



STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF METFORMIN AND GLIPIZIDE

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ABSTRACT

A simple, specific and fast reverse phase High Performance Liquid Chromatographic method was developed for the simultaneous estimation of Metformin and Glipizide in a combined tablet dosage form. Optimum chromatographic separation was achieved within 8 minutes by use of Microsorb-MV C₁₈ 250×4.6mm, 5µm column as stationary phase with mobile phase consisting of Acetate buffer (pH 4.0): Acetonitrile (60:40 v/v) and at a flow rate of 1 ml/min. Detection was carried out at 257nm in HPLC. The method was validated in accordance with ICH guidelines. Response was a linear function of concentration over the range of 60-140 µg/mL for Metformin and 10-50 µg/mL for Glipizide in HPLC. Calibration curve was plotted and correlation co-efficient for the drug was found to be 0.998 for Metformin and 0.999 for Glipizide. The accuracy studies showed % recovery of Metformin in the range 100.42% and for Glipizide 100.39%. The developed RP-HPLC method can be applied successfully for the determination Metformin and Glipizide in a combined tablet dosage form. All the validation parameters were found within limits and the method was found to be economic, precise, accurate and specific.

KEY WORDS

RP HPLC, Metformin, Glipizide, Validation, ICH guidelines.

INTRODUCTION

High Performance Liquid Chromatography is a technique used to separate, identify, and quantify each component in a mixture. It was developed by CsabaHorvat (1964), Kirkland & Huber in 1969. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. It works on the principle of hydrophobic interactions hence the more nonpolar the material is, the longer it will be retained.

Few analytical methods¹ have been reported for the single and simultaneous determination of Metformin and Glipizide in combined pharmaceutical dosage forms using Spectrophotometry, HPLC and different mathematical approaches. The main aim of present work to develop and validate a precise and accurate RP-HPLC method for the simultaneous determination of

Metformin and Glipizide in pharmaceutical solid dosage forms.

Metformin² is chemically 1, 1-Dimethylbiguanide. It consists of Molecular Formula C₄H₁₁N₅, Molecular Weight 129.1636 g/mol and pKa Value 12.4. It comes under the category of Biguanide anti Hyperglycaemic agent used for treating non-insulin dependent diabetes mellitus (NIDDM). Its pharmacologic mechanisms of action are different from other classes of oral antihyperglycemic agents. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.

Glipizide³, is chemically N- [2-(4- {[cyclohexyl carbamoyl] amino} sulfonyl) phenyl] ethyl]-5-methylpyrazine-2-carboxamide. It consists of Molecular formula C₂₁H₂₇N₅O₄S, Molecular weight 445.535 and pKa Value 5.9. It comes under the category of second-

generation sulfonylurea, is used with diet to lower blood glucose in patients with diabetes mellitus type II.

The main mechanism of action is Sulfonylurea likely bind to ATP-sensitive potassium channel receptors on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. Depolarization stimulates calcium ion influx through voltage-sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion, or exocytosis, of insulin.

MATERIALS AND METHODS

Instrumentation

High Performance Liquid Chromatography⁴ (Agilent 1260 infinity) is equipped with UV/PDA detector by using column Microsorb MVC₁₈ (250×4.6 mm, 5μ). Data processing was carried out by open lab software, Electronic balance (Infra), pH meter (Systronics), ultra sonicator (ANM industries), Syringe (Hamilton).

Reagents and chemicals

Acetonitrile (HPLC grade), Glacialaceticacid (AR grade), Triethylamine (AR grade), Water (HPLC grade). The solvents were filtered through 0.45μ membrane filter and Sonicate before use.

Solution Preparation

Preparation of Standard solution

Standard stock solutions of Metformin and Glipizide (μg/mL) were prepared by dissolving 500 mg of Metformin and 5 mg of Glipizide dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50 ml with mobile phase. Further dilutions of 140μg/mL of Metformin and 50 μg/mL of Glipizide were made by adding 1 ml of stock solution to 50 ml of mobile phase.

Preparation of sample solution

20 tablets (each tablet contains 500 mg of Metformin and 5 mg of Glipizide) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of Metformin and Glipizide (μg/mL) were prepared by dissolving weight equivalent to 500 mg of Metformin and 5 mg of Glipizide and dissolved in sufficient mobile phase. After that filtered the solution using 0.45 μ syringe filter and Sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 140μg/mL of Metformin and 50 μg/mL of Glipizide was

made by adding 1 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

Method Development

Column: Microsorb MV C₁₈ (250 × 4.6 mm, 5μ), Flow rate: 1.0 mL /min, Run time: 8min, Wavelength: 257nm, Column temperature: 25°C, Sample temperature: ambient, Injection Volume:20 μL, Run time: 8minutes as Shown in and Fig no.3.

Method Validation

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Standard sample

Standard stock solutions of Metformin and Glipizide were prepared by dissolving 50 mg of Metformin and Glipizide each dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron filter paper and Sonicated for 5min and dilute to 50 ml with mobile phase. 1ml from the resulting solution was transferred to 100ml volumetric flask and diluted to obtain 100μ/mL using mobile phase. The obtained is labelled as stock-2. Further dilutions of Metformin and Glipizide were made by from stock solution using mobile phase. It is observed from the above data, diluent are not interfering with the Metformin and Glipizide peaks as shown in fig no. 4(a) and 4(b).

