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STABILITY INDICATING RPHPLC METHOD DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF METFORMIN AND ACARBOSE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Acarbose in Tablet dosage form. Chromatogram was run through thermo BDS (250mm 4.6mm, 5µ). Mobile phase containing Buffer and Acetonitrile in the ratio of 35:65A was pumped through column at a flow rate of 1ml/min. Buffer used in this method was 0.02N KH_2PO_4 buffer at P^H 3.3. Temperature was maintained at 30ºC. Optimized wavelength for Metformin and Acarbose was 215nm. Retention time of Metformin and Acarbose were found to be 2.8min and 4.0min. %RSD of the Metformin and Acarbose were found to be 0.65 and 0.9 respectively. %Recover was Obtained as 99.83% and 99.97% for Metformin and Acarbose respectively. LOD, LOQ values are obtained from regression equations of Metformin and Acarbose were 0.4ppm, 1.3ppm and 0.8ppm, 2.5ppm respectively. Regression equation of Metformin is y = 13779x + 1840, and of Acarbose is y = 16828x + 4143.

KEYWORDS

Metformin, Acarbose, RP-HPLC, Buffer and Acetonitrile.

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INTRODUCTON

Metformin [Figure No.1] is an oral ant diabetic drug in the biguanide class. It is most widely prescribed ant diabetic drug in the world used to treat type 2 diabetes. Metformin helps to control the amount of glucose (sugar) in blood. It decreases the amount of glucose and also increases body's response to insulin, a natural substance that controls the amount of glucose in the

blood. It is not used to treat type 1diabetes[1]. It is also used for treatment of gestational diabetes, polycysticovary syndrome (PCOS)[1]. It works by decreasing hyperglycemia primarily by suppressing glucose production by the liver (hepatic gluconeogenesis). It helps to reduce LDL cholesterol and triglyceride levels, and is not associated with weight gain. Metformin comes as a liquid, as a tablet, and as an extended-release (long-acting) tablet taken orally. It is used alone or with other medications. Very rare but serious side effect with Metformin is lactic acidosis. Other than that common side effect are gastrointestinal irritations, including diarrhea, cramps, nausea, vomiting and increased flatulence. Literature survey revealed

The HPLC methods for estimation of metformin in Bulk, human plasma and pharmaceutical dosage forms[2–7]. LC-MS-MS method was reported for the determination of Metformin in human plasma[8]. Literature survey reveals several Analytical and Bioanalytical methods for the analysis of Metformin. These methods reported with Metformin alone or in combination with other drug. These include HPLC [9-11] and spectrophotometry analysis of Metformin in tablets.

Acarbose, Figure No.2 also known as BAY g 5421, is a α-glycosidase inhibitor that prevents absorption of sucrose and maltose. This compound has been found to delay digestion of complex disaccharides and carbohydrates. Further studies suggest that this compound can stimulate phosphorylase kinase (PHK) by binding to the glucoamylase-like domain new the amino termini and causing an alteration in the structure. Acarbose inhibits enzymes *(glycoside hydrolyses)* needed to digest carbohydrates, specifically, alpha-glycosidaseenzymes in the brush border of the small intestines and pancreatic alpha-amylase. Pancreatic amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, whereas the membrane-boundintestine *alphaglucosidaseshydrolyze oligosaccharides*, trisacch arides and disaccharides to glucose and othermonosaccharide's in the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules. In diabetic patients, the short-term effect of these drugs therapies is to decrease current blood glucose levels, the long-term effect is a reduction in HbAlc level. this reduction averages an absolute decrease of 0.7% which is a decrease of about 10% in typical HbAlcvalues in diabetes studies. Literature survey revealed that several Analytical and Bioanalytical methods for its estimation using Reversed Phase-High Performance Liquid Chromatography [RP-HPLC] with UV detection, HPLC - electro spray tandem mass spectrometry, LC-MS, liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry and RP-HPLC method. The developed method has various advantages over the above mentioned methods, as it is simple, economical, faster, precise and accurate and specificfor quantitative determination of Miglitol in pharmaceutical dosage form. As per our detailed literature survey as on date, there are very few reports using UV and RP-HPLC for the simultaneous quantitative estimation of Metformin and Migllitol in Bulk and Pharmaceutical dosage forms. We here in reported a new, simple, sensitive, precise, accurate, and linear and isocratic RP-HPLC method for the simultaneous quantitative estimation of Metformin and Migllitol in bulk and Formulation as per ICH Guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Metformin and Acarbose standard was obtained from reputed companies, formulation tablets were purchased from local pharmacy. HPLC grade Methanol, Water and Acetonitrile were purchased from Merck specialties Pvt. limited, Mumbai.

