RJC

RASĀYAN *J. Chem.*
Vol. 7 | No.1 | 55-63 | January - March| 2014
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF METFORMIN AND GLIBENCLAMIDE IN BULK AND TABLET DOSAGE FORM USING RP-HPLC

**Subhashini Edla and B. Syama Sundhar**
Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur
*E-mail: subhashini.edla@gmail.com

**ABSTRACT**

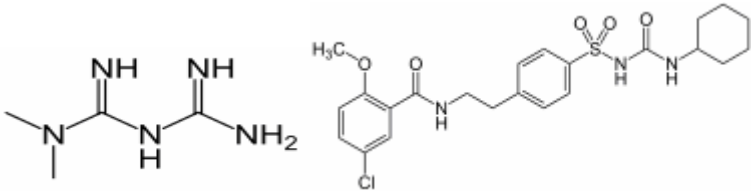
Metformin and Glibenclamide are prescribed for the treatment of patients with Diabetes.This study describes a rapid, simple, precise and accurate RP-HPLC method for simultaneous estimation of Metformin and Glibenclamide in their combined tablet dosage form. The separation was achieved on a Oyster BDS RP- C18 Column (150mm x 4.6mm, 5 µm) column with an isocratic mixture of Methanol: Acetonitrile: Water in 30:60:10 (v/v), at a flow rate of 1.0 ml/min and UV detection at 228 nm. The retention time for Metformin and Glibenclamide were 3.17 and 8.10min respectively. Good linear relation was observed with in a concentration range of 2-4.5µg/ml for Glibenclamide and 200-450µg/ml for Metformin with high $r^2$ value. The developed method was validated as per ICH guide lines. The developed method was found to be precise, accurate and was used for the simultaneous estimation of Metformin and Glibenclamide in fixed dosage forms.

**Keywords:** Metformin, Glibenclamide, HPLC estimation, analytical method validation.



## INTRODUCTION

Metformin is a potent anti-diabetic drug of class biguanide class considered as the first line treatment of diabetes mellitus particularly in overweight and obese people and people with normal kidney function[1-2]. It has been extensively used in the treatment of non-alcoholic fatty liver disease (NAFLD) and premature puberty, three other diseases that feature insulin resistance. It is used in the treatment of gestational diabetes and also polycystic ovary syndrome. Metformin is the only antidiabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. It also reduces LDL Cholesrol and triglceride levels and not associated with gaining weight. Upto 2010, Metformin is one of only two oral antidiabetics in the World Health Organization Model List of Essential Medicines (the other being glibenclamide. The drug is available in different forms as tablet, capsule and liquid formulations with different brand names[3-5]. Metformin is more commonly associated with gastrointestinal side effects than most other antidiabetic drugs[6]. The most common side effects of the drug is gastrointestinal irritation, including diarrhea, cramps, nausea, vomiting and increased flatulence. Among serious side effects lactic acidosis and vast majority of these cases seem to be related to comorbid conditions, such as impaired liver or kidney function[7]. Metformin has also been reported to decrease the blood levels of thyroid-stimulating hormone in people with hypothyroidism[8].



(a.) (b.)

Fig.-1: Structure of (a.) Metformin and (b.) Glibenclamide

Glibenclamide acts as anti-diabetic drug belongs to the class of sulfonylureas commonly known as sulfa drugs. The drug was first developed in 1966 in a cooperative study between Boehringer Mannheim (now part of Roche) and Hoechst (now part of Sanofi-Aventis)[9]. The drug is effectively used in the treatment of type 2 diabetes[10]. It is also helpful in improving the outcoming results on animal stroke models by preventing brain swelling and enhancing neuroprotection[11]. The mechanism of action of drug is illustrated by binding and activation of the sulfonylurea receptor 1 (SURI 1), which is considered as regulatory subunit of the ATP-sensitive potassium channels, in pancreatic beta cells ($K_{ATP}$)[12]. This leads to cell membrane depolarization opening voltage-dependent calcium channel due to inhibition. The increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release is resulted by the membrane depolarization of the cell membrane of pancreas[13,14]. It restricted to patients suffering with G6PD deficiency as it may cause acute haemolysis[15]. Recent studies reveled that Glibenclamide is associated with significantly higher annual mortality when combined with metformin than other insulin-secreting medications, and has potential to lower some of side effects[16]. The common side effects of the intaking drug are hypoglycemia and Cholestatic jaundice.

