Full Length Research Paper

Determination of gliclazide in a tablet dosage form in the presence of metformin hydrochloride by ion pair – reversed phase liquid chromatographic technique

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This study describes the development and validation of an isocratic HPLC method for the stability of gliclazide assay in the presence of metformin hydrochloride (MET) in pharmaceutical dosage forms using ion pair - reversed phase liquid chromatographic technique. The ion pairing agent used in this study is tetrabutyl ammonium hydrogen sulphate (TBHS). The TBHS 0.030 molar solution in water was adjusted to pH 6.0 with 1 N sodium hydroxide solution and was used as buffer. The composition of the buffer and acetonitrile used was 40:60 (v/v) on reverse phased column, bonded with octadecyl silane, while the wave length used was 225 nm. As such, the drug gave a linear response (r^2>0.999). The method was found to be selective, precise, accurate and robust, and can be used for quality control assay of the bulk and finished dosage form as a single dose in combination with metformin hydrochloride.

Key words: Ion pair, reversed phase, UV detection, gliclazide.

INTRODUCTION

Gliclazide is chemically known as 1-(hexahydrocyclopenta[c] pyrrol-2-(1H)-yl)-3-[(4-methylphenyl) sulfonyl] urea. Figure 1 shows an antidiabetic agent used as a single dose or with metformin hydrochloride in patients with Type 2 diabetes mellitus. The Type 2 diabetes mellitus is a progressive disorder, and although oral monotherapy is often initially successful, it is associated with a secondary failure rate, which contributes to the development of long-term diabetes complications resulting from persistent hypoglycemia. The accumulated evidence suggests that combination therapy, using oral anti diabetic agents with different mechanisms of action, may be associated with fewer side effects than higher dose monotherapy and may achieve similar or better glycemic control.

The literature survey reveals that a number of analytical methods have been developed for the quantitative determination of gliclazide in pharmaceutical dosage form.

Bansal et al. (2007) reported a stability that indicates the method of gliclazide tablet, whereas Lakshmi et al. (2010) reported a method for simultaneous determination of gliclazide and rosiglitazone in pharmaceutical dosage form. Rathnivel et al. (2009) reported RP-HPLC method for simultaneous determination of gliclazide and rosiglitazone in tablet, while Chowdary et al. (2009) developed a HPLC method for gliclazide in formulation. Rauini et al. (2001) developed a method for gliclazide in multi component drug in human syrum, while Kimura et al. (2007) reported a method for gliclazide using tolbutamide as an internal standard. Foroutan et al. (2006) reported a method for quantitation of gliclazide using monolithic column in human plasma, whereas Venkatesh et al. (2006) developed an extraction method for gliclazide along with five other antidiabetics by HPLC method in human plasma. Aburuz et al. (2005) developed a method for gliclazide with four other antidiabetics in human plasma, while Emmie et al. (2004) developed a method of LC-MS- MS in equin plasma and urine for gliclazide and nine other anti-diabetics. Maria-Cristina et al. (2009) reported HPLC method for the simultaneous

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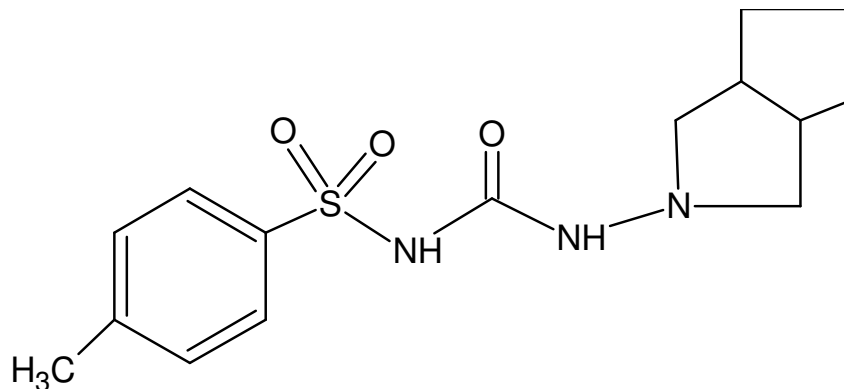*Corresponding author. E-mail: uday_basrur@indiatimes.com.

