BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **19**: 394–401 (2005)
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/bmc.465

ORIGINAL RESEARCH

Improved liquid chromatographic tandem mass spectrometric determination and pharmacokinetic study of glimepiride in human plasma

Constantinos Pistos,[1]* Maria Koutsopoulou[1] and Irene Panderi[2]

[1]Independent Research and Laboratory Solutions (ILS), 240 Klisthenous Str., 153 44, Gerakas, Athens, Greece
[2]School of Pharmacy, Division of Pharmaceutical Chemistry, University of Athens, Panepistimiopolis, Zografou 157 71, Athens, Greece

Received 26 July 2004; revised 4 October 2004; accepted 6 October 2004

ABSTRACT: An improved liquid chromatographic tandem mass spectrometric method for the determination of glimepiride in human plasma has been developed and fully validated. The article describes in detail the bioanalytical procedure and summarizes the validation results obtained. The samples were extracted using liquid–liquid extraction with a mixture of 1-chlorobutane–isopropanol–ethyl acetate (88:2:10, v/v/v). The chromatographic separation was performed on a reversed-phase Hypersil ODS column (250 × 4.6 mm i.d.; 5 μm particle size) using a mobile phase consisting of formic acid 0.05 M–acetonitrile (28:72, v/v), pumped at a flow rate of 0.3 ml min^{-1} heated to 25°C. The analytes were detected using an API 3000 triple quadrupole mass spectrometer with positive electrospray ionization in multiple reaction monitoring mode. Tandem mass spectrometric detection enabled the quantitation of glimepiride down to 0.50 ng mL^{-1}. Calibration graphs were linear (r better than 0.998, n = 11), in concentration range 0.50–1000 ng mL^{-1}, and the intra- and inter- day RSD values were less than 10.37 and 11.55% for glimepiride. The method was successfully applied to a kinetic study in order to assess the main pharmacokinetic parameters of glimepiride.

KEYWORDS: glimepiride; pharmacokinetic; human plasma; mass spectrometry

INTRODUCTION

Glimepiride is a new oral antidiabetic drug in the sulfonylurea class having a prolonged effect (Draeger, 1995; Geisen, 1988). The chemical formula of glimepiride is 1-H-pyroll-1-carboxamide-3-ethyl-2,5-dihidro-4-methyl-N-[2-[4-[[(4-methylsiklohexyl)amino] carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-trans and the two main biotransformation products are the metabolites M1 (-hydrohymethyl-) and M2 (-carboxylic acid-). Glimepiride is a white to yellowish white, crystalline, odorless powder, which is practically insoluble in water (*Physycians' Desk Reference*, 2001). In order to achieve appropriate control of blood glucose level, the treatment of non-insulin-dependent type II diabetes usually starts with diet and exersice. If this still results in insufficient metabolic control, oral hypoglygemic drugs or insulin are added to the non-pharmacological measures (Müller and Geisen, 1996; Eckert *et al.*, 1993). Glimepiride achieved metabolic control by administration of the lowest dose (1–8 mg daily) compared with the other sulfonylureas. In addition, it maintains a more physiological regulation of insulin secretion than other sulfonylureas during physical excersice, suggesting that there may be less risk of hypoglycemia with glimepiride (Kolterman *et al.*, 1984; Draeger *et al.*, 1996; Sonenberg *et al.*, 1997). The pharmacokinetic profile of glimepiride was assessed in healthy volunteers and non-insulin-dependent diabetes mellitus (NIDDM) patients after oral administration (Malerczyk *et al.*, 1994; Ratheiser *et al.*, 1993; Badian *et al.*, 1994).

Glimepiride is almost completely bioavailable from the gastrointestinal tract and is eliminated by metabolism in the liver (Pearson, 1998). According to *in vitro* studies, it is a substrate of the cytochrome P450 (CYP) 2C9 enzyme (Brian, 2000) which is polymorphically expressed (Miners *et al.*, 1998; Goldstein, 2001).

In the literature there are limited reported methods referring to the determination of glimepiride in biological fluids (Salem *et al.*, 2004; Mauer *et al.*, 2002; Lehr and Damm, 1990) and pharmaceutical preparations (Altinöz *et al.*, 2001), and studies of its bioavailability, pharmacokinetics and pharmacodynamics (Pistos *et al.*, 2004; Niemi *et al.*, 2001; Badian *et al.*, 1992).

Most of the published methods did not reach the level of sensitivity and selectivity that can be obtained today since the evolution of liquid chromatography–tandem mass spectrometry towards a routine technique

*Correspondence to: C. Pistos, ILS, 240 Klisthenous Str., 153 44, Gerakas, Athens, Greece.
E-mail: cpistos@ilsgr.com

Abbreviations used: CYP, cytochrome P450; MRM, multiple reaction monitoring; NIDDM, non-insulin-dependent diabetes mellitus.

Published online 14 January 2005

in the bioanalytical laboratory (Jemal, 2000; Niessen, 1999; Smyth, 2003).

