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High-performance liquid chromatography with electrochemical detection for analysis of gliclazide in plasma

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Abstract

A sensitive HPLC–electrochemical detection method was developed for the analysis of gliclazide (GL) in human plasma. After deproteination of 100 μL of plasma by acetonitrile, evaporation, and reconstitution, GL was separated on a C_{18} column (150 mm × 4.6 mm) by the mobile phase (70 mM disodium tetraborate, pH 7.5, containing 26.5% of acetonitrile). The regression equations were linear ($r > 0.9990$) over the range of 50 nM to 4.00 μM. The precision and accuracy of intra- and inter-day analysis were less than 5.3 and 0.93% for relative standard deviation and relative error, respectively. The limit of detection for plasma was 10 nM for GL (S/N = 3, 10 μL injection). This newly developed method was applied for monitoring blood levels with one healthy volunteer dosing with a GL tablet.

Keywords: Gliclazide

1. Introduction

Gliclazide (GL, Fig. 1), *N*-(4-methylbenzenesulfonyl)-*N*′-(3-azabicyclo-[3.3.0]oct-3-yl)urea, is a second-generation sulfonylurea commonly used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) [1]. GL undergoes extensive hepatic metabolic biotransformation to give seven inactive metabolites [1,2]. These metabolites are excreted in urine and feces [1]. However, in the plasma, the unchanged GL represents over 90% of the administered dose. In view of the widespread clinical usage of this drug, it is desirable to develop a sensitive and selective method for therapeutic drug monitoring.

Several HPLC and capillary electrophoresis (CE) methods were reported in the literature for the determination of GL in biological specimens [2–5]. Noguchi et al. reported the determination of GL in serum using HPLC–UV coupled with solid-phase extraction (SPE) at a limit of detection (LOD) of 60 nM [3]. Paroni et al. compared CE with HPLC for diagnosis of factitious hypoglycemia. SPE was used for pretreatment of serum, and limit of quantitation (LOQ) was shown to be 1.2 μM [4]. Najib et al. used liquid extraction and HPLC–UV for bioequivalence of two brands of GL. The LOD was reported as 0.6 μM in this study [2]. Rouini et al. described using toluene extraction method of GL in serum, and analysis was performed using HPLC–UV with a 90 nM LOD [5]. However, none of these reported methodologies involved the use of electrochemical detection (ED). The electrochemical detection is a very powerful detection system, which can detect weak currents of less than nA generated from oxidative or reductive reactions of interests. Fortunately, GL possesses an oxidative group, and can be detected by ED. In this study, we investigated the optimum conditions for the analysis of GL and successfully established one such HPLC–ED method with simple sample preparation and high sensitivity for the determination of GL in plasma. We also validate the applicability for analysis of GL with one healthy volunteer dosing with one 80-mg GL tablet of Diamicron.

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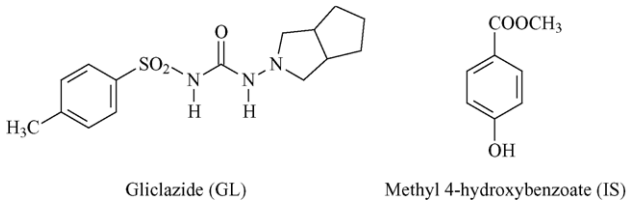

Fig. 1. Structures of gliclazide (GL) and methyl 4-hydroxybenzoate (IS).

2. Experimental

2.1. HPLC systems

A Waters 717 plus autosampler and a Waters system solvent module 501 pump were used. The Bioanalytical systems electrochemical detector consisted of a LC-4C amperometric detector, a glassy carbon electrode cell and an Ag/AgCl reference cell. A Beckman system Gold software was used for data processing.

Isocratic separation was achieved at ambient temperature using Apollo C_{18} column (150 mm × 4.6 mm; 5 μm, Alltech). The working electrode was set at an applied potential of 800 mV relative to an Ag/AgCl reference electrode, filter setting was 0.1 Hz, and range setting was 10 nA. The mobile phase was consisted with 70 mM disodium tetraborate (pH 7.5) containing 26.5% of acetonitrile, then filtered (Millipore, HVLP, 0.45 μm) under vacuum for degassing before using. Each run needed 10 min at a flow-rate of 0.8 mL/min.

2.2. Reagent, standard and plasma solutions

GL and methyl 4-hydroxybenzoate (internal standard, IS) were obtained from Sigma (St. Louis, MO, USA). Diamicron (Servier Ind., Giddy, France) is a commercial GL tablet (80 mg/tablet). Disodium tetraborate, acetonitrile (HPLC grade), methanol (HPLC grade) and hydrochloric acid were purchased from Merck (Darmstadt, Germany). Milli-Q water (Millipore, Bedford, MA, USA) was used for preparation of mobile phase and related aqueous solutions.