Instruments

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Metformin and Acarbose solutions. Ultrasonicator was used to sonicating the mobile phase and samples. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234) and pH of themobile phase was adjusted by using Systronics digital pH meter.

Chromatographic conditions

Separation of the drugs was achieved on a reverse phase BDS column, C18 (250 X4.6mm, 5µ). The mobile phase consists of a mixture Acetonitrile and potassium dihydrogen ortho phosphate buffer (pH adjusted to 3.3) in ratio of 65:35, v/v. The mobile phase was set at a flow rate of 1 ml/min and the volume injected was 10 µl for every injection. The detection wavelength was set at 215nm.

Mobile phase Preparation

The mobile phase was prepared by mixing Acetonitrile and buffer in the ratio of 65:35 v/v and later sonicated for 10 minutes for the removal of air bubbles.

Buffer Preparation

The buffer solution was prepared by weighing 2.72g of potassium dihydrogen orthophosphate (KH_2 PO_4) and transferring to 1000 ml of HPLC grade water to get 0.01M buffer strength, which was adjusted to pH 3.3 with dil. OPA solutions.

Preparation of Stock and working Standard Solution

Accurately Weighed and transferred 50mg of metformin and 5mg of Acarbose working Standards into 10ml and 10ml clean dry volumetric flasks, add 3/4 ml of diluents, sonicated for 5 minutes and make up to the final volume with diluents.1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

Preparation of Sample Solution

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 250mL volumetric flask, 200Ml of diluents added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 0.5ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

RESULTS AND DISCUSSION

A Reverse phase HPLC method was developed keeping in mind the system suitability parameters i.e. resolution factor (Rf) between peaks, tailing factor (T), number of theoretical plates (N), runtime and the cost effectiveness. The optimized method developed resulted in the elution of Metformin at 2.8min, Acarbose at 4.0min. Figures No.3 and 4 represent chromatograms of blank solution and mixture of working standard solutions respectively. All system suitability parameters meeting acceptable criteria for the mixture of standard solutions.

System Suitability

System Suitability tests are an integral part of method development and are used to ensure adequate performance of the chromatographic system. Retention time (RT), number of theoretical plates (N), peak resolution (Rs) and peak Tailing factor (T) were evaluated for six replicate injections of the mixture of standards at working concentration. The results were given in Table No.1 within acceptable limits. The sample peaks were identified by comparing the relative retention times with the standard drugs mixture. System suitability parameters were within the acceptance limits, ideal for the chromatographed sample. Integration of separated peak area was done and each drug concentration was determined by using

the peak area concentration relationship obtained in the standardization step.

Method validation

Validation of the analytical method is the process that establishes by laboratory studies in which the performance characteristics of the method meet the requirements for the intended analytical application. RP-HPLC method developed was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures. The method was validated for the parameters like system suitability, specificity, linearity, accuracy, precision, ruggedness, and robustness, limit of detection (LOD) and limit of quantification (LOQ)

Specificity

Figures No.3-4 of blank, mixture of standard drug solution and sample chromatogram reveal that the peaks generated in mixture of standard solution and sample solution at working concentrations are only because of the drugs as blank has no peaks at the retention times Metformin, and Acarbose. Hence the method developed is said to be specific.

Linearity

Linearity is determined by a series of three to six injections of five or more standards. Peak areas (or heights) of the calibration standards are usually plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve is evaluated through the value of the co-relation coefficient (r2). Because deviations from linearity are sometimes difficult to detect, two additional graphical procedures can be used. The first one is to plot deviations from regression line versus concentration or versus logarithm of concentration. For linear ranges, the deviations should be equally distributed between positive and negative values. Another approach is to divide signal data by their respective concentrations yielding the relative responses. A graph is plotted with the relative responses on Y-axis and the corresponding concentrations on X-axis on a log scale. The obtained line should be horizontal over the full linear range. At higher concentrations, there will typically be a negative deviation from linearity. Six linear concentrations of Metformin (125-750ppm) and Acarbose (12.5ppm to 75ppm) are prepared and injected. Regression equation of the Metformin and Acarbose are found to be, $y = 13779x + 1840$, $y = 16828x + 4143$. And regression co-efficient was 0.999.

Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation. Standard deviation (% RSD). The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Intermediate Precision

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug. Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered.

LOD

Limit of Detection was calculated by Metformin and Acarbose method and LOD for Metformin and Acarbose were found to be 0.4 and 0.8 respectively.

LOQ

Limit of Quantification was calculated by Metformin and Acarbose method and LOQ for Metformin and Acarbose were found to be 1.3 and 2.5 respectively.

Robustness

Capacity to remain unaffected by small but deliberate variations in method parameters. Comparison results under differing conditions with precision under normal conditions. The results are to determine the robustness of method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by 1 ± 0.2 ml/min. the percentage of organic modifier was varied by 65 ± 5% and column temperature was varied by 30 ± 5°C. Their effects on the retention time (TR), tailing factor (T), theoretical plate numbers (N) and repeatability of peak areas (n = 3) were studied. Considering the result of modifications in the system suitability parameters and the specificity of the method, it would be concluded that the method conditions are robust. Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Assay of Pharmaceutical Formulation

Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average % Assay was calculated and found to be 99.87 and 100.16 for Metformin and Acarbose respectively.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table No.1: System Suitability Studies Results

S.No	Parameter Property	Metformin	Acarbose
1	Repeatability	≤ 1%	≤ 1%
2	Resolution(Rs)	> 2	> 2
3	Tailing Factor(T)	≤ 2	≤ 2
4	Theoretical Plates(N)	> 2000	> 2000

Table No.2: Calibration Data of Metformin and Acarbose Method.

S.No	Concentration Metformin (µg/ml)	Response	Concentration Acarbose(µg/ml)	Response
1	0	0	0	0
2	125	1723238	12.5	217306
3	250	3397299	25	422317
4	375	5171877	37.5	639921
5	500	6938530	50	841890
6	625	8722078	62.5	1069972
7	750	10228763	75	1254977

Table No.3: Precision Data for Metformin and Acarbose

S.No	Metformin	Acarbose
1	4939343	837201
2	5008315	837349
3	4991606	822464
4	5029965	835401
5	5018074	823672
6	5012675	824740
Mean	4999996	830138
Std. Dev.	32261.7	7203.1
% RSD	0.65	0.9

Table No.4: Intermediate Precision /Ruggedness Result for Metformin and Acarbose

S.No	Metformin	Acarbose
1	4906406	823213
2	4950898	827037
3	4965558	821195
4	4911756	827225
5	4948329	828159
Mean	4936589	825366
Std. Dev.	26026.3	3003.4
%RSD	0.53	0.4

Table No.5: Accuracy Results of Metformin and Acarbose

S.No	Sample	Amount added (µg/ml)	Amount Recovered (µg/ml)	Recovery (%)	% RSD
1	Metformin	250	249.8	99.93	0.41
		500	498.2	99.63	0.44
		750	749.5	99.93	0.15
2	Acarbose	25	25.1	100.21	1.04
		50	49.9	99.87	0.7
		75	74.9	99.83	0.55

Table No.6: Robustness Data of Metformin and Acarbose Method

S.No	Robustness condition	Metformin %RSD	Acarbose % RSD
1	Flow minus	0.1	0.1
2	Flow Plus	0.1	0.1
3	Mobile phase minus	0.1	0.0
4	Mobile phase Plus	0.3	0.2
5	Temperature minus	0.0	0.3
6	Temperature Plus	0.2	0.1

Table No.7: Results Data for Pharmaceutical Tablet Dosage form

S.No	Metformin % Assay	Acarbose %Assay
1	98.89	100.93
2	100.28	100.94
3	99.94	99.15
4	100.71	100.71
5	100.47	99.29
6	100.36	99.42
AVG	100.11	100.07
STDEV	0.65	0.87
% RSD	0.65	0.87

Table No.8: Degradation Data of Metformin

S.No	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.86	0.177	0.469
2	Alkali	6.96	0.176	0.503
3	Oxidation	5.83	0.175	0.469
4	Thermal	4.99	0.159	0.442
5	UV	1.83	0.173	0.474
6	Water	0.44	0.163	0.412

Table No.9: Degradation Data of Acarbose

S.No	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	7.56	0.305	0.503
2	Alkali	6.78	0.271	0.459
3	Oxidation	5.90	0.277	0.442
4	Thermal	4.81	0.451	0.485
5	UV	1.65	0.276	0.480
6	Water	0.86	0.357	0.504