Literature survey reveals that only one HPTLC[17] study, one UPLC[18], few HPLC[19-22] and Speectrophotometry[23-27] methods have been described for analysis Metformin and Glibenclamide. So the present work is aimed to achieve a new RP-HPLC method determination estimation of Metformin and Glibenclamide API and tablet dosage forms.

## EXPERIMENTAL

### Instrumentation

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; Rheodyne injector with 20µl fixed volume loop, variable wavelength programmable UV detector UV7000 and the output signal was monitored and integrated by PEAK Chromatographic Software version 1.06. Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis and the data was recorded by Hitachi software. Sonicator (1.5L) 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 the mobile phase was adjusted by using Systronics digital pH meter.

### Chemicals and Solvents

The drug samples, Metformin and Glibenclamide working standard was obtained as gift sample from a research laboratory. The pharmaceutical formulation was procured from local market. Methanol, Acetonitrile and water used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Orthophosphoric acid and reaming buffer solutions used were AR Grade and purchased from Merck Specialties Private Limited, Mumbai, India.

### Preparation of standard stock solution

Standard stock solution of Metformin and Glibenclamide pure drug (1mg/ml) was prepared by accurately weighing about 10mg of each drug in 10ml volumetric flask. The drugs were dissolved with few ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent. The contents were mixed well and filtered through Ultipor $N_{66}$ Nylon 6, 6 membrane sample filter paper. From this stock solution selected concentrations were prepared by selected dilutions. 1ml from the selected concentrations of both the drugs were mixed and used as a combined standard solution for the estimation in pharmaceutical formulations.

### Preparation of Formulation Solution

Marketed formulation of Metformin and Glibenclamide were purchased (Ben-Q-Met Formt: Metformin 500mg and Glibenclamide5mg). From this 20 tablets were powered and the average weight of the power was calculated. Forms the power, an average weight of 10mg of Metformin was weighed and was dissolved in little amount of methanol and mixed well and then make up to 10ml with methanol. It was

filtered through 0.45µm nylon membrane filter paper. This was further diluted to 350µg/ml of Metformin by selected dilutions. Based on the label claim of the two drugs, 3.5µg/ml of Glibenclamide was obtained. This solution was used for formulation estimation of Metformin and Glibenclamide in combined dosage forms.

**Method Development**

To develop a simple and robust method for the simultaneous determination of Metformin hydrochloride and Glibenclamide in combined tablet dosage form using HPLC. The spectra of diluted solution of the Metformin hydrochloride and Glibenclamide in methanol were recorded separately on UV spectrophotometer. The peaks of maximum absorbance wavelengths were observed. The spectra of' the both Metformin hydrochloride and Glibenclamide were showed that a balanced wavelength was found to be 228nm.

Initial development trials have performed with octyl and octadecyl columns with different types, configurations and from different manufacturers. Finally good separation of both the drugs Metformin hydrochloride and Glibenclamide was achieved in Oyster BDS C18 column (250x 4.6 mm, 5µm). In this column both the drugs were resolved and symmetric peaks with high theoretical plates and low tailing factor was observed.

To effect ideal separation of the drug under isocratic conditions, mixtures of solvents like water, methanol and acetonitrile with or without different buffers in different combinations were tested as mobile phases on Oyster BDS C18 stationary phase. A mixture of Methanol, Acetonitrile, and water in the ratioof 30:60:10(v/v) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and almost tree from tailing.

Flow rates of the mobile phase were changed from 0.5-1.2 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 1.0mL/min flow rate was ideal for the successful elution of the analyte.

No interference in blank and placebo solutions for both drug peaks in the trail injections with a runtime of 12min. The above optimized chromatographic conditions were followed for the simultaneous determination of Metformin hydrochloride and Glibenclamide in bulk samples and its combined tablet formulations.

**Method Validation**

A new optimized method suitable for the simultaneous routine analysis of Metformin and Glibenclamide in bulk and pharmaceutical formulation samples was successfully developed. The developed method was validated as per ICH guidelines.

Calibration curve was stabled by preparing six different concentrations of Metformin and Glibenclamide based on the label claim of the formulation dosage form. Metformin of 200-500µg/ml and Glibenclamide of 2-5µg/ml solutions were mixed, from this 20µl of the sample was injected in to HPLC system. Peak area responses of the corresponding standards were used in constructing the calibration curve.

Accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 50%, 100%and 150% levels of standard concentration for both Metformin and Glibenclamide. The solutions were analyzed in triplicate at each level as per the proposed method.