Figure 1. Chemical structure of gliclazide.

analysis of metformin and gliclazide in human plasma, whereas Roohi (2002) developed a method for analysis of gliclazide in human plasma by using HPLC. However, Jia-Feng (2004) developed a method for gliclazide in human plasma by HPLC. All the aforementioned reported methods can be used for routine quality control analysis of gliclazide either in biological fluids or in pharmaceutical dosage form. The gliclazide is mostly prescribed with metformin hydrochloride as an extended release tablet form. None of these methods described the stability indicating the nature of gliclazide in the presence of metformin hydrochloride. The present work illustrates the development of the simple, rapid and precise stability indicating the ion pair-reversed phase liquid chromato-graphic techique for quantitation of gliclazide in combination with metformin hydrochloride. Nonetheless, all the degraded products are separated and they do not interfere with the principal peak of gliclazide.

EXPERIMENTAL

Materials and Reagents

A sample of gliclazide (assigned 99.7% purity) and metformin hydrochloride (assigned 99.8% purity) of pharmaceutical grade was received from Panacea Biotech Ltd- Bengaluru-India. Gliclazide Glix tablet (with 80 mg strength), Glizide-M tablet (with 500 mg metformin hydrochloride strength) and gliclazide (80 mg) were procured from the market. Tetrabutyl ammonium hydrogen sulphate of analytical grade was purchased from Merck-Mumbai, India, while HPLC grade acetonitrile was purchased from Qualigens – Mumbai, India. However, high purity water was prepared by Millipore milli Q plus purification system.

HPLC instrumentation and condition

The HPLC system consisted of Shimadzu LC- 2010CHT with quaternary gradient pumps attached to the UV and PDA detector. The chromatographic separations were performed on micro-bondapak C-18 column, 300 × 3.9 mm, particle size 10 μ maintained at 25°C using column oven, eluted with mobile phase at the flow rate of 1.0 ml/min. The mobile phase consisted of a mixture

of 0.03 molar TBHS in water and the pH was adjusted to 6.0 with 1 N sodium hydroxide and acetonitrile 40:60 (v/v), before it was filtered through 0.45 μm nylon filter and degassed in ultrasonic bath prior to use. The diluent used was a mixture of methanol and acetonitrile 50:50 (v/v). Measurements were made with injection volume 10 μl and ultraviolet (UV) detection at 225 nm. The output signal was integrated using LC-solution soft ware.

Standard and sample preparation

The standard stock solutions (1000 μg/ml for each gliclazide) were prepared separately by dissolving working standards in diluent and diluted to the desired volume with the same diluents. The standard calibration solution of gliclazide, which has a concentration in the range of 100 to 300 μg/ml, was prepared by diluting stock solution with diluent.

Analysis of dosage form

Twenty tablets were separately weighed, and their mean weight was determined and crushed in mortar. An amount of powdered mass, equivalent to 10 mg of gliclazide was transferred separately into a 50 ml volumetric flask containing 25 ml diluent, after which it was mechanically shaken for 10 min, ultrasoniccated for 5 min, and then diluted to volume with diluent before it was filtered. The first 10 ml of the filtrate was rejected, but the subsequent one was used.