System suitability

The main purpose of the system suitability is to ensure the system including instrument, analyst, chemicals and electronics are suitable to the intended application. One standard injection and five replicate system suitability solution injections were injected. All the system suitability parameters are within range and satisfactory as per ICH guidelines as shown in Table No. 1.

Linearity and Range

Preparation of calibration standards solution

Five calibration standards for Metformin (60-140 μg/mL) and Glipizide (10-50 μg/mL) were prepared by using standard stock solution. Regression equation of the Metformin and Glipizide are found to be, $y = 6508x + 13439$ and $y = 16236x + 1436$. The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Metformin and Glipizide is 0.998 and 0.999. The

relationship between the concentration of Metformin and Glipizide and area of Metformin and Glipizide is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits as shown in table no. 2(a) and 2(b) and fig no. 5(a) and 5(b).

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 80%, 100%, 120%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%. The percentage mean recovery of Metformin and Glipizide is 100.42% and 100.39% respectively as shown in table no. 3(a) and 3(b) and fig no. 6(a) - 6(i).

Method precision

Prepared sample preparations of Metformin and Glipizide as per test method and injected 6 times in to the column. Test results for Metformin and Glipizide are showing that the %RSD of Assay results are within limits. The results were shown in Table no. 4(a) and 4(b) and Fig no. 7(a) – 7(f).

Limit of Detection

The detection limit of an individual analytical procedure is the lowest amount of the analyte in a sample which can be detected but not necessarily quantitated as an exact value. The LOD for this method was found to be 0.287 µg/mL & area 45865.92 for Metformin and 0.065 µg/mL & area 16937.03 for Glipizide as shown in table no.5.

Limit of Quantification

The detection limit of an individual analytical procedure is the lowest amount of the analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOQ for this method was found to be 0.870 µg/mL & area 47471.05 for Metformin and 0.196 µg/mL & area 17383.08 for Glipizide.

Robustness

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 as shown in fig no. 8(a) -8(f) and Table No.6.

Assay

The amount of Metformin and Glipizide present in the taken dosage form was found to be 98.99 % and 99.28 % respectively as shown in Table No.7 And Fig No.9.

Stability indicating 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 as shown in Table No.8 (a) and 8 (b).

Acid Degradation Studies

To 1ml of stock solution Metformin and Glipizide, 1ml of 2N hydrochloric acid was added and refluxed for 30minutes at 60°C. The resultant solution was diluted to obtain 140 µg/mL & 50 µg/mL solution and 20µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample as shown in Fig No. 8(a).

Alkali Degradation Studies

To 1ml of stock solution Metformin and Glipizide, 1ml of 2N sodium hydroxide was added and refluxed for 30minutes at 60°C. The resultant solution was diluted to obtain 500µg/mL & 50 µg/mL solution and 20 µl were injected into the system and the chromatograms were recorded to assess the stability of sample as shown in Fig no. 8(b).

Oxidative Degradation studies

To 1 ml of stock solution of Metformin and Glipizide, 1 ml of 20% hydrogen peroxide (H₂O₂) was added separately. The solutions were kept for 30 minutes at 60°C. For HPLC study the resultant solution was diluted to obtain 500µg/mL & 50 µg/mL solution and 20 µl were injected into the system and the chromatograms were recorded to assess the stability of sample as shown in Fig no. 8(c).

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 6 hours to study dry heat degradation. For HPLC study the resultant solution was diluted to 500µg/mL & 50 µg/mL solution and 20µl were injected into the system and the chromatograms were recorded to assess the stability of the sample as shown in Fig no. 8(d).

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at temperature of 60°C. For HPLC study the resultant solution was

diluted 500µg/mL & 50 µg/mL solution and 20µL were injected into the system and the chromatograms were recorded to assess the stability of the sample as shown in Fig no. 8(e).

