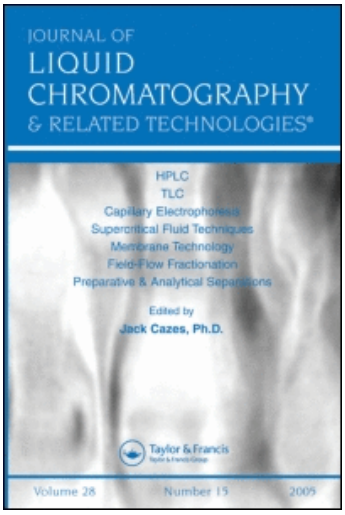

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Abstract: Glipizide is an oral hypoglycemic agent widely used to treat type 2 diabetes mellitus. In this study, an efficient HPLC-UV assay method for determining the plasma glipizide level was developed, validated, and used to assess the pharmacokinetic profile of the glipizide in healthy Korean volunteers. After extraction with diethyl ether, the chromatographic separation of glipizide was carried out using a Bondclone C_{18} column (10 μm, 300 × 3.9 mm) with a mobile phase of 10 mM potassium phosphate monobasic and methanol (40:60 [vol/vol], pH 3.5) and UV detection at 225 nm. The flow rate of the mobile phase was 1.0 mL/min and the retention time of glipizide and internal standard (I.S.) was approximately 11.5 and 8.6 minutes, respectively. The quantification limit was 15 ng/mL and the linear range of the calibration curve ranged from 15 to 800 ng/mL in plasma with a correlation coefficient >0.9999. The mean accuracy was 86–101%. The coefficient of variation (precision) in the intra- and inter-day validation was 1.8–14.2 and 1.7–8.1%, respectively. The pharmacokinetics of oral glipizide was evaluated after administering 5 mg to each of 13 healthy Korean subjects. The AUC_{inf}, C_{max}, T_{max}, and $T_{1/2}$ were 3432 ± 886 ng·h/mL, 629.0 ± 94.2 ng/mL, 2.8 ± 1.8 h, and 3.9 ± 0.9 h, respectively. The results showed large inter-individual differences in the AUC_{inf}, C_{max} and $T_{1/2}$.

Keywords: Glipizide, HPLC, Pharmacokinetics

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INTRODUCTION

Glipizide is a hypoglycemic agent and a second generation sulfonylurea antidiabetic that increases the level of insulin secretion by blocking the ATP sensitive potassium channel (K_{ATP} channel) in pancreatic beta-cells.[1,2] It is administered once daily to regulate the postprandial blood glucose level. It is completely absorbed after oral administration with essentially no first pass biotransformation of the drug. The peak plasma levels are achieved within 1 to 3 hours and the terminal half lives has been reported to range from 2.4 to 7 hours.[1,2] The plasma glipizide concentration is generally measured by high performance liquid chromatography (HPLC) with UV detection.[3–7] However, there are many limitations with the previously reported methods. Benzene, which is a carcinogen, has been used as an extraction solvent.[3,4] In addition, the extraction procedure is too complex due to the large number of steps,[5] and the detection limit is too high.[6] In the solvent drying process, special equipment is needed due to the high boiling point of toluene.[7] Therefore, this study developed and validated a simple and efficient HPLC-UV method to determine the plasma glipizide levels. This method was applied to a pharmacokinetic study in healthy Korean subjects.

EXPERIMENTAL

Chemicals and Reagents

Glipizide and tolbutamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Glipizide tablets (Digrin®, 5 mg) were purchased from Yuhan Corporation (Seoul, Korea). HPLC grade methanol and diethyl ether were purchased from Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) and Fisher Scientific (Waltham, MA, USA), respectively. All other chemicals were of the highest purity available. HPLC grade water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA) and used throughout the study. The mobile phase components, such as phosphate buffer and methanol, were filtered through a 0.2 μm pore size membrane filter prior to mixing and degassed ultrasonically after mixing.

Calibration Standards

Stock solutions of glipizide and tolbutamide were prepared in 50% methanol. The working solutions were prepared by diluting each stock solution with 50% methanol. Calibration standard samples of 15, 50,

100, 200, 400, 600, and 800 ng/mL were prepared by spiking 50 μL of the appropriate working solution into 450 μL of drug free human plasma.