RESULTS AND DISCUSSION

Method development

The primary target for developing the LC method is to achieve good separation between metfomin hydrochloride and gliclazide, thereby estimating the content of this drug by the single analytical method. Separation between metformin hydrochloride and gliclazide was observed using various other mobile phases and different organic modifiers, but the degraded product of gliclazide and metformin hydrochloride interfered with gliclazide peak. After choosing an ion pairing agent, tetrabutyl ammonium hydrogen sulphate of
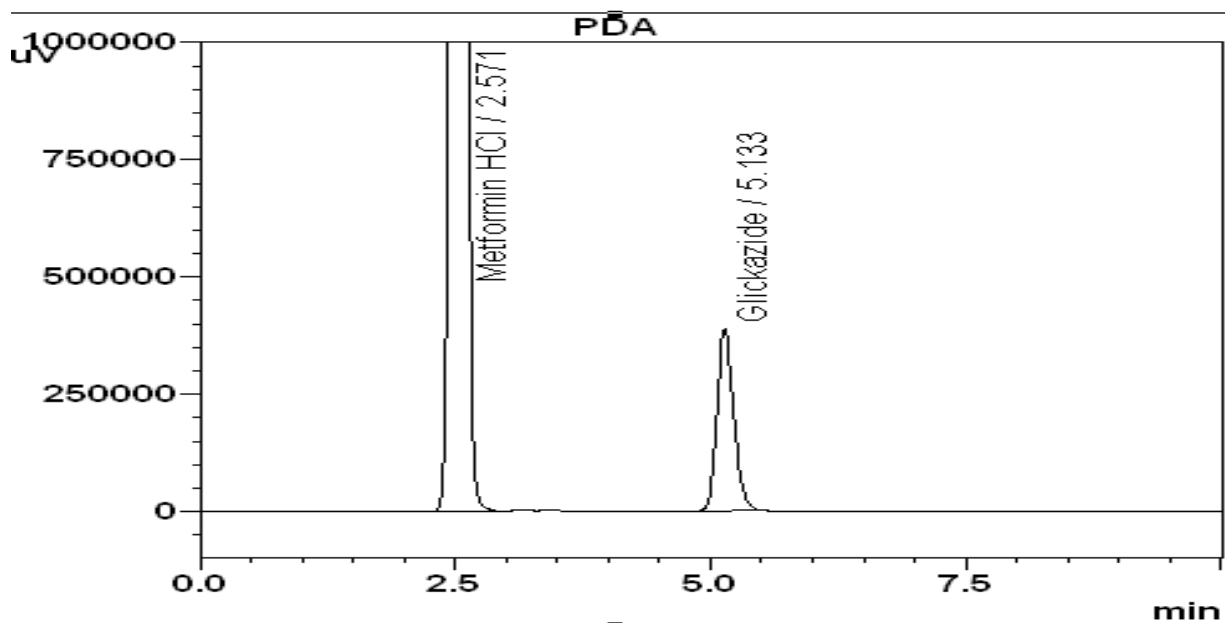
Figure 2a. Chromatograms of untreated Metformin hydrochloride and Gliclazide in tablet sample.

0.030 molar was adjusted to pH 6.0, and it was found that the metformin hydrochloride was separated without retention. Likewise, gliclazide and its degradants were separated well in the given condition and the mobile phase composition of 40:60 buffer and acetonitrile. Finally, the mobile phase, which consists of the said composition adopted, produces good resolution, reasonable retention and acceptable peak shape for gliclazide. The tablet matrix was also determined to see if any interference from them existed. No significant peaks from the matrix were observed in chromatogram, indicating that there was no interference from the formulation matrix. The validation was carried out for gliclazide in combination with metformin hydrochloride. A typical chromatogram of metformin hydrochloride and gliclazide tablet is shown in Figure 2a.

Degradation studies

Forced degradation of gliclazide tablet sample in combination with metformin hydrochloride carried out under different stress conditions (Heat, light, hydrogen-peroxide, acid and base) were prepared for further evaluation of the selectivity of the proposed LC method. The selectivity was studied only for gliclazide and not for metformin hydrochloride, as this was not retained in the column. For preparing acid and base induced degradation, product 5 ml of 0.1 M HCl and 0.1 M NaOH were separately added to 10 mg each of gliclazide equivalent tablet powder and were exposed at 80°C for 6 h. The degraded samples were then neutralized and placed in

50 ml volumetric flask and prepared as described in the sample preparation.

For preparing hydrogen peroxide induced degradation, product 0.5 ml hydrogen peroxide (30%v/v) was added to 10 mg each of gliclazide equivalent tablet powder and exposed at 80°C for 6 h. The degraded sample was placed in 50 ml volumetric flask and prepared as descrybed in the sample preparation. The forced degradation in acidic, basic and oxidation media was performed in the dark in order to avoid the possible effect of light. For preparing dry heat degradation, product 10 mg each of gliclazide equivalent tablet powder and stored at 80°C for 6 h under dry heat condition in the dark and then cooled to room temperature. The degraded sample solution was prepared as described in the sample preparation.

