metformin HCl, RP- HPLC and Validation.

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INTRODUCTON

Alogliptin is an Oral Hypoglycemic drug mainly to treat Type-II Diabetes Mellitus belongs to the class DPP-4 inhibitor[1]. Molecular formula for Alogliptin is $C_{18}H_{21}N_5O_2$ with IUPAC name 2-({6-[(3R)-3-aminopiperidin-1-yl]-3-methyl-2, 4-dioxo-3, 4-dihydropyrimidin-(2H) yl} methyl) benzonitrile. It is an orally administered anti-diabetic drug, which is accessible in 6.25mg, 12.5mg and 25mg in the form of tablets[2].

Metformin is the oldest insulin sensitizer and is first line therapy used in the management of type 2 diabetes mellitus (Non-Insulin Dependent Diabetes Mellitus)[3]. Metformin hydrochloride is (N, N-dimethyl imidodicarbonimidic diamide hydrochloride with the molecular formula $C_4H_{11}N_5$. HCl, molecular weight 165.63g/mole and white to off-white crystalline compound[4].

Literature survey revealed that several RP-HPLC based methods (5-15) have been reported for the estimation of simultaneous estimation of Alogliptin and Metformin. The aim of the present work was to develop simple, rapid, sensitive, specific, accurate, precise, economic and reliable RP-HPLC method for the simultaneous estimation of Alogliptin and Metformin in bulk and tablet dosage form suitable for quality control analysis.

MATERIAL AND METHODS

Chemicals

Alogliptin and Metformin HCl working standards were received as gift sample from NATCO Pharma pvt ltd, Hyderabad and sample tablets (Label claim: 12.5mg (Alogliptin) and 500mg (Metformin); Kazano tablets) were procured from a local medical shop. HPLC grade methanol and water, Potassium di hydrogen phosphate, Ortho Phosphoric acid were purchased from Merck Specialities Private Ltd., Mumbai and SD Fine Chem. Limited, Mumbai.

Chromatographic Conditions

HPLC-Shimadzu LC- 2010 binary system with PDA detector was used for the method development. The output signal was monitored and processed using LC solutions software. Chromatographic separation was performed on Agilent C_{18} G (250 X 4.6., 5µ) column at ambient temperature. The mobile phase containing phosphate buffer of pH adjusted to 3.0 with 0.1% OPA and methanol in the ratio of 20:80 v/v was pumped at 0.7 ml/min and detection was carried out at 242 nm. The injection volume for standard and sample was 10µl (fixed loop) and the total run time was 20min Table No.1.

Preparation of standard stock solution

About 10 mg of Alogliptin and Metformin HCl working standards were accurately weighed and transferred into two different 10 ml volumetric flasks, dissolved in diluent. The solutions were filtered through 0.45µm Ultipor N66 nylon filter and the volume was made up to the mark with the diluent to get 1000µg/ml (stock solution-I) of Alogliptin and Metformin HCl. Mobile phase was used as diluent.

Preparation of standard solution

Alogliptin and Metformin HCl (100µg/ml) were prepared from the standard stock solutions (stock solution-II) with the diluent. 1ml of Alogliptin and 1ml of Metformin HCl (from stock solution-II) were pipetted out and transferred to a 10ml volumetric flask and made upto the mark with the diluent to get 10µg/ml concentrations and filtered through 0.45µm Ultipor N66 nylon filter. Accurately 10µl was injected into the HPLC system and chromatogram was recorded.

Preparation of Sample solution

Twenty tablets were weighed, average weight determined and finely powdered. An accurately weighed quantity of powder equivalent to 10 mg of Alogliptin was transferred into a 10 ml volumetric flask. The tablet powder was dissolved in sufficient volume of diluent, sonicated for 20 minutes and degassed. The volume was made up to the mark with the diluent and the sample solution was filtered through 0.45µm nylon filter. From this sample solution appropriate aliquot was prepared using the diluent. Accurately 10µl was injected into the HPLC system and the peak area was recorded at 242nm.