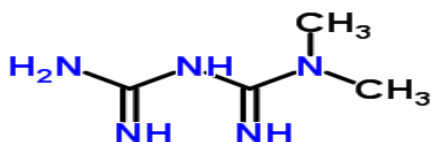


Fig no: 1 Structure of Metformin

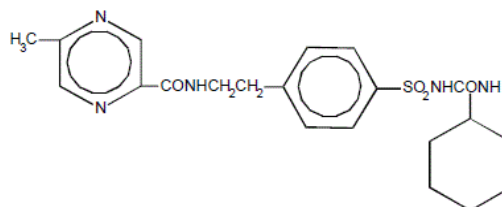


Fig no: 2 Structure of Glipizide

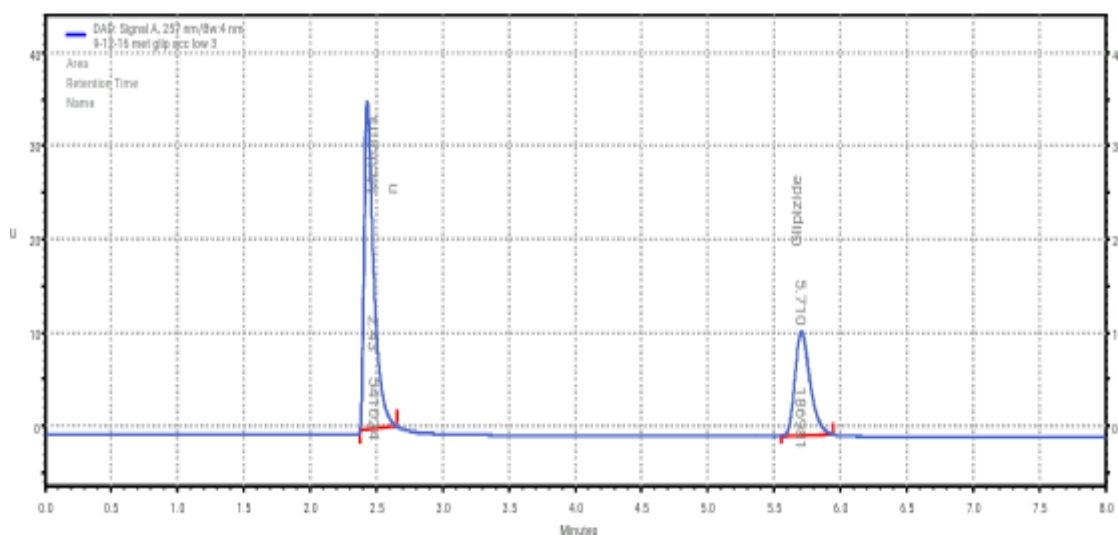


Fig no: 3 chromatograms of Metformin and Glipizide

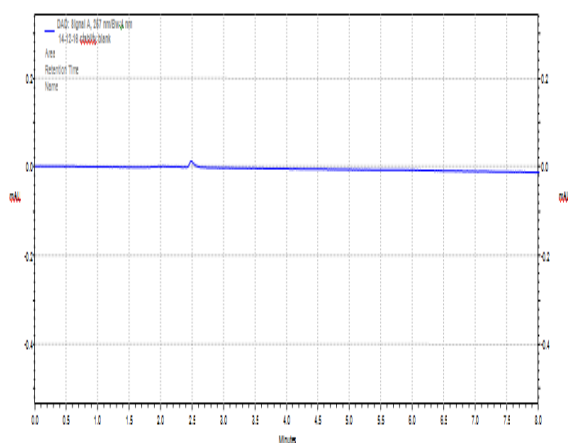


Fig no: 4(a) Blank chromatogram

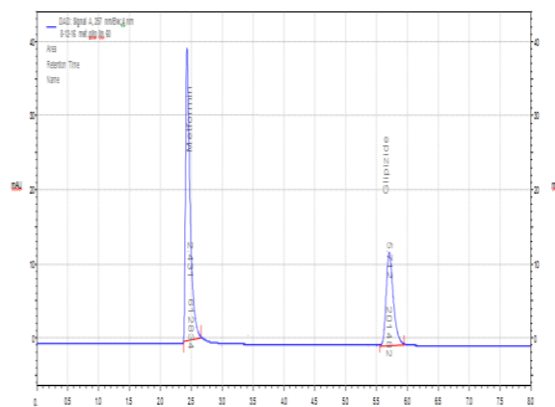


Fig no: 4(b) Standard chromatograms

Table No: 1 System suitability

Property	Metformin	Glipizide
Retention time (tr)	2.431	5.712
Theoretical plates(N)	10205	11067
Tailing factor (T)	1.27	1.25

