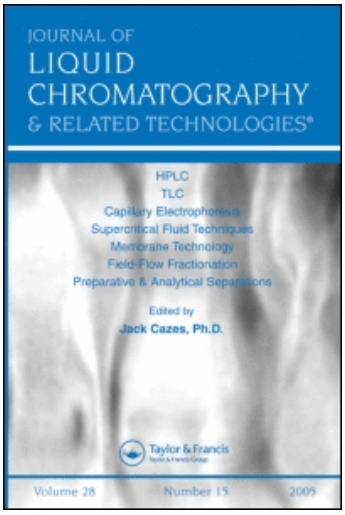

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A Simple and Sensitive Method for Determination of Glimepiride in Human Serum by HPLC

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Abstract: A simple and sensitive high performance liquid chromatographic (HPLC) method for glimepiride determination in human serum is described. The assay involves one-step liquid-liquid extraction with dichloromethane in acidified serum. Glibenclamide is used as the internal standard. Detection is done at 228 nm and limit of quantification is less than 10 ng/mL for glimepiride. The calibration curves are linear over the concentration range tested (10–1000 ng/mL). Accuracy, precision, and stability studies are performed.

This method is applied to the analysis of glimepiride serum samples of 41 Lebanese male volunteers after oral administration of a single glimepiride 3 mg tablet. Pharmacokinetic analysis of the data is done using a noncompartmental approach with WinNonlin software (WinNonlin® Professional, version 4.1).

Keywords: Glimepiride, Human serum, HPLC

INTRODUCTION

The hypoglycaemic agent Glimepiride is a second-generation sulphonylurea. It lowers plasma glucose levels by stimulating the release of insulin from the

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pancreas; it also increases insulin secretion in response to fuels such as glucose. It can be used in combination with metformin, the thiazolidinediones, alpha-glucosidase inhibitors, and insulin.[1–3]

Glimepiride is completely bioavailable and highly protein bound (99.4%).[4]

The therapeutic dosage in humans is low (1–4 mg per day in general). Maximum serum concentration (Cmax) achieved in healthy volunteers following a single oral dose ranges between 103.2 ng/mL with the 1 mg dosage form to 307.8 ng/mL with the 4 mg dosage form.[5,6]

The measurement of glimepiride in serum or plasma samples requires very sensitive and specific methods. High performance liquid chromatography (HPLC) techniques used for the determination of related sulfonylurea fail for glimepiride determination with respect to sensitivity and specificity.[7,8] Relatively complicated and expensive techniques for glimepiride assay in biological samples have been described: reversed phase HPLC after pre-column derivatization with UV detection,[9] liquid chromatographic tandem mass spectrometric method,[10] semi-microbore HPLC with column-switching,[11] and liquid chromatography-electrospray ionization tandem mass spectrometry.[12]

A simple and sensitive HPLC method with UV detection was developed in our laboratory after one-step liquid-liquid extraction of glimepiride from serum. The method was successfully applied to the pharmacokinetic analysis of glimepiride, administered orally as a single 3 mg tablet of Amaryl® to Lebanese male volunteers.

EXPERIMENTAL

Reagents and Standards

All chemicals used were reagent grade.

The following solvents and reagents were used: acetonitrile and water (HPLC grade, ROMIL-SpS), dichloromethane stabilized with ethanol analytical grade (Lab-Scan). HCl 1 N was prepared in the lab using concentrated HCl (Merck).

Glimepiride and glibenclamide standards were supplied by local manufacturer (Pharmaline).

Stock solutions of glimepiride or glibenclamide were prepared by dissolving 15 mg in 25 mL methanol. All stock solutions were protected from light and kept at 4°C. They were stable for at least one month.

Serum calibration samples were prepared using a 1/20 dilution of glimepiride stock solution with drug-free serum. Stability of the drug in serum at −20°C over 4 weeks was documented.

The internal standard solution was diluted 1/10 with methanol and used in samples preparation.

Instrumentation

The HPLC system consisted of an Agilent (Hewlett-Packard, USA) 1100 series quaternary pump, degasser, automatic injector, thermostated column compartment, and diode array detector. A Vortex TecnoKartell TK3; shaker BIOSAN Multi Bio RS-24, innovative mixing cycle was used. The data were collected using the system software (Chemstation, copyright© 1990–2002, Agilent technologies). Pharmacokinetic analysis was done using WinNonlin software (WinNonlin® Professional, version 4.1, copyright© 1998–2003, Pharsight Corporation).