VALIDATION OF THE DEVELOPED METHOD

The method developed was validated as per ICH guidelines [16] for linearity, accuracy, precision, LOD, LOQ, ruggedness and specificity.

Linearity

The linearity of the developed method was demonstrated over the concentration range of 10-30µg/ml of Alogliptin and Metformin HCl prepared from the stock solution. Calibration curve of the drugs was plotted for concentration v/s peak area. The regression equations of calibration curve was

y= 51081x +44044 and R^2 = 0.999 for Alogliptin and y= 84530x+1000000 and R^2= 0.999.

Accuracy

The accuracy of the method was determined by recovery studies in triplicate for each level. Fixed amount of sample was taken and Alogliptin and Metformin HCl equivalent to 80, 100 and 120 % of the standard was injected into the HPLC system. The method was repeated three times for each level. The average % recovery was calculated.

Precision

The precision of the method was studied by estimation of multiple samplings from the homogeneous sample of the drug at three different concentrations on the same day and on three different days. The precision was expressed as %RSD and was calculated for intraday and inter day precision.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calibration curve of the drug was prepared using 10- 30µg/ml concentrations of Alogliptin and Metformin HCl. LOD and LOQ was determined by signal to noise ratio. Limit of Detection (LOD) =3:1 S/ N and Limit of quantitation (LOQ) = 10:1S/N. In this equation, S is the signal (response of the drug) and N is the noise (response of the impurities). The LOD for Alogliptin and Metformin HCl were found to be 9.97 and 5.15µg/ml respectively and LOQ for Alogliptin and Metformin HCl were found to be 12.68 and 8.68µg/ml respectively.

Robustness

Robustness of the method was determined by making slight changes in the composition of mobile phase ± 2%, flow rate by ± 0.2ml/min and detection wavelength by ±2nm. Retention time and chromatograms were determined for the drug.

Specificity

Commonly used excipients such as starch, lactose and magnesium stearate were spiked into weighed quantity of the drug. The chromatograms were recorded by making suitable dilutions and the amount of drug present in the sample was determined.

RESULTS AND DISCUSSION

In the present study, simultaneous RP-HPLC method developed for the estimation of Alogliptin and Metformin HCl in bulk and tablet dosage form using Agilent C_{18} column (150 mm x 4.6 mm x 5µ particle size) at ambient temperature. To develop an effective method for the simultaneous estimation of Alogliptin and Metformin HCl, conditions such as detection wavelength, ideal mobile phase and concentration of the standards were optimized in preliminary trials. Alogliptin and Metformin HCl standard concentration was scanned in UV- region between 200-400 nm. Detection wavelength of Alogliptin and Metformin HCl was found to be at 242nm Figure No.3. The Alogliptin and Metformin HCl peaks in the sample was identified by comparing with the Alogliptin and Metformin HCl standards and the retention time was found to be around Figure No.4 and 5.

The estimation of Alogliptin and Metformin HCl tablets was carried out by RP-HPLC using mobile phase, phosphate buffer of pH adjusted to 3.0 with 0.1% OPA and methanol in the ratio of 20:80 v/v with flow rate of 0.7ml/min. The retention time was found to be 1.727min for Metformin HCl and 2.900 minutes for Alogliptin. System suitability parameters such as RSD for six replicate injections were carried out on freshly prepared standard solution and parameters were given in Table No.2. %RSD found to be less than 2%, theoretical plates >2000, and tailing factor 1.02 and 1.13 for Metformin HCl and Alogliptin indicating the suitability of the system for the estimation of the drug. The resolution was found to be 2.378.

The typical optimized chromatogram of Metformin HCl and Alogliptin is shown in Table No.1. The calibration curve of the drugs were constructed by plotting peak area of the drug (Y-axis) and concentration of the drug on (x-axis). A good linear relationship was observed between concentration of the drugs and the respective ratio of peak areas in the range of 10-30mcg/ml with a correlation coefficient of 0.999 reflecting that good correlation exists between peak areas and the concentrations Figure No.6 and 7.