Table No: 2 Linearity

Table no: 2(a) Linearity of Metformin

S. No.	Concentration (µg/ml)	Area
1	60	617813
2	80	672456
3	100	721269
4	120	786922
5	140	836059

Table no.:2(b) Linearity of Glipizide

S. No.	Concentration (µg/ml)	Area
1	10	202048
2	20	234061
3	30	269332
4	40	303857
5	50	337429

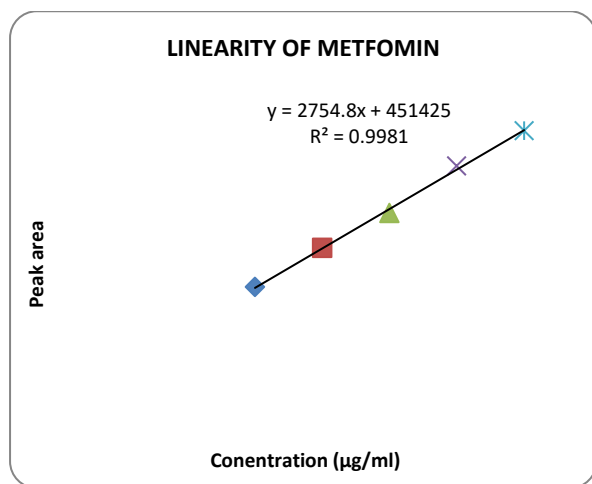


Fig no: 5(a) Linearity graphs of Metformin

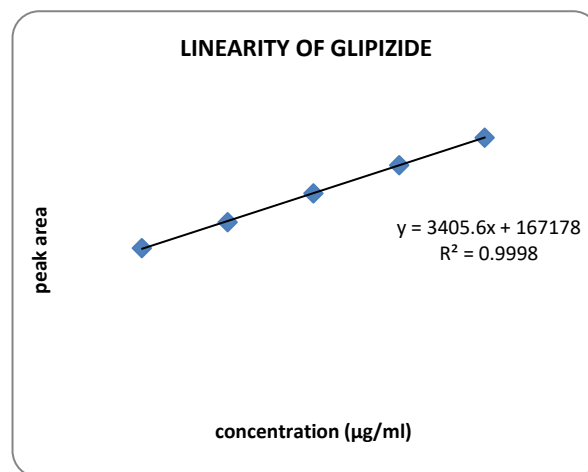


Fig no: 5(b) Linearity graphs of Glipizide Accuracy

Accuracy

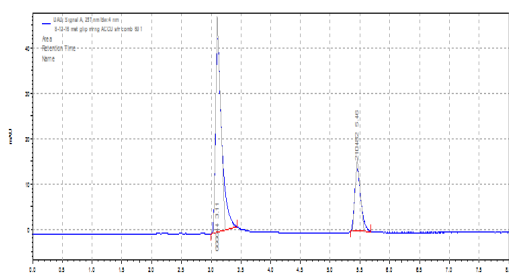


Fig no: 6(a) Chromatogram of 80% recovery (injection 1)

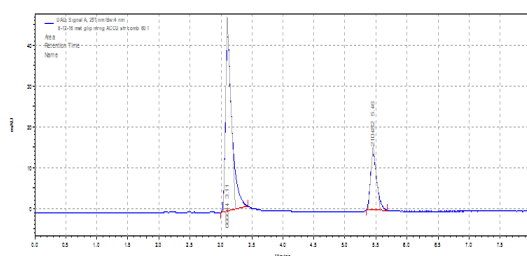


Fig no: 6(b) Chromatogram of 80% recovery (injection 2)

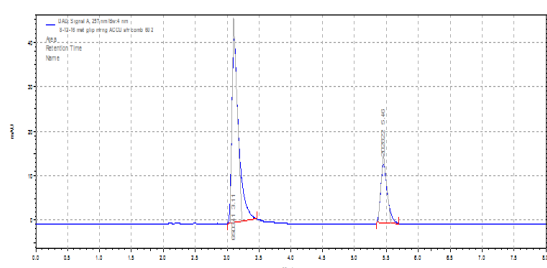


Fig no: 6(c) Chromatogram of 80% recovery (injection 3)

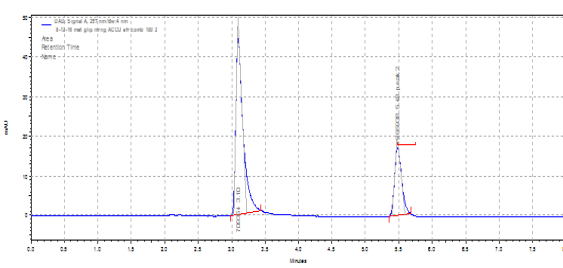


Fig no: 6(d) Chromatogram of 100% recovery (injection 1)

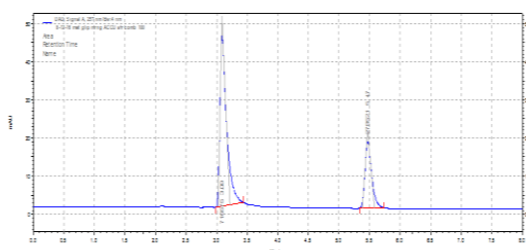


Fig no: 6(e) Chromatogram of 100% recovery (injection 2)

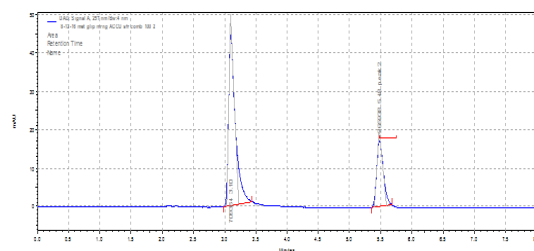


Fig no: 6(f) Chromatogram of 100% recovery (injection 3)

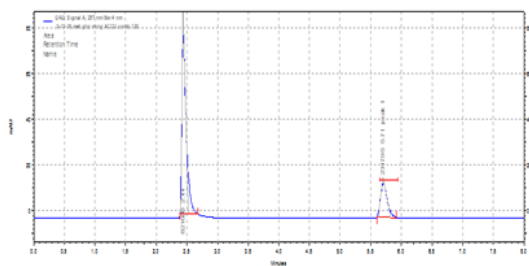


Fig no: 6(g) Chromatogram of recovery 180% (injection 1)

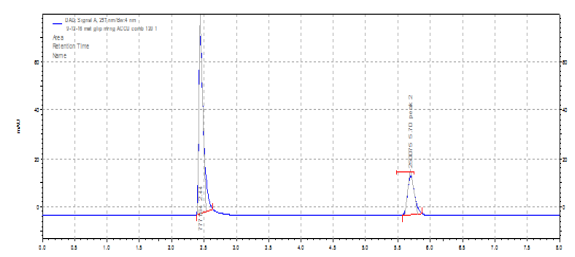


Fig no: 6(h) Chromatogram of recovery 180% (injection 2)

