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NOVEL RP-HPLC METHOD FOR METFORMIN HCl, GLIPIZIDE AND REPAGLINIDE PHARMACEUCTIAL DRUG PRODUCTS

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ABSTRACT

Novel RP-HPLC method has been developed for simultaneous determination of Meformin HCl, Glipizide and Repaglinide in dosage forms. The separation was achieved on a 3.5-micron C18 column (150 X 4.6 mm) using mobile phase consisting of buffer (1.0gm of Potassium dihydrogen phosphate in 1000mL, pH 3.0 with diluted orthophospharic acid .The flow rate was maintained at 1.0 ml/min. The detection of the constituents was done using UV detector at 210 nm. The retention time of metformin, glipizide and repaglinide were approximately 1.49, 3.71 and 9.84 min respectively. Recovery study values of three actives were 103% to 99% respectively, relative standard deviation of less than 2%. Linear response obtained for three actives correlation coefficient is not less than 0.999. The proposed method was applied for regular analysis and results found to be satisfactory.

Key words: Metformin hydrochloride, Glipizide, Repaglinide, RP-HPLC and dosage forms.

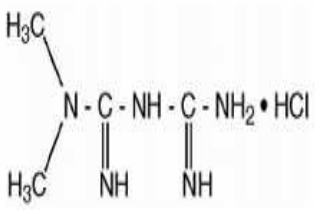
INTRODUCTION

Metformin hydrochloride [1-10] chemically, N, N-dimethylimidodicarbonimidic diamide hydrochloride is an antidiabetic agent. It is an oral biguanidine, which reduces the elevated blood glucose concentration in patients with diabetes but does not increase insulin secretion. It does not lower the blood glucose in nondiabetic subjects. Augmentation of muscular glucose uptake and utilization and reduction of increased hepatic glucose production through an antigluconergic action explain the blood glucose lowering effect. Metformin is safe and not teratogenic in many of the species studied. Oral bioavailability of metformin is about 50 - 60% and fecal recovery is about 30%. The rate of absorption was slower than that of elimination, which resulted in a plasma concentration profile of "flip-flop" type for oral metformin.

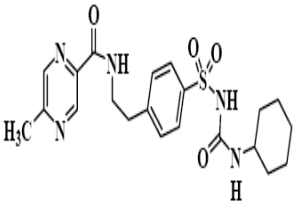
Glipizide [11-17] chemically is *N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl] phenethy)-5 methylpyrazine-2-carboxamide. It has antidiabetic properties, appears to lower blood glucose acutely by stimulating the release of insulin from the pancreas, an effect dependent upon functioning beta cells in the pancreatic islets. Mechanism of action is produced by blocking potassium channels in the beta cells of the islets of Langerhans. By partially blocking the potassium channels, it will increase the time the cell spends in the calcium release stage of cell signaling leading to an increase in calcium. The increase in calcium will initiate more insulin release from each beta cell. Glipizide comes as tablets and extended-release tablets to take by mouth. The regular tablet is usually taken one or more times a day, 30 minutes before breakfast or meals. The extended-release tablet is usually taken once a day with breakfast.

Repaglinide [18-19] is an oral antidiabetic drug. It is a meglitinide antidiabetic used in the management of type 2 diabetes mellitus, chemically *S*(+)2-ethoxy-4(2((3- methyl-1-(2-(1-piperidinyl) phenyl)-butyl) amino)-2-oxoethyl) benzoic acid. It works by stimulating the body to produce more insulin. Insulin is a natural substance that allows the body to properly use sugar from the diet.This medication should not be used to treat people with type 1 diabetes (insulin-dependent diabetes). Repaglinide is used alone or with other medications to control high blood sugar along with a proper diet and exercise program. It is used in people with type 2 (non-insulin-dependent) diabetes. Controlling high blood sugar helps prevent kidney damage, blindness, nerve problems, loss of limbs, and sexual function problems. Proper control of diabetes may also lessen your risk of a heart attack or stroke.

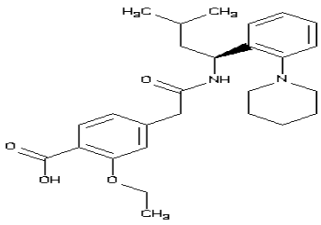
Figure-1 represents the chemical structrue of the three active ingredients and table-1 represents the available dosage forms.



Metformin HCl



Glipizide



Repaglinide

Figure-1: Chemical structure of three actives

Table-1: Available tablet dosage forms

Composition
Metformin HCl-250mg,500mg and 1000mg (SR)
Metformin HCl-250mg and Glipizide-2.5mg
Metformin HCl-500mg and Glipizide-5mg
Metformin HCl-400mg and Glipizide-2.5mg
Repaglinide-0.5mg, 1mg and 2mg
Metformin HCl-500mg and Repaglinide-1mg/ 2mg

Literature survey reveals that Spectrophotometric HPLC, RP-HPLC methods are available for individual and other combination products determination of Metformin, Glipizide and Repaglinide in pharmaceutical preparations and biological formulation so far, no single method has been reported for estimation of three actives in combined dosage forms, hence single RP-HPLC have been developed for simultaneous estimation.

MATERIALS AND METHODS

Materials:

All the chemicals and solvents used were of AR grade. The pure drugs of three actives were used as reference standards. Waters make Alliance HPLC equipment used for this study. All market samples were procured and analysed with the proposed method.

Chromatographic conditions: The following Chromatographic conditions were established for separation of three active igredients.

Waters HPLC:

Column Zodiac C18, 150mm x 4.6mm, 3.5µ

Buffer	Weighed accurately 1gm of Potassium di-hydrogen orthophosphate in to 1000mL of water and adjusted the pH to 3.0 with orthophospharic acid.
Mobile phase	Sol-A: Buffer; Sol-B Acetonitrile and gradient program: (0-4min, sol-A: 45-45; 4-8min- sol-A:45-30; 8-10min- sol-A:30-20; 10-12min- sol-A:20-45 and 120-16min-sol-A:45-45)
Detection	wavelength 210 nm
Sample size	10µL
Temperature	35°C
Runtime	16min

Standard Solution: Weighed and transferred 40mg of each active ingredient standard in to 100ml of Class-A volumetric flask and 70ml of diluent added and sonicated to dissovle the contents and dilueted to volume with diluent. Resulting solution further diluted to 5ml of into 50ml with diluent (each active 40ppm).

Marketed Formulation: Prepared the marketed all dosage forms to get the known concentration of 40ppm for all three ingredients.

System suitability parameters: Tailing factor of three active peaks in standard solution is not more than 2.0; Resolution between three actives is not less than 3.0 and %RSD of five replicate standard solutions area is not more than 2.0%.

Calculation:

$$\textbf{\% of content} = \frac{\text{Area of test solution x Std. Concentration x average x Potency of standard}}{\text{Area of standard solution x sample concentration x Label claim}}$$

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized with a view to develop a stability indicating assay method for three active ingredients. Various columns like octa-silane column, Octadecasilyl column, amide column and ion exchange column have been tried with different buffer in combination with acetonitrile and methanol. However, good resolution was obtained by using the optimized chromatographic conditions (materials and methods). Three active ingredients were well separated and resolution between each ingredient peak is not less than 4.0 and system suitability results were tabulated in table-2.