The quantitative estimation of the drugs in tablet was determined by taking concentration of the drug same to that of standard solution and the assay result was found to be 99.83% for Alogliptin and 99.89% for Metformin HCl Table No.3. The acceptance criterion of repeatability is RSD, and should not be more than 2.0 %. The method repeatability was 0.308% shows that the method was precise. The developed method was validated for its intra-day and inter-day precision. The results obtained were within the acceptable limit Table No.3. Estimation of the drugs by the developed RP-HPLC method for finding out intra and inter day variations show low coefficient of variation values which indicate that the developed method is highly precise.

By spiking various concentrations of the drugs ranging from 80-100-120% into previously analyzed samples the amount of the drug recovered was calculated and the results were shown in Table No.4. The Accuracy limit was the % recovery and was in the range of 99.5% to 100.9% for Alogliptin and 100.2% to 104.1% for Metformin HCl. From the validation of the developed method, the accuracy was within the limit, indicating that the proposed RP-HPLC method was highly accurate. LOD 9.97µg/ml and 5.15µg/ml for Alogliptin and Metformin HCl and LOQ 12.68µg/ml and 8.68µg/ml for Alogliptin and Metformin HCl Table No.2 suggest the sensitivity of the drugs.

Robustness of the method was studied by changing the chromatographic conditions slightly and results were presented in Table No.5. From the method developed it was observed that there were no significant changes in the retention time and area of the chromatograms by making slight alterations in detection wavelength, composition and flow rate of the mobile phase. The %RSD was less than 1%, which demonstrated that the RP-HPLC method developed was robust.

The RP-HPLC method developed in the present study was used to quantify Alogliptin and Metformin HCl in bulk and tablet dosage form and the results were comparable with the corresponding labeled quantity Table No.3. High recovery values and no additional peaks in the chromatogram indicate that the developed method was free from interference of the commonly used excipients in the tablet dosage form. So the developed RP-HPLC method is accurate and specific and could be used in routine analysis of Alogliptin and Metformin HCl in bulk and tablet dosage form.

Table No.1: Optimized chromatographic conditions

S.No	Parameter	Optimized condition
1	Chromatograph	HPLC (Shimadzu prominence with PDA detector)
2	Column	Agilent C_{18} G 150mm x 4.6mm, 5µ
3	Mobile Phase	phosphate buffer of pH adjusted to 3.0 with 0.1% OPA and methanol in the ratio of 20:80 v/v
4	Flow rate	0.7 ml/min
5	Detection wavelength	242 nm
6	Injection volume	10µl
7	Column temperature	Ambient

Table No.2: System suitability and validation parameters of the developed method

S.No	Parameter	Metformin HCl	Alogliptin
1	Theoretical plates	4789	2698
2	Tailing factor	1.02	1.13
3	Retention time (min)	1.727	2.900
4	Linearity range (µg/ml)	10-30µg/ml	10-30µg/ml
5	Regression equation Y=mx=c	Y =42935x+1E+06	y=26171x+31754
6	Slope (m)	42935	26171
7	Intercept (c)	1E+06	31754
8	Correlation coefficient	0.999	0.999
9	Percent RSD	<2	< 2
10	Precision Intraday (n=)		0.726
11	Precision Intraday (n=)		0.697
12	LOD (µg/ml)	5.15	9.97
13	LOQ (µg/ml)	8.68	12.68

Table No.3: Results of Analysis of the tablet dosage form

S.No	Formulation	Label claim	Amount Found ± SD (n=5)		% recovery		%RSD	
			MET	ALO	MET	ALO	MET	ALO
1	Kazano	12.5mg(Alo), 500mg (Met) **Intra day** Session- 1 Session- 2 Session- 3 **Inter day** Day 1 Day 2 Day 3	498.29mg ± 0.032 —	12.37mg ± 0.045 —	99.68% —	98.96% —	0.088 0.130 0.729 0.682 0.0068 0.627 0.215 0.123	0.435 0.356 0.268 0.582 1.163 0.948

