Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytica Chimica Acta 514 (2004) 185–191
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Rapid, simple, specific liquid chromatographic-electrospray mass spectrometry method for the determination of glibenclamide in human plasma

Ben-Mei Chen [a], Yi-Zeng Liang [b,*], Fang-Qiu Guo [b], Lan-Fang Huang [b], Fu-Liang Deng [a], Xin Chen [a], Ya-Li Wang [b]

[a] *Analytical Testing Center of Xiangya Medical School, Central South University, Changsha, Hunan 410078, China*
[b] *Research Center of Modernization of Chinese Medicine, College of Chemistry and Chemical Engineering, Central South University, Yue Lu Shan Nan Lu 154, Changsha, Hunan 410083, China*

Received 11 December 2003; received in revised form 8 March 2004; accepted 17 March 2004

Abstract

A sensitive liquid chromatographic-electrospray ionization mass spectrometric method was developed and validated for fast determination of glibenclamide in human plasma. After deprotienation with methanol, centrifugation, evaporation to dryness and dissolving in mobile phase, samples were separated using a Hypersil-Keystone C18 reversed-phase column (150 mm × 2.1 mm ID, 5 μm), together with a mobile phase containing of acetonitrile–10 mM ammonium acetate (55:45, v/v), 0.2% acetic acid and 0.1% trifluoracetic acid. Glibenclamide and its internal standard, glipizide, were measured by electrospray ion source in positive selective ion monitoring mode. The method demonstrated linearity from 1 to 400 ng/mL ($r = 0.998$). The limit of quantification for glibenclamide in plasma was 1 ng/mL with good accuracy and precision. The recovery of the method was over 91.5%. Intra- and inter-day precision ranged from 3.42 to 5.57 and 7.49 to 8.64% (RSD), respectively.

Keywords: Glibenclamide; LC–MS; Electrospray ionization

1. Introduction

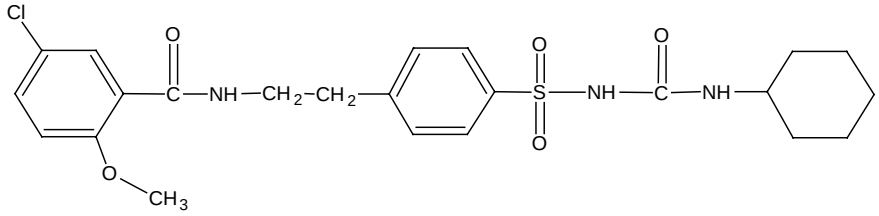
Glibenclamide (Fig. 1) is a sulfonylurea-type oral antidiabetic agent widely used for treatment of hyperglycemia in patients with type-II non-insulin dependent diabetes mellitus. It is rapidly and completely absorbed from the gastrointestinal tract. Sulfonylureas of the first generation used in human therapy have been almost completely substituted by those of more potent second generation for which much lower dosage was required. Diagnosis of hypoglycemic crises of unknown origin includes testing the possibility that the patient has taken a sulfonylurea drug.

Many analysis methods have been developed for measurement of glibenclamide in plasma such as HPLC coupled with UV detection [1–7], HPLC with fluorescence detection [8,9], capillary electrophoresis [10,11] and LC–MS [12–15]. HPLC with UV or fluorescence detection methods were not suitable for pharmacokinetic studies in human plasma after administration of therapeutic doses of glibenclamide since they did not have sufficient sensitivity and specify. HPLC–MS has become the popular analytical tool of the pharmacokinetic study for its high sensitivity and selectivity. The advent of atmospheric pressure ionization (API) mass spectrometry has revolutionized the analysis of drugs and metabolites in biological samples. With their ease of coupling directly with HPLC, high sensitivity and suitability for a wide range of analytes, including polar involatile molecules, an API source is an ideal interface to mass spectrometry.