Precision is the measure of how close the data values to each other for a number of measurements under the same analytical conditions. Precision of the method was determined by performing interday variation, intraday variation and repeatability studies.Standard concentration of Metformin and Glibenclamide was prepared in six times and %RSD was calculated in both intraday and interday precision.

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. Robustness test was carried out by small variation in the chromatographic conditions and % change in the results was calculated. Here robustness was performed by change in

mobile phase ratio, mobile phase pH and wavelength of the detector. Standard solution was analyzed under these changed experimental conditions. % change in the results was calculated

Inter day variations were performed by using six replicate injections of standard solution which were prepared and analyzed by different analyst on three different days over a period of one week. The percent relative standard deviation (% RSD) was calculated.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit.

The quantification limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

**Analysis of tablet formulation**

The prepared formulation solution was injected into HPLC system. Peak areas of the corresponding peak area are compared with the standard results and formulation assay was calculated for the proposed method.

## RESULTS AND DISCUSSION

The development of an analytical method for the determination of drugs by HPLC has received considerable attention in recent years because of their importance in quality control of drug products. The objective of this study was to develop a rapid and sensitive HPLC method for estimation of Metformin and Glibenclamide in tablet formulations using the most commonly employed RP C-18 column with UV detection. The mobile phase was optimized with Methanol: Acetonitrile: Water in 30:60:10 (v/v). From the overlain spectrum of Metformin and Glibenclamide, wavelength was selected, at 228nm, iso-absorptive point for both the drugs. Good resolution was carried out at 228nm and both drugs showed good absorbance at this wavelength with minimum interference of the other drug. Optimized chromatographic conditions were shown in table-1. Standard and blank and formulation chromatograms were shown in figures-2 and 3. All parameters of these proposed method was validated as per the ICH guidelines.

Table-1: Optimized chromatographic conditions for Metformin and Glibenclamide

| S.No. | Parameter | Results |
|---|---|---|
| 1 | MP | Methanol: Acetonitrile: Water in 60:20:20 (v/v) |
| 2 | Wavelength | 228nm |
| 3 | Stationary Phase | Oyster BDS RP- C18 Column |
| 4 | pH of MP | 4.3 |
| 5 | Flow Rate | 1.0ml/min |
| 6 | Run Time | 12min |
| 7 | Pump Mode | Isocratic |
| 8 | Pump Pressure | 15.5±5MPa |
| S.No. | Parameter | Results |
| 1 | Api Concentration | Metformin – 350µg/ml<br>Glibenclamide- 3.5µg/ml |
| 2 | RT | Metformin – 3.17min<br>Glibenclamide- 8.10min |
| 3 | Resolution | Metformin --------<br>Glibenclamide- 39.20 |
| 4 | Area | Metformin – 438543<br>Glibenclamide- 9497 |
| 5 | Theoretical Plates | Metformin – 7412<br>Glibenclamide- 96983 |
| 6 | Tailing Factor | Metformin – 1.28<br>Glibenclamide- 1.03 |

Blank and Placebo solutions show no peak at the retention time of Metformin and Glibenclamide, hence proving the specificity of the method. A good linear relation was observed with in the concentration range of 2-4.5µg/ml for Glibenclamide with regression equation y = 1274.7x –2552.9[$r^2$ 0.9989] and 200-450µg/ml for Metformin with regression equation y = 27726x –329.16[$r^2$ 0.9986]. Linearity results were shown in table-2 and graphs were shown in figure-4.

