RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Metformin Hydrochloride and Gliclazide in Bulk and Tablet Formulation

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ABSTRACT

The current study's goal was to develop and validate an easy-to-use, highly-sensitive HPLC approach for simultaneously quantifying metformin and gliclazide in dosage forms on a single chromatographic run. Water symmetry Shield RP 8 column, 250 mm x 4.6 mm x 5 m, detection wavelength of 230 nm, mobile phase of buffer of pH 2.5 adjusted with orthophosporic acid and 1-mL triethylamine and methanol in a gradient programme for 25 minutes at 1.0-mL/min flow rate, 10 L injection volume, and 25 °C column temperature were the chromatographic conditions that were used. Retention times for metformin and gliclazide were found to be 2.23 and 14.88 minutes, respectively. According to ICH criteria, the method's accuracy, precision, and resilience were evaluated.

Keywords: Metformin hydrochloride, Gliclazide, RP-HPLC, Simultaneous estimation, Validation, ICH.

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INTRODUCTION

Biguanide-class antihyperglycemic medication metformin is used to treat type II diabetes. Currently, metformin is the first medication of choice for treating type II diabetes, and at least 120 million patients worldwide are given this prescription. It is often referred to as an insulin sensitizer that significantly lowers plasma fasting insulin levels and insulin resistance.[1]

Gliclazide is a member of the group of medicines called as second-generation sulfonylureas. It promotes the release of the body's natural insulin by binding to a particular receptor on the pancreatic β cells.[2]

The literature reveals a plethora of metformin estimate techniques in dosage forms or biological samples,[3] gliclazide in dosage forms or biological samples,[4] a combination of metformin with other drugs,[5] a combination of gliclazide with other drugs[6] and a combination of metformin and gliclazide.[7-9] These methods claim to be rapid, sensitive, specific and simple but there is always a need to offer a better method for routine estimation of the drug content in dosage forms.

For the concurrent measurement of metformin as well as gliclazide in dosage formulae on a single chromatographic run, the current study sought to design and validate a straightforward, sensitive, highly effective HPLC approach.

MATERIAL AND METHODS

Material and Reagents

Metformin hydrochloride (99.9% purity) and gliclazide (99.8% purity) were obtained as gift sample for research work from Herman finochem Ltd and Bal Pharma Ltd, respectively. HPLC grade acetonitrile was obtained from Finar Scientific while HPLC grade methanol, analytical reagent grade triethylamine, and orthophosphoric acid were obtained from Rankem. Analytical reagent grade potassium dihydrogen orthophosphate and ammonium dihydrogen orthophosphate have been bought from Merck. HPLC grade water was arranged in the laboratory with the Milli Q water purifier.

Instrument

A Shimadzu HPLC Prominence-I LC-2030 3D Plus Series system, equipped with a quaternary pump, autosampler, and PDA- detector, was used for the study.

Preparation of Buffer Solution

Add 1-mL of triethylamine and 1-mL of orthophosphoric acid to 1000 mL of water. Use orthophosphoric acid to bring the pH to 2.5. Place a 0.45 m nylon membrane filter over the solution to be filtered.

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Diluent

A mix of acetonitrile and methanol in the proportion of 60:40 was employed to prepare the drug solutions.

Preparation of Metformin Hydrochloride Standard Stock Solution (625 ppm)

The working standard of 125 mg of metformin hydrochloride was accurately weighed and then moved to a 200 mL volumetric flask. The solution was sonicated for full dissolution after 130 mL of diluent was added. With diluent, the volume was adjusted to the desired level.

Preparing Gliclazide Standard Stock Solution (100 ppm)

A 200 mL volumetric flask was filled with a working standard of 20 mg of gliclazide that had been precisely weighed. The solution was sonicated for full dissolution after 130 mL of diluent was added. With diluent, the volume was adjusted to the desired level.

Mix Standard Solution

A volumetric flask of 50 mL capacity was occupied with 10 mL of the standard stock solutions of the drugs metformin hydrochloride and gliclazide. With the aid of the diluent, the volume was brought up to par.