The photochemical stability of the drugs was also studied by exposing the tablet powder to 1,200 K lux of visible light and 200 W h m^{-2} of UV light by using photo stability chamber. The same procedure was followed as indicated for dry heat degradation. The resulting solutions were used as the degraded sample solution and determined under the described chromatographic condition. Typical chromatograms of all the degraded tablet samples are shown in Figures 2b to d.

The degraded samples were compared to a tablet sample without degradation. The spectral homogeneity (with peak purity from 200 to 400 nm) was determined in the forced degraded samples. The threshold was set at≥0.990. The peak purity, peak threshold and percent degradation (Table 1) of gliclazide (10 mg each) demonstrated that the proposed LC method was able to separate each drug from degradants generated during
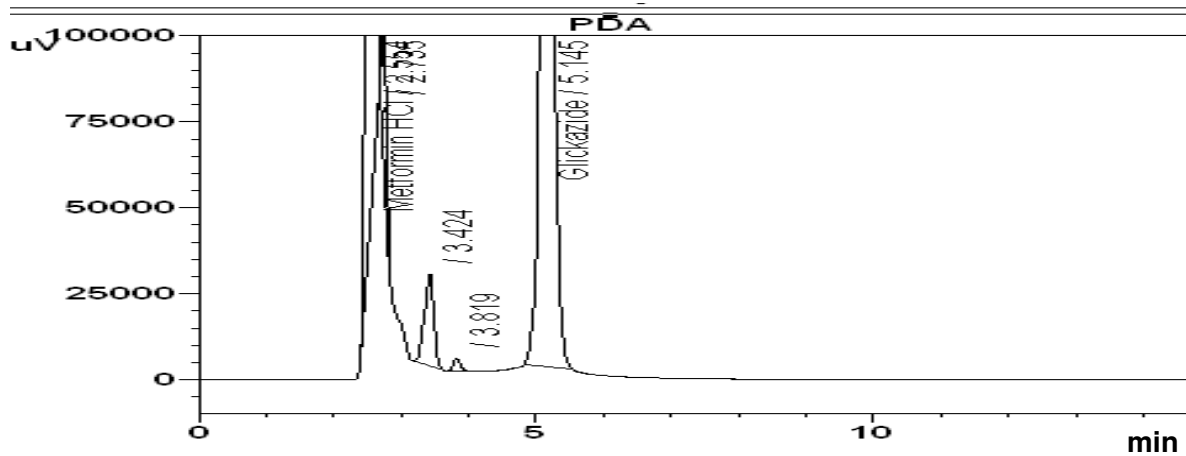
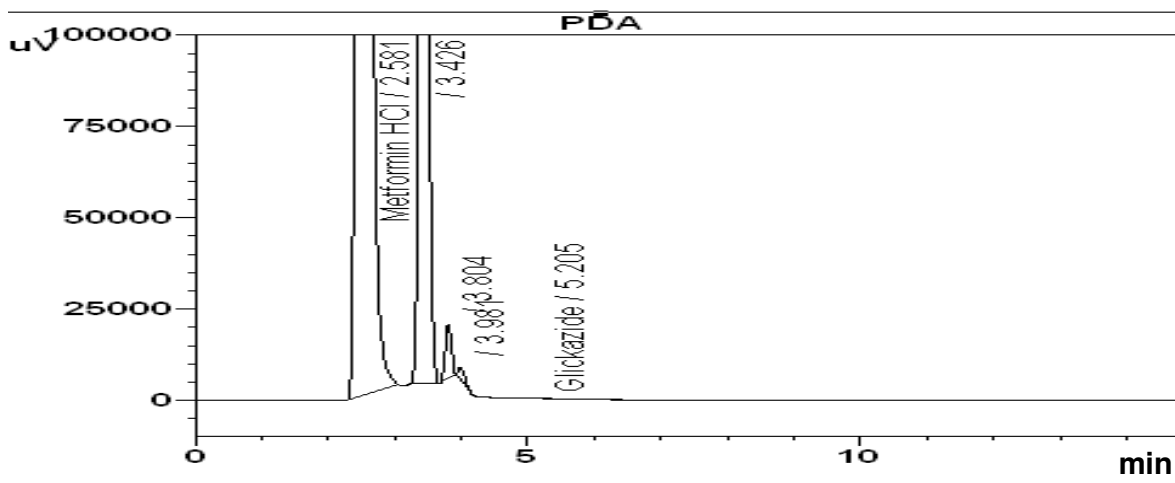**Figure 2b.** Chromatograms of base hydrolysis-degraded Metformin hydrochloride and Gliclazide in tablet sample.