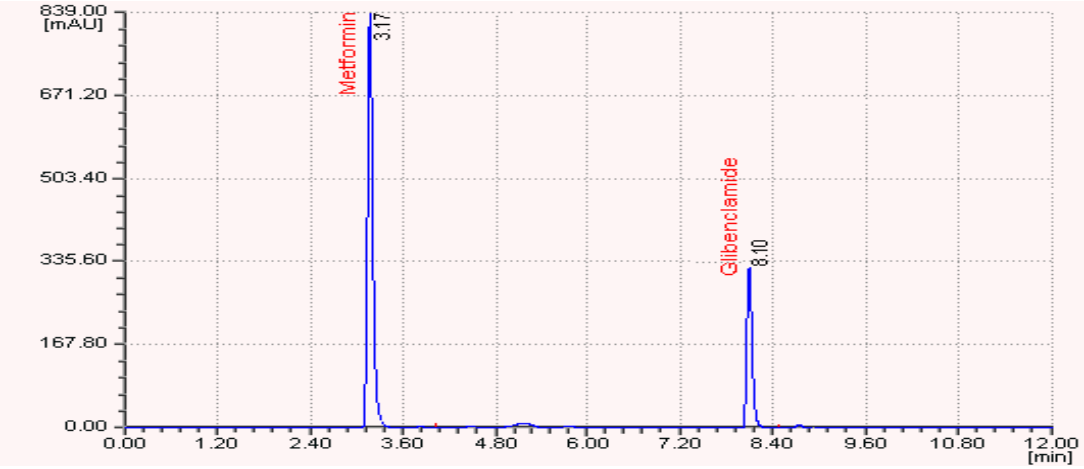



Fig.-2: Standard chromatogram of Metformin and Glibenclamide

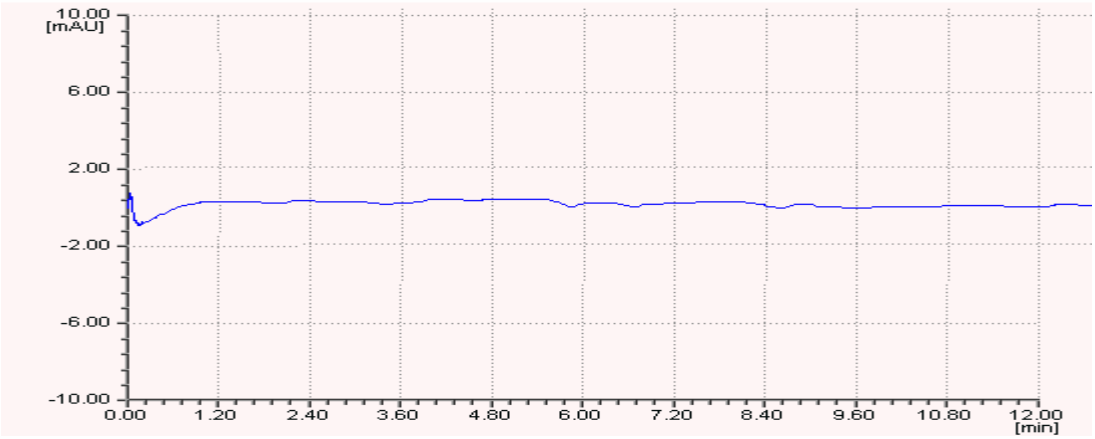



Fig.-3 : Blank chromatogram of Metformin and Glibenclamide

Recovery was carried out by standard addition method of 50%, 100% and 150% addition to standard, pre analyzed sample of 200µg/ml for Metformin and 2µg/ml for Glibenclamide in triplicates. % recovery for each case was calculated and was found to be within the acceptance criteria of 98-102% for both drugs. This showed that the recoveries of Metformin and Glibenclamide by the proposed methods are satisfactory. Recovery results were shown in table-3 and 4 for both Metformin and Glibenclamide.

The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and was found to be 0.59 and 0.72 for Glibenclamide in inter day and interday precision, 0.62 and 0.78 for Metformin in inter day and interday precision. This was lying within the acceptable range of ±2. This showed that the precision of the methods are satisfactory.

Ruggedness performed by using six replicate injections of standard solution of concentrations which were prepared and analyzed by different analyst on three different days over a period of one week. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.64 and 0.71 for Metformin and Glibenclamide respectively, which are well within the acceptable criteria of not more than 2.0. It was concluded that the analytical technique showed good repeatability. Results were shown in table-5.
The limit of detection values was found to be0.01µg/ml Glibenclamide and 0.30µg/ml for Metformin and limit of quantification values was found to be0.3µg/ml Glibenclamide and 1.0µg/ml for Metformin. This indicates that the proposed method is very sensitive.

