Development and Validation of a Simple Method for Simultaneous Estimation of Metformin Hydrochloride and Gliclazide in Tablets by using Reversed Phase High Performance Liquid Chromatography

Kanij Fatema, Md. Zakiur Rahman, Tasnuva Haque, Mohammad Abul Kalam Azad and Md. Selim Reza

Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka
Dhaka-1000, Bangladesh

ABSTRACT: The objective of this study was to develop a simple, efficient, precise and accurate reverse-phase HPLC method for the simultaneous determination of metformin in combination with gliclazide in newly formulated tablets. Chromatographic determination was performed on a reversed phase C_{18} column (2.6 mm x 250 mm; 5 µm particle size) using a mixture of buffer (1 ml of orthophosphoric acid with 1 ml triethylamine upto 1000 ml with HPLC grade water) and methanol at the ratio of 60:40 as mobile phase at a flow rate of 1ml/min. The UV detection was set at 230 nm. Under the developed conditions, good separation of the analytes was achieved. The calibration curves were linear with coefficient correlation between 0.998 to 1.0 for both drugs over a concentration range of 1 to 50 µg/ml for metformin hydrochloride and 0.16 to 8 µg/ml for gliclazide. The method was also validated in terms of precision (RSD = 0.06 to 3.22%) and accuracy (percent deviation = 0.049 to 2.602%). The proposed method was applied for the analysis of these analytes in newly formulated tablets and potencies were found to be 99.41±0.24% for metformin hydrochloride and 99.77±0.37% for gliclazide which were acceptable.

Key words: HPLC, metformin hydrochloride, gliclazide, combination dosage form, tablet.

INTRODUCTION

Metformin hydrochloride, an insulin-sensitizing biguanide used to treat type-2 diabetes, has been shown to be as effective as insulin or sulfonylureas when used as monotherapy.[1-5] For many patients with type 2 diabetes, monotherapy with an oral antidiabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control.[6] In such cases a combination of metformin hydrochloride and one of the sulfonylureas is used.[7] The fixed dose combination of gliclazide (80 mg) and metformin hydrochloride (500 mg) once or twice daily with meals to a maximum of 4 tablets per day (depending upon the glycemic control) showed significant efficacy in improving the glycemic control in type 2 diabetics.[8]

Gliclazide is a second generation sulphonylurea that is widely used in the treatment of patients with type 2 diabetes because it has similar efficacy to other sulphonylureas but a lower risk of hypoglycaemia.[9-10]

Many methods have been reported in the literature for the estimation of metformin hydrochloride and gliclazide individually.[11-15] However, there is no simple method has been reported for the simultaneous estimation of metformin hydrochloride with gliclazide. The complexity of the multicomponent dosage forms is that multiple entities

Correspondence to:
Md. Selim Reza
Tel: +880-2-9661920-73 Exnt. 8182; Fax: +880-2-8615583
Email: selimreza_04@yahoo.com

Dhaka Univ. J. Pharm. Sci. **9**(2): 83-89, 2010 (December)

and excipients poses considerable challenge to the analytical chemist during the development of assay procedure. In the early part of this century, colorimetric and spectrophotometric methods were used for drug analysis due to reasons of economy and easy availability. These methods, however, are used to a lesser extent today because they lack specificity, sensitivity and accuracy. For the simultaneous estimation of the drugs present in multicomponent dosage forms, HPLC method is considered to be most suitable since this is a powerful and rugged method.[16]

Analysis of the ingredients of the combined dosage form sometimes requires two separate sample preparations due to large differences in the label claims. In the present work, however, although large differences existed in the label claims of the ingredients of this dosage form, the analysis were performed with the same sample preparation.

MATERIALS AND METHODS

Active drugs and Reagents. Metformin hydrochloride (Aicon Bioscience Limited, India) and Gliclazide (Zhejiang Huayi Pharmaceuticals Co. Limited, China) were kind gift from Eskayef Bangladesh Ltd. Potassium dihydrogen phosphate, sodium hydroxide, methanol, triethyl amine were purchased from merck, Germany, ortho-phosphoric acid and acetonitrile were purchased from sigma-Aldrich, Switzerland.