Preparation of Sample Solution (125 ppm Metformin HCl and 20 ppm of Gliclazide)

A 200 mL volumetric flask was filled with a precisely weighed amount of tablet powder that contained 125 mg of metformin HCl and 20 mg of gliclazide. The solution was sonicated for full dissolution after 130 mL of diluent was added. With diluent, the volume was adjusted to the proper level. This solution was further diluted by 10 to 50 mL with diluents, well mixed, and sonicated.

Method Development and Optimization

In a single run, several mobile phases and columns were tested for eluting metformin and gliclazide from the mix standard solution. The column and mobile phase suitability were decided using the peak evenness, tailing aspect, separation of the peaks and the theoretical plate.

Columns Tested for Separation

Inertsil ODS 3V of dimension 150 X 4.6 mm, particle size 5 µm Shimadzu, 250×4.6 mm, 5 µm water symmetry Shield RP 8, 250×4.6 mm, 5 µm

Mobile Phase Tested

Mobile phase - A (buffer)
Buffer phosphate- pH 3.0
Orthophosphoric acid and triethylamine mix, pH 2.5
Mobile phase - B (organic solvent)
Methanol
Acetonitrile-Methanol (60:40)

Selection of Wavelength of Detection

Metformin solution exhibited an absorption maximum of 233.4 nm while gliclazide has an absorption maxima of 220 nm. Hence a wavelength of 230 nm that depicted an acceptable absorption was used as the detection wavelength for both pharmaceutical substances.

Table 1: Gradient-time schedule

S. No.	*Time (min)*	*Mobile Phase A*	*Mobile Phase B*
1	0.01	75	25
2	10	30	70
3	18	30	70
4	20	75	25
5	25	75	25

Experimental Conditions for the Optimized Method

The chromatographic conditions used for the study included
Column: Water symmetry Shield RP 8, 250×4.6 mm, 5µm
Flow rate: 1.0 mL/min
Injection volume: 10 µL
Wavelength: 230 nm
Run time: 25 minutes
Mobile Phase A: Buffer solution
Mobile Phase B: Methanol

Method Validation[10-12]

System suitability parameters

Six replicate injections of a 10 L solution of the mix standard were injected after the column had been equilibrated with the mobile phase. Peak response, or peak area, was measured after the chromatograms were recorded. Calculated was the percent relative standard deviation (%RSD).

Precision

Prepared six test solutions of metformin hydrochloride 500 mgand gliclazide 80 mg. Tablets and assayed according to the test method. The %RSD for analysis of six replicate test solutions was calculated.

Linearity

A total of 125 mg metformin hydrochloride and 20 mg gliclazide standard were precisely measured by weighing and placed inside a 200 mL capacity flask. A 130 mL of diluent was poured in it, and content was dissolved using a sonicator. The flask was filled up to the required level with diluent. Different concentration ranges of solutions, such as 50 to 150%, were made.

Recovery study

Known amount of metformin hydrochloride and gliclazide accuracy stock solution has been spiked in placebo at 50, 100 and 150% of test concentration in a single preparation. According to the test technique, the concentrations of gliclazide and metformin hydrochloride were measured. The amount recovered and the actual amount added was used to compute the recovery percentage.

Preparation of accuracy stock solution

Accurately weighed measures of 125 mg of metformin HCl and 20 mg of gliclazide were kept in a 200 mL flask. A 130 mL

of diluting solution was topped up and the solution was sonicated for complete dissolution.

Preparation of accuracy

- *50% solution*

Accurately measured and put into a 200 mL volumetric flask the amount of placebo that is comparable to 125 mg metformin hydrochloride and 20 mg gliclazide. The solution had been sonicated for full dissolution after 130 mL of diluent was added. In the same flask, 5 mL of accuracy stock solution was topped up and further topped with diluent to reach the desired volume. After being sonicated, the mixture was filtered through a 0.45-inch nylon filter.

