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Research Article

SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND GLIBENCLAMIDE BY RPHPLC METHOD FROM COMBINED TABLET DOSAGE FORM

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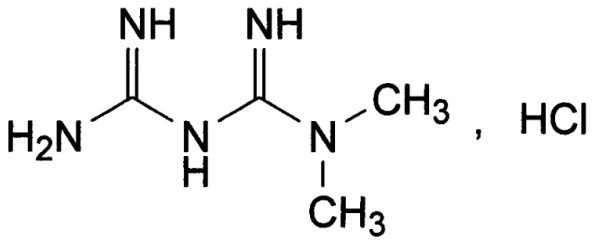
ABSTRACT

A high performance reverse phase liquid chromatographic procedure is developed for simultaneous estimation of Metformin hydrochloride and Glibenclamide in combined tablet dosage form. The method was carried out on a Agilent Hypersil ODS (25cm x 4.6mm, i.d. 5µ) column with a mobile phase used consisting of acetonitrile**:** mono basic sodium phosphate Buffer (50:50) and the pH of buffer was adjusted to 2.5 using 2M Orthophosphoric acid. The detection of the combined dosage form was carried out at 228 nm and a flow rate employed was 1 ml/min and column oven temperature at 30^0C. The retention times of Metformin HCl & Glibenclamide were 2.709& 9.216 minutes respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantification as per ICH norms. The proposed method can be used for the estimation of these combined drugs.

Keywords: Glibenclamid, Metformin Hydrochloride, HPLC

INTRODUCTION

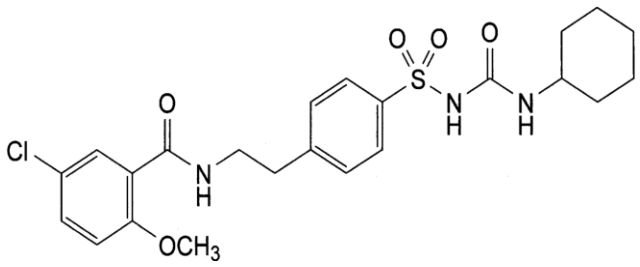
Metformin Hydrochloride is 1,1-Dimethylbiguanide hydrochloride [1] and is used in the treatment of diabetes mellitus. It is completely different from the hypoglycemic sulfonamides [2] both in its structure and its mode of action. It possibly interferes with mitochondrial respiratory chains and promotes peripheral glucose utilization by enhancing anaerobic glycolysis or it enhances binding of insulin to its receptors and potentiates its action. Other explanation is that it suppresses hepatic gluconeogenesis and inhibits intestinal absorption of glucose. It causes little or no hypoglycemia in non diabetic patients [3,4,5].



Metformin HCL

Dimethylbiguanide hydrochloride

Glibenclamide is 5-chloro-*N*-(4-[*N*-(cyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide is an anti-diabetic drug in a class of medications known as sulfonylureas. It was developed in 1966 in a cooperative study between Boehringer Mannheim (now part of Roche) and Hoechst (now part of sanofi-aventis). It is used in the treatment of type II diabetes. As of 2007, it is one of only two oral anti-diabetics in the World Health Organization Model List of Essential Medicines (the other being metformin). As of 2003, in the United States, it was the most popular sulfonylurea [6,7].



Glibenclamide

1-[[4-[2-[(5-Chloro-2-methoxybenzoyl)amino]ethyl]phenyl]sulphonyl]-3-cyclohexylurea

The combination containing Metformin Hydrochloride and Glibenclamide available in the market of Metformin HCl 500 mg & Glibenclamide 2.5 mg and Metformin HCl 500 mg & Glibenclamide 5 mg.

There is no Pharmacopoeial method for simultaneous analysis of Metformin HCl and Glibenclamide as fixed dosage combination. Literature survey revealed that chromatographic methods for determination of Metformin HCl and Glibenclamide alone or in combination of other are available [8]. Literature search also revealed LC methods for simultaneous determination of Metformin HCl and Glibenclamide in tablet dosage forms with different mobile phase composition and using wavelength programming technique [9]. The objective of the present study was to develop a simple, accurate, precise and selective reverse phase HPLC method for simultaneous determination of Metformin HCl and Glibenclamide from tablet dosage forms available in the market.