Figure 2c. Chromatograms of acid hydrolysis-degraded Metformin hydrochloride and Gliclazide in tablet sample.
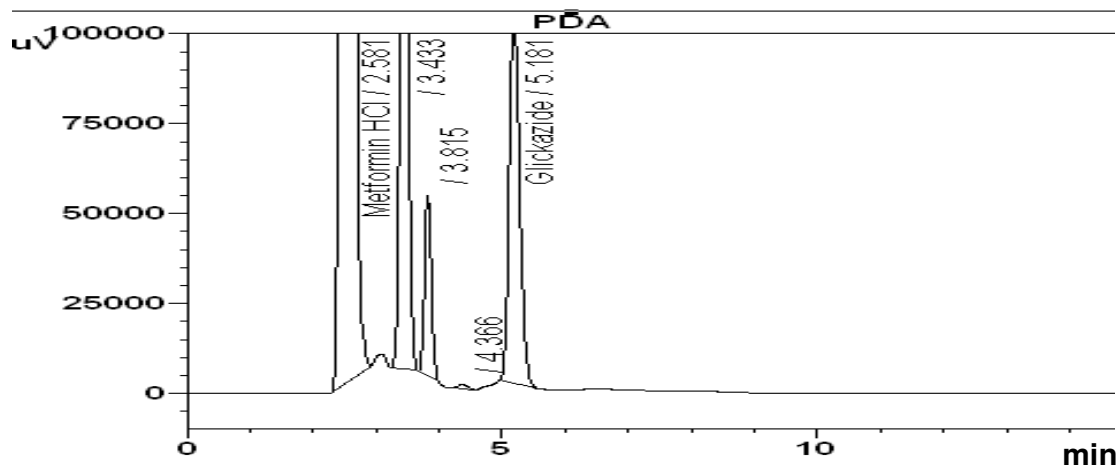

Figure 2d. Chromatograms of oxidative-degraded Metformin-hydrochloride and Gliclazide in tablet sample.

Table 1. Results of the forced degradation study of gliclazide.

Stress condition	Peak purity	Single point threshold	% degraded (both metformin and gliclazide)
Light	1.0000	0.9999	Nil
Heat	1.0000	0.9999	Nil
Acid	1.0000	0.9999	100%
Base	1.0000	0.9999	14%
Oxidation	1.0000	0.9999	73%

forced degradation studies from both metformin hydrochloride and gliclazide.

Linearity

The linearity of the response of drugs was verified at five concentration levels ranging from 100 to 300 µg/ml for gliclazide tablets. The calibration curve was constructed by plotting mean area response A against concentration C of each drug. The regression equations obtained for gliclazide were $A = 41911C$ ($r^2 = 0.9999$, n = 5). The result shows that an excellent correlation existed between peak area and concentration of gliclazide within the concentration range tested.

Limit of quantitation and detection

The limit of quantitation (LOQ) was defined as the lowest concentration that can be determined with acceptable accuracy and precision, which can be established at a signal to noise ratio of 10. LOQ of each drug was experimentally verified by six injections of each drug at its LOQ concentration. The LOQ of gliclazide was found to be 0.10 µg/ml. The limit of detection (LOD) was defined as the lowest concentration that can be detected and established at a signal to noise ratio of 3. As such, the LOD of gliclazide was found to be 0.08 µg/ml.