***Average of 6 determinations**

Table No.4: Recovery studies of the developed method

S.No	Drug	Preanalysed Sample Conc(µg/ml)	Recovery Level	Amount Added (µg/ml)	Total Amount Found (µg/ml)	%Recovery
1	Metformin	20	80% 100% 120%	16 20 24	35.58 40.12 43.65	98.83% 100.30% 99.20%
2	Alogliptin	20	80% 100% 120%	16 20 24	36.04 40.92 43.86	100.11% 102.3% 99.68%

Table No.5: Robustness data of the developed method for Metformin

S.No	Parameter	Proposed	Modification	%RSD	Retention time (min)	Tailing factor
1	Flow Rate (± 0.1ml/min)	0.7	0.6 0.8	0.727 0.681	1.789 1.716	1.048 1.150
2	Mobile Phase (±2 %) (W:M)	20:80	18:82 22:78	1.035 0.557	1.726 1.735	1.198 1.191
3	Wavelength (±2nm)	242nm	240nm 244nm	0.832 0.377	1.724 1.737	1.128 1.210

Table No.6: Robustness data of the developed method for Alogliptin

S.No	Parameter	Proposed	Modification	%RSD	Retention time (min)	Tailing factor
1	Flow Rate (± 0.1ml/min)	0.7	0.6 0.8	0.727 0.681	3.010 2.889	1.048 1.250
2	Mobile Phase (±2 %) (M:W)	20:80	18:82 22:78	1.138 0.557	2.928 2.900	1.198 1.191
3	Wavelength (±2nm)	242nm	240nm 244nm	0.891 1.372	2.900 2.912	1.128 1.210