An ever-increasing need exists within the pharmaceutical industry to shorten the drug development timeline and increase the sensitivity. Maximizing the throughput of assays and sensitivity that support clinical studies is, therefore, a key concern in a bioanalytical laboratory. For adequate support of clinical studies, an analytical method is required for the determination of plasma levels of glimepiride.

Our laboratory was particularly interested in the concurrent assay of a glimepiride mixture in plasma that would be both rapid and sensitive for further application of the method in a bioequivalence study. This paper describes the development and validation of an assay with a short time of analysis (4.0 min) and improved sensitivity for the determination of glimepiride in human plasma. The *in vivo* data described in this paper provides a valuable application and useful insight into the pharmacokinetics of glimepiride.

EXPERIMENTAL

Chemicals. Glimepiride of pharmaceutical purity grade was provided by Neuland Laboratories Limited (India). All solvents and water were of HPLC grade and were purchased from Lab-Scan Analytical Sciences Ltd, Ireland. HPLC type I water from a Milli-Q system (Millipore, Bedford, MA, USA) was used. Formic acid (analytical reagent grade) was purchased from Panreac Quimica SA (Barcelona, Spain).

Liquid Chromatographic conditions. The HPLC Agilent 1100 Series (Agilent Technologies, Waldbronn, Germany) is equipped with G1312A binary pump, G1379A degasser, G1367A autosampler equipped with G1330B thermostat, G1316A thermostatted column compartment and G1323B control module. Twenty-microliter aliquots of the processed samples were injected on an Hypersil ODS column (250 × 4.6 mm i.d., 5 µm particle size), obtained from Thermo Hypesil (Cheshire, UK). The mobile phase consisted of formic acid 0.05 M–acetonitrile (28:72, v/v), pumped at a flow rate of 0.3 mL min^{-1} heated to 25°C.

Mass spectrometric conditions. Mass spectrometry was performed on an API 3000 triple quadrupole mass spectrometer (ABI-SCIEX, Toronto, Canada) using multiple reaction monitoring (MRM). A turbo electrospray interface in positive ionization mode was used. The main working parameters of the mass spectrometer are summarized in Table 1. Data processing was performed on Analyst (version 1.4) data system (SCIEX).

For the MS–MS analysis of the compounds the most intense peak in the mass spectrum, $[M+H]^+$, was chosen, providing the product ions mass spectrum. In particular, detection of the ions was performed in the MRM mode, monitoring the transition of the precursor ions at *m/z* 490.65 to the product ion at *m/z* 351.98 for glimepiride.

Table 1. Tandem mass spectrometer main working parameters

Parameter	Value
Curtain gas (psi)	12
Collision energy (V)	47
Collision cell exit potential (V)	12
Declustering potential (DP) (V)	150
Entrance potencial (DP) (V)	10
Ion source gas (gas 1) (psi)	30
Auxiliary gas (psi)	20
Ion spray voltage (V)	5000
Ion transition for glimepiride (*m/z*)	490.65/351.98
Mode of analysis	Positive
Source temperature (°C)	150

Stock and working standard solutions. Stock standard solutions of glimepiride, 100 µg mL^{-1}, were prepared by dissolving appropriate amount of the compound in acetonitrile–water (50:50, v/v). All stock standard solutions were stored frozen in the dark (–20°C), and were found to be stable for several weeks.

A series of working standard solutions of glimepiride were prepared by the appropriate dilution of the stock standard solution in acetonitrile–water (50:50, v/v), to reach concentration ranges of 5.0–10,000 ng mL^{-1} for glimepiride. The working standard solutions were freshly prepared every week and stored in the dark and under refrigeration.

Drug-free human plasma was screened prior to use to ensure that it was free of endogenous interference at the retention time of the analyte. A 100 µL aliquot of the appropriate working standard solution of glimepiride was added to a 1 mL aliquot of human plasma. Calibration standard solutions were prepared freshly every day over the concentration range of 0.50, 0.75, 1.0, 4.0, 10, 40, 100, 250, 500, 750 and 1000 ng mL^{-1} for glimepiride. Quality control samples were prepared in human plasma at three concentration levels (10, 40 and 250 ng mL^{-1}) and additional levels near to the limit of quantitation (LLOQ; 0.75 ng mL^{-1}) and the 80% of the upper limit of quantitation (ULOQ; 750 ng mL^{-1}). All QC solutions were stored at –20°C. Calibration standard solutions and quality control samples were prepared from separate stock solutions prepared with separate weighing of the analyte.

Sample preparation. The clinical frozen samples were thawed for 30 min at room temperature. After a slight vortex of the samples, 1 mL of each of the plasma samples was transferred into a 10 mL glass test tube. First 250 µL of 1 M hydrochloric acid were added and the sample was vortex mixed. Subsequently 5 mL of extraction solvent (1-chlorobutane/ethyl acetate/propan-2-ol 88:10:2) were added and the sample was shaken for 20 min at 800 rpm in an orbital shaker. After centrifugation at 3500 rpm for 10 min at 20°C, the aqueous phase (bottom layer) was discarded and the organic phase (upper layer) was transferred into a clean 10 mL glass test tube. The organic phase was then evaporated to dryness under a steam of nitrogen. The residue was reconstituted in 200 µL acetonitrile–formic acid 0.05 M 50:50. After vortex mixing 50 µL were injected into the HPLC.