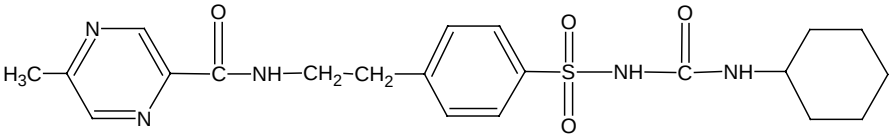
The aim of present study was therefore to develop and validate a sensitive LC–MS method for determining glibenclamide in human plasma. An LC–single quadrupole mass spectrometry system with an electrospray ionization source was used for the quantification of glibenclamide using 0.2 mL plasma sample. The utility of the methodology was

* Corresponding author. Tel.: +86-731-8830831; fax: +86-731-8825637.
E-mail addresses: yizeng_liang@263.net, yzliang@public.cs.hn.cn (Y.-Z. Liang).

0003-2670/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2004.03.044



Glibenclamide



Glipizide

Fig. 1. Structures of glibenclamide and glipizide (IS).

demonstrated by analyzing plasma samples from selected subject participating in pharmacokinetic study. This method was sensitive, specific, rapid and accuracy, and adequate to provide pharmacokinetic concentration–time profile.

2. Experimental

2.1. Reagents and chemicals

Glibenclamide and glipizide were purchased from the China Supervise Institute of Drug and Biological Preparation (Beijing, China). All solvents were HPLC grade and were obtained from Dikma (Scarorough, USA). Trifluoracetic acid (TFA) was purchased from Merck (Darmstadt, Germany). Other reagents were of analytical grade, and all water used was Milli-Q grade (Millipore, Bedford, MA, USA).

2.2. Equipment

The HPLC system consisted of a Shimaduz LC-10Advp pump, an SCL-10Advp system controller, a CTO-10Avp column oven, an FCV-10Alvp low pressure gradient unit, a DGU-14A degasser and an SPD-M10Avp diode array detector (Shimaduz, Kyoto, Japan). The samples were dried on a Savant drier (USA). The mass spectrometer was an LCMS-2010 single quadrupole equipped with electrospray ionization interface (Shimaduz). The data were collected and processed using LCMSSolution software.

2.3. Chromatographic conditions

Chromatographic separations were performed using a Thermo Hypersil-Keystone Hypurity C18 (150 mm × 2.1 mm, 5 μm) analytical column. The oven temperature was set at 40 °C. The mobile phase consisted of acetonitrile–10 mM ammonium acetate (55:45, v/v) containing 0.2% acetic acid and 0.1% (v/v) TFA and was isocratically eluted at a flow rate of 0.2 mL/min. The wavelength of the SPD-M10Avp diode array detector was set at 200–350 nm.

2.4. Mass spectrometer conditions

An LCMS-2010 quadrupole mass spectrometer was interfaced with an electrospray ionization (ESI) probe. The temperatures were maintained at 250, 250, and 200 °C for the probe, CDL, and block, respectively. The voltages were set at 4.5 kV, −50 V, 25 V, 150 V, and 1.6 kV for the probe, CDL, Q-array 1, 2, 3 bias, Q-array radio frequency (RF) and detector, respectively. The flow rate of nebulizer gas was 4.5 L/min. The ions of selection monitoring were decided by positive scanning from *m*/*z* 100–600. For the quantification of glibenclamide, the analysis was carried out in selection ion monitoring in positive ion mode at *m*/*z* 446 (glipizide, IS) and 494 (glibenclamide).

Tuning of mass spectrometer was performed with the help of autotuning function of LCMSSolution software (Version 2.02) using tuning standard solution (polypropylene glycol). Optimization and calibration of mass spectrometer were achieved with autotuning.