Chromatographic Conditions

The separation was achieved on an Agilent LiChrospher 100, C_{18} column, 5 μm particle size, 250 × 4 mm I.D., with a 2 μm precolumn filter. The mobile phase consisted of 50% water acidified with glacial acetic acid (0.1 mM, pH = 2.5–2.7) and 50% acetonitrile. The flow rate was 0.7 mL/min and UV detection done at 228 nm. All analyses were performed at room temperature. The injection volume was 25 μL and a small volume of air was bubbled through each sample before injection during analysis of large series.

Plasma Extraction Procedure

An internal standard solution of 50 μL was added to 1.5 mL serum in a 15 mL polypropylene centrifuge tubes with flat cap (15 × 118 mm, Corning, USA) and mixed for 10 seconds on vortex. HCl 1 N (40 μL) was immediately added drop by drop using a micropipette while gently vortexing the tubes. To each tube, dichloromethane (7 cc) was added and the tubes were vortexed for 10 sec at high speed. The sample was finally shaken on a rotating shaker (30 rotation/minute) for 30 min.

After centrifugation for 20 min at 3000 rpm and 4°C, 6 cc of the clear dichloromethane layer was transferred with a calibrated pipette to a disposable glass tube (10 × 75 mm) and evaporated under a gentle stream of nitrogen. The dried residue was taken up with 30 μL of mobile phase and 25 μL of this mixture was injected.

Subjects and Protocol

Forty-one healthy white male adults participated in the study. All subjects were Lebanese and within −15% to +10% of normal body weight (according to Broca's formula).

Subjects were non smokers, drink alcohol occasionally, or not at all. Volunteers were excluded if they had clinically or biological significant

Table 1. Intra-day variability of the assay for glimepiride in serum (n = 3). Correlation coefficients are 0.9979, 0.9984, and 0.9994 for each of the 3 curves

Concentration added (ng/mL)	Concentration found (ng/mL)	S.D	Coefficient of variation (%)
10	9.346	1.052	10.274
20	18.306	0.451	8.469
50	45.440	0.465	9.118
100	101.169	1.710	1.697
200	206.815	3.066	3.407
400	386.592	2.455	3.349
800	840.886	12.864	5.108
1000	971.441	9.447	2.852

abnormalities, history of allergy to glimepiride or to any of the excipients, or to sulfa drugs, addiction or history of addiction to drugs, any chronic disease or any chronic intake of medicine and heavy caffeine consumption.

The protocol was approved by the ethical committee of Hôtel Dieu de France Hospital and Saint-Joseph University, and the study was conducted in accordance with the guidelines on good clinical practice and with ethical standards for human experimentation established by the declaration of Helsinki.

Each volunteer received a single 3 mg tablet of glimepiride (Amaryl®, Aventis, German, batch number 40D458). Blood (3 mL) was drawn via an indwelling intravenous catheter for determination of glimepiride concentrations. The blood was immediately centrifuged and serum stored at −20°C degrees until analysis (maximum 1 month).

Table 2. Inter-day variability of the assay for glimepiride in serum (n = 4). Correlation coefficients are 0.9995, 0.9979, 0.9988, and 0.9988 for each of the 4 curves

Concentration added (ng/mL)	Concentration found (ng/mL)	S.D	Coefficient of variation (%)
10	10.021	1.452	12.571
20	20.122	2.199	9.523
50	48.838	3.913	6.327
100	103.446	3.185	3.447
200	206.351	3.047	3.176
400	377.087	8.859	5.728
800	832.626	17.046	4.077
1000	981.504	15.718	1.849

RESULTS

Assay Validation

For assay validation, glimepiride was mixed with drug-free human serum over the concentration ranges 10–1000 ng/mL.

Each mixture was divided into several portions. Intra-day variability was examined using 3 series and inter-day variability was examined using 4 series, done on 4 separate days.