Table No.10: Results Data of Validation Parameters As Per ICH

S.No	Parameters	Metformin	Acarbose
1	Calibration range (mcg / ml)	125-750ppm	12.5-75ppm
2	Optimized wavelength	215nm	215nm
3	Retention time	2.8min	4min
4	Regression equation (Y*)	y = 13779x + 1840	y = 16828x + 4143
5	Correlation coefficient(r2)	0.999	0.999
6	Precision (% RSD*)	0.65	0.9
7	% Recovery	99.83%	99.97%
8	Limit of Detection (mcg / ml)	0.4ppm	0.8ppm
9	Limit of Quantitation (mcg / ml)	1.3ppm	2.5ppm

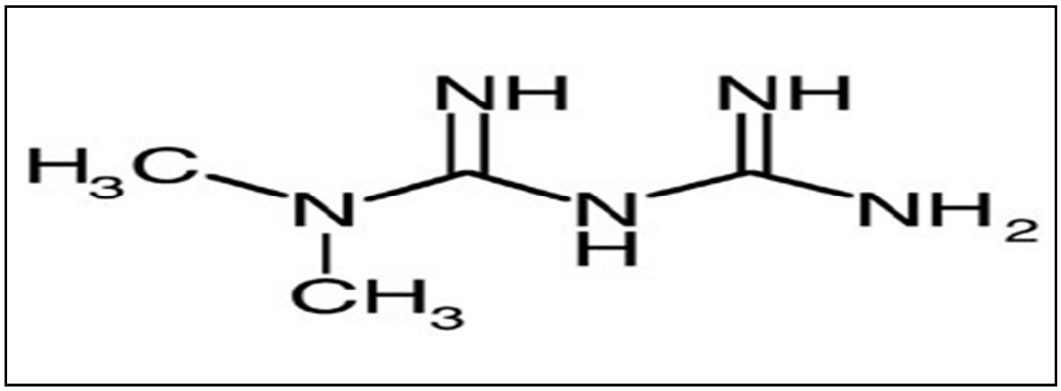

Figure No.1: Structure of Metformin

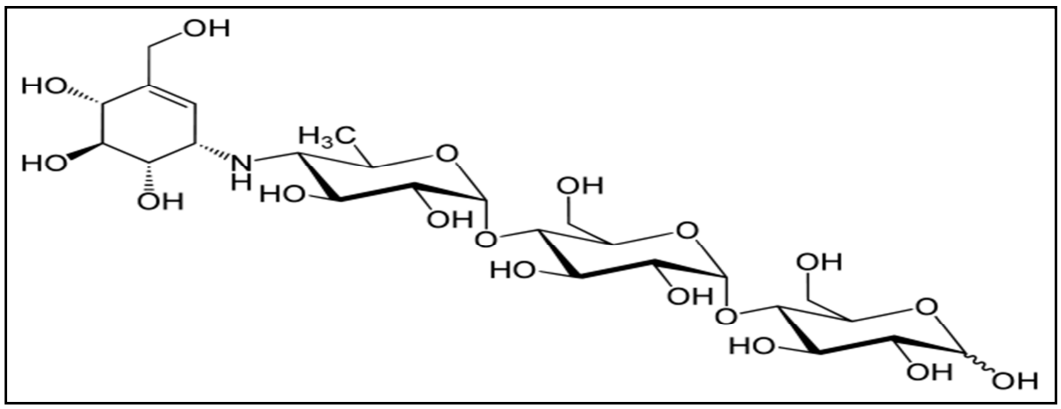

Figure No.2: Structure of Acarbose

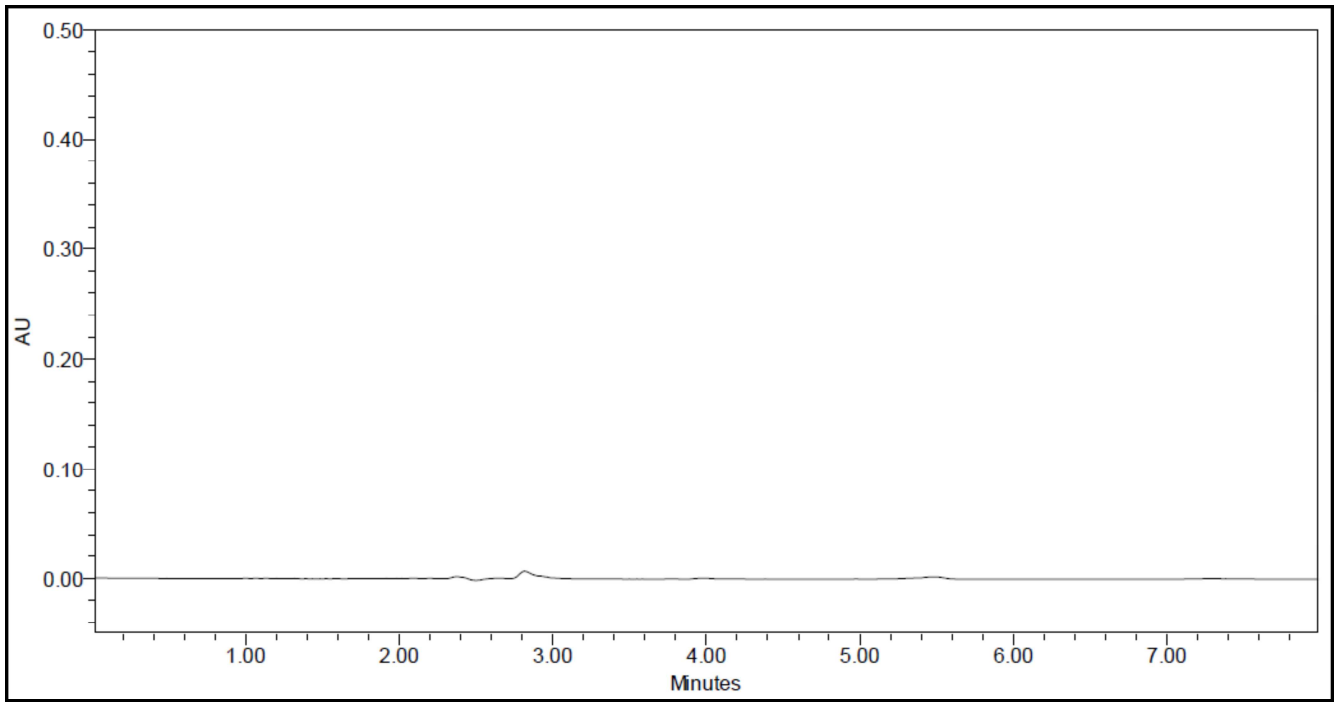

Figure No.3: Typical Chromatogram of Blank Solution

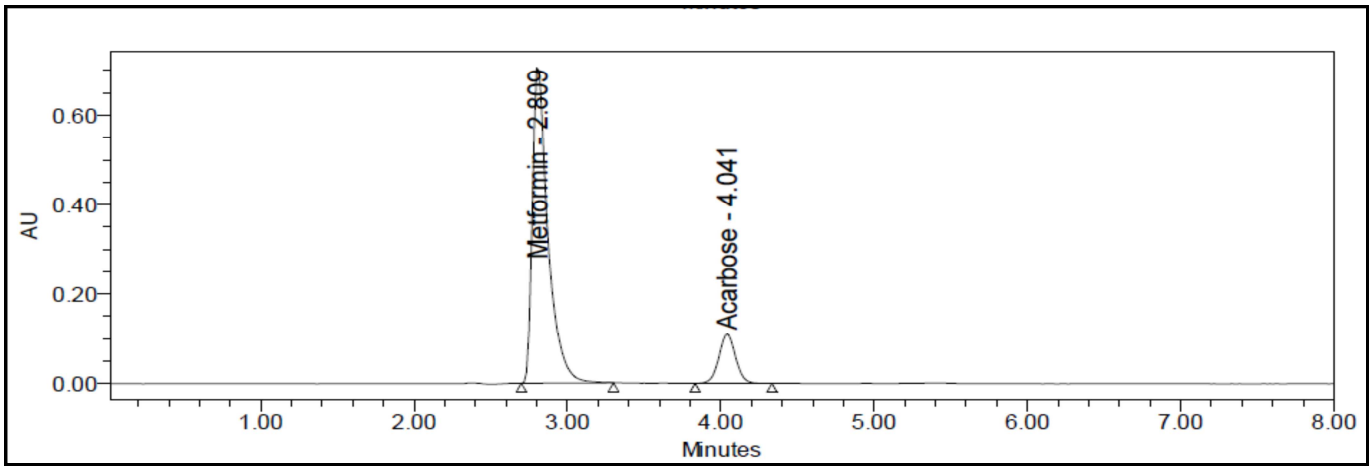