2.5. Preparation of stock solutions, calibration standard and quality control samples

A stock solution of glibenclamide at concentration of 100 μg/mL was prepared in methanol. This solution was further diluted with methanol to give serial concentrations of 4, 8, 20, 40, 200, 400, 800 and 1600 ng/mL of glibenclamide to obtain calibration solutions. The internal standard (glipizide) was also prepared as a stock solution (1 mg/mL) in methanol and was further diluted with methanol to give a concentration of 100 ng/mL and used for all analyses. The

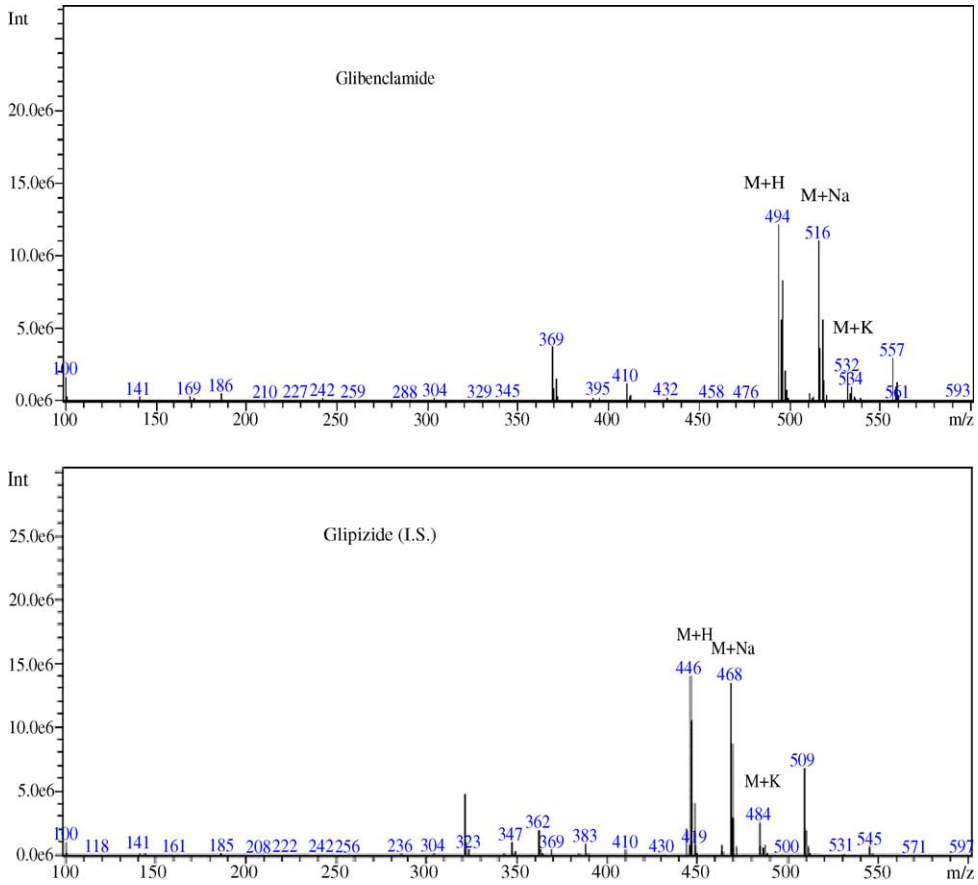


Fig. 2. ESI–MS positive ion scanning spectra of glibenclamide and glipizide (IS). Chromatographic conditions—column: Thermo Hypersil-Keystone Hypurity C18 (150 mm × 2.1 mm, 5 μm), oven temperature: 40 °C, mobile phase: acetonitrile–10 mM ammonium acetate (55:45, v/v) containing 0.2% acetic acid and 0.1% (v/v) TFA, flow rate: 0.2 mL/min. Mass spectrometer: ESI source, positive scan *m/z*: 100–600, temperature—probe: 250 °C, CDL: 250 °C, block: 200 °C, voltages—capillary: 4.5 kV, CDL: −50 V, Q-array 1, 2, 3 bias: 25 V, Q-array RF: 150 V, detector: 1.6 kV. Nebulizer gas flow rate: 4.5 L/min.

calibration curve samples were freshly prepared by adding 50 μL of calibration solutions and 50 μL of internal standard solution to 0.2 mL of blank plasma to yield concentrations of 1, 2, 5, 10, 50, 100, 200 and 400 ng/mL of glibenclamide. The quality control (QC) samples were prepared by adding 50 μL of calibration solutions to 0.2 mL lank plasma to form concentrations of 1, 50 and 400 ng/mL of glibenclamide, and the amount of internal standard added was as same as the calibration curve samples. The further processing of both calibration curve samples and quality control samples was as described in Section 2.6. All standard stock solutions were prepared once a month and stored at 5 °C.