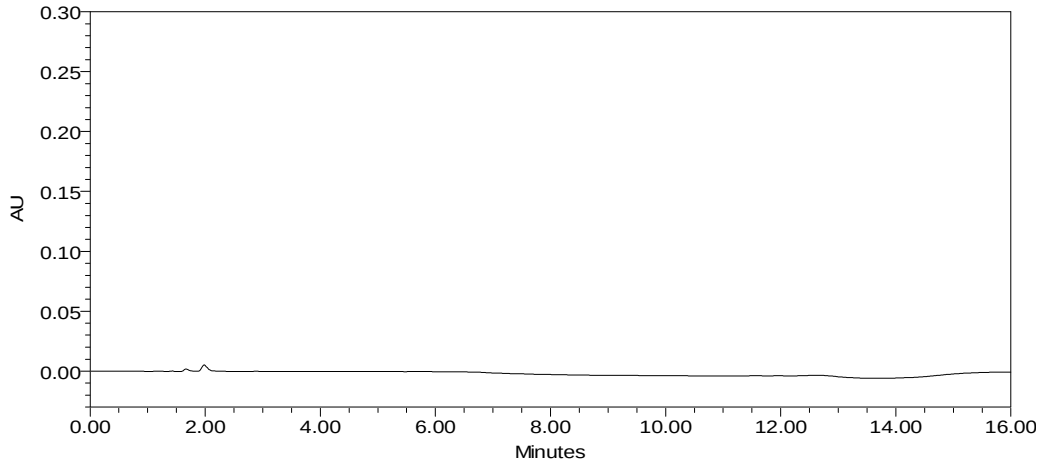


Figure-2: Diluent chromatogram.

Table-2: System suitability (Area %RSD)

Standard solution Area							
Active Name	**Inj-1**	**Inj-2**	**Inj-3**	**Inj-4**	**Inj-5**	**Average**	**%RSD**
Metformin HCl	1033033	1029947	1025052	1032345	1036856	1031447	0.42
Glipizide	1197777	1201447	1201358	1209026	1210038	1203929	0.44
Repaglinide	2995081	3000014	3000085	3016736	3022019	3006787	0.39
Retention time (min)							
Active Name	**Inj-1**	**Inj-2**	**Inj-3**	**Inj-4**	**Inj-5**	**Average**	**%RSD**
Metformin HCl	1.48	1.47	1.48	1.48	1.48	1.48	0.30
Glipizide	3.71	3.7	3.7	3.7	3.71	3.70	0.15
Repaglinide	9.82	9.81	9.81	9.81	9.82	9.81	0.06
Tailing factor							
Active Name	**Inj-1**	**Inj-2**	**Inj-3**	**Inj-4**	**Inj-5**	**Average**	**%RSD**
Metformin HCl	1.1	1.1	1.1	1.1	1.1	1.1	0
Glipizide	1.4	1.4	1.4	1.4	1.4	1.4	
Repaglinide	1.0	1.0	1.0	1.1	1.0	1.0	

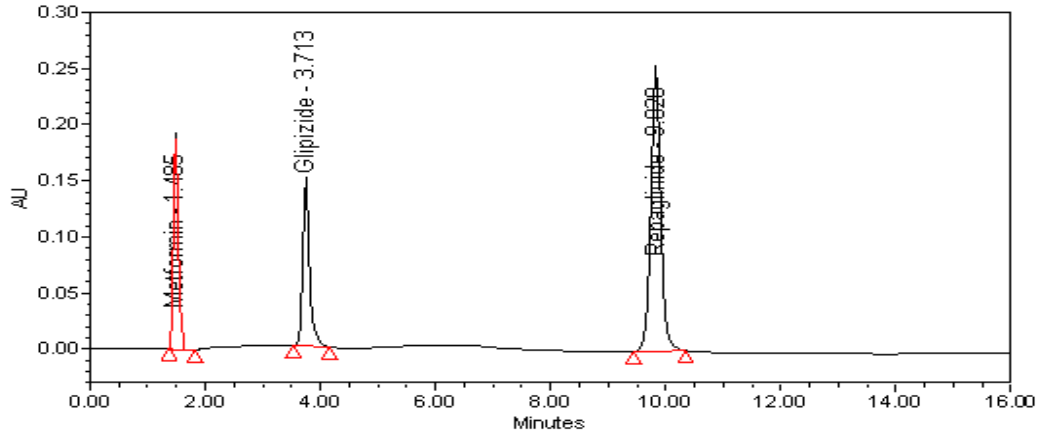


Figure-3: Standard solution.