Stock solutions of GL and IS at 1 mM were prepared and suitably diluted in methanol and stored at −70 °C and room temperature, respectively. Plasma solutions were prepared by spiking various volume of the diluted stock solution of GL into the healthy plasma.

2.3. Extraction procedure

A 100-μL aliquot of plasma sample was added 1 mL acetonitrile to precipitate protein and vortexed for 1 min. After centrifugation (12 000 rpm, 10 min), supernatant was transferred to another tube, then dried under nitrogen atmosphere. The residues were reconstituted with 200 μL IS solution (5 μM) and vortexed for 1 min. A 10 μL aliquot was injected into HPLC–ED for analysis.

2.4. Method validation parameters

The LOD and LOQ were first determined by spiked into plasma with decreasing concentrations of GL until a ratio of single to noise equaled 3 (S/N = 3, injection 10 μL) and S/N ≧ 10, respectively. Known levels of GL-spiked plasma sample (0.05, 0.30, 0.50, 2.00 and 4.00 μM) were prepared for calibration curves. Following extraction process, the calibration graphs were established with the peak area ratio of GL to IS as ordinate (y) versus concentration of GL as abscissa (x). The regression equations of intra-day analysis were calculated from the assay values of triplicate prepared plasma solutions on the same period ($n = 3$). The regression equations of inter-day analysis were calculated from the assay values of prepared plasma solutions on five different periods ($n = 5$).

Extracted recoveries of GL spiked in plasma matrix were determined by comparing the calculated concentrations with the spiked concentrations. Meanwhile, pretreated plasma samples were analyzed during 12 h for evaluating the stability of GL-spiked plasma after extraction under room temperature.

Intra- and inter-day accuracy and precision of proposed method were evaluated by analyzing three different concentration of GL-spiked plasma (0.08, 0.80 and 3.00 μM) under the conditions described previously in a single and five different days, respectively.

2.5. Application in one volunteer's plasma

Informed consent was obtained from one volunteer in this study. The male volunteer (27-year-old, 70 kg) was administered one 80-mg GL tablet of Diamicron (Servier Ind.). Venous blood samples were collected in heparinized tubes during the 0.5–48-h time interval after administration. The plasma collected before dosing was employed as a blank. All blood samples were centrifuges immediately, the plasma separated and stored at −70 °C until analysis.

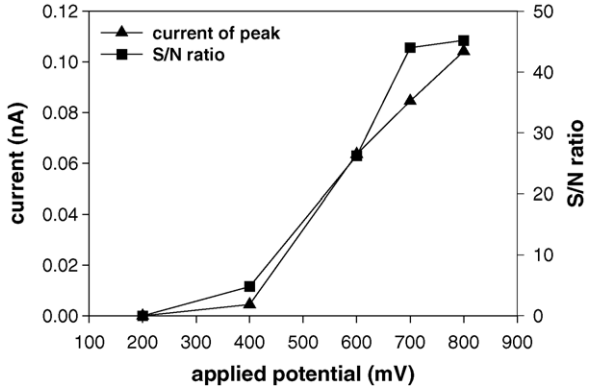

Fig. 2. Hydrodynamic voltammogram and plot of S/N ratio for gliclazide at different applied potentials.