Validation procedure. Eleven plasma calibration standards were prepared and analyzed in duplicate in three separate analytical runs. Calibration curves were calculated based on the measurement of the peak area signal of glimepiride. Least-squares linear regression was used to fit the measured signal versus the theoretical concentration; a weighting factor of $1/(\text{concentration})^2$ was also applied.

For the validation of the assay, quality control samples were prepared with concentrations of 0.75, 10, 40, 250 and 750 ng mL^{-1}. The latter was diluted 10-fold in drug-free human plasma prior to sample processing so as to demonstrate the ability to dilute samples above the ULOQ. Six replicates of each sample were analyzed in three analytical runs. The accuracy assessed by the relative percentage error, E_r%, which was determined as the percentage difference between the mean concentration and the theoretical concentration. Moreover, precision was assessed by the relative standard deviation (RSD%). The intra- and inter-assay accuracy was required to be within ±20% at the LLOQ level and within ±15% for other concentrations. The precision was required to be less than 20% at the LLOQ level and less than 15% at other concentrations.

Drug-free human plasma was processed according to the sample preparation procedure (liquid–liquid extraction). The organic layer was evaporated to dryness under a gentle stream of nitrogen. Dry extracts were reconstituted in the mobile phase, adding appropriate aliquots (100 µL) of the 1000 ng mL^{-1} working standard solution of glimepiride representing 100% recovery. Ion suppression was determined by comparing the analytical response of this sample with that of the unextracted standard. The recovery of the liquid–liquid extraction procedure was evaluated at 250 ng mL^{-1} for glimepiride. It was determined by comparing the peak areas obtained from the plasma calibration samples after standard analysis with the peak areas obtained from extracts of blank plasma samples reconstituted with working standard solutions as described above.

Stability of glimepiride in human plasma was evaluated by assaying spiked plasma samples containing 100 ng mL^{-1} of glimepiride. The spiked plasma samples were stored at ambient temperature for 6 h, at 6°C for 40 days, at –20°C for 75 days and after three complete freeze–thaw cycles at –20°C. Peak area measurements obtained from the analysis of the stored samples were compared with the peak measurements that were obtained from the analysis of freshly prepared plasma samples. The analyte was considered stable in the biological matrix when 80–120% of the initial concentration was found.

Sampling procedure. The method was applied to a pharmacokinetic study for the evaluation of the pharmacokinetics of glimepiride after oral administration of low doses of glimepiride. Six non-smokers, healthy male and female volunteers from the local population, were screened and enrolled in the trial. The mean age of the volunteers was 25.5 ± 3.8 years, the mean height was 175.3 ± 10.2 cm while the body weight was 73.34 ± 9.4 kg. On the first day a single dose of 4 mg glimepiride was administered with 200 mL of bottled water. No intake of alcohol, caffeine or xanthene-containing food or drink or smoking was allowed within 72 h before each dose. The subjects fasted for at least 10 h (food) and 3 h (drink) before receiving their morning breakfast. No alcohol, caffeine or xanthene-containing food or drink or smoking was allowed during the post-dosing confinement period. Subjects received standardized meals approximately 6 h after dosing during the confinement period. The subjects were not allowed to lie down or sleep for 12 h after dosing. No strenuous activity was allowed during the confinement periods. Blood samples were collected just before drug administration (t_1 = 0.00) and 14 times after drug administration (n = 15): t_2 = 0.50 h, t_3 = 1.00, t_4 = 1.50, t_5 = 2.00, t_6 = 2.50, t_7 = 3.00, t_8 = 3.50, t_9 = 4.00, t_{10} = 6.00, t_{11} = 8.00, t_{12} = 10.00, t_{13} = 12.00 t_{14} = 16.00 and t_{15} = 24.00 h after drug administration.

The blood samples were obtained from a short intravenous catheter (Abbocath®) and collected into 12 mL tubes, using heparin as an anticoagulant. Blood samples were immediately centrifuged (3500 rpm, 10 min), and the separated plasma was transferred into 2.0 mL Eppendorf tubes and stored under –70°C until analysis.

RESULTS AND DISCUSSION

Optimization of MS detection and chromatographic conditions

Mass spectrometric parameters were optimized so as to achieve the maximum abundance of the product and fragmented ions of the analyzed compounds. Full-scan mass spectra and product ion scan spectra of the analyte were obtained by direct infusion into the mass spectrometer of a 250 ng mL^{-1} solution of the analyte diluted in the mobile phase at a flow rate of 20 µL min^{-1}. Glimepiride was evidenced by its dominant mass peak at ($[M + H]^+$, m/z = 490.65), which was chosen as the precursor ion. The compound was fragmented to produce an intense product ion signal at *m/z 351.98*. Ionization and fragmentation were found to be highly efficient and, as a result, a substantial detection response was found at the lower limit of quantitation (0.50 ng mL^{-1}). Full-scan API 3000 mass spectra and product ion mass spectra of glimepiride are displayed in Fig. 1.