Instrumentation and chromatographic condition. A Shimadzu integrated high performance liquid chromatographic system was used for this experiment. This system equipped with a SCL-10AVP system controller, LC-10ATVP quaternary gradient pump, SPD10AVP detector, CPO-10ASVP column oven, DGU-14A degasser and a SIL-10ADVP auto sampler controlled by CLASS-VP software. The octadecyl silyl (C18) – Xterra (Waters, Ireland), pH resistant (2.6 mm x 250 mm; 5 µm) column was used. Initially a photodiode array detector (Waters Alliance HPLC systems, Waters, USA) was used and the wavelength for both drugs (metformin hydrochloride and gliclazide) were determined at 230 nm simultaneously. The run time was set for 20 minutes at a flow rate of 1 ml/ minute.

Selection of mobile phase. Initially water and acetonitrile were used at 45:55 and 55:45 ratio as mobile phase. Finally mixture of prepared buffer and methanol were used as mobile phase at the ratio of 60:40 at ambient temperature using a flow rate of 1.0 ml/min and run for 20 minutes.

Preparation of buffer solution. The buffer solution was prepared in 1000 ml volumetric flask with 900 ml of HPLC grade water (Sartorious, arium 611, Ultrapure water systems, Germany), 1 ml of triethylamine, and 1 ml of orthophosphoric acid. The final volume was made upto 1000 ml with HPLC grade water and mixed well. This solution was filtered through 0.22 µm filter paper and degassed properly. Then the prepared buffer and methanol were used at different ratios for the selection of desired mobile phase.

Preparation of diluent (Phosphate buffer: pH 7.4). 27.22 g of potassium dihydrogen phosphate was taken into a 1000 ml volumetric flask to dissolve and diluted up to the mark with HPLC grade water. The concentration of this solution was 0.2 M. Then 50 ml of this solution was taken into a 200 ml volumetric flask and 39.1 ml of 0.2M sodium hydroxide was added. Finally the volume was made 200 ml with HPLC grade water.

Preparation of standard curve for metformin hydrochloride and gliclazide. 100 mg of metformin hydrochloride and 16 mg of gliclazide were weighed accurately into two separate 100 ml volumetric flasks to mix with 5 ml methanol to dissolve and were diluted up to 100 ml with phosphate buffer (pH 7.4) in each volumetric flask. From these solutions of concentrations of 1, 5, 10, 15, 20, 30, 40, and 50 µg/ml of metformin hydrochloride and 0.16, 0.80, 1.60, 2.40, 3.20, 4.80, 6.40, 8.0 µg/ml of gliclazide were prepared. The samples were analyzed by HPLC at 230 nm for metformin hydrochloride and gliclazide separately. Finally a standard curve was prepared by plotting above mentioned concentration versus corresponding area under the peak (Fig. 2).

Simultaneous determination of metformin hydrochloride and gliclazide from developed formulation (tablet). Required amount of powdered tablets which contain 500 mg of metformin hydrochloride and 80 mg of gliclazide were weighed accurately and taken into 100 ml volumetric flask. 10 ml of methanol was added to dissolve and volume was made up to 100 ml with phosphate buffer (pH 7.4). 5 ml of this solution was diluted to 100 ml. The solution was filtered through 0.2 µ membrane filter before analysis by HPLC.

RESULTS AND DISCUSSION

Selection of mobile phase. A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of metformin hydrochloride and gliclazide in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers. Separation obtained between metformin hydrochloride and gliclazide were not good, for the mobile phase consisting of water: acetonitrile = 45:55. Separated peaks were obtained after change the ratio of water and acetonitrile of mobile phase to 55:45 but the obtained peak for gliclazide was not sharp enough. Finally well distinct separated peaks were obtained when analysis was done by mobile phase consisting of buffer and methanol at the ratio of (60:40) shown in Fig. 1 (a). Distinct and symmetric peaks were also obtained for metformin hydrochloride and gliclazide from developed formulation (Fig. 1 b). The retention time of metformin hydrochloride and gliclazide were 2.60 and 17.04 minutes respectively.

Specificity of the method. Representative chromatograms are illustrated in fig. 1(a), 1(b). These chromatograms included separate samples of metformin hydrochloride, gliclazide and mixture of these simultaneously from standard solution and developed formulation. It showed that developed analytical method was specific for the analysis of metformin hydrochloride and gliclazide from standard mixture as well as tablet formulation.