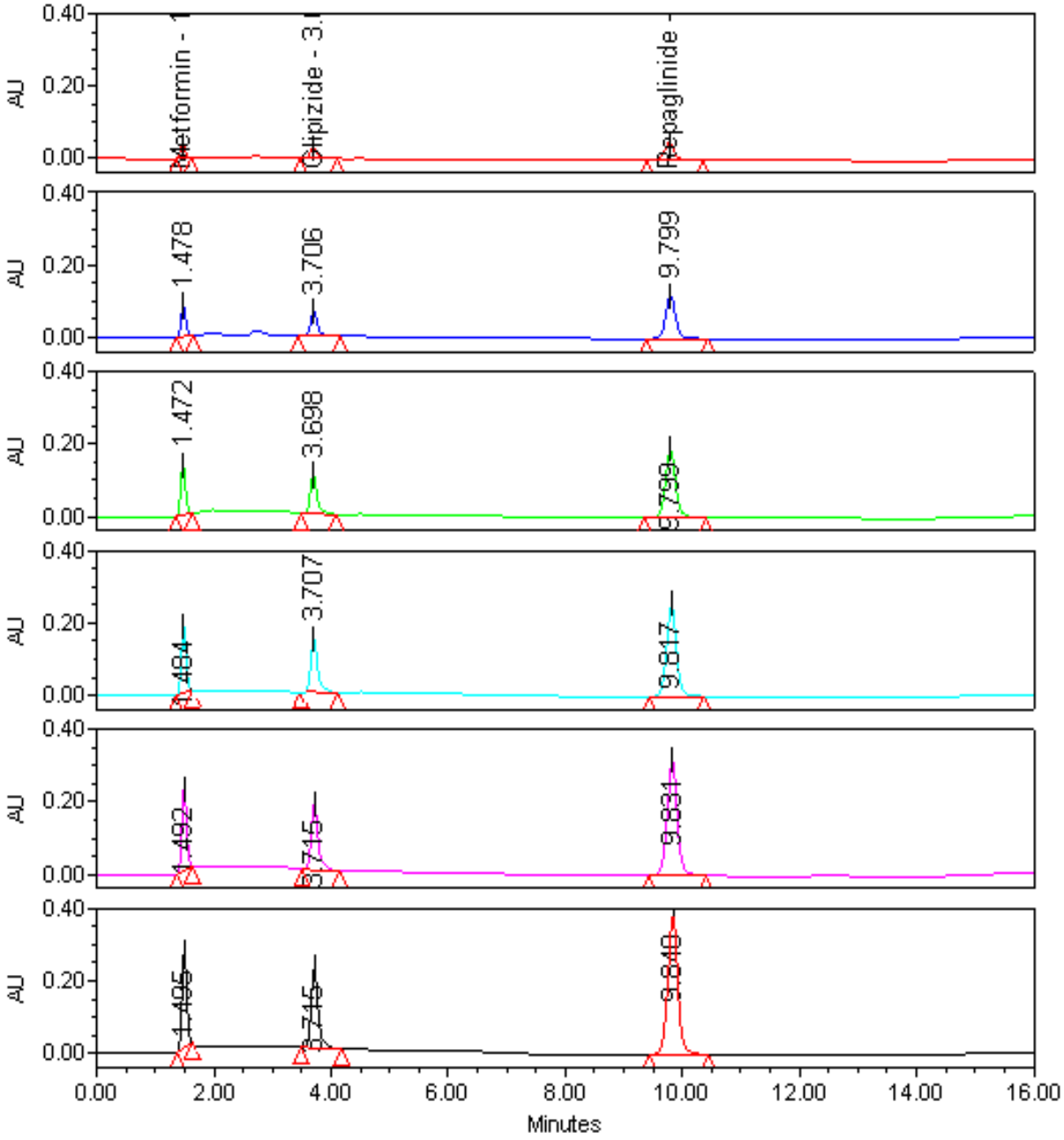


Figure-4: Linearity chromatograms

Linearity: The linearity of the response of three actives was verified at six concentration level ranging from 10ppm to 60ppm. The calibration curve was constructed by plotting mean area response A against concentration C. The result show that an excellent correlation existed between peak area and concentration of three active ingredients. Results were tabulated in table-3 and chromatograms were represented in figure-4.

