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Original article

# A rapid hydrophilic interaction liquid chromatographic determination of glimepiride in pharmaceutical formulations

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ABSTRACT

Glimepiride is one of the most widely prescribed antidiabetic drugs and contains both hydrophobic and hydrophilic functional groups in its molecules, and thus could be analyzed by either reversed-phase high performance liquid chromatography (HPLC) or hydrophilic interaction liquid chromatography (HILIC). In the literature, however, only reversed-phase HPLC has been reported. In this study, a simple, rapid and accurate hydrophilic interaction liquid chromatographic method was developed for the determination of glimepiride in pharmaceutical formulations. The analytical method comprised a fast ultrasound-assisted extraction with acetonitrile as a solvent followed by HILIC separation and quantification using a Waters Spherisorb $S_5NH_2$ hydrophilic column with a mobile phase consisting of acetonitrile and aqueous acetate buffer (5.0 mM). The retention time of glimepiride increased slightly with decrease of mobile phase pH value from 6.8 to 5.8 and of acetonitrile content from 60% to 40%, indicating that both hydrophilic, ionic, and hydrophobic interactions were involved in the HILIC retention and elution mechanisms. Quantitation was carried out with a mobile phase of 40% acetonitrile and 60% aqueous acetate buffer (5.0 mM) at pH 6.3, by relating the peak area of glimepiride to that of the internal standard, with a detection limit of 15.0 μg/L. UV light absorption responses at 228 nm were linear over a wide concentration range from 50.0 μg/L to 6.00 mg/L. The recoveries of the standard added to pharmaceutical tablet samples were 99.4–103.0% for glimepiride, and the relative standard deviation for the analyte was less than 1.0%. This method has been successfully applied to determine the glimepiride contents in pharmaceutical formulations.



## 1. Introduction

Glimepiride, 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea (Fig. 1), is a sulfonylurea derivative and one of the most widely prescribed oral drugs for the treatment of non-insulin dependent type II diabetes mellitus (T2DM) (Davis, 2004; Harper et al., 2013). Glimepiride acts as an insulin secretagogue. It stimulates the secretion of insulin by pancreatic β-cells and increases sensitivity of intracellular insulin receptors, which lowers blood glucose level (Kabadi and Kabadi, 2004; Shukla et al., 2004). Glimepiride is usually used after diet and exercise measures fail to achieve appropriate control of blood glucose level. Like all other sulfonylurea medicines, glimepiride is normally given to diabetic patients over a long period of time. The overdose of glimepiride can cause hypoglycemia and other side effects, such as gastrointestinal tract disturbance, allergic reactions, liver dysfunction, chest pain, irregular heartbeat, endocrine disruption, and hemolytic anemia (Adachi and Yanai, 2015; Chounta et al., 2005; Harper et al., 2013; Papathanassiou et al., 2009). Thus, the amount of glimepiride in pharmaceutical dosage formulations administrated to the patients is critical in achieving high level of efficacy and safety of the anti-diabetic medication. Therefore, an accurate, simple and fast analytical method for monitoring glimepiride in pharmaceutical formulations is needed for the quality control.

Many analytical methods have been reported for the determination of glimepiride. Altinöz and Tekeli (2001) used a simple derivative UV spectrophotometric method for the determination of glimepiride in pharmaceutical tablets. Fahim et al. (2014)

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described a transmission Fourier transform infrared spectroscopy (FTIR) technique for analysis of metformin and glimepiride in drug samples. Badawy et al. (2010) performed quantitative measurement of glimepiride, and three other anti-diabetic drugs, rosiglitazone, pioglitazone and glyburide, using cyclic voltammetry and differential pulse voltammetry. Several research groups developed and validated analytical methods based on high performance liquid chromatographic (HPLC) and high performance thin layer liquid chromatography (HPTLC) for the determination of glimepiride individually or with some other anti-diabetic drugs (Jain et al., 2008; Kovaříková et al., 2004; Ni et al., 2014; Dash et al., 2016; Sane et al., 2004a, b; Shaodong et al., 2010). Among all reported analytical methods, HPLC has been the most commonly used method with high selectivity and accuracy, especially for complex biological samples. However, all previously reported HPLC methods for the analysis of glimepiride were based on reversed phase separation; and no hydrophilic interaction (aqueous normal phase) liquid chromatographic (HILIC) technique has been reported on the analysis of glimepiride, a drug containing both hydrophilic and hydrophobic functional groups, in the literature. In contrast to reversed phase HPLC, which employs a nonpolar stationary phase (SP) and a polar mobile phase (MP), HILIC uses a polar hydrophilic (normal) SP and an aqueous-polar organic solvent MP (Chen and Zuo, 2007; Jiao and Zuo, 2009; Zuo et al., 2002; Zuo et al., 2014). Hence, HILIC provides a different elution order and selectivity from reversed phase HPLC and has been increasingly applied to the separation and determination of polar pharmaceutical drugs and metabolites in recent years (Qin et al., 2008; Dejaegher and Heyden, 2010; Ares and Bernal, 2012; Zuo et al., 2011, 2014, 2015). In this study, an accurate, simple and rapid HILIC method has been developed for the determination of glimepiride in pharmaceutical formulations.

## 2. Experimental

### 2.1. Chemicals

Glimepiride and 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfo nic acid) diammonium salt (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (ACN) and acetic acid (95%) were supplied by Fisher Scientific (Fair Lawn, NJ, USA). Sodium hydroxide was obtained from CMS. Inc. (Houston, Texas, USA). Except where noted, all reagents were of analytical grade and all solutions were prepared using distilled-deionized water. The mobile phase solvents were degassed by vacuum filtration through 0.45 μm nylon membranes (Fisher Scientific, Fair Lawn, NJ, USA) before HPLC analysis.

### 2.2. Chromatographic conditions

An Alliance HPLC system (Waters Corporation, Milford, MA. USA) equipped with a Waters 2695 Separation Module, a Waters 486 Tunable UV-Visible Absorbance Detector and Empower 2 software was employed for analyses. The analytical column used was a Waters Spherisorb $S_5NH_2$ column (250 mm × 4.6 mm, 5 μm; Waters, Milford, MA). The detection of glimepiride was carried out by UV absorbance at 228 nm. The flow rate was 1.0 mL/min. The injection volume was 20 μL.

### 2.3. Standard solution preparation

Standard stock solution of glimepiride was prepared in acetonitrile with concentration of 250 mg/L. Internal standard stock solution was prepared by dissolving 2,2′-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt in distilled-deionized water with concentration of 1000 mg/L. Working standard solutions were prepared by adding appropriate amount of standard stock solutions into mobile phase (40% ACN and 60% of 5.0 mM sodium acetate buffer solution, pH 6.3).

### 2.4. Optimization of mobile phase

Mobile phase consisted of acetonitrile and acetate buffer solution (5.0 mM) was tested for separation of the standard mixtures and samples. To examine the effect of pH value on the analyte retention, the pH value of mobile phase was varied from 5.8 to 6.8 while other mobile phase compositions remain constant (50:50 ACN and acetate buffer). The effect of organic solvent percentage on the retention was tested by changing the content of acetonitrile in mobile phase from 40% to 60% in intervals of 10%, while pH of mobile phase was maintained at 6.3.

### 2.5. Calibration curve

Standard working solutions (10.0 mL) of 0.00, 1.00, 2.00, 3.00, 4.00, 5.00, and 6.00 mg/L glimepiride were prepared by mixing the desired volume of standard stock solution with constant amount (25 μL) of internal standard stock solution. Calibration curves were constructed by linear regression of the peak area ratio of glimepiride standards to the internal standard versus the concentration of glimepiride. The accuracy (recovery) was tested at two concentration levels by spiking known amount (2.00 and 3.00 mg/L) of glimepiride stock solution into the ground samples and determining the amounts of standard recovered.

### 2.6. Sample preparation

Glimepiride tablets were obtained from Walgreen (1.0 mg pink tablet, Sanofi Pharmacy, Deerfield, IL, USA) and Sanofi (2.0 mg green tablet, Beijing, China), respectively. Tablets of each brand were weighed and finely grinded, a quantity of around 5.0 mg powder was accurately weighed and transferred into 8.00 mL of acetonitrile. To increase extraction efficiency, the mixture of tablet powder and acetonitrile was ultra-sonicated for 5 min and then centrifuged at 2500 rpm for 15 min. The supernatants were filtrated through 0.45 μm membrane filters. 4.0 mL of the filtrates were mixed with 6.0 mL aqueous acetate buffer solution (5.0 mM). The pH of final solution was adjusted to 6.30 by using 2.0 M NaOH. To minimize local acid-base concentration change during the pH adjustment, micro syringe was used for adding pH adjusting reagent. 25 μL of internal standard stock solution was added into prepared sample solutions before injection into HPLC.

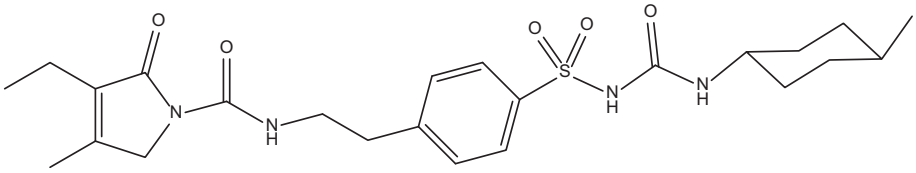

**Figure 1.** Chemical structure of glimepiride.

### 2.7. Method validation

The method was validated in accordance with the ICH guidelines for linearity, accuracy, precision, specificity, limit of determination, limit of quantification, system suitability and robustness (ICH Q2[R1], 2005).

## 3. Results and discussion

### 3.1. HILIC method development and optimization

Glimepiride contains both hydrophobic and hydrophilic functional groups and thus could be analyzed by either RP-HPLC or HILIC. In the literature, only RP-HPLC methods have been reported (Jain et al., 2008; Kovaříková et al., 2004; Ni et al., 2014; Dash et al., 2016; Sane et al., 2004a; Shaodong et al., 2010). This study focused on the development of an appealing alternative HILIC technique for the determination of glimepiride in pharmaceutical formulations. In HILIC, separation is hypothetically achieved by partitioning the analytes between the stagnant adsorbed aqueous layer on the surface of a polar stationary phase and a less polar aqueous organic mobile phase. Adsorption of the solutes on the stationary phase, hydrophobic, and dipole-dipole interactions as well as ionic attraction/repulsion between solutes and stationary phase active functional groups may also play a significant role in the retention of HILIC (Zuo et al., 2011, 2014). Based on our previous studies on the separation and determination of creatinine, uric and ascorbic acid in pharmaceutical and biological fluid samples (Zuo et al., 2011, 2015), a Waters Spherisorb $S_5NH_2$ column was selected for this study with a mobile phase consisting of acetonitrile and an aqueous buffer of 5.0 mM sodium acetate. To optimize the mobile phase composition, the effects of organic solvent content and the pH value of mobile phase were tested.

#### 3.1.1. Effect of mobile phase pH

Mobile-phase pH plays an important role in HILIC retention since it can influence the electric charge state of both ionizable solutes and stationary phase, which may affect the thickness of the stagnant enriched aqueous layer on the surface of the stationary phase and also lead to an additional ionic interaction mechanism for the retention. To examine the effect of mobile phase pH on the analyte retention, the pH value of mobile phase was varied from 5.80 to 6.80 while other mobile phase composition was kept constant at 50% acetonitrile and 50% of 5.0 mM acetate buffer. The pH was adjusted after mixing acetonitrile and the buffer. As shown in Fig. 2, the retention time of glimepiride decreased slowly with increasing pH values. In the pH range from 5.8 to 6.8, the amino stationary phase (with a $pK_a \sim 9.8$) was positively charged, which could induce the electrostatic attraction with the negatively charged solutes and affect the retentions. Glimepiride has a p*K*a ~ 6.2 and its negative charge density increased as the mobile phase pH increased from 5.8 to 6.8. However, the increasing formation of acetate anions through the dissociation of acetic acid with the increasing pH in the mobile phase reduced the electrostatic attraction of the amino stationary phase with the negatively charged glimepiride, thus resulting in a drop in the retention time, as presented in Fig. 2.

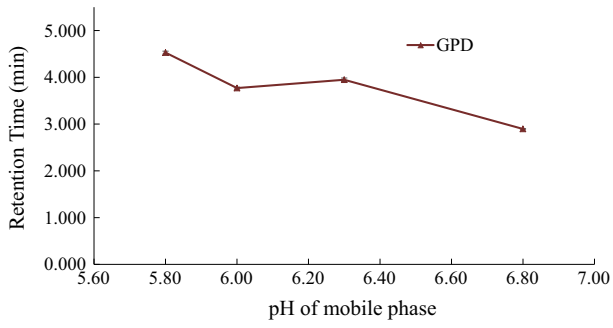


**Figure 2.** Effect of mobile phase pH on the retention of glimepiride.

#### 3.1.2. Effect of acetonitrile content in mobile phase

A typical HILIC mobile phase containing acetonitrile and an aqueous acetate buffer was employed in this study. The effects of acetonitrile content on the retention was investigated by varying the percentage of acetonitrile in the mobile phase while keeping the acetate buffer concentration (5.0 mM), pH value (6.3) and other chromatographic parameters constant with a mobile phase flow rate of 1.0 mL/min. As showed in Fig. 3, the increase of acetonitrile in mobile phase from 40% to 60% caused slight reduction in retention time. This observation probably reflects a mixed mode retention mechanism. In addition to the hydrophilic interaction, there were stronger electrostatic interaction between the negatively charged solutes and positively charge amino phase in the mobile phase with lower percentage of acetonitrile, and higher solubility of glimepiride in acetonitrile than in water.

After extensive preliminary experimental trials, a baseline separation of glimepiride from internal standard and pharmaceutical formulation matrices was achieved with symmetrical peaks in less than 9 min when using an isocratic elution with a mobile phase consisting of 40% acetonitrile and 60% of 5.0 mM aqueous acetate buffer of pH 6.3 at a flow rate of 1.0 mL/min. Fig. 4 showed a separation of a standard mixture of glimepiride and internal standard, 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), and Fig. 5 presented a typical chromatogram of a pharmaceutical formulation.

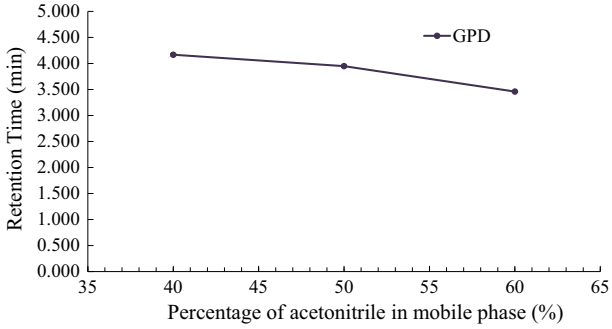


**Figure 3.** Effect of content of acetonitrile in mobile phase (pH 6.30) on the retention of glimepiride.

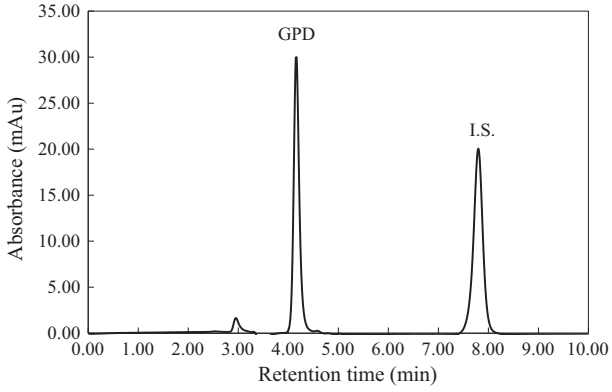


**Figure 4.** A HPLC chromatogram of a standard mixture of glimepiride (4 mg/L) and internal standard (5 mg/L). Injection volume, 20.0 μL.

### 3.2. Method validation

The calibration curve for glimepiride was obtained using a series of standard solutions over the concentration range of 0.00–6.00 mg/L. Three replicate injections of standard solutions at each concentration were performed. A linear relationship between the ratio of the peak area of the standard to that of the internal standard ($y$) and the concentration of the standard ($x$) was obtained over the range of 0.00 μg/L to 6.00 mg/L. The calibration curve followed the equation: $y = 0.2183x$ with $R^2 = 1.000$ (Fig. 6). The limit of determination (LOD) was 15 μg/L, which was calculated as the concentration of glimepiride that gives rise to peak height with a signal-to-noise ratio (S/N) of 3. The limit of quantification (LOQ) was 50 μg/L, determined as the concentration of glimepiride that gives rise to peak height with a S/N of 10.

The analytical accuracy and recovery of the described method was tested by adding two levels of known amounts of glimepiride standard into a grinded tablet sample, and the percentage recoveries were found to be 99.4–103.0% (Table 1). The reproducibility of the retention time of glimepiride (4.13 min) and internal standard (7.72 min) were determined from 11 consecutive injections during an analysis of a series of glimepiride samples. The relative standard deviation (R.S.D.%) was found to be 0.01 and 0.02% for glimepiride and 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid), respectively. The precision in the peak area was better than 2.0% for ten consecutive injections of the same glimepiride sample. Good peak area precision was achieved without adding any internal standard. Glimepiride was identified by matching the retention times against that of authentic standards.

Day-to-day precision was evaluated by performing six injections of standard solutions and formulation extracts each day on four different days within 2 weeks period. Day-to-day precision (R.S.D.) on the basis of retention time and peak area were better than 0.04 and 2.2%, respectively. Repeatability of the method was performed by three analysts (six determinations by each analyst) using the proposed method and two different HPLC instruments. The results showed no significant differences: R.S.D.% = 0.90.