Glimepiride plasma concentrations were determined by means of reversed-phase HPLC method. The mobile phase was formic acid 0.05 M–ACN 28/72. It was pumped isocratically at 0.3 mL/min through Hypersil ODS 5 µm, 250 × 4.6 mm column, heated to 25°C. The selectivity of the method towards endogenous plasma compounds was found to be fully satisfactory. In none of the tested lots of analyte-free plasma was any unacceptable interference seen. A chromatogram obtained from the analysis of a plasma sample spiked with 100 ng mL^{-1} of the analyte is displayed in Fig. 2 and illustrates the selectivity of the proposed chromatographic procedure. Under the current chromatographic conditions glimepiride eluted at 3.24 min.

Sample preparation procedure for the analysis of biological samples in LC-MS/MS is a matter of great

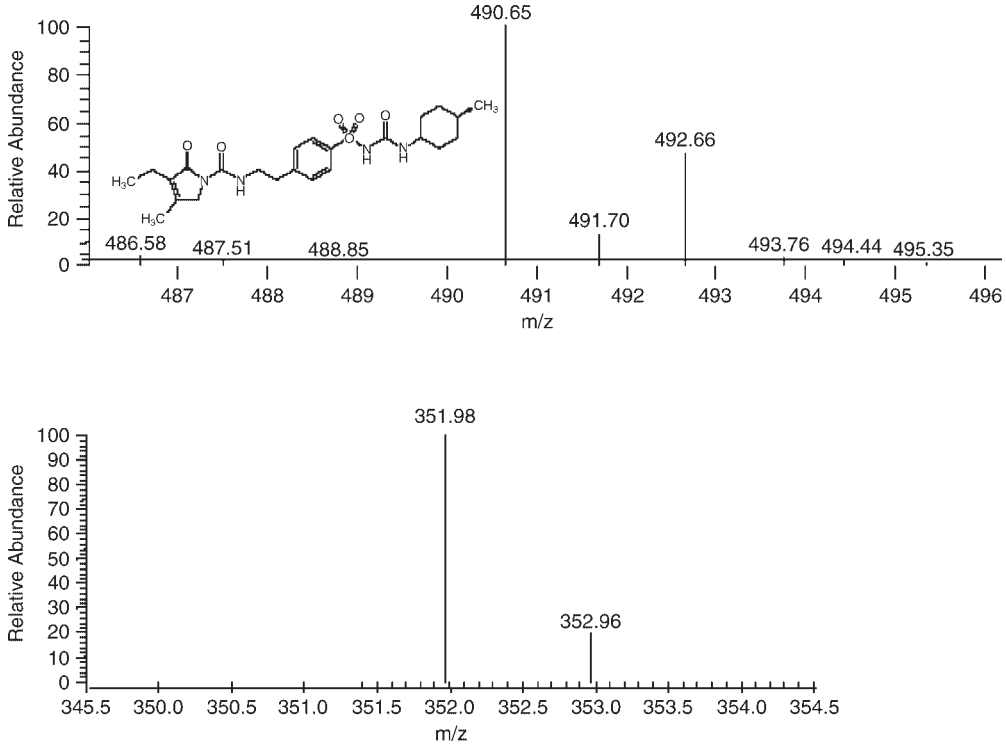

Figure 1. Full-scan spectrum of glimepiride and product ion scan spectra of its dominant mass peak at ($[M + H]^+$, m/z = 490.65) in formic acid 0.05 M–ACN, 28:72. It was pumped isocratically at 0.3 mL min^{-1} through Hypersil ODS 5 µm, 250 × 4.6 mm column, heated to 25°C.

Table 2. Analytical parameters of the calibration equations for the determination of glimepiride by liquid chromatography electrospray ionization tandem mass spectrometry

Concentration range (ng mL^{-1})	Regression equations[a]	r[b]	SD[c] Slope	SD[c] Intercept	S_r^d	α/S_α^e
0.50–1000	$R_{glm} = 1.931 \times C_{glm} + 0.18$	0.998	0.21	0.14	0.09	1.29
Mean of three calibration curves over a period of 3 weeks						
0.50–1000	$R_{glm} = 2.121 \times C_{glm} + 0.22$	>0.997	0.24	0.12	<0.13	<1.83

[a] Ratio of the peak area amplitude of glimepiride, R_{glm}, vs. the corresponding concentration; C_{glm} is the concentration of glimepiride.
[b] Correlation coefficient.
[c] Standard deviation of slope and intercept.
[d] Standard error of the estimate.
[e] Theoretical value of t at p = 0.025 level of significance, for $f = n - 2 = 9$ df, 2.26.

concern. In order to improve the robustness of the method and the sensitivity and recovery of the analytes, a selective sample preparation approach was required. A mixture of 1-chlorobutane–isopropanol–ethyl acetate (88:2:10, v/v/v) was chosen as the optimum solvent mixture for the liquid–liquid extraction procedure and led to almost complete extraction recovery for the analyte. The selectivity of sample preparation is complementary to the selectivity of the subsequent chromatographic separation, which is advantageous for the sensitivity of the total analytical procedure.