Figure No.4: Typical Chromatogram for the Sample

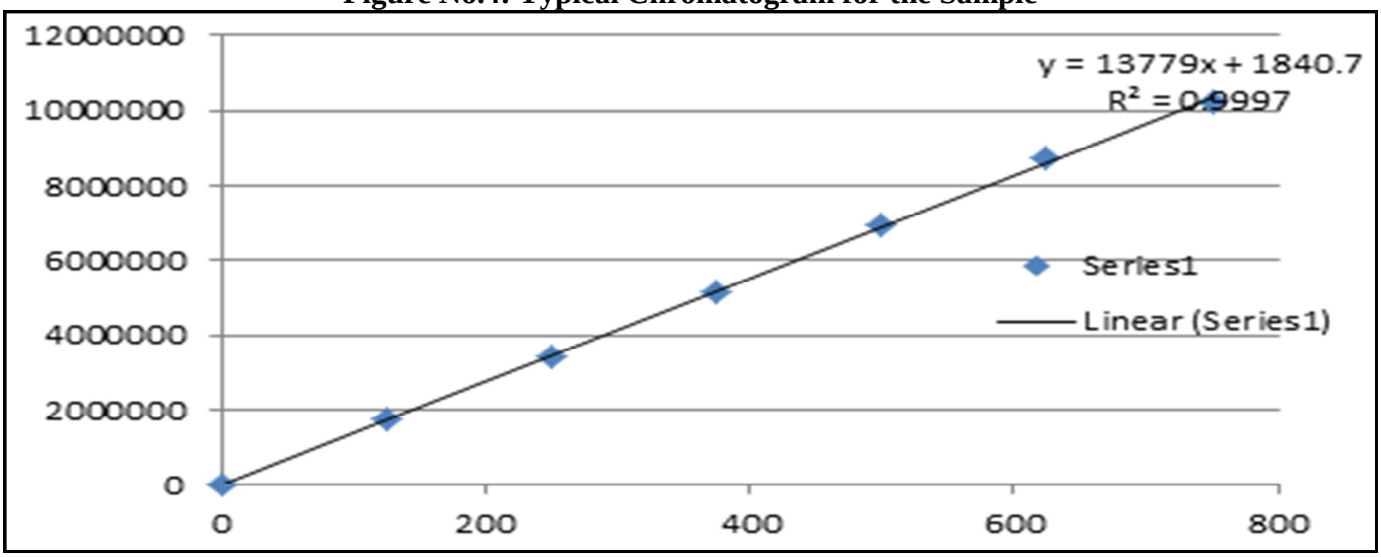

Figure No.5: Calibration Curve of Metformin

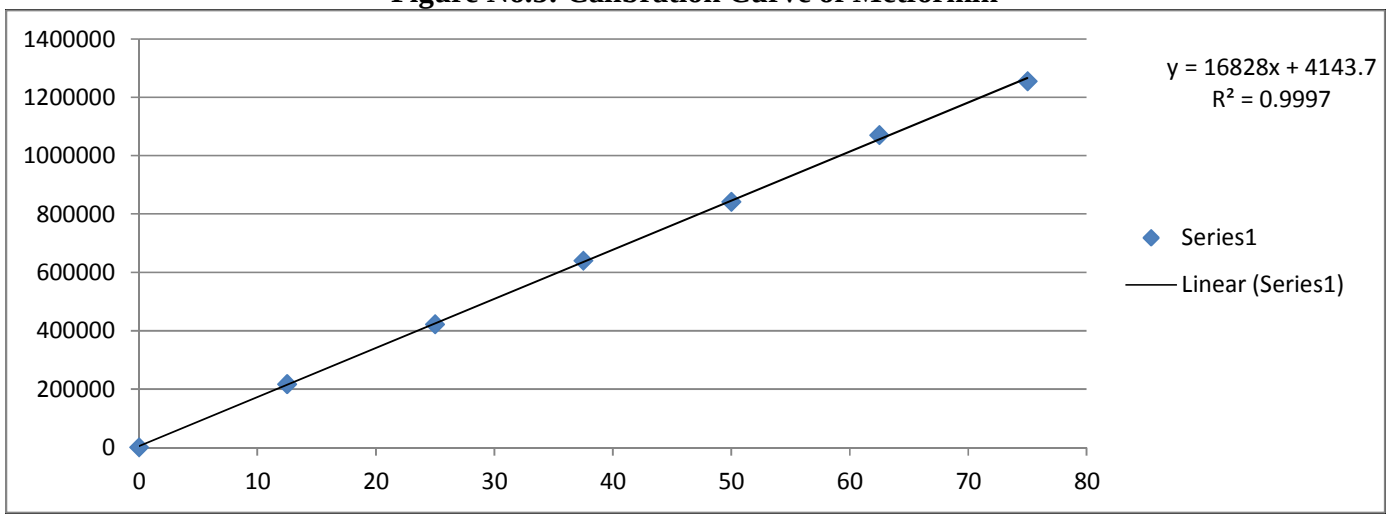

Figure No.6: Calibration Curve of Acarbose

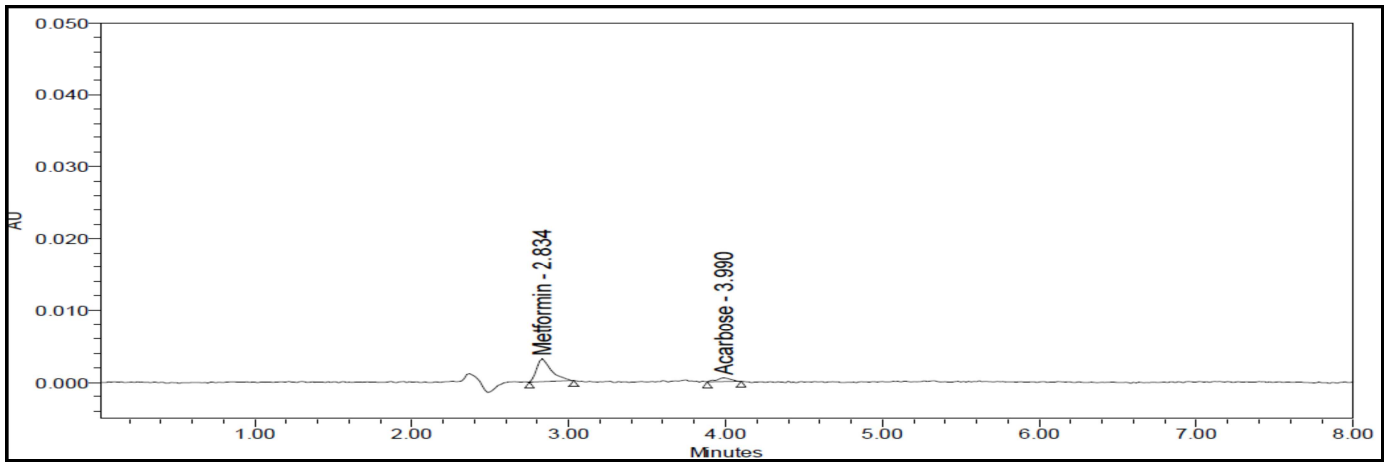

Figure No.7: Chromatogram of LOD

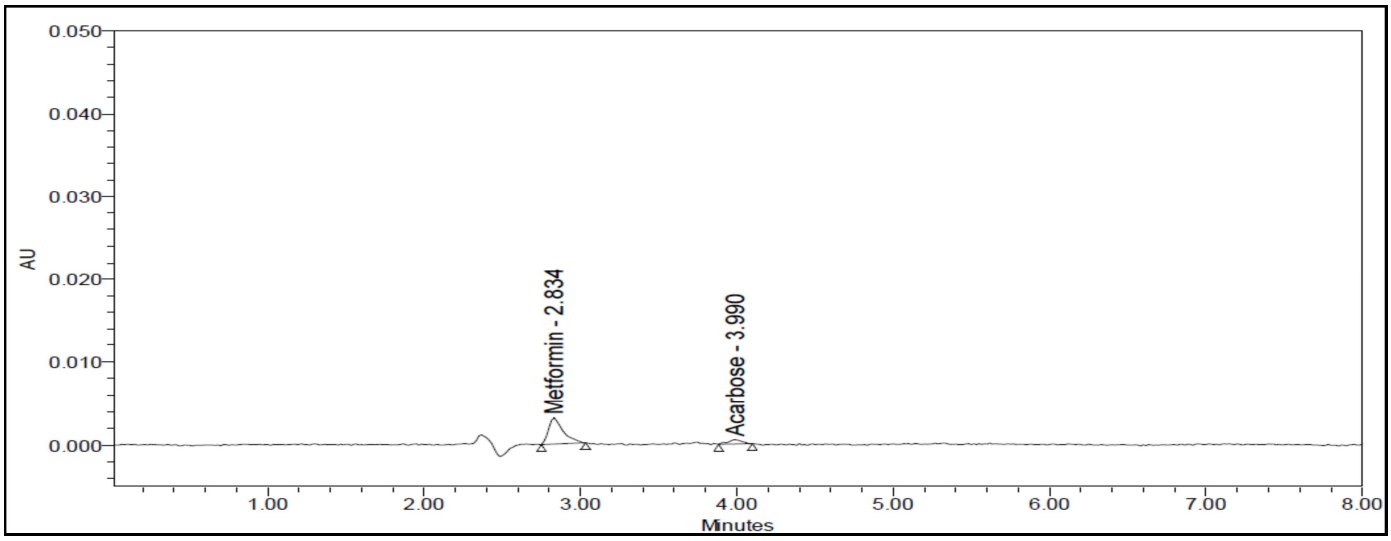

Figure No.8: LOQ Chromatogram of Metformin and Acarbose