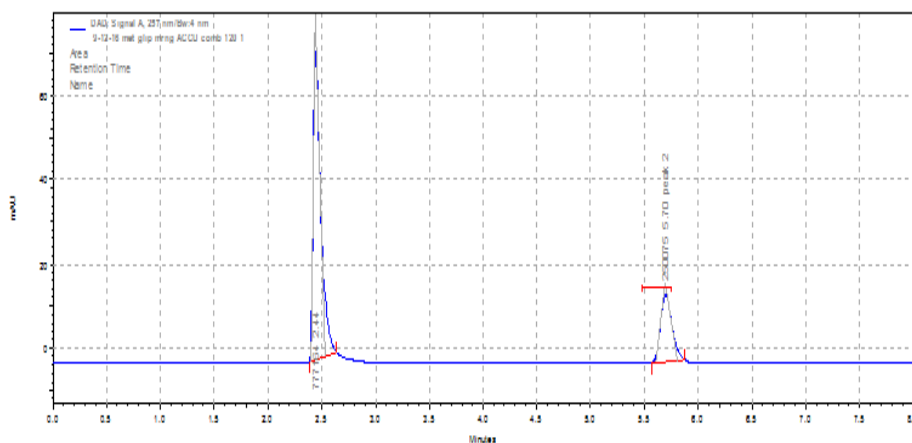


Fig no: 6(i) Chromatogram of recovery 180% (injection 3)

Table no: 3(a) Recovery results of Metformin

Recovery level	Amount taken (µg/ml)	Area	Average area	Amount recovered (µg/ml)	%Recovery	Mean % Recovery
80%	80	539581	541146	80	100.87	100.42
	80	542834				
	80	541024				
100%	100	596575	586936	100	100.22	
	100	564879				
	100	599354				
120%	120	651605	654468	120	100.17	
	120	657164				
	120	654636				

Table no: 3(b) Recovery results for Glipizide

Recovery level	Amount taken (µg/ml)	Area	Average area	Amount recovered (µg/ml)	%Recovery	Mean% Recovery
80%	80	186679	187023	20	99.58	100.39
	80	187409				
	80	186981				
100%	100	201598	201314	30	100.80	
	100	200949				
	100	201395				
120%	120	217865	218786	40	99.50	
	120	219340				
	120	219153				

Precision

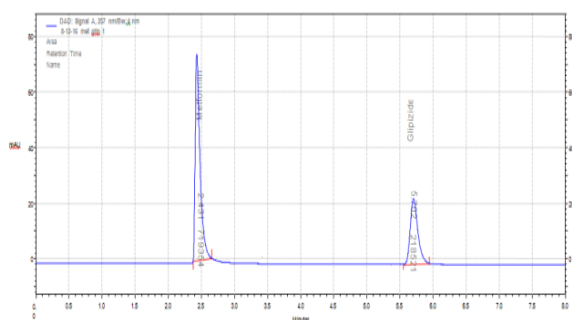


Fig no: 7(a) Chromatogram of Precision (injection 1)

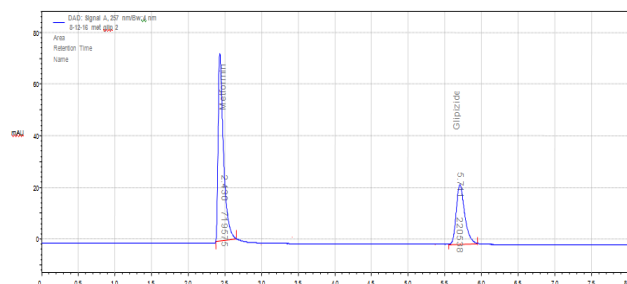


Fig no: 7(b) Chromatogram of Precision (injection 2)

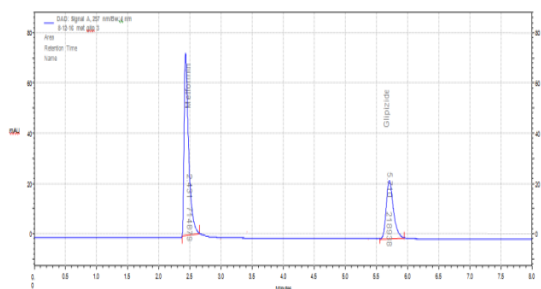


Fig no: 7(c) Chromatogram of Precision (injection 3)

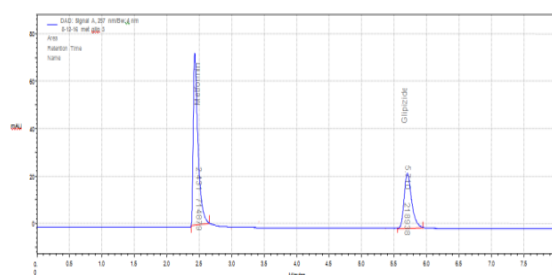


Fig no: 7(d) Chromatogram of Precision (injection 4)

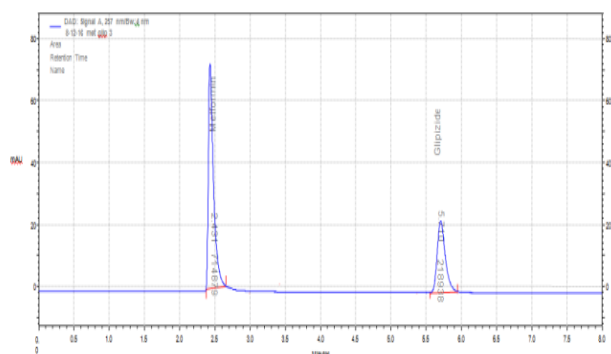


Fig no: 7(e) Chromatogram of Precision (injection 5)