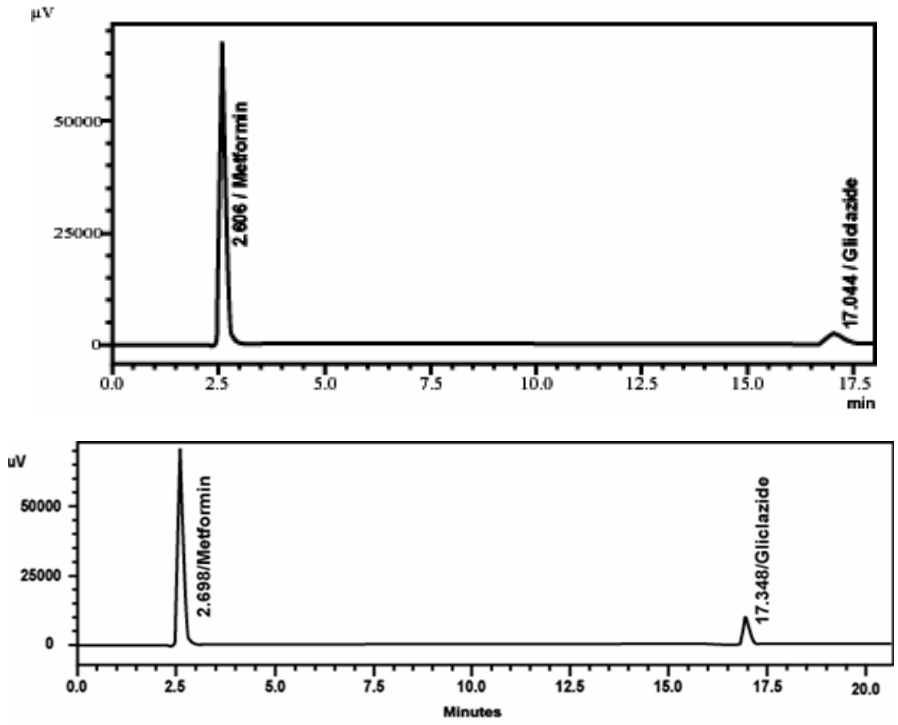

Figure 1. Chromatograms showing peaks of metformin hydrochloride and gliclazide (a) by using mobile phase buffer : methanol = 60:40 (b) for developed formulation (tablet).

Linearity. Table 1 presents the equation of the regression line, correlation coefficient (r^2), relative standard deviation (RSD %) values of the slopes. Excellent linearity was obtained for metformin hydrochloride between the range of 1.0 to 50 µg/ml with r^2 values of 0.998, 0.997, and 0.999 and for gliclazide the values were obtained 0.999, 0.996, and 0.998 between the range of 0.16 to 8.0 µg/ml (Fig. 2). Relative standard (%) values were obtained 0.058 % and 0.153 % for metformin hydrochloride and gliclazide respectively.

Table 1. Result of linearity of the developed method.

Compound	λ_{max}	Equation	r^2	Mean ± SD	RSD (%)
Metformin hydrochloride	230	Y= 96209X+3181	1.000	0.999±0.001	0.100
	230	Y= 95705X−10072	0.999		
	230	Y= 95870X−57210	0.998		
Gliclazide	230	Y= 53620X−51	0.999	0.999±0.000	0.058
	230	Y= 53220X+75	1.000		
	230	Y= 52914X−295	0.999		

X = Concentration (µg/ml); Y = Area; RSD (%) = (Standard deviation / Mean) x 100

Precision. The precision of the method (within-day variation of replicate determination) was checked by repeatedly injecting the mixture of metformin hydrochloride and gliclazide 5 times. The precision of the method, expressed as the RSD % (Table 2) and the values were found to be 0.08 – 3.22 %.

Accuracy. A standard working solutions-containing metformin hydrochloride and gliclazide, having the final concentration of 10 µg/ml, 30 µg/ml, 50 µg/ml of metformin hydrochloride and 1.6 µg/ml, 4.8 µg/ml, 8.0 µg/ml of gliclazide were prepared. The prepared mixtures of standard solutions were injected 5 times as a test sample. From the respective area counts, the concentration of metformin hydrochloride and gliclazide were calculated simultaneously by using the detector response (Table 3).

Table 2. The precision of the developed method (n=5) (λ_{max} = 230 nm).

Compound	Standard concentration (µg/ml)	Peak Area (Mean ± SD)	RSD (%)
Metformin hydrochloride	10.0	968058 ± 29578	3.06
	30.0	2760348 ± 90371	3.22
	50.0	4807433 ± 3852	0.08
Gliclazide	1.6	82371 ± 330	0.40
	4.8	255678 ± 3148	1.23
	8.0	422712 ± 1188	0.28

Table 3. The accuracy of the developed method (n=5).