EXPERIMENTAL

Reagents and Chemicals:

Acetonitrile used was of HPLC grade of Merck and Milli Q water was used for the preparation of the mobile phase. All other reagents used were of HPLC or AR grade.

Drugs used:

Metformin HCl (potency: 99.1%) and Glibenclamide (potency: 100%) were in House Reference Standard Central Drugs Laboratory. Tablet formulation GLUCORED FORTE containing Metformin HCl (500 mg) and Glibenclamide (5 mg) was purchased from market for analysis.

Instrumentation:

The HPLC system consisted of a solvent delivery module of Agilent HPLC 1100 Series Quaternary Gradient pump equipped with 20 µl loop and G1365B Multi Wavelength Detector and C18 column (Agilent Hypersil ODS 2: 15cm x 4.6mm, 5µm) were used for the analysis.

Chromatographic Condition:

The mixture of 0.1% w/w Sodium dihydrogen Phosphate Buffer, pH 2.5 (adjusted with H_3PO_4) and Acetonitrile (50:50 v/v) as mobile phase in an isocratic elution mode was found to be suitable to resolve Metformin HCl and Glibenclamide satisfactorily on a C18 column. The mobile phase was filtered through 0.45 µ membrane filtered and then ultrasonicated for 10 minutes. The mixture of Acetonitrile and Water (4:1) used as diluent. The flow rate of 1.0 ml/min was set for elution and detection was carried out using UV-Visible detector set at 228 nm. All determinations were performed at a constant column temperature of 30ºC with a load of 20µl.

Preparation of working standard solutions:

A standard stock solution of Glibenclamide was prepared by dissolving 25.0 mg Glibenclamide in 100 ml with Diluent. A mixed standard solution was prepared by weighing 25.0 mg Metformin HCl and 1 ml standard stock solution of Glimepiride in a 50 ml volumetric flask and volume make up with the diluent . The final concentration of the standard solutions was Metformin HCl 500 mcg/ml and 5 mcg/ml of Glibenclamide.

Preparation of Sample Solution:

Twenty tablets each containing 500 mg of Metformin Hydrochloride and 5 mg of Glibenclamide were weighed and finely powdered, a quantity of 50 mg of Metformin Hydrochloride and 0.5 mg of Glibenclamide was weighed and transferred to 100 ml volumetric flask and 50 ml diluent was added to it. The solution was then sonicated for 10 minutes and finally volume was made upto the 100 ml mark with diluent. Solution was filtered through 0.22 micron membrane filter. The final concentration of Metformin HCl 500mcg/ml and Glibenclamide 5mcg/ml and this solution was used for the estimation.

Assay Method:

A steady baseline was recorded and diluent was injected as blank, then mixed standard solution injected and the chromatogram recorded. The retention times of Metformin HCl & Glibenclamide were found to be 2.709 & 9.216 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The peak area of Standard & sample solution were calculated. The estimation of the drugs was calculated using following formula:-

$$\text{Content of Drugs (mg/tab)} = \frac{\text{Sample area} \times \text{Standard Conc}}{\text{Standard area} \times \text{Sample Conc}} \times \text{Claim of tab}$$

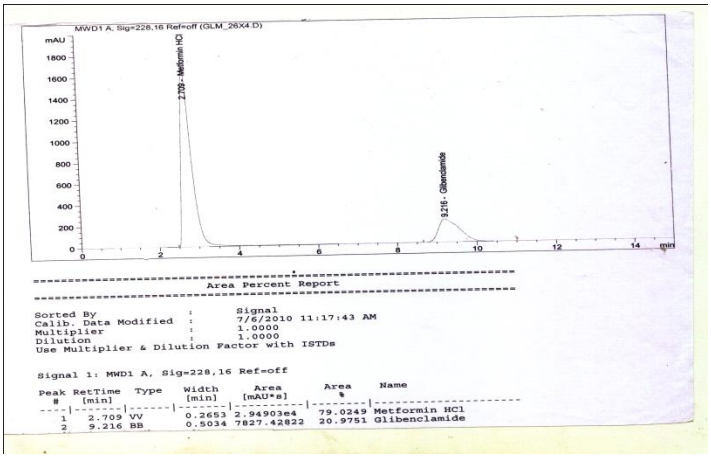


Figure 1: Chromatogram of Metformin HCl (Retention time 2.709 min,) and Glibenclamide (Reention time 9.216 min,)