- *100% solution*

Accurately measured and put into a 200 mL volumetric flask the amount of placebo that is comparable to 125 mg metformin hydrochloride and 20 mg gliclazide. The solution was sonicated for full dissolution after 130 mL of diluent was added. In the same volumetric flask, 10 mL of accuracy stock solution was topped up and further topped with diluent to reach the desired volume. After being sonicated, the mixture was filtered through a 0.45-inch nylon filter.

- *150% solution*

Accurately measured and put into a 200 mL volumetric flask the amount of placebo that is comparable to 125 mg metformin hydrochloride and 20 mg gliclazide. The solution was sonicated for full dissolution after 130 mL of diluent was added. In the same flask, 15 mL of accuracy stock solution was topped up and further topped with diluent to reach the desired volume. After being sonicated, the mixture was filtered through a 0.45-inch nylon filter.

Robustness

Purposefully changing the following instrumental circumstances has confirmed the method's robustness.

- Modifying the flow rate by 1.0 to 0.1 mL/min.
- By 230 + 2 nm wavelength adjustment.
- By raising the temperature of the column oven by 25 or 5°C.

RESULTS AND DISCUSSION

Chromatographic Conditions

The mobile phase and gradient programming was selected after several trial runs. The chromatogram obtained for estimating metformin and gliclazide simultaneously using the selected chromatographic conditions and the chromatogram of the blank mobile phase are presented in Figures 1 and 2. The resolution of peaks was found to be good and a complete separation of both the drugs from the mixture was obtained.

System Suitability

The system suitability parameters prove that the system works perfectly and is in condition for analysis of samples. The result obtained from system appropriateness are presented in Table 1.

The %RSD for the retention time and peak area for both drugs was less than 2% indicating that the system and parameters are suitable for analysis.

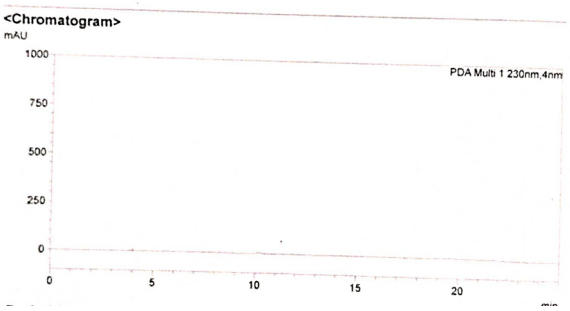

Figure 1: Chromatogram of the blank mobile phase

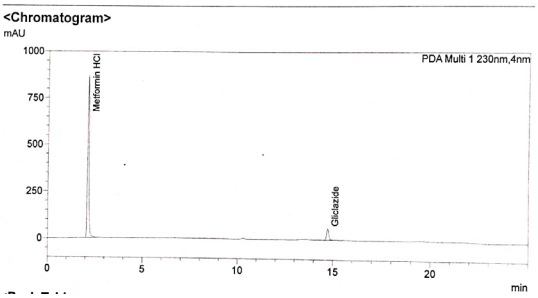

Figure 2: Chromatogram of metformin and gliclazide mix standard

Table 2: System appropriateness parameters

S. No.	*Retention time metformin (min)*	*Peak area*	*Retention time gliclazide (min)*	*Peak area*
1	2.25	4726724	14.97	488839
2	2.19	4724929	14.83	490920
3	2.22	4727051	14.92	491855
4	2.24	4722431	14.88	491491
5	2.31	4721873	14.81	492192
6	2.22	4721956	14.91	492557
Mean	2.23833333	4724161	14.8866667	491309
SD	0.04070217	2391.68	0.0595539	1335.71
%RSD	1.81841416	0.05	0.40004857	0.27

Linearity

The linear concentration range of metformin hydrochloride (62–187 µg/mL) as well as gliclazide (10–30 µg/mL) were evaluated. The method has been found to exhibit linearity in the above concentration range (Figure 3a and b).

Accuracy

The accuracy was assessed by spiking the preanlayzed test solutions with precalculated concentration of the pure drug and determining the concentration of the added solution. Table 2 represents the recovery study results at different spiked samples levels. The mean recovery of metformin was 99.8% while that of gliclazide was found to be 99%.