Compound	Standard concentration (µg/ml)	Measured concentration (µg/ml) (Mean ± SD)	Deviation (%)
Metformin hydrochloride	10.0	10.07 ± 0.308	0.650
	30.0	29.22 ± 0.939	2.602
	50.0	49.98 ± 0.040	0.049
Gliclazide	1.6	1.59 ± 0.006	0.343
	4.8	4.78 ± 0.059	0.502
	8.0	7.99 ± 0.022	0.150

Deviation (%) = (standard concentration - measured concentration) / standard concentration x 100.

Sensitivity. The lowest concentration of analyte detectable or quantifiable with a stated degree of reliability is commonly called sensitivity.[17] The detection limit (LOD) is the smallest quantity of analyte of which it can be said, with a given level of confidence, that it is present in the sample. The limit of detection (LOD) and the limit of quantitation (LOQ) can be determined by the equation LOD ≈ 3σ and LOQ ≈ 10σ, where, σ is the standard deviation of the response that is measured from the standard deviation of a regression line or the standard deviation of the y-intercepts of regression line.[18]

Table 4. Parameters of Linear Ordinary Least-Squares Regression.

Parameters	Notation	Estimates for Metformin	Estimates for Gliclazide
Observations		24	24
Correlation coefficient	N	0.999	1.000
Residual standard deviation	R	62261.8005	2877.5224
Slope	σ	96145.929	53257.005
Intercept	a	-28695.9	-121.415

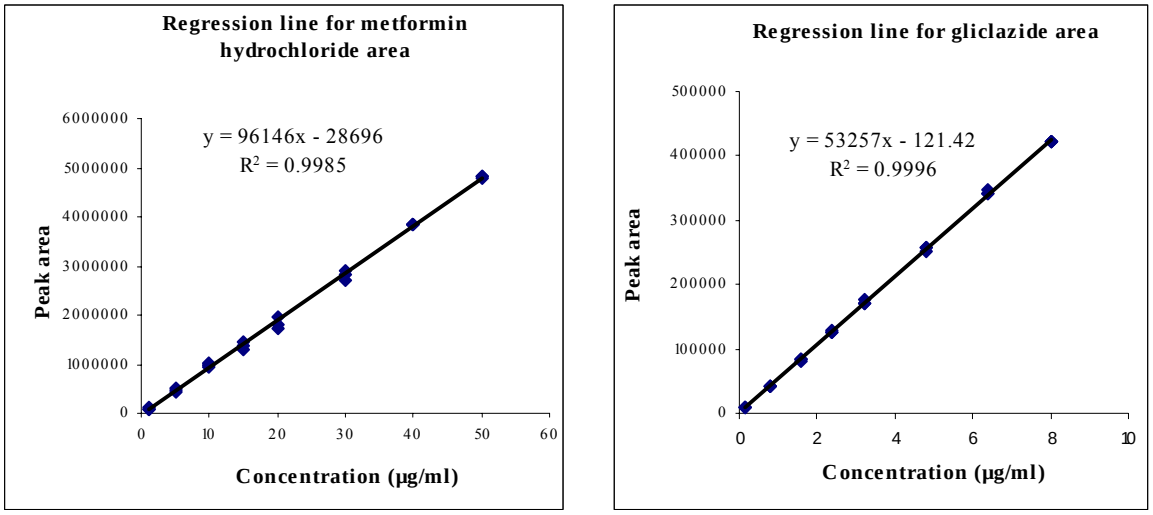

Figure 2. Experimental data and regression line for metformin hydrochloride and gliclazide area

Table 5. LOD and LOQ of metformin and gliclazide obtained with the Ordinary Least-Squares Regression.

Drug	Criteria	Area	Level (µg/ml)
Metformin	LOD area = 3 σ	186785.4015	2.2412
	LOQ area = 10 σ	622618.005	6.7742
Gliclazide	LOD area =3 σ	8632.5672	0.1643724
	LOQ area =10 σ	28775.224	0.5425885

Results of metformin hydrochloride and gliclazide determination in the developed formulation. The detection wavelength of 230 nm was chosen for the determination of metformin hydrochloride and gliclazide in new formulated tablet. The isocratic program throughout HPLC method was adopted to analyze both components in a single run. No significant peak was observed from

the tablet excipient (Figure 1 d). The potencies were found to be 99.41±0.24% and 99.77±0.37% of metformin hydrochloride and gliclazide respectively from the developed formulation.

Table 6. Results in developed formulation (n=6).