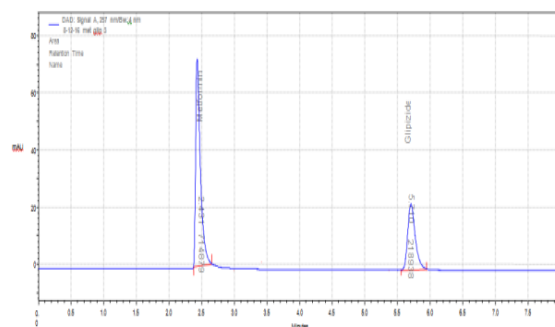


Fig no: 7(f) Chromatogram of Precision (injection 6)

Table no: 4(a) Results for method Precision of Metformin

S. No.	Concentration (µg/ml)	Inter-day	Intra-day	Standard Metformin
1.	100	719354	715899	723456
2.	100	719575	716892	703877
3.	100	714879	719354	733478
4.	100	720046	720101	71756
5.	100	719678	714879	720158
6.	100	715899	717681	721098
7.	AVG	718238.5	717467.7	720137
8.	STD	2241.843	2001.575	9552.139
9.	%RSD	0.312131	0.278978	1.3264

Table no: 4(b) Results for method Precision of Glipizide

S. No.	Concentration (µg/ml)	Inter-day	Intra-day	Standard Glipizide
1.	30	218521	219871	221056
2.	30	220538	219396	219984
3.	30	218938	218521	220571
4.	30	220456	220945	221984
5.	30	219871	219887	218057
6.	30	219936	219680	219783
7.	AVG	219710	219266.7	220239.2
8.	STD	815.9414	1368.826	1330.926
9.	%RSD	0.371372	0.624275	0.604309

Limit of Detection

Table no: 5 Limit of detection of Metformin and Glipizide

S. No.	Metformin Concentration (µg/ml)	Peak Area	Glipizide Concentration (µg/ml)	Peak Area
1	60	617813	10	202048
2	80	672456	20	234061
3	100	721269	30	269332
4	120	786922	40	303857
5	140	836059	50	337429
S.D.	239.57	726903.8	66.576	2153434
Slope	2754.33		3405	

Robustness

Table no: 6 Robustness data of Metformin and Glipizide

S. No.	Robustness condition	Metformin %RSD	Glipizide %RSD
1.	Flow minus	0.7	0.9
2.	Flow plus	1.2	1.4
3.	pH minus	0.7	1.3
4.	pH plus	0.8	0.6
5.	Mobile phase minus (10 ml)	0.9	1.2
6.	Mobile phase plus (10 ml)	1.2	0.7