HPLC Instruments and Conditions

The HPLC system consisted of a Waters 515 HPLC pump, a Waters 717 Plus Autosampler and a Waters 2487 Dual λ Absorbance Detector (Waters, Milford, MA, USA). The analytic column was a Bondclone C_{18} column (10 μm, 300 × 3.9 mm, Phenomenex, Torrance, CA, USA). The data module and data software were Autochro Data Module and Autochro Win-Chromatography Data System (Young Lin Instruments, Korea), respectively. The detection wavelength, 225 nm, was determined by scanning the maximum absorbance wavelength of glipizide and tolbutamide in 50% methanol using a UV spectrophotometer (Agilent 8453, Agilent Technologies Inc., Santa Clara, CA, USA). A mixture of 10 mM potassium phosphate monobasic and methanol (40:60 [vol/vol], pH 3.5) was used as the mobile phase, and eluted at a flow rate of 1.0 mL/min. The pH was adjusted with ortho-phosphoric acid. The column temperature was 30°C.

Pharmacokinetic Study

Thirteen healthy male Korean volunteers, weighing 64.9 ± 5.6 kg and aged 23.4 ± 2.0 years, were enrolled in this pharmacokinetic study of oral glipizide. The study was performed according to the Declaration of Helsinki, and was approved by the Institutional Review Board of College of Pharmacy, Sungkyunkwan University. Written informed consent was obtained from each subject. The subjects were not on any concomitant medications and were free from significant cardiac, hepatic, renal, pulmonary, gastrointestinal, neurological, or hematological diseases, which were determined within four weeks before beginning the study from their medical history, physical examinations, and laboratory screenings. They received a single oral dose of a 5 mg glipizide tablet at 7 AM after an overnight fast. Glucose, 75 g, was administered 30 min after dosing. Meals were served 5 and 9 hours after administering glipizide. Blood samples were collected in heparinized glass tubes from an antecubital vein immediately before administration as well as at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 7, 9, 12, and 15 hours after dosing. The blood samples were centrifuged immediately and the plasma samples were stored at −70°C until needed.

Determination of Glipizide Plasma Concentrations

Five hundred microliters of plasma or calibration standards, 50 μL of internal standard solution (5 μg/mL tolbutamide), and 850 μL of

0.05 M HCl were added to a glass tube. After mixing, 5 mL of diethyl ether was added and the mixture was stirred for 30 seconds. Each sample was centrifuged at 2,500 rpm for 10 minutes. The organic layer was transferred to a new tube and evaporated to dryness under a nitrogen stream at 50°C. The residue was reconstituted with 500 μL of 50% methanol and an 80 μL aliquot was injected for HPLC analysis.

Assay Validation

The specificity of the method was determined by comparing the chromatograms obtained from the samples containing glipizide and internal standard (I.S.) with those obtained from blank samples. The limit of quantification (LOQ) was defined as the lowest concentration at which the precision expressed by the relative standard deviation (RSD) was >20%. The accuracy, expressed by the relative difference between the measured and the true value, was also <20% and the signal to noise ratio (S/N ratio) was >10. The precision was determined as the coefficient of variation (CV), and the accuracy was determined as the percentage relative error (RE). The intra-day reproducibility was determined by analyzing 5 sets of spiked human plasma, and the inter-day reproducibility was determined over a 5 day period. The extraction recovery of glipizide was determined by comparing the peak area of three extracted samples at 50, 200, and 800 ng/mL with the mean peak area of the recovery standards.

Calculations of Pharmacokinetic Parameters

The pharmacokinetic parameters of glipizide were estimated using non-compartmental methods on the BA-Calc 2002 program from the Korea Food & Drug Administration.[8] The actual blood sampling times were used, and the maximum plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were the observed values. The elimination rate constant (k_e) was determined by linear regression analysis of the log linear part of the plasma concentration time curve. The $T_{1/2}$ was calculated using the equation, $T_{1/2} = \ln 2/k_e$. The area under the curve (AUC) was calculated using the linear trapezoidal rule and extrapolated to infinity (AUC_{inf}).

RESULTS

Specificity

Figure 1 shows the UV spectrum of glipizide and the internal standard (I.S.), tolbutamide. The wavelength, 225 nm, was used to detect glipizide

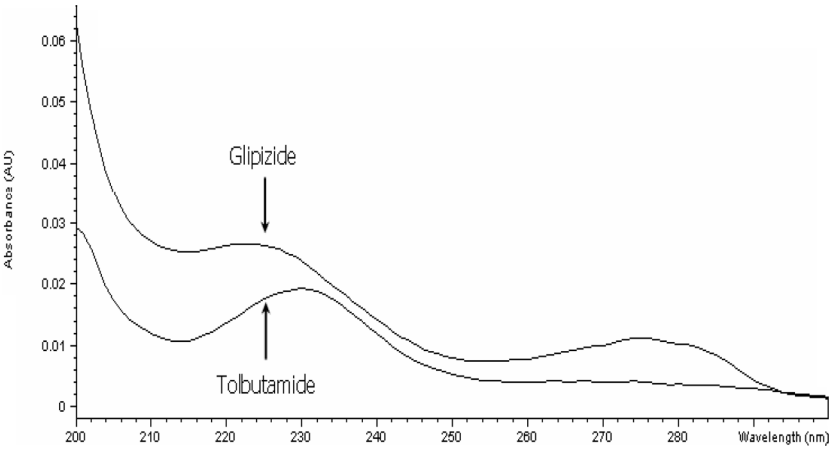

Figure 1. Absorbance spectrum of glipizide and tolbutamide.