Compound	Retention time	Area	Equation	Concentration (µg/ml)	Claimed amount mg/tablet	Amount found mg/tablet	% potency
Metformin hydrochloride	2.698 ± 0.012	4791503 ± 11675	Y=96209 X +3181	49.77 ± 0.12	500	497.70 ± 1.21	99.54 ± 0.24
Gliclazide	17.348 ± 0.001	424245 ± 1561	Y= 53220 X +75	7.97 ± 0.03	80	79.70 ± 0.29	99.63 ± 0.37

CONCLUSION

The validation study shows that the developed method is accurate, rapid, precise, reproducible and inexpensive with acceptable correlation co-efficient, RSD(%) and standard deviations which make it versatile and valuable for simultaneous determination of metformin hydrochloride and gliclazide from pharmaceutical dosage form, especially new formulated tablet. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed method is simple and do not involve laborious time-consuming sample preparation. So this HPLC method can be used in the quality control department.

ACKNOWLEDGEMENTS

The authors are thankful to Eskayef Bangladesh Limited for providing reference standards and all facilities to complete this research work.

REFERENCES

1. Hermann, L.S., Scherstén, B., Bitzén PO, Kjellström, T., Lindgärde, F. and Melander, A. 1994. Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes Care* **17**, 1100-1109.
2. De Fronzo, R.A. and Goodman, A.M. 1995. Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N. Engl. J. Med*. **333**, 541-549.
3. United Kingdom Prospective Diabetes Study Group. 1995. Relative efficacy of randomly allocated diet, sulphonylurea, insulin, or metformin in patients with newly diagnosed non-insulin dependent diabetes followed for three years. United Kingdom Prospective Diabetes Study (UKPDS 13) *B.M.J.* **310,** 83-88.
4. Johansen, K. 1999. Efficacy of metformin in the treatment of NIDDM. Meta-analysis. *Diabetes Care*. **22**, 33-37.
5. United Kingdom Prospective Diabetes Study Group. 1998. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet.* **352**, 854-865.
6. De Fronzo, R.A. 1999. Pharmacologic therapy for type 2 diabetes mellitus. *Ann. Intern. Med.* **131**, 281-303
7. Tack, C. and Smits, P. 2006. Thizolidinedione derivatives in type 2 diabetes mellitus. *Netherland J. Med*. **64**, 166.
8. Bajpai, N., Gulati, A. and Chatterjee, A. 2005. A 4-6 months open labeled, multicentric, non-comparative study for evaluation of efficacy, safety and tolerability of glizid M in type 2 diabetes. *Ind. Pract.* **58**, 283-291.
9. Garber, A.J. 2000. Using dose–response characteristics of therapeutic agents for treatment decisions in type 2 diabetes. *Diabetes Obes. Metab*. **2,** 139-147.
10. Lindblad, U. and Melander, A. 2000. Sulphonylurea dose–response relationships: relation to clinical practice. *Diabetes Obes. Metab*. **2**, 25-31.
11. Indian Pharmacopoeia. 1996. The Controller of Publications, New Delhi.
12. British Pharmacopoeia. 2005. Vol 1 The Stationary Office, MHRA British Pharmacopoeial Commission office, London.
13. Huupponen, R., Ojala Karlsson, P., Rouru, J. and Koulu, M. 1992. Determination of metformin in plasma by High Performance Liquid chromatography. *J. Chromatogr. Biomed. Appl.* **583,** 270-273.
14. Vasudevan, M., Ravi, J., Ravisankar, S. and Suresh, B. 2001. Ion-pair liquid chromatography technique for the estimation of metformin in its multicomponent dosage forms. *J. Pharm. and Biomed. Analysis* **25**, 77-84.
15. Porta, V., Schramm, S.G., Kano, E.K., Koono, E.E., Armando, Y.P., Fukuda, K. and Serra, C.H.R. 2008. HPLC-UV determination of metformin in human plasma for application in pharmacokinetics and bioequivalence studies. *J. Pharm. and Biomed.. Analysis* **46**, 143-147.

16. ICH Expert working Group. 1996. Validation of analytical procedures: text and methodology. *ICH Harmonised Tripartite Guideline* **Q2** (R1), 1-8.

17. Armbruster, D.A., Margaret, D., Tillman and Hubbs, L.M. 1994. Limit of Detection (LOD) / Limit of Quantitation (LOQ): Comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs. *Clinic.Chem.* **40**, 1233-1238.

18. Vial, J. and Jardy, A. 1999. Experimental Comparison of the Different Approaches to Estimate LOD and LOQ of an HPLC Method. *Anal. Chem*. **71,** 2672-2677.