Statistical analysis of data

Spiked plasma samples of glimepiride, prepared as described in the Experimental section, were injected into the LC-MS/MS system for the calibration procedure. Calibration graphs were constructed at 11 concentration levels in the range 0.50–1000 ng mL^{-1} for the analyte. A linear relationship of the peak area signals of the analyte was observed, as shown by the equations presented in Table 2. In all cases, back-calculated concentrations in the calibration curves were within

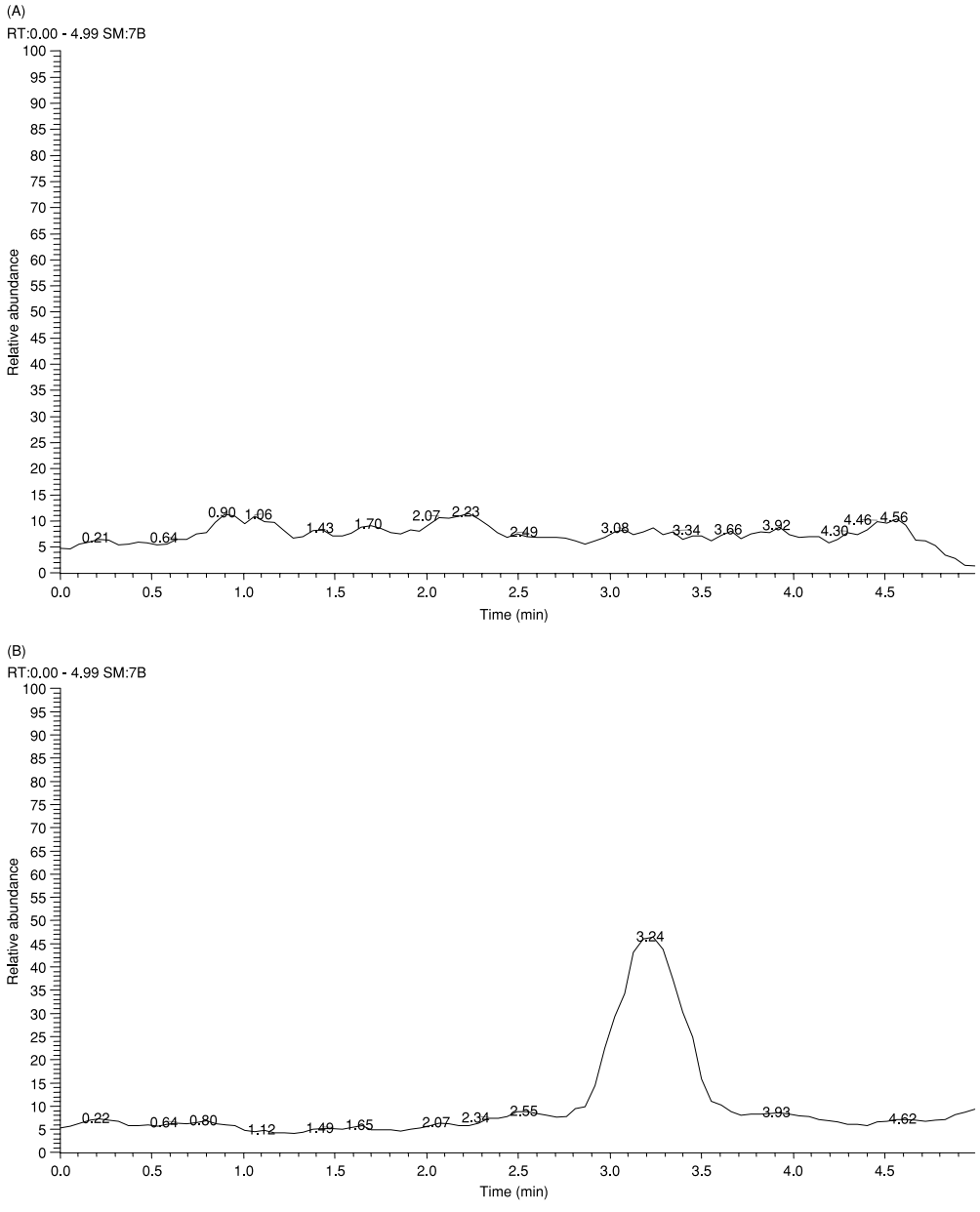

Figure 2. (A) Representative MS chromatogram obtained from the analysis of a blank plasma sample. (B) Representative MS chromatogram obtained from the analysis of a sample spiked at the LOQ level with glimepiride (t_R = 3.24 min). Chromatographic conditions: formic acid 0.05 M–ACN, 28:72. It was pumped isocratically at 0.3 mL min^{-1} through Hypersil ODS 5 μm, 250 × 4.6 mm column, heated to 25°C.

15% of the nominal values, which is in agreement with international guidelines (CPMP, 1998) and indicate that the linear model acceptably describes the relationship between concentration and response. The average regression equation for the analyte is also presented in Table 2, along with the RSD values of the slope and intercept; the correlation coefficient invariably; exceeded 0.997.