2.6. Sample preparation

A plasma sample (0.2 mL) was placed in a 1.5 mL Eppendorf tube. After the addition of 50 μL of a 100 ng/mL solution of internal standard, the tube was briefly vortexed and 0.2 mL of methanol was added into the tube. After vortexing for 30 s, the tube was centrifuged at 12 000 rpm for 10 min at 5 °C and the supernatant was transferred to another clear Eppendorf tube. The extract was evaporated to dryness in a Speed Vacplus Model vacuum drier. The residue was redissolved with 100 μL of mobile phase, vortexed for 30 s and centrifuged at 12 000 for 5 min, and 5 μL of supernatant was injected onto the analytical column.

2.7. Precision, accuracy, linearity and recovery

The precision and recovery of the method were estimated by replicating analysis ($n = 5$) of QC samples at concentrations 1, 50 and 400 ng/mL. Intra-day precision was evaluated by analyzing QC samples five times over 1 day, while inter-day precision was evaluated by analyzing QC samples in five different days. For checking the accuracy of the method, human plasma after administration of glibenclamide spiked with standard solutions was assayed. The accuracy was estimated by comparing the concentrations from spiked plasma samples with assayed concentration. The calibration curve was constructed by plotting the peak area ratios of glibenclamide to internal standard against glibenclamide concentrations. The unknown sample concentrations were calculated from the regression equation of the calibration curve.

3. Results and discussion

3.1. Selection of HPLC and MS conditions

To select an appropriate ionization mode in LC–MS analysis, the mass spectra were measured in ESI and APCI mode using injection glibenclamide and internal standard solutions. In both ionization modes, the base peak intensity of positive ion was higher than those of negative ion. Fig. 2 shows the positive ion mass spectra of glibenclamide and internal standard by ESI scanning from *m/z* 100 to 600. Therefore, the ESI positive ion mode was selected in this study. Maurer et al. [14] screened the antidiabetics of the sulfonylurea-type in plasma by APCI-mass spectrometry. The limits of detection and quantification were 2 and 10 ng/mL, respectively. Those of our experiment, however, were 0.5 and 1 ng/mL, respectively. This sensitivity was also higher than Magni's [13]. This shows that the polar groups (three amino groups) in glibenclamide molecule were easily ionized by ESI mode than APCI mode. So the sensitivity of glibenclamide in ESI mode was higher than APCI. Hsieh and Selinger [15] determined glibenclamide in human serum by HPLC–MS with ionspray source interface, and a high limit of quantification (10 ng/mL) was reported in his method. This high limit of quantification cannot meet with the need of quantification in lower plasma concentration of glibenclaminde after administration of low dosage.

The separation and ionization of glibenclamide were affected by the components of mobile phase. The amino groups of glibenclamide molecule were easily protonized in stronger acidic mobile phase before entering the ionization ambient (chamber). The protonated amino groups of different position in the molecule have the different chromatographic behavior on the column. Thus several peaks with same *m/z* were observed in the mass spectrum (Fig. 3A) in stronger acidic mobile phase. When the acidity of mobile phase was appropriate, the only specified amino group can be protonated in column, while other amino groups were not protonated before entering the ionization

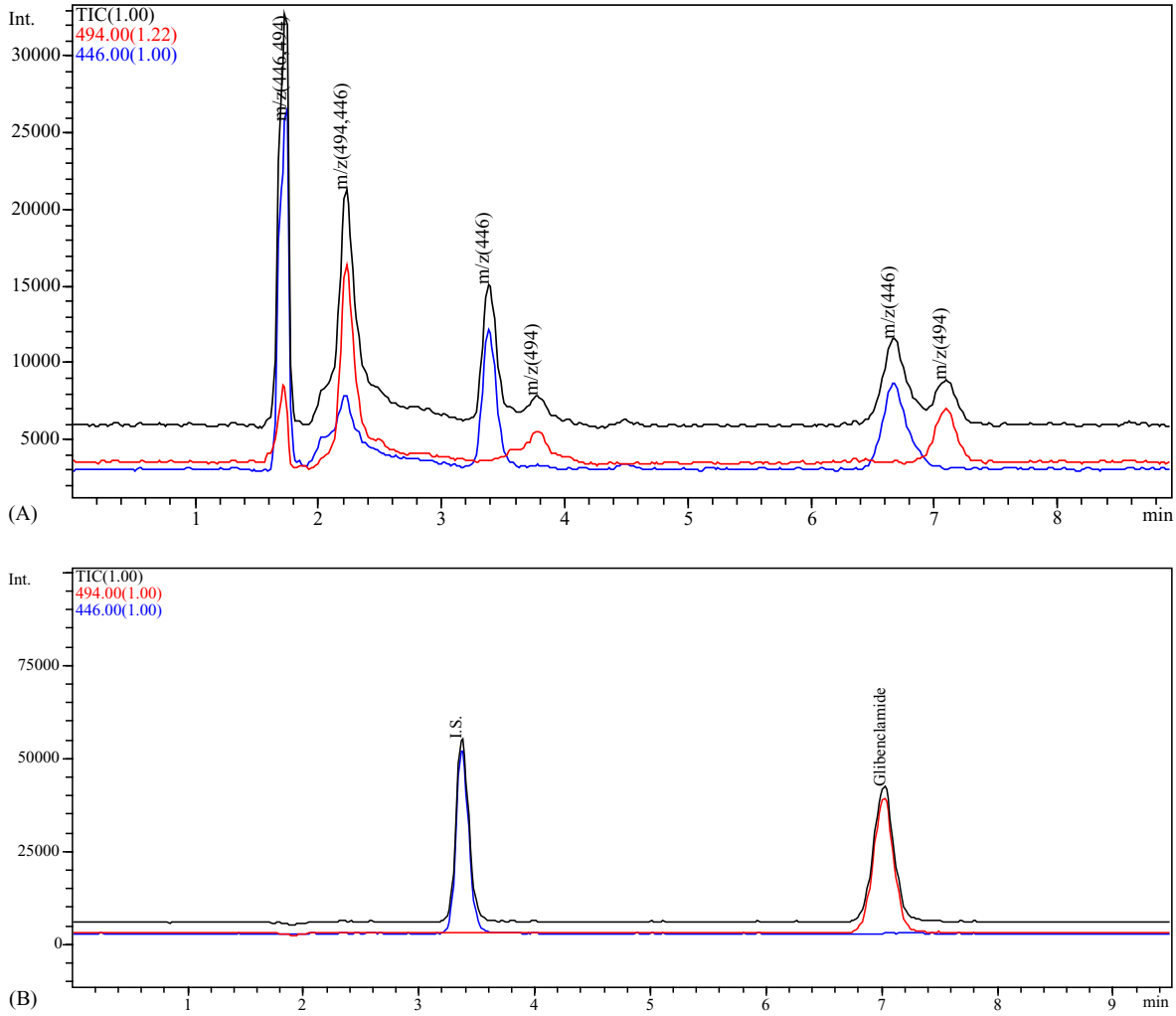


Fig. 3. Selective and total ion chromatograms of glibenclamide and its IS. Positive ion monitored at *m/z* 446 (glipizide, IS), 494 (glibenclamide): (A) mobile phase containing 0.2% of TFA; (B) mobile phase containing 0.1% of TFA; other conditions as in Fig. 2.