RESULTS AND DISCUSSION

The HPLC as optimized with a view to develop precise and stable assay method. Both the pure drugs Metformin HCl and Glibenclamide were run in different composition of mobile phases and different columns (Zorbax eclipse 25cm x 4.6mm i.d., 5µ),(phenomenex Luna 25cm x 4.6mm i.d., 5µ)[10,11,12]. The mixture of Sodium diHydrogen Phosphate Buffer, pH 2.5 (adjusted with H_3PO_4) and Acetonitrile (50:50 v/v) as mobile phase was found as optimal for obtaining well defined and resolve peaks at a flow rate of 1.0 ml/min at a column oven temperature 30°C. The optimum wavelength for detection was used at 228 nm, at which best detector response gave sharp and symmetrical peaks with 2.709 and 9.216 for Metformin HCl and Glibenclamide respectively. The typical chromatogram of sample solution is shown in Fig.1. The percentage of individual drugs was calculated. The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

Component	Amount present (mg/Tab)	Amount Found (mg/Tab)	% Estimation	%Recovery
Metformin HCl	500 mg	496.83 mg	99.36	98.24
Glibenclamide	5 mg	4.91 mg	98.20	96.25

Table 1

METHOD VALIDATION

Linearity and Range:

The linearity of the method was determined at five concentration levels having concentration range 125 mcg/ml to 450 mcg/ml for Metformin HCl and 0.25mcg/ml to 2.0 mcg/ml Glibenclamide. The calibration curve was constructed by plotting Area against Concentration of drugs. The slope and intercept value for calibration curve for Metformin Hydrochloride and Glibenclamide are shown given below.

Ruggedness and Robustness:

To evaluate the robustness of the developed method was determined by making slight changes in the chromatographic conditions.

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC(LC-20AT),Agilent HPLC(1200 series) and Waters Breeze HPLC by different operators using different C_{18} columns like Zorbax Stable bond, Poroshell 120 and Kromasil with a Flow rate 1.00 ± 0.02 ml/min and detection 228 ± 8 nm. It was observed that there were no marked changes in the chromatograms, which demonstrated that the

RP-HPLC method developed, are robust and rugged.

System Repeatability:

To check the degree of repeatability of the method six injections of mixed standard were carried out and % RSD was calculated for the peak areas for both the components.

Accuracy:

To study the accuracy of the developed method, recovery study was carried out by external addition of standard of Metformin HCl and Glibenclamide to the pre-analyzed sample at three different levels 50%, 100% and 150%.

Precision:

The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six repeated injections of standard and sample solutions were made and the area of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and the area of drugs peaks and percentage RSD were calculated. From the data obtained, the developed RP-HPLC method was found to be precise.

Limit of Detection and Limit of Quantification:

The limit of detection (LOD) and limit of quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for Metformin Hydrochloride and Glibenclamide was found to be 0.019µg/mL and 0.033 µg/mL, respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 0.058µg/mL and 0.1µg/mL for Metformin Hydrochloride and Glibenclamide, respectively.

System suitability studies:

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table II). The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3.0 % standard deviation range and % RSD less than 2.0 during routine performance of the method.

Validation Parameters	Metformion HCl	Glibenclamide
Linearity Range	125-450 mcg/ml	0.25-2.0 mcg/ml
Regression equation Y=mx+C	Y=74.056X+33.36	Y=57.229X+3.0571
Correlation Coefficient	0.9997	0.9959
Theoretical plate/meter	4520	5161
Resolution factor	-	7.7
Asymmetric factor	2.35	2.8
LOD(µg/ml)	0.019 µg/ml	0.033 µg/ml
LOQ(µg/ml)	0.058 µg/ml	0.1 µg/ml

Table 2: Summery of Analytical Method Validation

CONCLUSION

The developed RP-HPLC method was proved to be simple, fast and reliable. The method was validated for its performance parameters e.g. Linearity, Repeatability, Accuracy, Precision, Ruggedness, Robustness etc. The developed method offers several advantages in terms of simplicity in mobile phase, isocratic mode of elution and sample preparation steps and comparative short run time makes the method specific, repeatable and reliable for its intended use in simultaneous determination of Metformin HCl and Glibenclamide in tablet dosage form as well as in other formulations.

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Calibration Curve of Glibenclamide: