Short communication

Development and validation of RP-HPLC-UV method for the determination of Glipizide in human plasma

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ABSTRACT

A simple, sensitive and selective HPLC method with UV detection for determination of Glipizide in human plasma was developed. Liquid–liquid extraction method was used to extract the drug from the plasma samples. Chromatographic separation of Glipizide was achieved using C_{18} column (ZORBAX ODS 4.6 × 150 mm). The mobile phase was comprised of 0.01 M potassium dihydrogen phosphate and acetonitrile (65:35, v/v) adjusted to pH 4.25 with glacial acetic acid. The analysis was run at a flow rate of 1.5 mL/min with an injection volume was 20 μL. The detector was operated at 275 nm. The calibration curve was linear over a concentration range of 50–1600 ng/mL. Intra-day and inter-day precision and accuracy values were below 15%. The limit of quantification was 50 ng/mL and the mean recovery was above 98%. Freeze-thaw, short-term, long-term and post-preparative stability studies showed that Glipizide in plasma sample was stable. The method may be successfully applied to analyze the Glipizide concentration in plasma samples for bioavailability and bioequivalence studies.

1. Introduction

Glipizide is a second generation sulfonylurea[1] that is used in the treatment of noninsulin-dependent diabetes mellitus.[2] This compound appears to be the most potent among other sulfonylurea derivatives.[3] It produces action by blocking potassium channels in the beta cells of islet of langerhans.[4]

Existing literature reveals that analytical methods including Gas Chromatography (GC)[5] and High Performance Liquid Chromatographic (HPLC) have been developed for determination Glipizide in human serum[6] as well as in plasma or urine.[7–9] GC method requires derivatization process to form a thermally stable product and thus time consuming.[5] Besides this, GC method lacks specificity as structurally similar sulphonylureas may form identical derivatives. The HPLC methods reported in the literature[6,7] have retention time (RT) of 25 and 10.5 min, respectively. High running time is both time and solvent consuming. Therefore, the present study was aimed to develop and validate an efficient new RP-HPLC method for the determination of Glipizide in human plasma that can be subsequently applied to understand its pharmacokinetics in biological system.

2. Materials and methods

Glipizide was purchased from Chunghwa Chemical Synthesis and Biotech Co., Ltd., (Taiwan). Hydrochloric acid (35%) and potassium dihydrogen phosphate were of analytical grades purchased from R & M (Essex, United Kingdom). Glacial acetic acid and acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade.

Study was approved by board of advance studies and an ethical committee on human use at Department of Pharmacy, The Islamia University of Bahawalpur, Punjab, Pakistan.

2.1. Instrumentation (specification of HPLC)

Analysis was performed using Agilent Liquid Chromatography with a pump series 1100, Agilent UV Visible Detector set at 275 nm. A reverse phase system was used consisting of C_{18} column (ZORBAX ODS 4.6 × 150 mm, 5 μm).

2.2. Chromatographic Conditions

The mobile phase consisted of 0.01 M potassium dihydrogen phosphate buffer and acetonitrile in a ratio of 65:35 (v/v) adjusted

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to pH 4.25 using glacial acetic acid. It was eluted at a flow rate of 1.5 mL/min with an injection volume of 20 μL. The effluent was monitored at a wavelength of 275 nm with a run time of 10 min.

2.3. Preparation of standard drug solutions

Stock drug solution of 20 ug/mL was prepared by dissolving 10 mg of Glipizide in 500 mL of methanol:dichloromethane mixture (10:90). The solution was sonicated for 5 min and allowed to equilibrate at room temperature. The working standard drug solutions of 300, 2500, and 10,000 ng/mL were prepared by serial dilution of the stock drug solution. All solutions were stored at 2−8 °C.

2.4. Preparation of quality control samples

Three quality control (QC) plasma samples at concentrations of 150, 850, and 1300 ng/mL were prepared by spiking 500 μL blank plasma with appropriate working standard drug solutions.

2.5. Preparation of standard plasma samples

Standard plasma samples ranged from 50 to 1600 ng/mL were prepared by spiking 500 μL blank human plasma with suitable working standard drug solutions.

2.6. Plasma sample treatment

An aliquot of 500 μL of Glipizide spiked plasma samples were measured accurately into 10 mL glass tubes followed by adding 100 μL of 0.05 M HCL to precipitate the plasma protein. The mixture was vortexed for 45 s and extracted with 3 mL of toluene and again vortexed for 45 s. Samples were then centrifuged at 4000 rpm for 15 min. The supernatant was collected and transferred to glass tubes and concentrated to dryness using sample concentrator using nitrogen. Dried residue was reconstituted with 75 uL of mobile phase. Plasma sample of 20 μL was injected into the chromatographic system.

2.7. Preparation of calibration curve

Calibration curve in the range of 50−1600 ng/mL was constructed to encompass anticipated range of Glipizide concentration in plasma. The extraction and reconstitution of Glipizide was done as per method described earlier. Twenty micro liter of each of the above reconstituted samples were injected and peak area was obtained.

2.8. Method validation

The proposed method was validated as with respect to selectivity, linearity, accuracy, precision, recovery, limit of quantification (LOQ), and stability.[10] The quality control samples at concentrations of 150, 850, and 1300 ng/mL, as well as 50 ng/mL (LOQ) were used to determine the within-day and between-day precision, accuracy, and recovery of the assay method. The freeze and thaw stability, short-term temperature stability, postoperative stability, and long-term stability were carried out at concentrations of 150 and 1300 ng/mL, whereas the stock solution stability was performed at concentration of 20 μg/mL.

2.9. Selectivity

The selectivity of the method was determined to ensure the analyte peak was well separated and free from interference of endogenous compounds in the plasma using blank plasma samples spiked with working standard solution.

2.10. Linearity

Standard plasma samples with concentration range of 50−1600 ng/mL were prepared by spiking plasma with different concentration of Glipizide. Standard calibration curve was constructed by plotting the peak area of Glipizide against the corresponding concentrations of standard plasma samples. Linear regression equation and correlation coefficient were determined. The linearity was determined from six standard calibration curves ($n = 6$)[10] over a concentration range of 50−1600 ng/mL.

2.11. Precision and accuracy

For inter-day precision and accuracy, six replicates of each analyte concentrations were injected over 6 consecutive days, whereas for intra-day precision and accuracy, six replicates of each analyte concentrations were injected on the same day. The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for LOQ where it should not exceed 20% of the CV. For accuracy, the mean value should be within 15% of the actual value except at LOQ where it should not deviate more than 20%.

2.12. Recovery

The recovery was calculated by comparing the peak area obtained from the plasma sample after treatment with that of the aqueous drug solution of the corresponding concentration. Each concentration was analyzed six times.
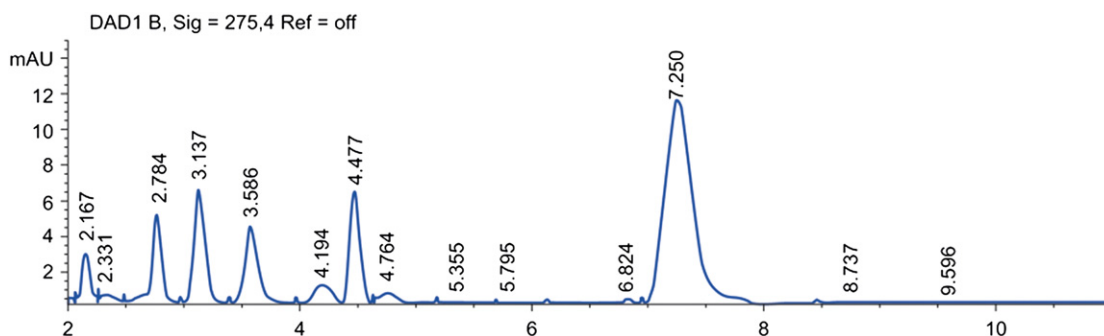

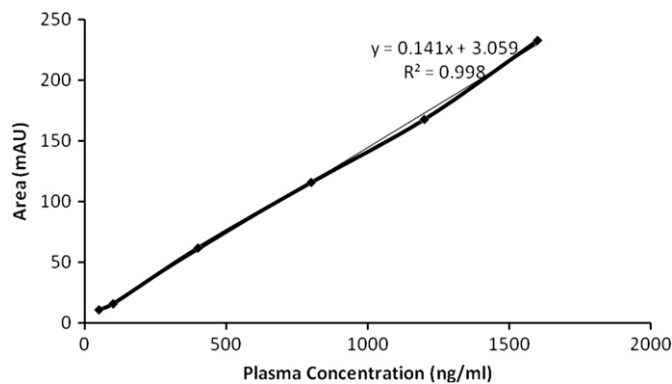**Fig. 1.** Chromatogram of Glipizide spiked plasma at 1600 ng/mL.

Fig. 2. Standard curve for Glipizide in human plasma.

2.13. Limit of quantification (LOQ)

The limit of quantification was determined as the lowest concentration of Glipizide in the standard calibration curve that could be quantified with a value of bias below 20% and a signal-to-noise ratio of at least 10.

2.14. Stability studies

2.14.1. Freeze and thaw stability
The analyte stability was determined after three freeze and thaw cycles. Three aliquots at each of the lower and high concentrations were stored at −20 °C for 24 h and thawed unassisted at ambient room temperature of 25 °C. When completely thawed, the samples were refrozen for 24 h under the same conditions. The freeze-thaw cycles were repeated two more times and analyzed on the third cycle.[10]

2.14.2. Short-term stability
Three aliquots of each of the lower and high concentrations were thawed unassisted at ambient room temperature of 25 °C and kept at this temperature for 8 h and analyzed.

2.14.3. Post-preparative stability
The stability of the processed samples, including the residence time in the auto-sampler was determined. The stability of Glipizide was assessed over the anticipated run time of 8 h for the batch size.

2.14.4. Long-term stability
Three aliquots of each of the lower and high concentrations of Glipizide stored at −20 °C over a period of 3 months were analyzed.

2.14.5. Stock drug solution stability
The stability of stock solution was evaluated at an ambient room temperature of 25 °C for 8 h. The stability was tested by comparing the instrument response to stored stock drug solution with that of freshly prepared solutions.

Table 2
Stability studies of Glipizide (Mean ± standard deviation, $n = 3$).

| Stability study | Glipizide concentration (ng/mL) | | | | | | | | |
| | 150 | | | 1300 | | | 20000 | | |
	Mean	SD	RSD	Mean	SD	RSD	Mean	SD	RSD
Freeze and thaw	94.12	6.20	6.58	92.97	6.13	6.59	–	–	–
Short-term	93.53	7.69	8.22	90.52	4.45	4.91	–	–	–
Long-term	97.06	5.29	5.45	95.59	4.68	4.89	–	–	–
Post-preparative	90.70	6.04	6.65	94.44	2.52	2.66	–	–	–
Stock solution	–	–	–	–	–	–	99.78	0.33	0.33

3. Results and discussion

3.1. Method development and sample preparation

Different organic solvents such as methanol, acetonitrile, and chloroform were used for extraction of Glipizide from human plasma. Among these solvents, toluene was found to be most suitable with high recovery of more than 90%. Internal standard was not required since the method showed high selectivity and sensitivity.

3.2. Selectivity

The chromatogram of Glipizide spiked plasma at 16,000 ng/mL is shown in Fig. 1. It can be seen that the peak of analyte was well resolved and separated.

3.3. Linearity

The standard calibration curve was linear over a concentration range of 50–1600 ng/mL. The correlation coefficient (r^2) was 0.9989 [Fig. 2], which is well within acceptable limit.[10]

3.4. Precision and accuracy

Both inter- and intra-day precision and accuracy values were well within the acceptable limits [Table 1].

3.5. Recovery

The mean recovery values of 93.47% (±9.99), 98.57% (±7.34), 98.68% (±6.72), and 101.72% (±4.67) were obtained at 50, 150, 850, and 1300 ng/mL, respectively. Average recovery of the analyte was above 98%.

3.6. Limit of quantification

The LOQ was 50 ng/mL, which is acceptable to quantify Glipizide in plasma samples.

Table 1
Intra-day and inter-day precision and accuracy data (mean ± standard deviation, $n = 6$).

| Known concentration spiked (ng/mL) | Intra-day | | | Inter-day | | |
	Concentration found (ng/mL)	Precision (% RSD)	Accuracy (% error)	Concentration found (ng/mL)	Precision (% RSD)	Accuracy (% error)
50	49.50 (±4.03)	8.14	−0.99	50.70 (±5.42)	10.69	1.40
150	144.81 (±11.85)	8.18	−3.46	142.13 (±10.59)	7.45	−5.25
850	873.93 (±36.79)	4.21	2.82	839.23 (±57.18)	6.81	−1.27
1300	1316.12 (±81.18)	6.17	1.24	1348.56 (±61.90)	4.59	3.74

3.7. Stability study

The results of short-, long-, and post-preparative stability results were more than 90%, which are well within the acceptable limit.[10] The stock solution was stable at an ambient room temperature of 25 °C for more than 8 h with mean value of 99.78% ($\pm$0.33) at 20,000 ng/mL [Table 2].

4. Conclusion

A rapid, simple, selective, and sensitive HPLC method for estimation of Glipizide in human plasma was successfully developed that can be used in studies aiming at pharmacokinetics of Glipizide.

Conflicts of interest

All authors have none to declare.

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