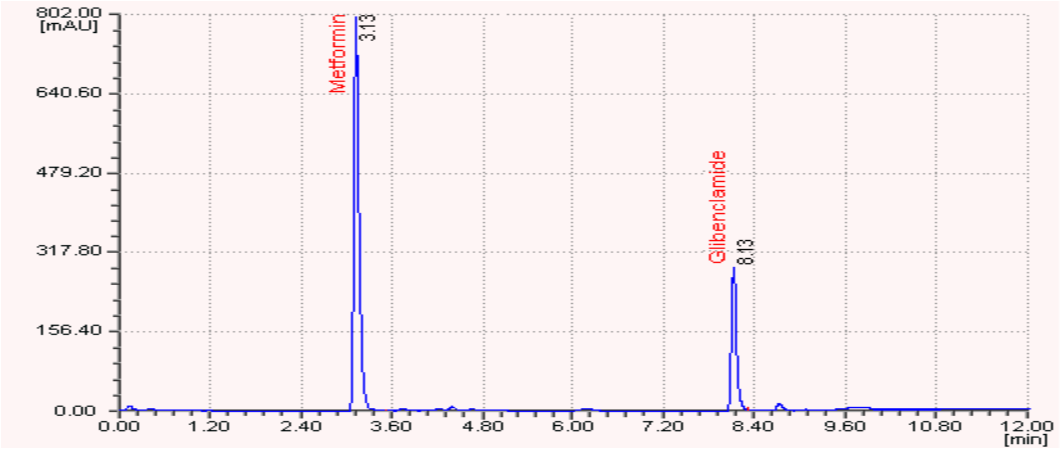



Fig.-4: Formulation chromatogram of Metformin and Glibenclamide

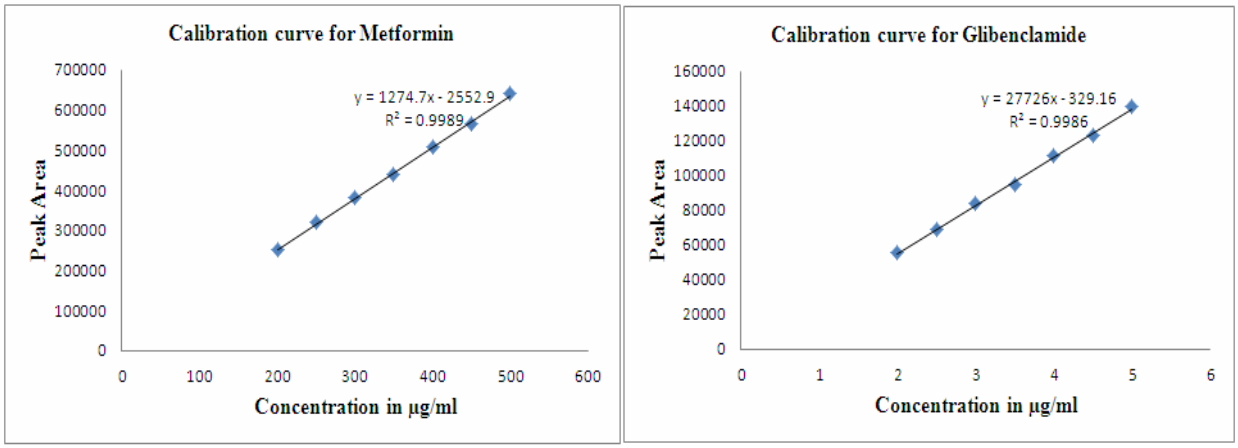



Fig.-5: Linearity of Metformin and Glibenclamide

Table-2: Linearity results of Metformin and Glibenclamide

| S.No. | Metformin | | Glibenclamide | |
|---|---|---|---|---|
| | Concentration [µg/ml] | Peak Area | Concentration [µg/ml] | Peak Area |
| 1 | 200 | 252235 | 2 | 55334.5 |
| 2 | 250 | 319817 | 2.5 | 69207 |
| 3 | 300 | 380004 | 3 | 83667.2 |
| 4 | 350 | 438543 | 3.5 | 94976.1 |
| 5 | 400 | 509275 | 4 | 111033 |
| 6 | 450 | 564322 | 4.5 | 123106 |

Table-3: Recovery results of Metformin

| S.No. | %Recovery | Concentration in µg/ml | | | Amount Found | % recovery |
|---|---|---|---|---|---|---|
| | | Target | Spiked | Total | | |
| 1 | 50% | 200 | 100 | 300 | 296.65 | 98.88 |
| 2 | | 200 | 100 | 300 | 298.84 | 99.61 |
| 3 | | 200 | 100 | 300 | 297.05 | 99.02 |
| 4 | 100% | 200 | 200 | 400 | 395.48 | 98.87 |
| 5 | | 200 | 200 | 400 | 395.48 | 98.87 |
| 6 | | 200 | 200 | 400 | 396.24 | 99.06 |
| 7 | 150% | 200 | 300 | 500 | 500.35 | 100.08 |
| 8 | | 200 | 300 | 500 | 498.47 | 99.69 |
| 9 | | 200 | 300 | 500 | 498.08 | 99.61 |