ambient. So the single peak occurred in the mass spectrum (Fig. 3B). The retention times of glibenclamide and IS were also affected by the acidity of mobile phase, and they were decreased with increasing acidity of mobile phase. The sensitivity of glibenclamide was improved by increasing acidity of mobile phase because of raising the rate of ionization. The acidity over 0.1% of TFA and 0.2% of acetic acid in mobile phase, however, can bring about multi-peaks of glibenclamide and IS (Fig. 3A). Thus the concentrations of 0.1% of TFA and 0.2% of acetic acid in mobile phase were appropriate for separation and ionization of glibenclamide. Fig. 4 shows the total ion chromatograms of blank plasma, blank plasma spiked with glibenclamide and internal standard, and plasma sample after administration of glibenclamide. A typical UV (260 nm) chromatogram extracted from human plasma is shown in Fig. 5. Much interference

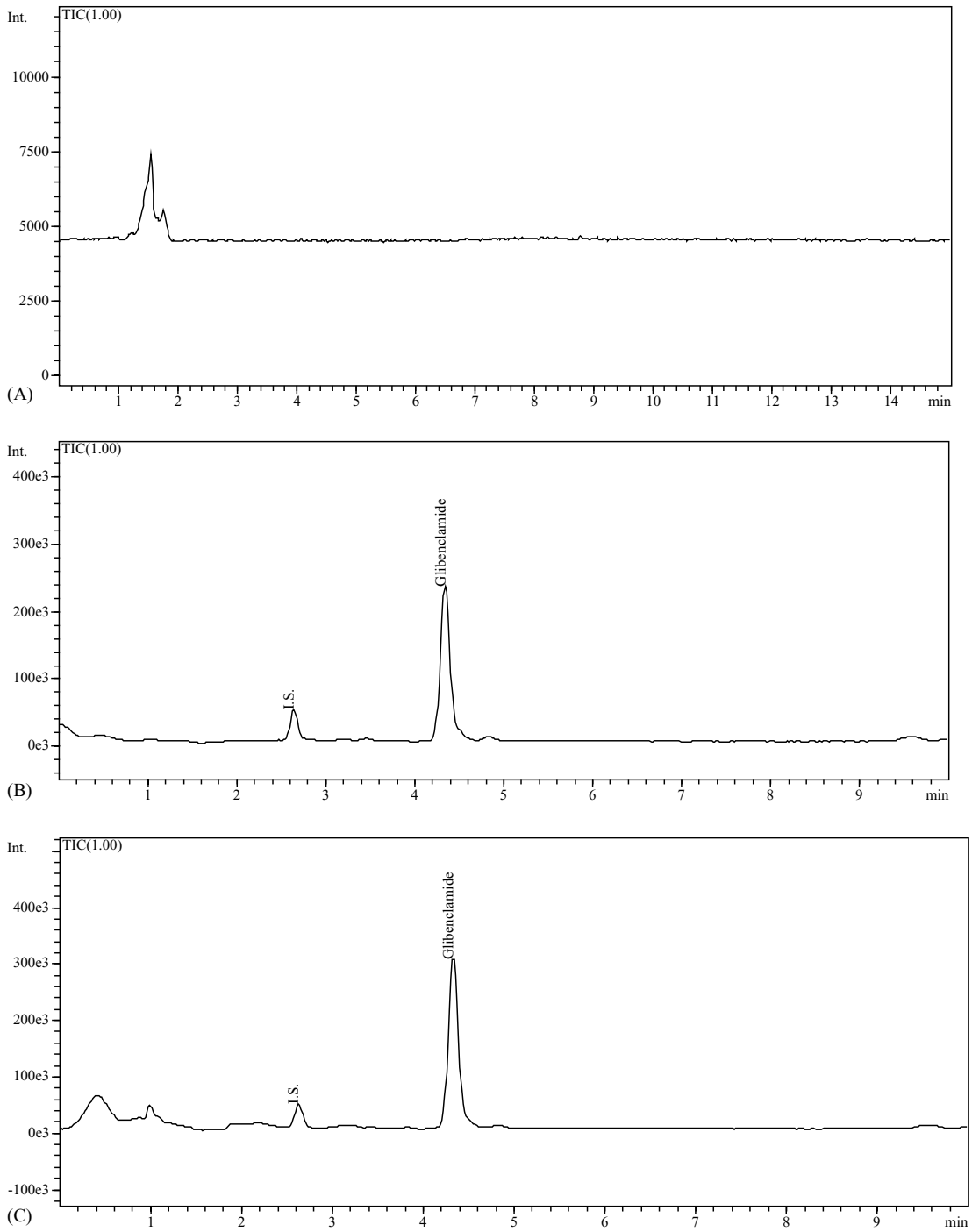


Fig. 4. Total ion chromatograms of blank plasma (A), blank plasma spiked with standard and IS (B) and human plasma sample after administration of glibenclamide (C). Positive ion monitored at *m*/*z* 446 and 494; other conditions as in Fig. 2.