Precision

The precision of the method has been determined as system precision and method precision. The system precision represented the ability of the method to produce reproducible results using the pharmaceutical substance, whereas the method precision indicates the ability of the method to analyze

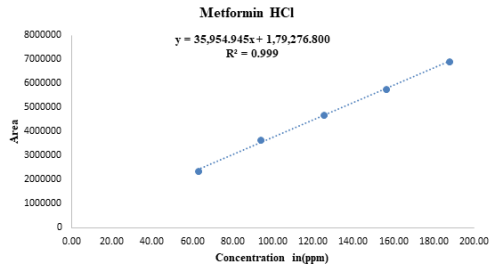

Figure 3a: Linearity curve of metformin

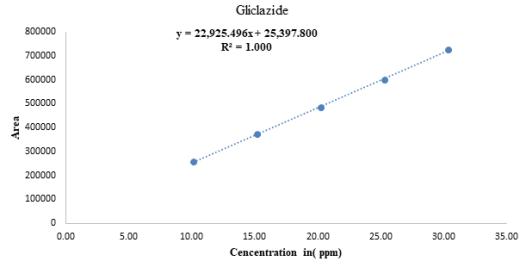

Figure 3b: Linearity curve of gliclazide

Table 3: Statistical evaluation of recovery study

Level (Spike in %)	*%Recovery of metformin*	*%Recovery of Gliclazide*
Level -1(50%)	100.3	98.6
Level -2(100%)	100.6	99.5
Level -3(150%)	98.6	99
Overall Mean	99.8	99
Overall SD	1.0762	0.4404
Overall %RSD	1.08	0.44

Table 4: Statistical data for precision

	System Precision		*Method Precision*	
S. No.	*Peak area of Metformin Hydrochloride*	*Peak area of gliclazide*	*%Assay of Metformin in tablet*	*%Assay of gliclazide in tablet*
1	4726724	488839	99.2	99.7
2	4724929	490920	99.9	100.4
3	4727051	491855	98.8	98.8
4	4722431	491491	99.5	99.4
5	4721873	492192	100.9	101
6	4721956	492557	99.3	99.1
Mean	4724161	491309	100.1	99.7
SD	2391.68	1335.71	0.9786	0.8221
%RSD	0.05	0.27	0.98	0.82

the drug with repeatability in the tablet sample. The results were found to have a %RSD of less than 2%, suggesting a precise method and system (Tables 3 and 4).

Robustness

Small deliberate variations in mobile phase, detection wavelength and column oven temperature were made to study the effect of the changes on the results obtained. Table 4 presents the robustness of the method.

Table 5: Robustness of the method

Variable Parameter	*%Assay of metformin hydrochloride*	*%Assay of gliclazide*
Plus Flow (1.1 mL/min)	97.6	99.1
Minus Flow (0.9 mL/min)	97.5	98.6
Plus wavelength (232 nm)	99.2	98.5
Minus Wavelength (228 nm)	99	98.1
Plus Column oven Temperature (30ºC)	98.3	98.7
Minus Column oven Temperature (20ºC)	98.2	99.4

Table 6: Result of tablet analysis

Parameter	*Metformin hydrochloride*	*Gliclazide*
Mean area	4724161	491309
SD	2391.68	1335.71
%RSD	0.0506	0.2718
%Assay	99.20%	99.70%

Analysis of Combination Tablet

The endorsed method has been applied to determine the amount of metformin and gliclazide in the marketed tablet combination (Aristo Pharma) and the results obtained are presented in Table 6.

CONCLUSION

For the concurrent measurement of metformin hydrochloride and gliclazide, a modest, linear, accurate, as well as exact reversed-phase HPLC approach has been created and validated. The proposed method was proven to be efficient and practical for laboratory quality control analysis of tablet dosage forms containing the two pharmaceutical compounds in bulk and tablet form. The brand of tablet that included the two APIs that were examined using the validated method had satisfactory quality since the contents of the two APIs were within acceptable bounds.

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