Table-4: Recovery results of Glibenclamide

| S.No. | %Recovery | Concentration in µg/ml | | | Amount Found | % recovery |
|---|---|---|---|---|---|---|
| | | Target | Spiked | Total | | |
| 1 | 50% | 2 | 1 | 3 | 2.97 | 99.09 |
| 2 | | 2 | 1 | 3 | 2.98 | 99.35 |
| 3 | | 2 | 1 | 3 | 2.98 | 99.21 |
| 4 | 100% | 2 | 2 | 4 | 3.99 | 99.69 |
| 5 | | 2 | 2 | 4 | 3.99 | 99.73 |
| 6 | | 2 | 2 | 4 | 3.99 | 99.80 |
| 7 | 150% | 2 | 3 | 5 | 5.01 | 100.20 |
| 8 | | 2 | 3 | 5 | 4.93 | 98.64 |
| 9 | | 2 | 3 | 5 | 5.03 | 100.71 |

Table-5: Ruggedness of Metformin and Glibenclamide

| S.No. | Metformin at 350µg/ml | | | Glibenclamide at 3.5µg/ml | | |
|---|---|---|---|---|---|---|
| | Intraday | Inter day | Ruggedness | Intraday | Inter day | Ruggedness |
| 1 | 439593 | 438761 | 440137 | 94282 | 93491.7 | 95407.6 |
| 2 | 438994 | 431739 | 437358 | 94579.9 | 94625.7 | 94093.5 |
| 3 | 433319 | 433712 | 433495 | 95016.8 | 94706.9 | 95506.5 |
| 4 | 435044 | 438734 | 439857 | 94789.7 | 94322 | 94401 |
| 5 | 437105 | 432115 | 433968 | 94690.3 | 95605.8 | 95717 |
| 6 | 440114 | 438425 | 436863 | 93438.5 | 94587.7 | 94582 |
| RSD | 0.62 | 0.78 | 0.64 | 0.59 | 0.72 | 0.71 |

Small deliberate variation in the chromatographic condition does not show influence on the results indicates that the proposed method is robust. Results of the robustness were shown in table-6.

The validated method was applied for the assay of commercial tablets of Metformin and Glibenclamide (Ben – Q- Met Fort: Metformin 500mg and Glibenclamide5mg). Peak area of the detector response was used to calculate % assay. The results presented good agreement with the labeled content. Results were shown in table-7 and formulation chromatogram was shown in figure- 3.

Table-6: Robustness results of Metformin and Glibenclamide

| S.No. | Condition | Change | Metformin at 350µg/ml | | Glibenclamide at 3.5µg/ml | |
|---|---|---|---|---|---|---|
| | | | Area | % Change | Area | % Change |
| 1 | Standard | - | 295655 | - | 171735 | ……. |
| 2 | MP 1 | 35:55:10 (v/v) | 440487 | 0.44 | 95506 | 0.56 |
| 3 | MP 2 | 25:65:10 (v/v) | 433112 | 1.24 | 94061 | 0.96 |
| 4 | WL 1 | 233nm | 430440 | 1.85 | 93959 | 1.07 |
| 5 | WL 2 | 223nm | 431553 | 1.59 | 95306 | 0.35 |
| 6 | pH 1 | 4.2 | 436515 | 0.46 | 94952 | 0.02 |
| 7 | pH 2 | 4.4 | 437077 | 0.33 | 95269 | 0.31 |

Table-7: Formulation results of Metformin and Glibenclamide

| S.No. | Drug | Brand | Dosage | Amount Prepared | Amount Found | %Assay |
|---|---|---|---|---|---|---|
| 1 | Metformin | Ben – Q- Met Fort | 500mg | 350µg/ml | 393.44µg/ml | 98.36 |
| 2 | Glibenclamide | | 5mg | 3.5µg/ml | 3.48µg/ml | 99.40 |

## CONCLUSION

A simple isocratic RP-HPLC method has been developed for the simultaneous determination of Metformin and Glibenclamide using a UV detector. The developed method does not contain any buffers in the mobile phase with simple uv detection with less mobile phase flow rate. Hence the method is simple, sensitive, accurate, rugged, robust, rapid and precise. The absence of additional peaks in the chromatogram indicated that there is no interference of the common excipients used in the tablets. The method has a relatively short run time that allows quantifying a large number of samples in routine and quality control analysis of fixed dose combination tablets. Hence, the above said method can be successfully applied for the simultaneous estimation of Metformin and Glibenclamide in tablet dosage forms.

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[RJC-1091/2014]