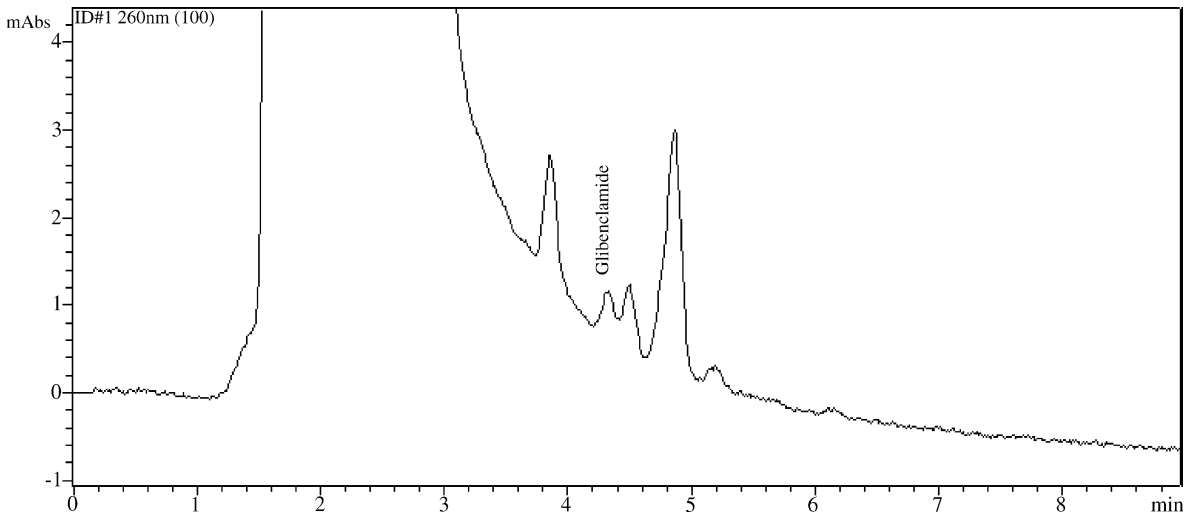


Fig. 5. UV chromatogram of human plasma sample after administration of glibenclamide. Simultaneously monitored at 260 nm with mass spectrometer. Chromatographic conditions as in Fig. 2.

Table 1
Precision and recovery for glibenclamide of quality control sample in human plasma ($n = 5$)

Nominal concentration (ng/mL)	Mean found concentration (ng/mL)	Recovery (%)	Precision (RSD, %)
Intra-day			
1	1.06	106.0	5.57
50	45.97	91.5	3.42
400	377.12	94.3	5.39
Inter-day			
1	1.02	102.0	8.15
50	46.72	93.1	7.49
400	378.20	94.6	8.64

was observed in the present conditions, thus UV detector was not good selectivity for the analysis of glibenclamide.

3.2. Linearity

The calibration curve of glibenclamide was linear over the range from 1 to 400 ng/mL with the correlation coefficient of 0.998. The data demonstrates acceptable accuracy, reproducibility and good fit to the non-weighted regression lines. The calibration curve had the regression equation of $y = 0.2771 + 0.08423x$, where y is the peak area ratio of glibenclamide to internal standard, x the concentration of glibenclamide.