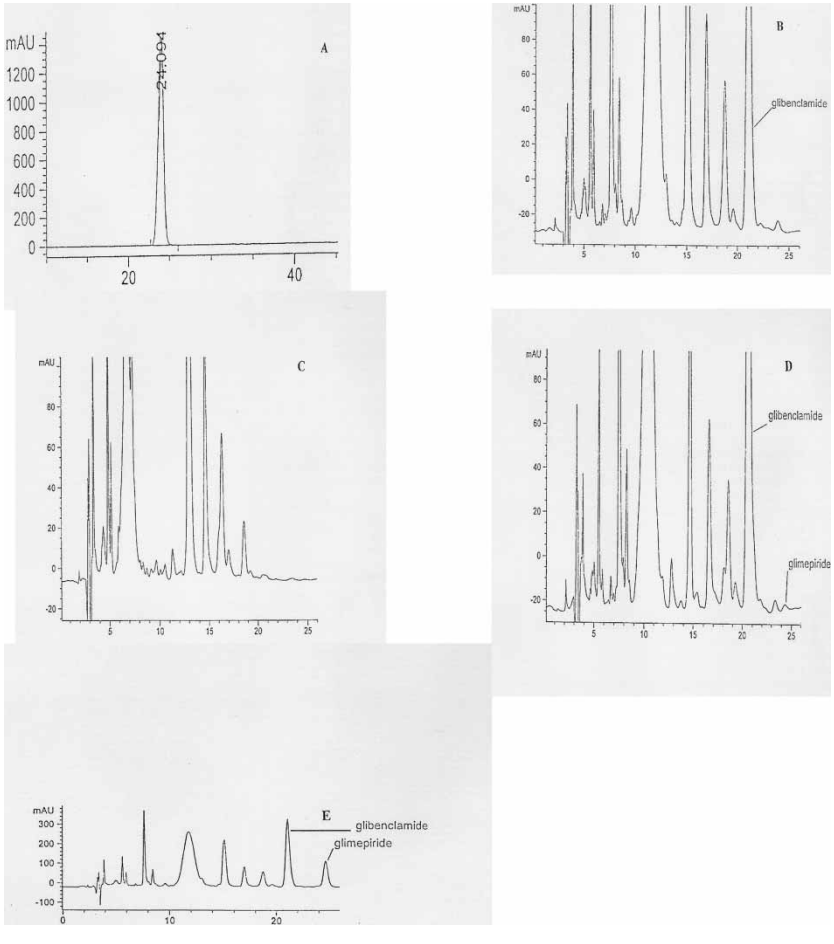

Figure 1. Chromatograms of (A) standard glimepiride solution, (B) blank serum with internal standard, (C) blank serum, (D) extracted sample at 10 ng/mL and (E) extracted sample at 1000 ng/mL.

Tables 1 and 2 illustrate the results of within-run and day to day precision studies. The precision was determined using coefficients of variation (CV) within the day and between days.

The calibration curves were linear over the concentration range studied (correlation coefficients 0.997 to 0.999). Higher than 1000 ng/mL concentrations were not tested for linearity.

The limit of quantification (LOQ) was calculated using the standard deviation of the intercepts and the mean slope of the calibration curves (LOQ = 3 × Standard Deviation of the intercepts/mean slope), and it was 8.2 ng/mL. Concentrations less than 10 ng/mL were not considered in the pharmacokinetic analysis because their coefficient of variation was higher than 15%. The detection limit (3 × signal-to-noise ratio) was 2.5 ng/mL.

Figure 1 shows typical chromatograms of standard glimepiride solution, blank serum containing the internal standard, blank serum, extracted sample at 10 ng/mL and at 1000 ng/mL.

Extraction Yield

Extraction recoveries determined by comparing the peak heights obtained by direct injection of standard glimepiride solution with those obtained after dichloromethane extraction of serum samples were not less than 80% over the 10–1000 ng/mL concentration range (Table 3).

Stability

The amount of glimepiride recovered over a period of 30 days in serum samples stored at −20°C did not differ from the initial concentrations (Table 4).

Table 3. Glimepiride recovery after extraction: drug-free human serum was spiked with glimepiride at different concentrations and extraction coefficient calculated

Concentration (ng/mL)	Average extraction coefficient (%) (n = 3 for each level)
10	85.6
20	84.7
50	82.4
100	85.5
200	81.8
400	84.5
800	84.3
1000	85.6

Table 4. Glimepiride stability Results: drug-free human serum was spiked with 3 different concentrations of glimepiride and stored at −20 °C over a period of 30 days

Concentration added (ng/mL)	Concentration obtained (ng/mL)		
	Day 1 Day 30	Day 14	Day 21
20	20.212 19.114	18.304	21.557
200	192.367 217.435	219.811	198.446
1000	965.117 1023.887	1041.647	944.443

Pharmacokinetics of Glimepiride

Figure 2 shows the average serum concentration time curve of glimepiride after oral administration of a single 3 mg Amaryl® tablet to 41 Lebanese healthy male adults. A peak serum concentration of 220.4 ng/mL was observed at 2.5 hours after dosing.