A Student's *t*-test was performed to determine whether the experimental intercepts (α) of the above-mentioned regression equations were significantly different from the theoretical zero value. The test is based on the calculation of the quantities $t = \alpha/S_\alpha$, where α is the intercept of the regression equations and S_α is the standard deviation of α, and their comparison with tabulated data of the *t*-distribution. The calculated *t*-values are also presented in Table 2; the values do not exceed the 95% criterion of $t_p = 2.26$ for $f = 9$ degrees of freedom, which denotes that the intercepts of all regression lines are not significantly different from zero.

A thorough investigation for the determination of the extraction efficiency of the proposed liquid–liquid

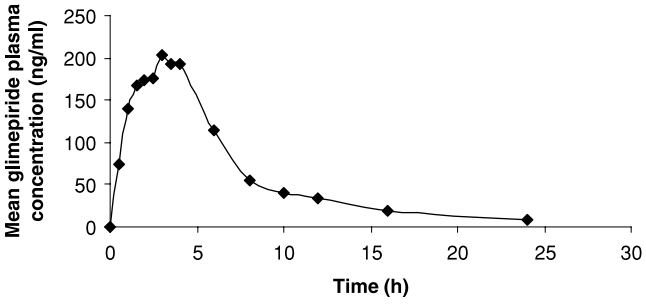

Figure 3. Mean plasma concentration-time curves of glimepiride after oral administration of 4 mg tablets of glimepiride to six healthy volunteers.

extraction procedure was conducted. The extraction recovery was evaluated at 250.0 ng mL^{-1} for glimepiride. The recovery was determined by calculating the ratio of the area of the spiked plasma samples to the area of the unextracted standards. The mean recovery was found to be 95.6 ± 5.6% for glimepiride.

The limit of detection, LOD, for glimepiride was determined according to the definitions of ICH Topic Q2B (ICH Topic Q2B, 1996). Therefore, it was calculated using the equations $y - \alpha = 3.3 \times S_\alpha$ and $y - \alpha = b \times \text{LOD}$ (where b is the slope and S_α is the standard deviation of the intercept of the regression line), and it was found to be 0.16 ng mL^{-1} for the analyte.

The limit of quantitation, LOQ, for glimepiride was also determined according to the definitions of ICH Topic Q2B (ICH Topic Q2B, 1996). Thus, it was calculated using the equations $y - \alpha = 10 \times S_\alpha$ and $y - \alpha = b \times \text{LOQ}$ (where b is the slope and S_α is the standard deviation of the intercept of the regression line). The limit of quantitation was found to be 0.50 ng mL^{-1} for glimepiride. A representative MS chromatogram obtained from the analysis of a sample spiked at the LOQ level for the analyte along with a chromatogram obtained from the analysis of a blank plasma sample are presented in Fig. 3.

Intra-day data for the precision and accuracy of the method given in Table 3 indicate RSD% ranging from 2.68 to 10.37% for glimepiride. Moreover, the accuracy was assessed by the relative percentage error, E_r%, which was ranged from –3.0 to 1.25% for glimepiride. Inter-day data for the precision and accuracy are also presented in Table 3. The inter-day RSD and E_r% values for glimepiride ranged from 2.76 to 11.55 and –0.5 to 4.0, respectively.

The ionization of the analyte in liquid chromatographic tandem mass spectrometric procedures is susceptible to interference from matrix components and thus the accuracy of the quantitative determinations may well be influenced by matrix effect. In this study, the concentrations in the quality control samples were calculated against a calibration curve, which was prepared in a different lot of plasma in each analytical batch. Thus, accuracy and precision data presented in Table 3 indicate that the proposed methodology exhibits reliable results for the analyte regardless of the composition of the human plasma.

The stability results presented in Table 4 indicate that the analyte can be considered stable under the various conditions investigated, since its concentration decreased by no more than 7.1% relative to the reference and no degradation products were observed.

In order to evaluate method robustness, the retention time for glimepiride was determined under slightly different chromatographic conditions. The factors selected for examination were the pH of the buffer, concentration of the buffer, the percentage of acetonitrile in the mobile phase and column temperature; each factor was charged at three levels (–1, 0 and 1).

Table 3. Accuracy and precision of within- and between-run analysis for the determination of glimepiride in human plasma by liquid chromatography electrospray ionization tandem mass spectrometry

Concentration added (ng mL^{-1})	Assayed concentration (ng mL^{-1})			
	Mean ± SD (n = 6)	RSD (%)[a]	Mean recovery (%)	E_r (%)[b]
Intra-day				
0.75	0.76 ± 0.06	7.89	105.4	1.3
10.0	9.70 ± 0.85	8.76	107.1	–3.0
40.0	40.50 ± 4.20	10.37	97.4	1.25
250.0	253.10 ± 6.80	2.68	95.6	1.24
750.0	744.80 ± 44.50	5.97	89.3	0.6
Inter-day				
0.75	0.78 ± 0.07	8.97	103.7	4.0
10.0	10.3 ± 0.92	8.93	105.8	3.0
40.0	39.8 ± 4.6	11.55	95.9	–0.5
250.0	253.3 ± 7.0	2.76	95.8	1.3
750.0	748.3 ± 39.4	5.26	91.1	0.2

[a] Percentage relative standard deviation.
[b] Relative percentage error.