and I.S. Figure 2 shows the chromatograms of blank human plasma, human plasma spiked with glipizide and I.S., and plasma sample from the volunteers taking glipizide. The retention time of glipizide and I.S. was approximately 11.5 and 8.6 minutes, respectively. There were no interfering peaks from any endogenous substances in the blank plasma.

The calibration curves of glipizide were linear over the range, 15–800 ng/mL. The relative coefficient (r^2) was 0.9999 with the equation, $y = 0.0027x + 0.0106$. The limit of quantification was 15 ng/mL.

The mean recovery was 81.8%, 79.9%, and 82.2% in the low (50 ng/mL), medium (200 ng/mL), and high (800 ng/mL) levels, respectively.

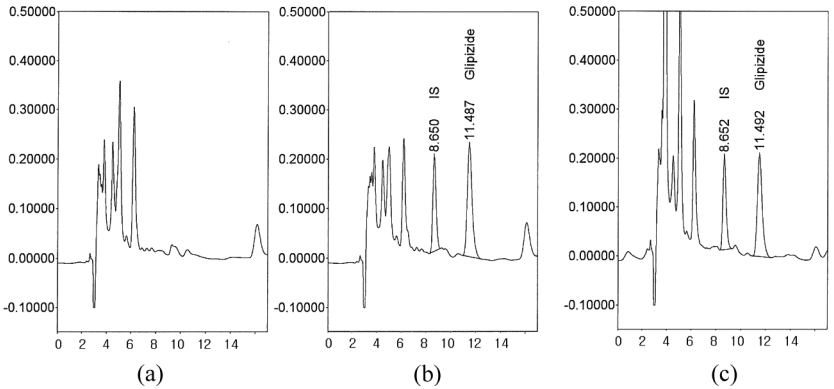

Figure 2. HPLC chromatogram of plasma glipizide. (a) blank human plasma; (b) human plasma spiked with glipizide (15 ng/mL) and I.S. (tolbutamide 5 μg/mL); and (c) human plasma sample at 1 hr after administration of glipizide 5 mg.

Table 1. Precision and accuracy of the HPLC assay method for human plasma glipizide

Concentration (ng/mL)	Precision (C.V.%)		Accuracy (%)
	Intra-day	Inter-day	
15	14.2	8.1	86.2
50	10.1	7.2	98.0
100	6.8	5.5	99.5
200	5.2	3.3	101.3
400	1.8	4.0	101.4
600	2.3	1.7	99.4
800	2.8	1.9	100.0

The intra-day precision (C.V.) and inter-day precision was <10.1% and <7.2%, respectively. The intra-day and inter-day precision at LOQ was 14.2% and 8.1%, respectively (Table 1). The accuracy was 98.0–101.4%, and the accuracy at the LOQ was 86.2% (Table 1).

Pharmacokinetic Study

Figure 3 shows the plasma glipizide concentrations after the oral administration of glipizide, 5 mg, in 13 healthy Korean subjects. The time to

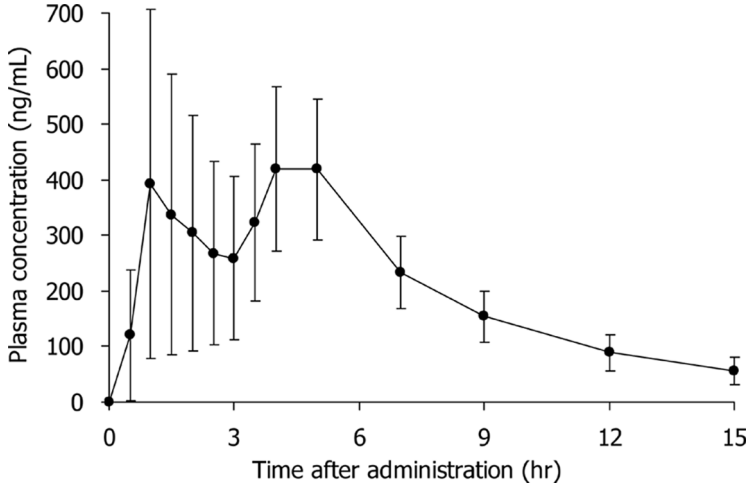

Figure 3. Plasma concentration of glipizide after the oral administration of glipizide 5 mg. Each value represents the mean ± S.D. of 13 volunteers.