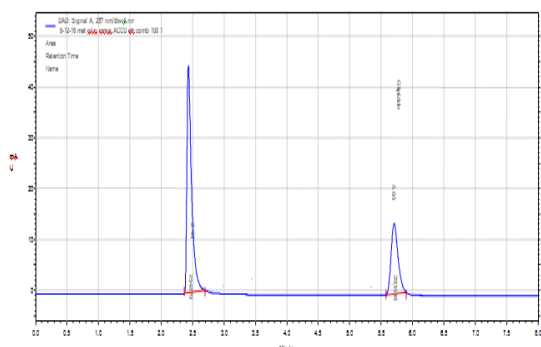


Fig no: 8(a) Mobile phases plus

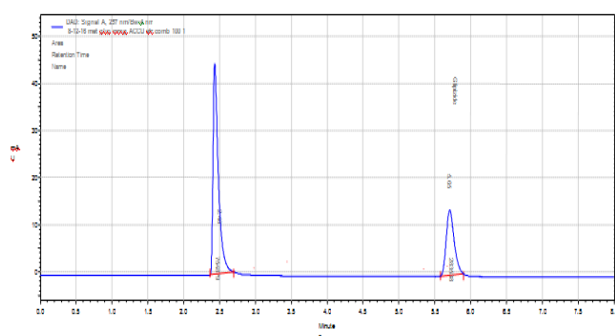


Fig no: 8(b) Mobile phase minus

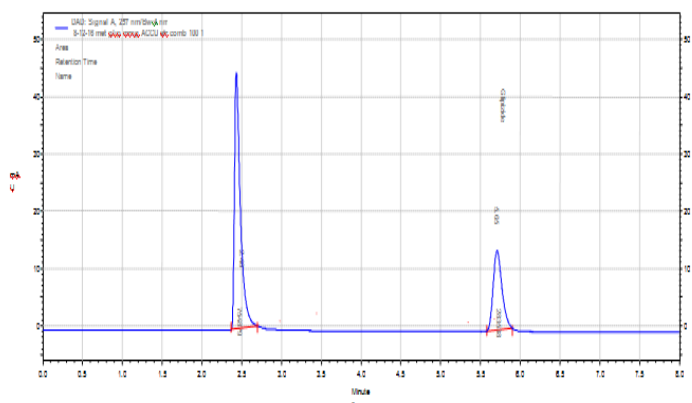


Fig no: 8(c) flow minus

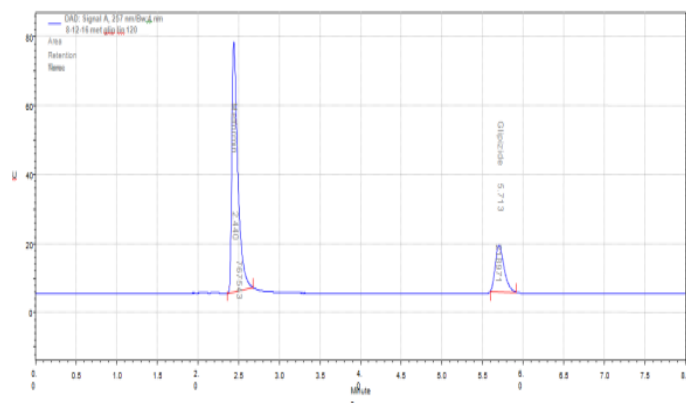


Fig no: 8(d) flow plus

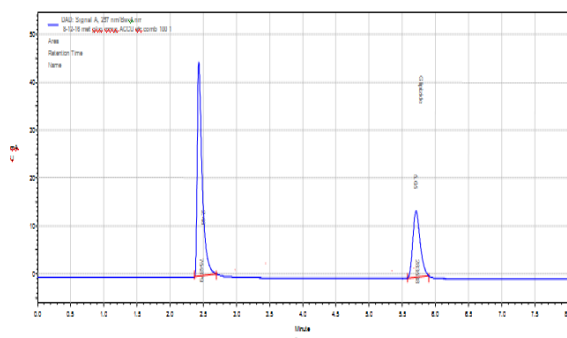


Fig no: 8(e) pH minus

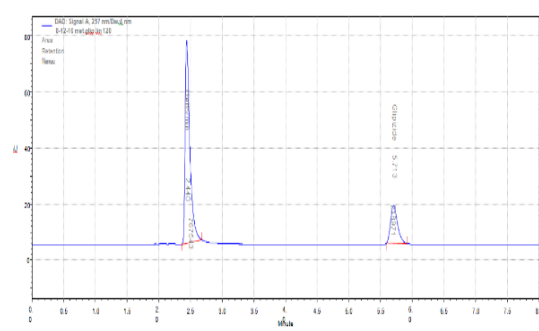


Fig no: 8(f) pH plus

Assay

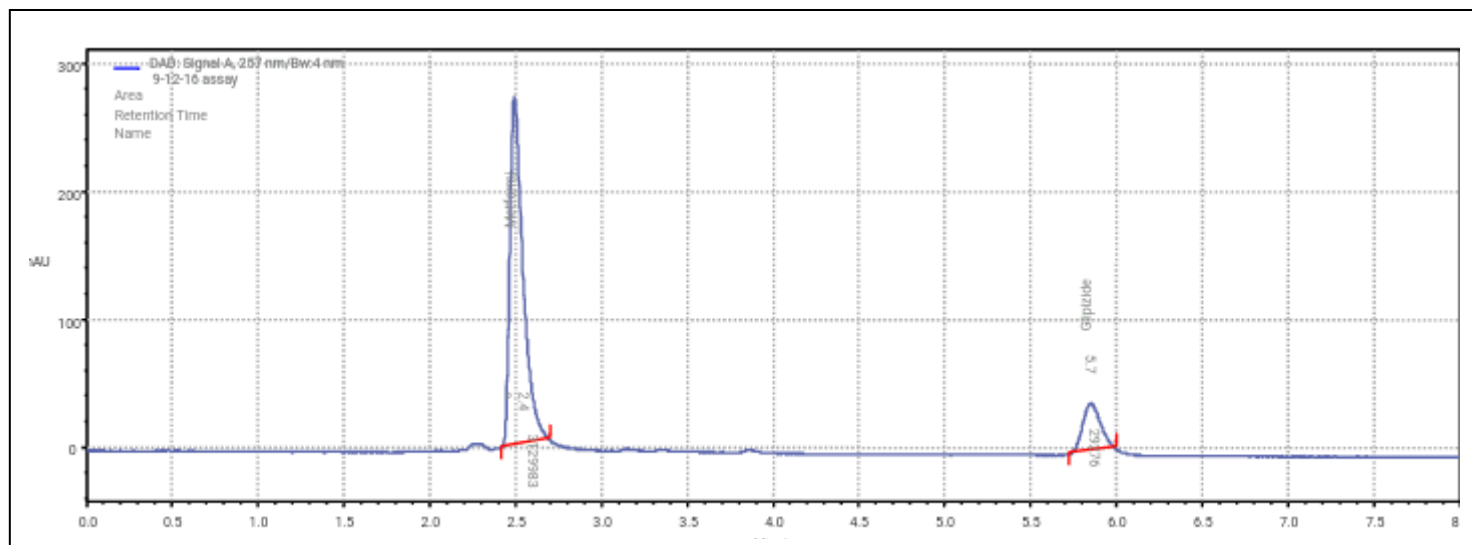


Fig no: 9 Chromatogram of assay

Table no: 7 Assay Results

S. No.	Metformin %Assay	Glipizide %Assay
1	99.65	99.46
2	98.41	100.01
3	99.39	100.97
4	98.16	98.33
5	98.77	98.39
6	99.59	98.51
AVG	98.99	99.28
STD DEV	0.68	1.07
%RSD	0.64	1.08