Table 4. Stability data for glimepiride in human plasma under various storage conditions

Storage conditions	Time	Concentration (ng mL^{-1}) Initial	Found	(Percentage) deviation[a]	RSD (%),[b] $n = 3$
Ambient temperature	6 h	98.2	97.3	−0.9	3.2
6°C	40 days	99.3	102.4	3.12	7.1
−20°C	75 days	100.1	102.6	2.49	4.6
Freeze–thaw cycles; −20°C	3 cycles	98.7	99.1	0.4	4.2

[a] Percentage of mean deviation from $t = 0$.
[b] Percentage relative standard deviation.

Table 5. Retention time for glimepiride during robustness testing

Proportion A:B (%)	Buffer molarity	Column temperature	Mean r_t ($n = 3$)
28:72	50.0	22.0	1.5
28:72	50.0	25.0	1.6
28:72	50.0	28.0	1.5
26:74	50.0	25.0	1.4
30:70	50.0	25.0	1.6
28:72	50.0	25.0	1.6
28:72	50.0	25.0	1.4
28:72	42.5	25.0	1.4
28:72	52.5	25.0	1.5

One factor at a time was changed to estimate the effect. Thus, replicate injections ($n = 3$) of a mixed standard solution containing 100 ng mL^{-1} of glimepiride were performed under small changes of four chromatographic parameters (factors). As shown in Table 5, in all cases the retention time was well above 1.0, which indicates that slight differences in chromatographic conditions do not unacceptably affect the performance of the method.

Application to pharmacokinetic studies

The method was further applied to a pharmacokinetic study for the evaluation of the main pharmacokinetic parameters of glimepiride. Six non-smokers, healthy male and female volunteers from the local population, were screened and enrolled in the trial. Figure 3 represents mean plasma concentration profile of glimepiride vs time following a single *per os* administration of 4 mg of the drug under fasting conditions.

The pharmacokinetic parameters were calculated by the EquivTest™ Statistical Solutions software. The area under the curve from first until last sampling, $AUC_{0\text{-last}}$, area under the curve extrapolated to infinity, $AUC_{0\text{-inf}}$, maximum concentration, C_{max}, time of the maximum concentration, T_{max}, and elimination half life, $T_{1/2}$, were the pharmacokinetic parameters, which were computed according to EMEA guidelines (CPMP, 1998). Descriptive statistics of the pharmacokinetic parameters are summarized in Table 6. Results indicate maximum glimepiride plasma concentration, C_{max}, of 244.37 ± 71.60 ng mL^{-1} with an elimination half-life, $T_{1/2}$, of 4.61 ± 0.83 h.

CONCLUSION

The proposed liquid chromatographic triple quadrupole mass spectrometric method enables a rapid and accurate assay for the determination of glimepiride in human plasma with a run time lower than 4.0 min. The method consists of a simple liquid–liquid extraction

Table 6. Pharmacokinetic parameters for glimepiride preparation (4 mg/tb)

Parameter	$AUC_{0\text{-last}}$[a] (ng h/mL), reference	$AUC_{0\text{-inf}}$[b] (ng h/mL), reference	C_{max}[c] (ng/mL), reference	T_{max}[d] (h), reference	$T_{1/2}$[e] (h), reference
Median	1380.20	1425.87	241.45	2.92	—
Geometric Mean	1370.44	1448.05	234.12	2.69	—
Arithmetic Mean	1479.93	1549.98	244.37	2.90	4.61
SD	562.59	299.54	71.60	0.98	0.83
Maximum	3112.70	3359.64	437.24	4.00	—
Minimum	507.06	554.34	105.93	1.00	—
CV (%)	38.01	38.68	30.57	36.32	—

[a] Area under the curve from first until last sampling.
[b] Area under the curve extrapolated to infinity.
[c] Maximum concentration.
[d] Time of the maximum concentration.
[e] Elimination half-time.

procedure and an isocratic chromatography condition using a reversed-phase column. MS/MS detection increases the sensitivity and selectivity of the proposed method. Recovery and precision studies successfully quantified glimepiride in spiked plasma samples. Furthermore, the method has also been successfully applied to a pharmacokinetic study for the evaluation of the main pharmacokinetic parameters in the plasma of six non-smokers healthy volunteers. The plasma concentrations detected during the kinetic study were well within the limitations of the assay. As a result the sensitivity of the assay was adequate for the determination of kinetic parameters of glimepiride in the described study.

REFERENCES

Altinöz S and Tekeli D. Analysis of glimepiride by using derivative UV spectrophotometric method. *Journal of Pharmaceutical and Biomedical Analysis*, 2001; **24**: 507–515.

Badian M, Korn A, Lehr KH, Malerczyk V and Waldhäusl W. Determination of the absolute bioavailability of glimepiride (HOE 490), a new sulphonylurea. *International Journal of Clinical Pharmacological Therapy and Toxicology* 1992; **30**(11): 481–482.