Precision

The repeatability method (intra-day precision) was evaluated by assaying six samples, prepared as described in the sample preparation. The mean % assay and percentage R.S.D. for assay values of gliclazide were found to be 100.2 and 0.57%, 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 not be more than 2.0%. The intermediate precision (inter-day precision) was performed by assaying six samples prepared by different analysts, different HPLC systems and different HPLC columns in different days as described in the sample preparation. The mean % assay and percentage R.S.D. for assay values of gliclazide

were 98.6 and 0.3%, respectively which is well within the acceptance criteria. Thus, the results of intra-day precision and inter-day precision were evaluated with respect to student's t- test and F-test. The student t-test and F-test [Tcal (T_{tab}) = -1.66(2.571) and F_{cal} (F_{tab}) = 0.19(3.05)] were therefore accepted. Consequently, the result shows the good precision of the method.

Accuracy

Accuracy was determined by applying the developed method to synthetic mixtures of excipients, to which known amounts of each drug corresponding to 80, 100 and 120% of label claim had been added. The accuracy was then calculated as the percentage of analyte recovered from the formulation matrix. Mean recovery (Mean ± S.D) of gliclazide was 100.55 ± 0.17%. The obtained result suggested the accuracy of the developed method for the determination of gliclazide in combination with metformin hydrochloride.

Robustness

The robustness of the method was determined by analyzing the same samples at standard operating conditions and also by changing the analytical conditions, such as: mobile phase composition, temperature and pH and flow rate. In all the deliberate varied chromatographic conditions carried out, that is, organic phase composition, column temperature, pH and flow rate in mobile phase, the system suitability parameter and % assay for gliclazide from the six replicate injections of the test solution was found to be within the acceptable limits. However, the robustness of the method is established as the percentage deviation from the mean assay value obtained from the precision study, which is less than ± 2%. Table 2 represents the robustness of the method.

Assay of formulation

The validated LC method was applied to the determination of gliclazide tablets as a single component combined with metformin hydrochloride. Two batches were assayed and

Table 2. Results of robustness study of gliclazide.

Set name	Initial precision (RSD%)	Tailing factor (≤ 1.5)	% purity
Standard condition	0.31	1.30	98.59
Flow rate (0.9 ml/in)	1.03	1.24	99.45
Flow rate (1.1 ml/min)	0.34	1.23	98.35
Mobile phase organic composition + 10% Acetonitrile	0.91	1.32	98.64
Mobile phase organic composition - 10% Acetonitrile	0.45	1.34	99.05
Column temperature + 5°C	0.48	1.22	100.3
Column temperature - 5°C	0.68	1.26	100.1
pH 5.8	0.54	1.34	98.30
pH 6.2	0.29	1.32	99.26

Table 3. Results of gliclazide in marketed product.

Marketed tablet	Drug	% Amount found ± SD	% RSD
Glix	Gliclazide (80 mg)	100.19 ± 0.34	0.34
		100.53 ± 0.55	0.55
Glizid-M	Gliclazide (80 mg) with metformin hydrochloride (500 mg)	100.20 ± 0.57	0.57
		99.83 ± 0.45	0.45

the results are shown in Table 3, indicating that the amount of each drug in the tablet samples met the requirements (90 to 110% of the tablet claim).

Solution stability and mobile phase stability

The R.S.D. of gliclazide assay during solution stability and mobile phase stability experiments was within 1%. The solution and mobile phase stability experiments data confirmed that the sample solutions and mobile phase, used during assay determination, was stable up to 48 h.

Conclusion

An isocratic ion pair reversed phase liquid chromatographic method was developed and validated for the determination of gliclazide as a bulk drug and in pharmaceutical dosage form as a single component in combination with metformin hydrochloride. This chromatographic assay fulfilled all the requirements needed for it to be identified as a reliable and feasible method, including accuracy, recovery and precision data. It is highly accurate, precise and selective. The analytical procedure and its chromatographic run time is less than 6 min. Therefore, the HPLC method can be used as a routine sample analysis for stability study purposes.

ACKNOWLEDGMENTS

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