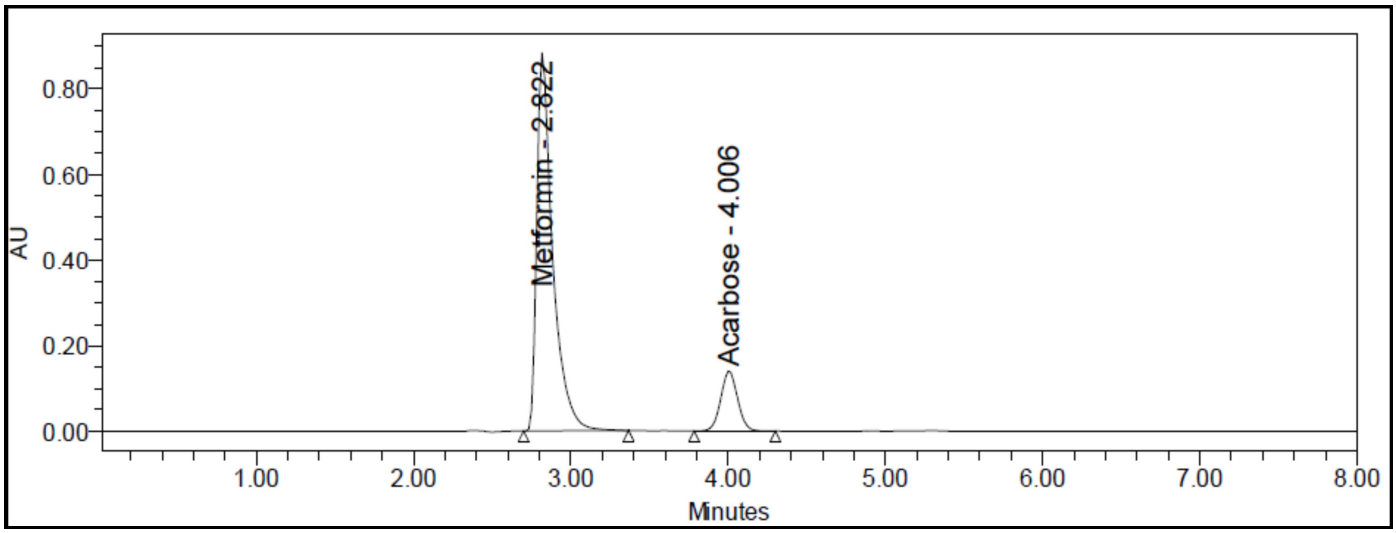

Figure No.9: Chromatogram for Sample (Tablet Formulation)

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Metformin and Acarbose in Tablet dosage form. Retention time of Metformin and Acarbose were found to be 2.8min and 4.0min. % RSD of the Metformin and Acarbose were found to be 0.65 and 0.9 respectively. % Recover was Obtained as 99.83% and 99.97% for Metformin and Acarbose respectively. LOD, LOQ values are obtained from regression equations of Metformin and Acarbose were 0.4ppm, 1.3ppm and 0.8ppm, 2.5ppm respectively. Regression equation of Metformin is y = 13779x + 1840, and of Acarbose is y = 16828x + 4143. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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