Stability indicating Studies

Table no: 8(a) Degradation data of Metformin

S. No.	Degradation Condition	Area	% Assay	Amount Degraded %
1.	Acid	1383287	95.00	5.00
2.	Alkali	1087654	98.91	1.09
3.	Oxidative	1035678	97.16	2.84
4.	Thermal	1399673	96.12	3.88
5.	Neutral	1414765	97.85	2.15

Table no: 8(b) Degradation data of Glipizide

S. No.	Degradation Condition	Area	% Assay	Amount degraded %
1.	Acid	283176	95.58	4.42
2.	Alkali	263176	96.12	3.88
3.	Oxidative	250591	95.98	4.02
4.	Thermal	321386	98.58	1.42
5.	Neutral	324896	97.22	2.78

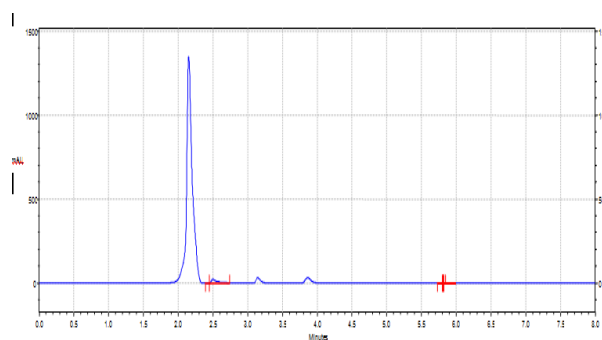


Fig no: 8(a) Acid Degradation

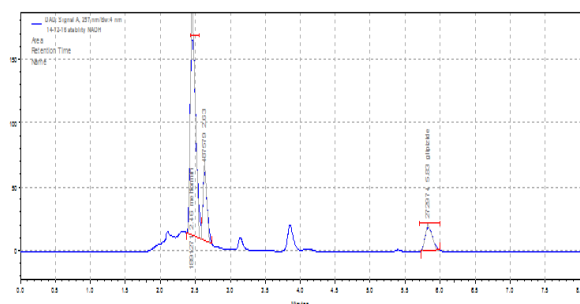


Fig no: 8(b) Alkali degradation

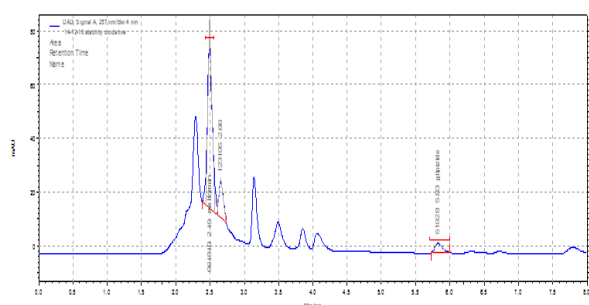


Fig no: 8(c) Oxidative Degradation

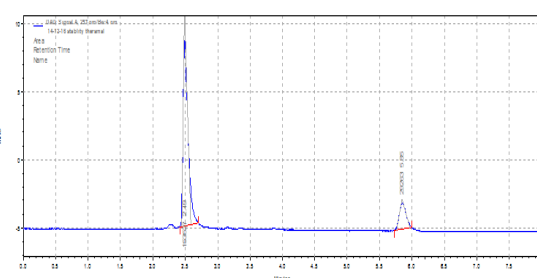


Fig no: 8(d) Thermal degradation

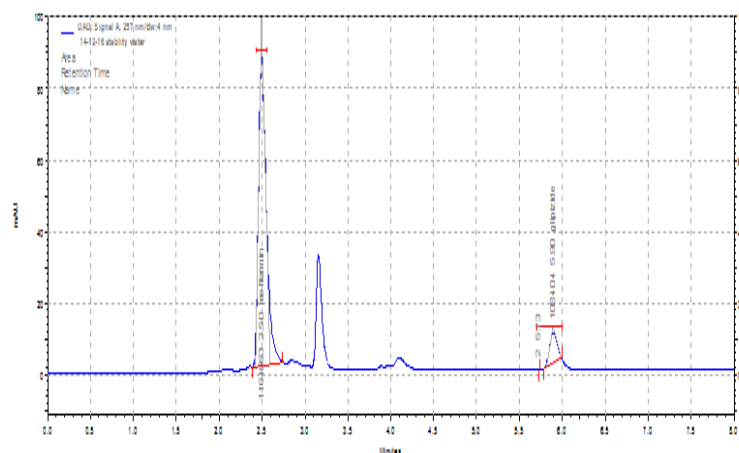


Fig no: 8(e) Neutral Degradation

SUMMARY AND CONCLUSION

Parameter	Metformin	Glipizide
Calibration range	60 - 140 µg/ml	10-50µg/ml
Optimized wavelength	257 nm	257 nm
Retention time	2.433 min.	5.710 min.
Regression equation	$y = 2754.x + 45142$	$y = 3405.x + 16717$
Correlation Coefficient (r^2)	0.998	0.999
Precision (%RSD)	0.37	0.31
% Assay	98.99	99.29
LOD	0.287 µg/ml	0.065 µg/ml
LOQ	0.870 µg/ml	0.196 µg/ml

From all the above validation conclusions, it is very clear that the Reverse Phase HPLC isocratic method developed and validated as per ICH guidelines is sensitive, accurate, precise, linear and convenient for intended applications in any pharmaceutical industries.

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Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

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