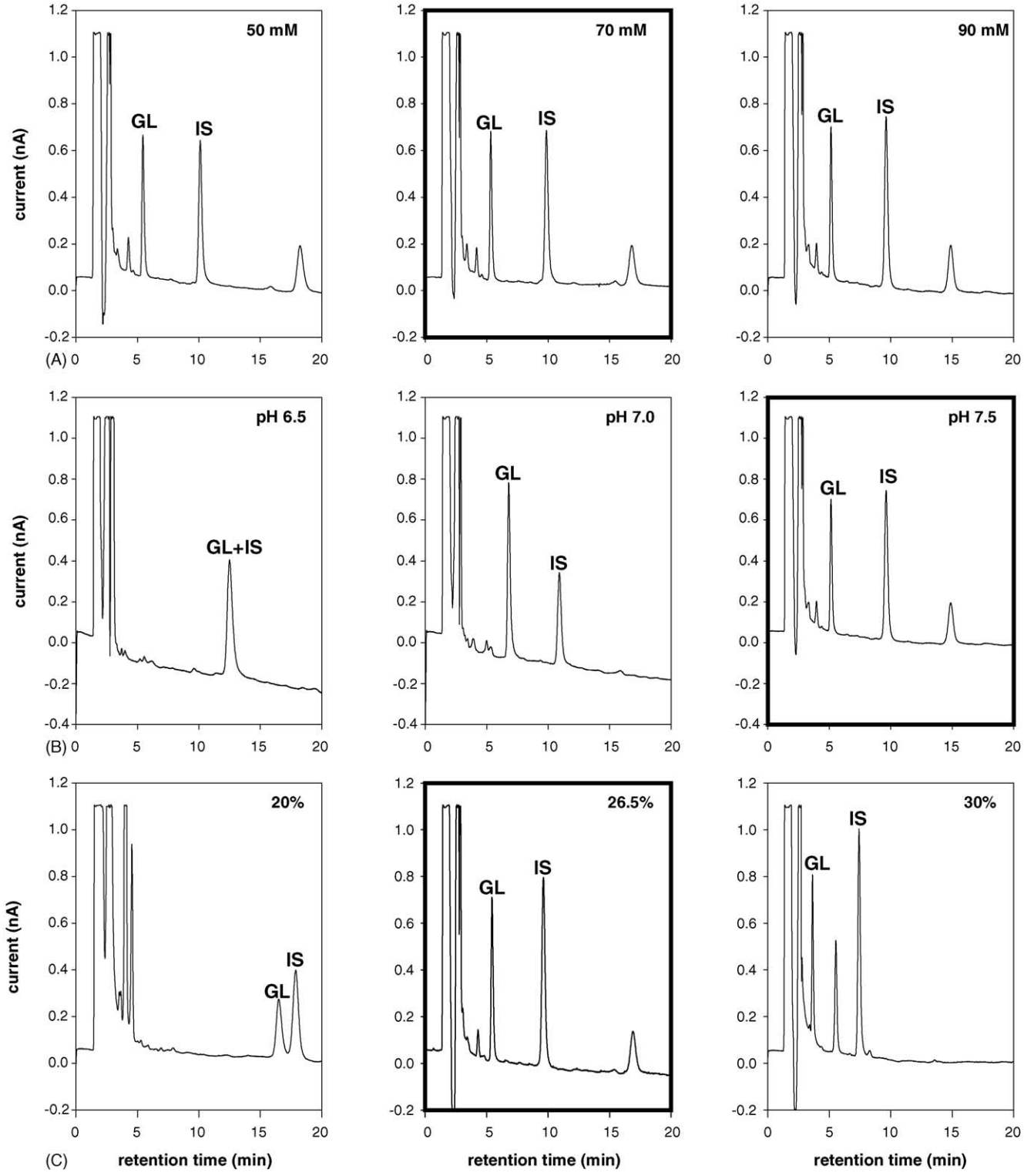

Fig. 3. Effects of parameters on the separation of GL. (A) Concentrations of borate buffer; (B) pH values of borate buffer; (C) proportions of acetonitrile. See Fig. 4 for other conditions.

3. Results and discussion

3.1. HPLC–ED method development

ED is a specific and sensitive detection system for analytes, which possess the characteristics of redox. Screening the detectibility of GL, glipizide and metformin, only GL presented the redox activity. In order to obtain the highest sensitivity, the applied potentials (200–900 mV) were examined for the performance of redox behavior of GL-spiked plasma sample at 0.3 μM, and shown as Fig. 2. It needed longer preconditioning time to reach stable status for the potential at 900 mV. At 800 mV, the highest peak current and S/N ratio could be obtained. Concerning about the sensitivity and balance time of ED, we selected 800 mV as working potential for this study.

As shown in Fig. 3, the analyte and IS showed dramatically different resolutions and responses in ED when adjusting the buffer conditions. Effects of concentrations (50–90 mM) and pH values (6.5, 7.0 and 7.5) of borate buffer on peak heights and retentions of GL were investigated and shown in Fig. 3A and B, respectively. Concentration of electrolyte displayed no apparent difference, and was arbitrarily chosen at 70 mM for elution. Due to pK_a of GL (5.98 and 2.21), there is less partition in the buffer with lower pH value, and it could save the separation time. We selected pH 7.5 for separation.

Effects of proportions (20–35%) of acetonitrile in borate buffer were optimized and shown in Fig. 3C. Using 20% of acetonitrile, it made GL broadening and time-consuming. In the buffer containing greater than 30% of acetonitrile, GL would coelute with interference peak. To prevent from interference of blank and achieve complete separation and shorter analysis time, the proportion of acetonitrile was set at 26.5%.

3.2. Method validation in plasma

The LOD (S/N = 3) and LOQ (S/N ≧ 10) were found to be 10 and 50 nM, respectively, when injecting 10 μL into HPLC. The regression equations were linear in the range of 50 nM to 4.00 μM ($r > 0.999$). The data indicates good linearity of this method for the intra- and inter-day assays.