Table-3: Linearity Results.

Linearity solutions	Active Ingredient Name		
	Metformin HCl	Glipizide	Repaglinide
Level-1 (10ppm)	206328	242672	607178
Level-2 (20.0ppm	496296	577587	1443294
Level-3 (30.0ppm)	785810	906218	2290334
Level-4 (40.0ppm)	1046794	1222364	3050207
Level-5 (50.0ppm)	1304300	1535436	3844783
Level-6 (60.0ppm)	1539112	1851679	4645485
Co-relation Coefficient	0.9992	0.9999	0.9999

Precision: Method repeatability (intra-day precision) was evaluated by market samples which were prepared as described in the sample preparation. The mean % assay and percentage R.S.D. for assay values were found to be 99.0% and 0.7%, respectively, which is well within the acceptance criteria that is, assay value should be between 97.0 and 103.0% and R.S.D. should be not more than 2.0%. The intermediate precision (inter-day precision) was performed by different analyst, different HPLC system and different HPLC column in different days as described in the sample preparation. The assay values were found to be satisfactory. The results indicated the good precision of the developed method.

Table-4: Precision Results.

Active Ingredient Name	Sample preparations						Average (%)
	Prep-1	Prep-2	Prep-3	Prep-4	Prep-5	Prep-6	
Metformin HCl	98.77	100.10	99.82	99.35	99.63	99.81	99.58
Glipizide	101.10	99.10	99.79	98.75	99.36	100.11	99.70
Repaglinide	100.10	99.27	101.00	99.90	99.61	99.64	99.92

Accuracy: Accuracy was determined by applying the developed method to synthetic mixtures of excipients to which known amounts of each drug 25% to 150% of std concentration. The accuracy was then calculated as the percentage of analyte recovered from the formulation matrix. Results found to be satisfactory and results tabulated in table-5

Table-5: Accuracy Results.

Active Ingredient Name	Spike level						Average % Recovery
	25%	50%	75%	100%	125%	150%	
Metformin HCl	100.16	100.03	100.92	100.02	100.65	99.25	100.17
Glipizide	99.89	99.86	100.59	100.44	100.54	99.88	100.20
Repaglinide	99.66	98.91	99.69	100.71	100.18	101.10	100.04

Robustness: To determine the robustness of the developed method, experimental conditions were purposely altered. One factor at a time was changed to estimate the effect. Thus, five replicate

injections of standard solution were injected under each parameter and observed the change on the tailing factor for three active peaks and the R.S.D. for peak area also within the limit. The flow rate of mobile phase was changed by ± 10% that is 0.9 to 1.1 mL/ min. The effect of column temperature was studied at 33 and 37°C instead of 35°C. system suitability results were within the limit.

Solution stability and mobile phase stability: The R.S.D. of assay of metformin, glipizide and repaglinide during solution stability andmobile phase stability experiments was within 1%. The solution stability and mobile phase stability experiments data confirms that sample solutions and mobile phase used during assay determination was stable up to 48hours at room temperature.

CONCLUSION

The developed method is selective, rapid, precise and accurate. The retention time is of Metformin hydrochloride is 1.4min, Glipizide is 3.7min and Repaglinide is 9.8min, repectively. Method validation results reveal the method is precise, accurate and rugged. This indicates that the proposed method could be used as a stability-indicating method for the determination of Metformin, Glipizide and Repaglinide in bulk powder and pharmaceutical formulations.

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