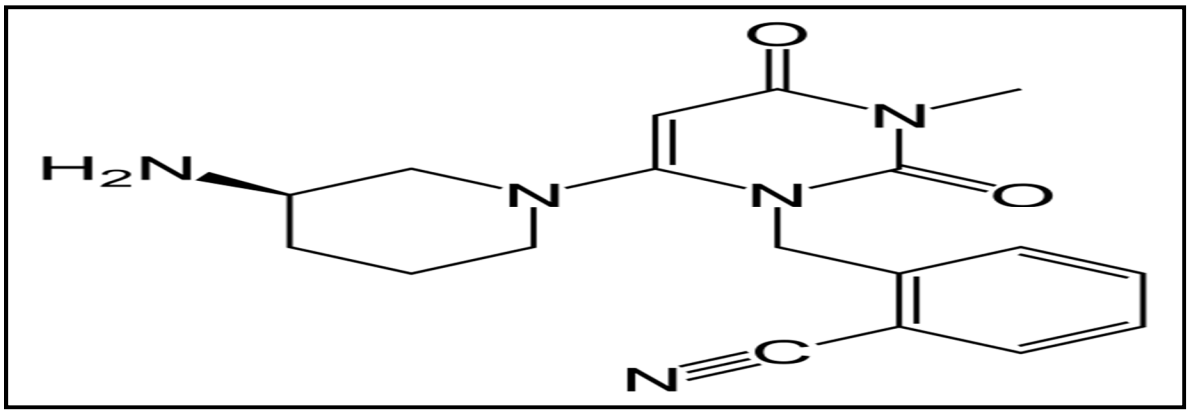

Figure No.1: Structure of Alogliptin

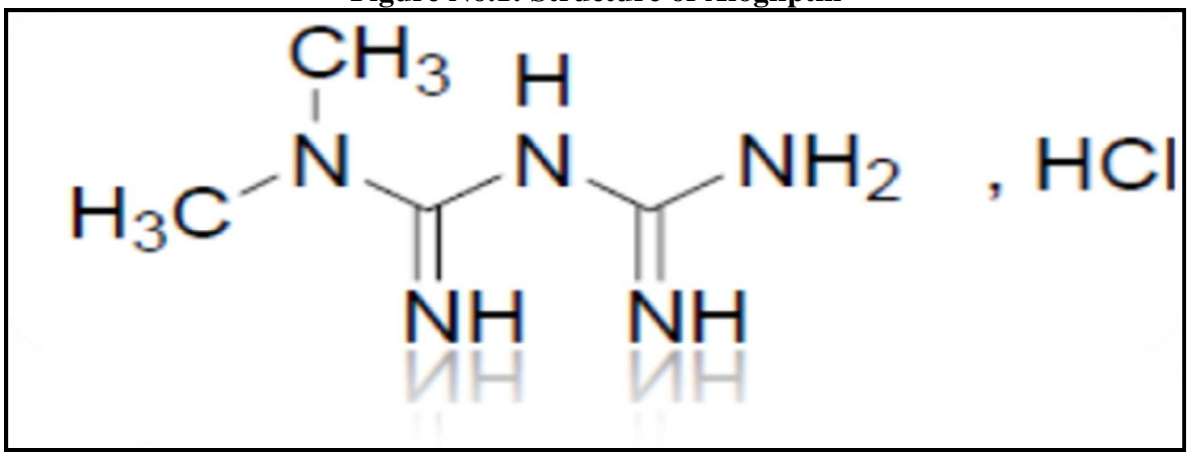

Figure No.2: Structure of Metformin HCl

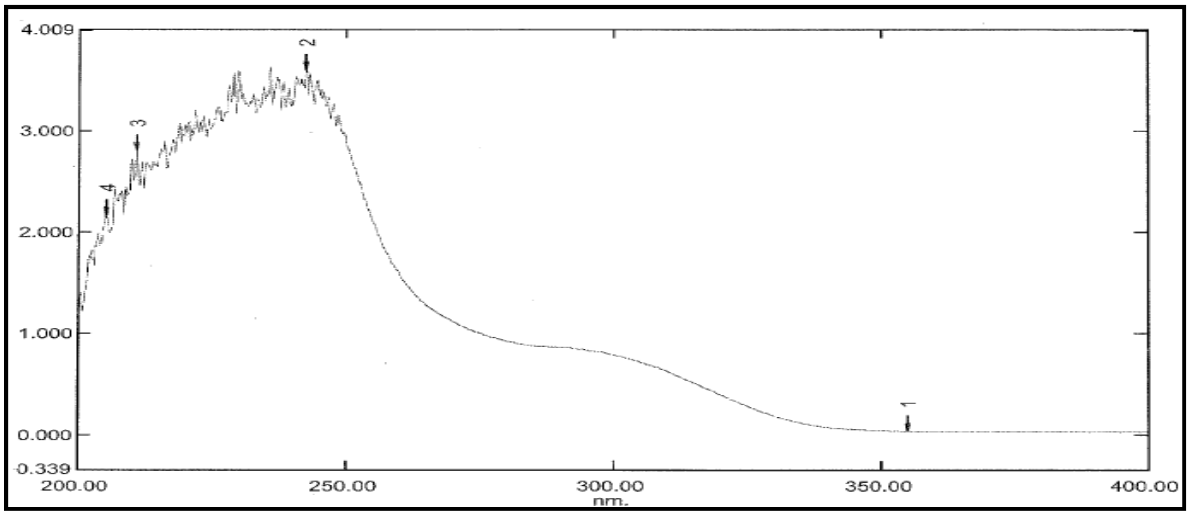

Figure No.3: UV Spectrum of Alogliptin and Metformin HCl

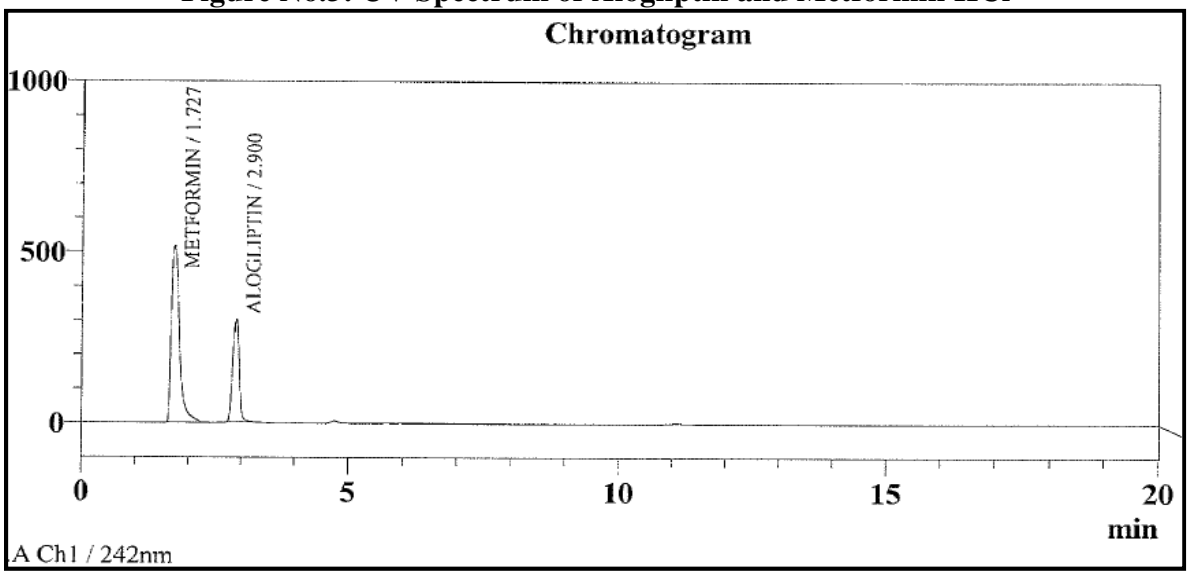

Figure No.5: Chromatogram of sample solution of Alogliptin and Metformin HCl