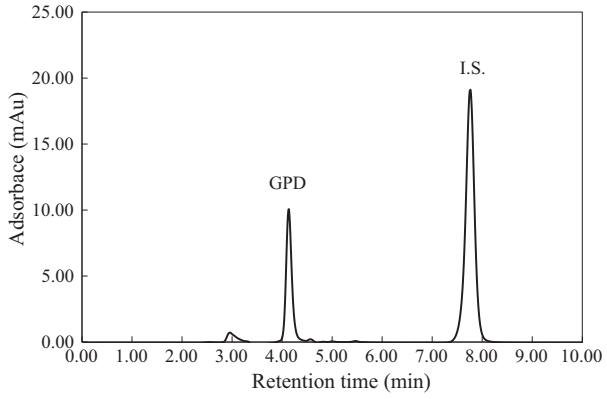


**Figure 5.** HPLC chromatogram of a pharmaceutical tablet sample with internal standard (5 mg/L). Injection volume, 20.0 μL.

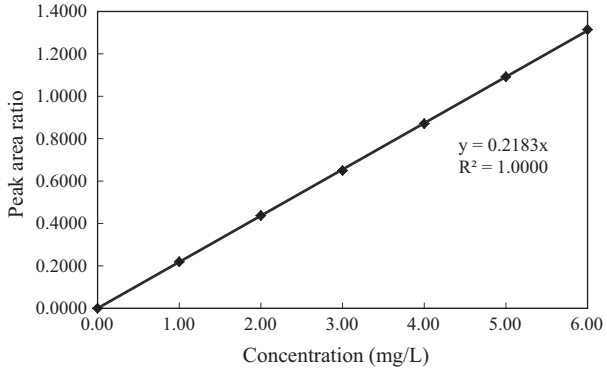


**Figure 6.** Calibration curve for glimepiride.

**Table 1**
The recoveries of glimepiride in pharmaceutical tablet samples.

| Std added (mg/L)[a] | Recovery (%) | | | RSD (%) |
|---|---|---|---|---|
| 2.00 | 101.4 | 101.7 | 103.0 | 0.84 |
| 3.00 | 99.6 | 100.7 | 99.4 | 0.71 |

[a] Concentration of standard are expressed as the equivalent concentrations added in the final injected solution.

### 3.3. Determination of glimepiride in pharmaceutical tablets

The HILIC method developed was applied to the determination of glimepiride contents in pharmaceutical formulations. Glimepiride was extracted from the grinded tablet powder using acetonitrile with ultra-sonication for 5 min. After centrifugation and filtration, the filtrate was diluted by using 5.0 mM aqueous acetate buffer to keep the solvent composition consistent with mobile phase. For fast analysis, the acetonitrile filtrate solution can be directly injected onto the HILIC column without the solvent mismatching problem with the mobile phase and chromatographic peak shape deterioration (Zuo et al., 2014). The amounts of glimepiride determined were 1.00 ± 0.02 mg/tablet for 1.0 mg Walgreen pink tablets and 1.98 ± 0.05 mg/tablet for 2.0 mg Sanofi green tablets. According to U.S.P. (2010), the tablet formulation should contain not less than 90% (w/w) and not more than 110% (w/w) of the labeled amount of glimepiride. Both these tablet formulations were found to satisfy the U.S.P. standards.

## 4. Conclusions

In this study, a fast, sensitive, and accurate HILIC method for the determination of glimepiride has been developed for the first time. The developed HILIC method has shown different retention mechanisms from previously reported RP-HPLC techniques. The retention time of glimepiride slightly decreased with increase in pH from pH 5.8 to 6.8, and in acetonitrile content from 40% to 60% on an amino stationary phase, signifying a mix-mode hydrophilic interactions between solutes, stationary phase and mobile phase of acetonitrile and 5.0 mM aqueous acetate buffer. A baseline separation of glimepiride from internal standard and sample matrices was achieved in less than 9 min using a mobile phase consisting of 40% acetonitrile and 60% of 5.0 mM aqueous acetate buffer at pH 6.3 at a flow rate of 1.0 mL/min. Good linearity and sensitivity were obtained with this developed method. The described method was successfully applied to the determination of glimepiride in pharmaceutical formulations. No pharmaceutical matrix component was found to interfere the glimepiride determinations. Given the advantages of HILIC in the analysis of small polar compounds, the method developed in this study could also be used in the separation and quantitative measurements of glimepiride in pharmaceutical stability and pharmacokinetic studies.

## Conflict of interest

The authors are not aware of any conflict of interest.

## Acknowledgment

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