DISCUSSION AND CONCLUSION

A sensitive, simple, reliable, and accurate reversed phase HPLC method was described in this paper for determination of glimepiride in human serum. This

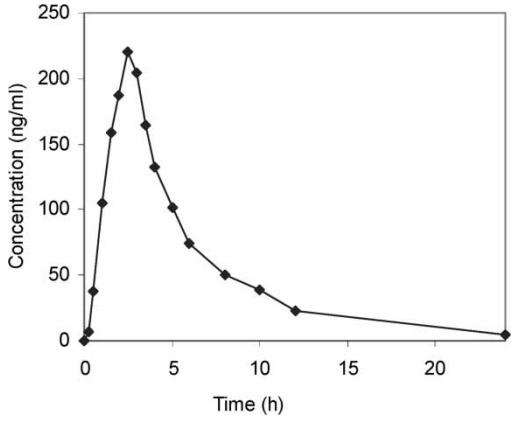

Figure 2. Average serum concentration-time curve of glimepiride after administration of a single oral dose of 3 mg to Lebanese male volunteers.

analytical procedure is inexpensive and simple because it requires fewer preparation steps, is less time consuming than methods using pre-column derivatization, and is particularly suitable when tandem mass spectrometric detection is not available. Even though we don't yet have a clear explanation for this, acidifying the serum samples drop by drop improves the extraction of glimepiride when compared to instant addition of the total acid volume. The assay was unaffected by the presence of heparin or sodium ethylenediaminetetraacetic acid in the collection tube.

This method was successfully applied for glimepiride assay and pharmacokinetic analysis after oral administration of a single 3 mg tablet to healthy adults, and it is currently being tested for glimepiride determination in urine. Further development of the method is, however, necessary to adequately measure concentrations reached after 3 half-lives of the drug when a low oral dosage form of glimepiride is administered, particularly the 1 mg tablet.

REFERENCES

1. Davis, S.N. The role of glimepiride in the effective management of type 2 diabetes. J. Diabetes Compl. **2004**, *18* (6), 367–376.
2. Bell, D.S. Practical considerations and guidelines for dosing sulfonylureas in monotherapy or combination therapy. Clin. Ther. **2004**, *26* (11), 1714–1727.
3. McCall, A.L. Clinical review of glimepiride. Expert Opin. Pharmacother. **2001**, *2* (4), 699–713.
4. Badian, M.; Korn, A.; Lehr, K.H.; Malerczyk, V.; Waldhausl, W. Detrmination of the absolute bioavailability of glimepiride (HOE490), a new sulfonylurea. Clin. Pharmacol. Ther. Toxicol. **1992**, *30*, 481–482.
5. Hoechst, A.G. Glimepiride. Drugs Future **1992**, *17*, 774–778.
6. Malerczyk, V.; Badian, M.; Korn, A.; Lehr, K.H.; Waldhausl, W. Dose linearity assessment of glimepiride (Amaryl) tablets in healthy volunteers. Drug Metabol. Drug Interact. **1994**, *11* (4), 341–357.
7. Uihlein, M.; Sistovaris, N. High performance liquid column and thin-layer chromatographic determination of human serum glibenclamide at therapeutic levels. J. Chromatogr **1982**, *227* (1), 93–101.
8. Emilsson, H.; Sjoberg, S.; Svedner, M.; Christenson, I. High performance liquid chromatographic determination of glibenclamide in human plasma and urine. J. Chromatogr **1986**, *383* (1), 93–102.
9. Lehr, K.H.; 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. J. Chromatogr **1990**, *526*, 497–505.
10. Pistos, C.; Koutsopoulou, M.; Panderi, I. Improved liquid chromatographic tandem mass spectrometric determination and pharmacokinetic study of glimepiride in human plasma. Biomed. Chromatogr. **2005**, *19* (5), 394–401.
11. Song, Y.K.; Maeng, J.E.; Hwang, H.R.; Park, J.S.; Kim, B.C.; Kim, C.K. Determination of glimepiride in human plasma using semi-microbore high performance liquid chromatography with column-switching. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. **2004**, *810* (1), 143–149.

12. Salem, I.I.; Idrees, J.; Al Tamimi, J.I. Determination of glimepiride in human plasma by liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. **2004**, *799* (1), 103–109.

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