Badian M, Korn A, Lehr KH, Malerczyk V and Waldhäusl W. Absolute bioavailability of glimepiride (Amaryl) after oral administration. *Drug Metabolism, Drug International* 1994; **11**(4): 331–339.

Brian WR. Hypoglycemic agents In *Metabolic Drug Interactions*, Levy HR, Thummel KE, Trager WF, Hansten PD and Eichelbaum M (eds). Lippincott Williams & Willkins, Philadelphia, PA, 2000; 529–543.

CPMP. *Note for Guidance on the Investigation of Bioavailability and Bioequivalence*. Committee for Proprietary Medicinal Products: London, 1998.

Draeger E. Clinical profile of glimepiride, *Diabetes Research and Clinical Practice* 1995; **28**(suppl.): 139–146.

Draeger KE, Wernicke-Panten K, Lomp HJ, Schuler E and Rosskamp R. Long-term treatment of type 2 diabetic patients with the new oral antidiabetic agent glimepiride (Amaryl): a double-blind comparison with glibenclamide. *Hormone Metabolism Research* 1996; **28**(9): 419–425.

Eckert HG, Kellner HM, Gantz D, Jantz H, Hornke I and Puttkamer GD. Pharmacokinetics and metabolism of glimepiride. *Clinical Reports* 1993; **27**: 61–92.

Geisen K. Special pharmacology of the new sulfonylurea glimepiride. *Arzneimittelforschung* 1988; **38**(8): 1120.

Goldstein JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *British Journal of Clinical Pharmacology* 2001; **52**: 349–355.

ICH Topic Q2B, *Pharmaeuropa* 1996; **8**: 108.

Jemal M. High-throughput quantitative bioanalysis by LC-MS/MS. 2000; **14**: 422–429.

Kolterman OG, Gray RS, Shapiro G, Scarlett JA, Griffin J and Olefsky JM. The acute and chronic effects of sulfonylurea therapy in type II diabetic subjects. *Diabetes* 1984; **32**: 346–354.

Lehr KH and Damm P. Simultaneous determination of the sulphonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after pre-column derivatization. *Journal of Chromatography* 1990; **526**: 497–505.

Malerczyk V, Badian M, Korn A, Lehr KH and Waldhäusl W. Dose linearity assessment of glimepiride (Amaryl) tablets in healthy volunteers. *Drug Metabolism, Drug International* 1994; **11**(4): 341–357.

Mauer HH, Kratzsch C, Kraemer T, Peters FT and Weber AA. Screening, library-assisted identification and validated quantification of oral antidiabetics of the sulfonylurea-type in plasma by atmospheric pressure chemical ionization liquid chromatography-mass spectrometry. *Journal of Chromatography B Analytical Technology in Biomedicine and Life Sciences* 2002; **773**(1): 63–73.

Miners JO and Birkett DJ. Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *British Journal of Clinical Pharmacology* 1998; **45**: 525–538.

Müller G and Geisen K. Characterization of the molecular mode of action of the sulfonylurea, glimepiride, at adipocytes. *Hormone Metabolism Research* 1996; **28**(9): 469–487.

Niemi M, Backman JT, Neuvonen M, Laitila J, Neuvonen PJ, Kivisto KT. Effects of fluconazole and fluvoxamine on the pharmacokinetics and pharmacodynamics of glimepiride. *Clinical Pharmacology and Therapy* 2001; **69**(4): 194–200.

Niessen WMA. *Liquid Chromatography–Mass Spectrometry*. Chromatographic Science Series, vol. 79. Marcel Dekker: New York, 1999; 405–459.

Pearson JG. Glimepiride. A review of its use in the management of type II diabetes mellitus. *Drugs* 1998; **55**: 563–584.

Physicians Desk Reference, 55th edn. Medical Economics: Montvale, NJ, 2001; 678.

Pistos C, Astraka C, Kalovidouris M, Vassilopoulos E and Koutsopoulou M. Bioequivalence Evaluation of two brands of glimepiride 4 mg tablets in healthy human volunteers. *International Journal of Clinical Pharmacology and Therapeutics* 2004 (in press).

Ratheiser K, Korn A, Waldhausl W, Komjati M, Vierhapper H, Badian M and Malerczyk V. Dose relationship of stimulated insulin production following intravenous application of glimepiride in healthy man. *Arzneimittelforschung* 1993; **43**: 856–858.

Salem II, Idrees J and Al Tamini JI. Determination of glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. *Journal of Chromatography* B 2004; **799**: 103–109.

Smyth WF. Electrospray ionisation mass spectrometric behaviour of selected drugs and their metabolites. *Analytica Chimica Acta* 2003; **492**: 1–16.

Sonenberg GE, Garg DC, Weidler DJ, Dixon RM, Jaber LA, Bowen AJ, Dechemey GS, Mullican WS, Stonesifer LD. Short-term comparison of once- versus twice-daily administration of glimepiride in patients with non-insulin-dependent diabetes mellitus. *The Annals of Pharmacotherapy* 1997; **31**(6): 671–676.