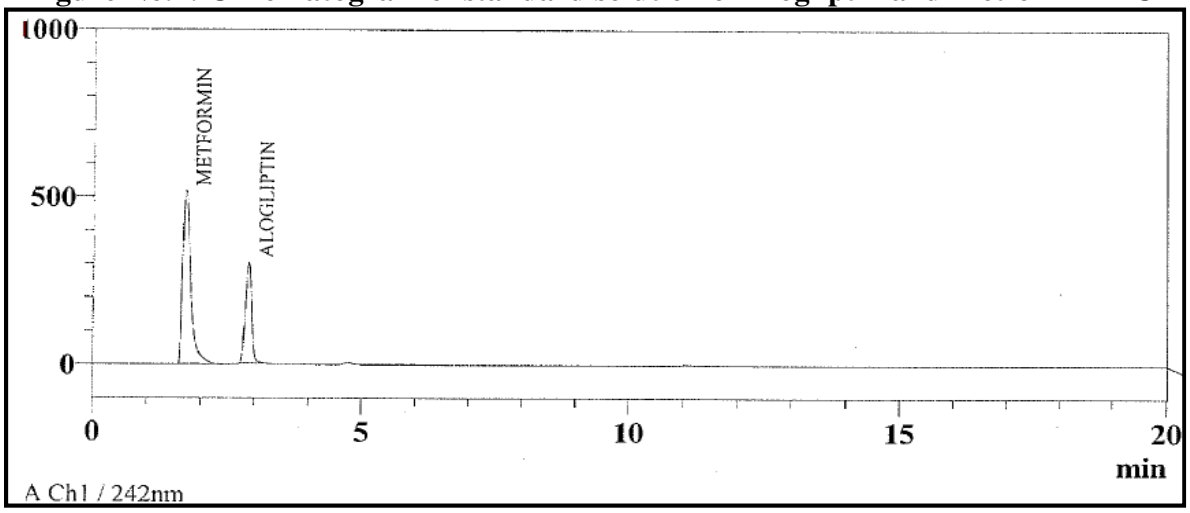

Figure No.4: Chromatogram of standard solution of Alogliptin and Metformin HCl

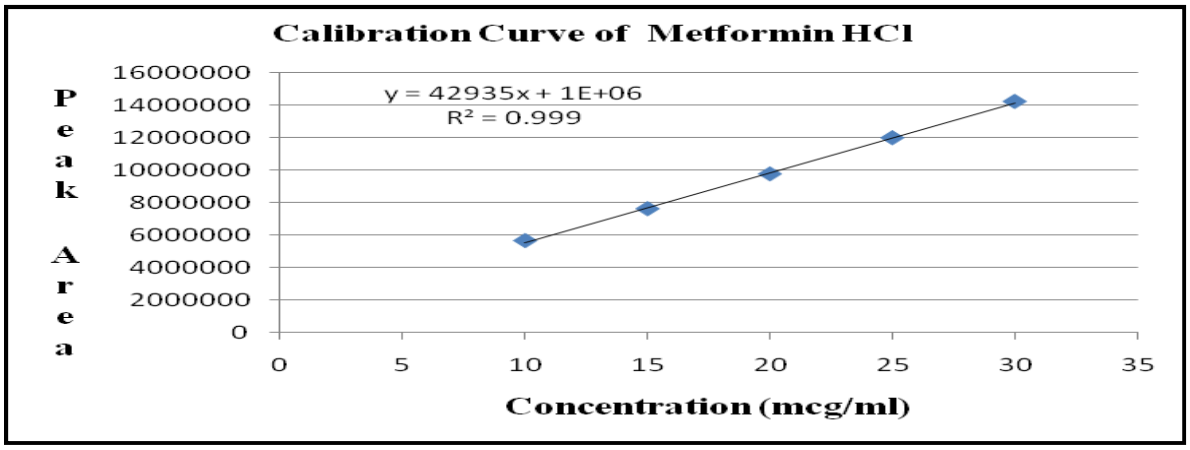

Figure No.6: Calibration curve of Metformin HCl

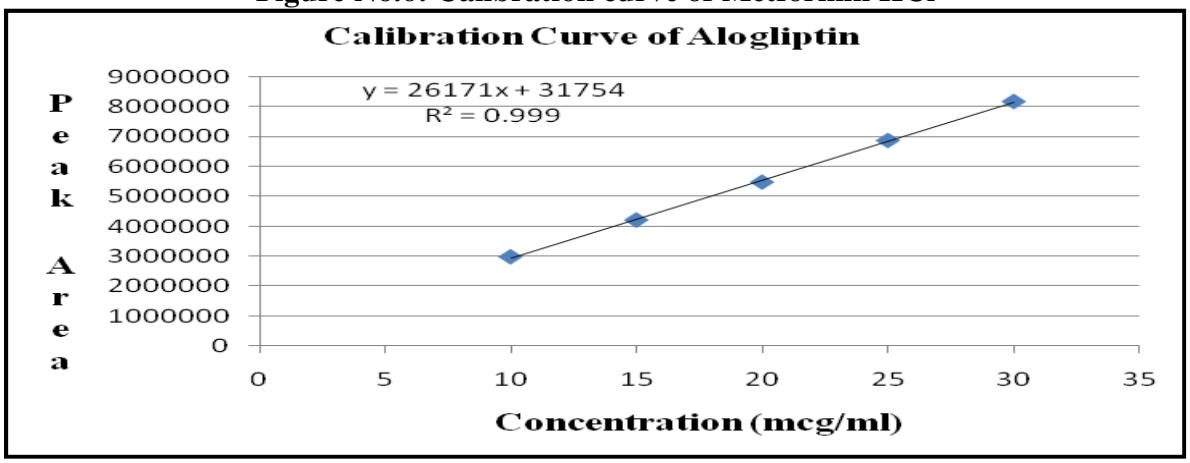

Figure No.7: Calibration curve of Alogliptin

CONCLUSION

The developed RP-HPLC method in the present study was found to be simple, rapid, specific, accurate, precise, linear and robust. Thus, the method is suitable for the simultaneous estimation of Alogliptin and Metformin HCL in raw material and tablet formulation in quality control with a high degree of Accuracy and Precision.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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