3.3. Precision, accuracy and recovery

Both the intra- and inter-day precisions of the method were determined by analysis of replicates ($n = 5$) of QC samples containing known concentrations of 1, 50 and 400 ng/mL of glibenclamide. The precision of the method was described as relative standard deviation (RSD) among each assay. The accuracy of the method was evaluated by analysis human plasma after administration of glibenclamide spiked with standard solutions. The accuracy was described as a percentage error of measured concentrations versus nominal concentrations and the RSD, respectively. Precision and accuracy were calculated at each concentration. Recovery was determined by comparing found con-

Table 2
Accuracy for glibenclamide of plasma samples spiked with standard solutions ($n = 5$)

Sample concentration (ng/mL)	Added concentration (ng/mL)	Mean found concentration (ng/mL)	Error (%)	RSD (%)
9.6	1	10.8	1.9	5.48
9.6	50	56.8	−4.7	4.36
9.6	300	301.2	−2.7	4.75

centrations with nominal concentrations of glibenclamide in QC samples. The mean absolute recoveries of glibenclamide ranged from 91.5 to 106.0% between 1 and 400 ng/mL of glibenclamide. The results were shown in Tables 1 and 2.

4. Conclusions

A fast, sensitive, specific LC–ESI–MS method for determination of glibenclamide in human plasma was developed and validated. Significantly lower limit of quantification (1 ng/mL) was achieved in plasma, compared with the previously published methods. The steps of sample preparation were uncomplicated using 0.2 mL plasma sample. The method has been successfully applied to the pharmacokinetics studies and produced satisfactory results demonstrate that the method is reproducible.

Acknowledgements

This project was supported in part by National Natural Science Foundation of China (Grants Nos. 20175036 and 20235020).

References

[1] W.J. Adams, D.S. Krueger, J. Pharm. Sci. 68 (1979) 1138–1140.
[2] L. Zecca, S. Trivulzio, A. Pinelli, R. Colombo, O. Tofanetti, J. Chromatogr. 339 (1985) 203–209.
[3] H. Emilsson, S. Sjoberg, M. Svedner, I. Christenson, J. Chromatogr. 383 (1986) 93–102.
[4] M.E. Abdel-Hamid, M.S. Suleiman, Y.M. El-Sayed, N.M. Najib, M.M. Hasan, J. Clin. Pharm. Ther. 14 (1989) 181–188.
[5] T. Rydberg, E. Wahlin-Boll, A. Melander, J. Chromatogr. 564 (1991) 223–233.
[6] J.R. Valdes Santurio, E. Gonzalez Porto, J. Chromatogr. B 682 (1996) 364–370.
[7] I. Niopas, A.C. Daftsios, J. Pharm. Biomed. Anal. 28 (2002) 653–657.
[8] R. Paroni, B. Comuzzi, C. Arcelloni, S. Brocco, S. de Kreutzenberg, A. Tiengo, A. Ciucci, P. Beck-Peccoz, S. Genovese, Anal. Chem. 54 (1982) 1287–1291.
[9] J. Khatri, S. Qassim, O. Abed, B. Abraham, A. Al-Lami, S. Masood, J. Pharm. Pharm. Sci. 4 (2001) 201–206.
[10] M. Nunez, J.E. Ferguson, D. Machacek, G. Jacob, R.P. Oda, G.M. Lawson, J.P. Landers, Anal. Chem. 67 (1995) 3668–3675.
[11] W.J. Adams, G.S. Skinner, P.A. Bombardt, M. Courtney, J.E. Brewer, Clin. Chem. 46 (2000) 1773–1780.
[12] P.R. Tiller, A.P. Land, I. Jardine, D.M. Murphy, R. Sozio, A. Ayrton, W.H. Schaefer, J. Chromatogr. A 794 (1998) 15–25.
[13] F. Magni, L. Marazzini, S. Pereira, L. Monti, M. Galli Kienle, Anal. Biochem. 282 (2000) 136–141.
[14] H.H. Maurer, C. Kratzsch, T. Kraemer, F.T. Peters, A.A. Weber, J. Chromatogr. B 773 (2002) 63–73.
[15] S. Hsieh, K. Selinger, J. Chromatogr. B 772 (2002) 347–356.