Table 2. Pharmacokinetic parameters of glipizide after a single 5 mg oral dose of glipizide

Populations	No. of subjects	T_{max} (h)	C_{max} (ng/mL)	AUC_{inf} (ng·h/mL)	$T_{1/2}$ (h)	Reference
Korean	13[a]	2.8 ± 1.8	629 ± 94	3432 ± 886	3.9 ± 0.9	Present study
Caucasian (American)	10[a]	2.1 ± 1.0	465 ± 146	2584 ± 1305	4.2 ± 2.7	[13]
Indian	12[a]	1.8 ± 0.3	523 ± 60	1897 ± 279	1.7 ± 0.5	[12]
Caucasian (Swedish)	6[b]	1.7 ± 0.9	326 ± 150	1708 ± 721	4.1 ± 2.5	[10]
Caucasian (American)	10[b]	2.3 ± 1.7	385 ± 150	1898 ± 664	4.2 ± 1.5	[11]
Caucasian (American)	8[b]	2.8 ± 1.6	421 ± 142	3139 ± 1847	5.2 ± 2.0	[14]

[a]healthy subjects.
[b]NIDDM(non-insulin-dependent diabetes mellitus) patients.

reach the peak plasma concentration (T_{max}) showed considerable variation. The T_{max} was 1, 4, and 5 hours in 6, 4, and 3 subjects, respectively (data not shown). The T_{max} and C_{max} was 2.8 ± 1.8 hr and 629.0 ± 94.2 ng/mL (range 441.7–777.5 ng/mL), respectively. The AUC_{inf} was 3432 ± 886 ng · h/mL (range 1917–4594 ng·h/mL). The k_e and mean half-life ($T_{1/2}$) was 0.187 $\pm 0.045\ h^{-1}$ and 3.9 ± 0.9 h, respectively. The individual values for C_{max}, $T_{1/2}$ and AUC_{inf} are shown in Figure 4. The C_{max} and AUC_{inf} in the Korean subjects were somewhat higher than in Caucasians and Indians (Table 2).[12–14]

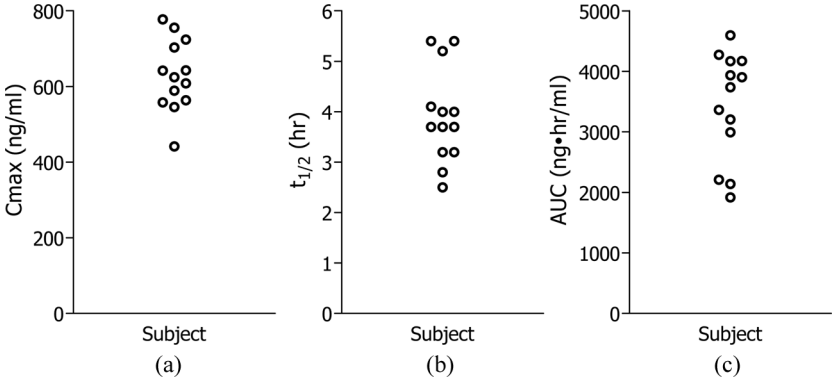

Figure 4. Individual values for C_{max} (a), $T_{1/2}$ (b) and AUC_{inf} (c) of glipizide.

DISCUSSION AND CONCLUSIONS

This paper reports a simpler and safer HPLC method for analyzing glipizde than the HPLC methods reported previously.[3–7] Diethyl ether was used as the extraction solvent instead of using carcinogenic benzene or non-volatile toluene.[3,4,7] The LOQ of this method was lower than previously reported methods using diethyl ether as the extraction solvent.[5,6] The specificity, linearity, and recovery were sufficient to measure the plasma concentrations of glipizide.

In the pharmacokinetic study, the plasma glipizide concentrations reached the primary peak concentration and then decreased thereafter. Subsequently, the plasma glipizide concentrations increased again 3 hours after administering the drug. A similar plasma concentration profile of oral glipizide has been reported in other studies. This was attributed to enterohepatic circulation or irregular absorption.[9–12] The C_{max} and AUC_{inf} in Korean subjects were somewhat higher than in Caucasians and Indians.[12–14] Large inter-individual variations in C_{max}, $T_{1/2}$, and AUC_{inf} were encountered.

In conclusion, this HPLC assay method for plasma glipizide is a convenient, safe, and useful method for determining the plasma glipizide concentration.

ACKNOWLEDGMENT

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