The precision (relative standard deviation, RSD) and accuracy (relative error, RE) of the proposed method for spiked samples were studied. The results (Table 1) show that the RSD and RE at these three concentrations were all below 5.3 and 0.93%, respectively.

The recoveries for GL in spiked plasma from three individuals were greater than 99.12%. The stability of GL-spiked plasma after extraction under room temperature could be kept at least 12 h.

3.3. Application to human plasma

One male volunteer (27-year-old, 70 kg) was orally administrated with one 80-mg GL tablet. The chromatogram resulting from the analysis of plasma sample at 48 h is shown in Fig. 4. The calculated GL level was 1.36 μM. The concentration–time profile of GL in plasma was monitored for 48 h, and shown as Fig. 5. From the results, the time (t_{max}) to reach peak plasma concentration (C_{max}) was 5 h and 6.07 μM, respectively. Substituting the plasma lev-

Table 1
Precision and accuracy for the determination of gliclazide in intra- and inter-day analysis

Concentration known (μM)	Concentration found (μM)	RSD (%)	RE (%)
Intra-day analysis ($n = 3$)			
0.08	0.081 ± 0.001	1.778	0.927
0.80	0.803 ± 0.042	5.306	0.427
3.00	2.989 ± 0.017	0.586	−0.346
Inter-day analysis ($n = 5$)			
0.08	0.081 ± 0.001	1.420	0.799
0.80	0.805 ± 0.028	3.473	0.569
3.00	3.006 ± 0.039	1.295	0.217

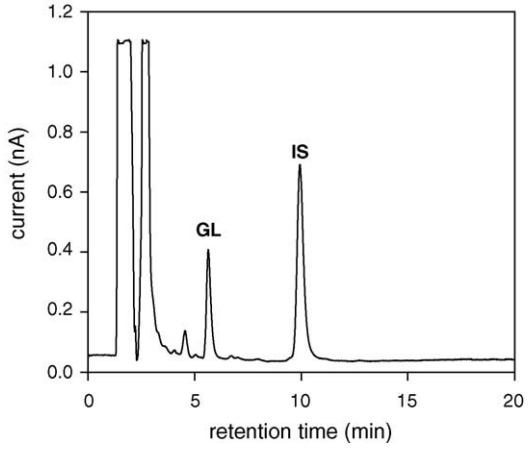

Fig. 4. A chromatogram of GL in plasma sampling at 48 h from one volunteer (27-year-old, 70 kg) who took one 80-mg GL tablet. Conditions: C_{18} column (150 mm × 4.6 mm; 5 μm); mobile phase: 70 mM disodium tetraborate (pH 7.5) containing 26.5% of acetonitrile; flow: 0.8 mL/min; applied ED potential: 800 mV; filter setting: 0.1 Hz; range setting: 10 nA.

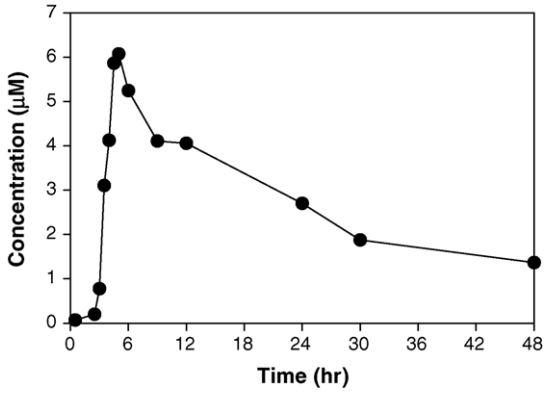

Fig. 5. Plasma concentration–time profile of gliclazide after dosing an 80-mg tablet to a healthy volunteer (27-year-old, 70 kg).

els at different sampling times into Eq. (1) [6], we estimated the half-life ($t_{1/2}$) of GL was about 18 h. One recent study demonstrated the pharmacokinetics of GL [1]. It showed that administration of a single oral dose of GL 40–120 mg to healthy volunteers and patients with NIDDM resulted in C_{max} of 6.8–24 μM within 2–8 h. The $t_{1/2}$ of GL is between 8.1 and 20.5 h. Our data seem to agree closely with this literature.

$$t_{1/2} = \frac{0.693t}{\ln(C_{p1}/C_{p2})} \quad (1)$$

where $t_{1/2}$ is half-life, t the time interval between the plasma samples, C_{p1} the first or higher plasma concentration and C_{p2} the second or lower plasma concentration.

We first established a HPLC–ED method to analysis the concentration of GL in human plasma. Further studies will try to clarify the pharmacokinetics and pharmacodynamics of GL especially in NIDDM patients.

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