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Short communication

Rapid determination of metformin in human plasma using ion-pair HPLC

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Abstract

A rapid, simple and sensitive ion-pair HPLC method has been developed for quantification of metformin in plasma. The assay enables the measurement of metformin for therapeutic drug monitoring with a minimum detectable limit of 20 ng/ml. The method involves simple, one-step extraction procedure and analytical recovery was complete. The separation was performed on an analytical 150 × 4.6 mm i.d. μbondapak C_{18} column. The wavelength was set at 235 nm. The mobile phase was 40% acetonitrile, 0.01 M sodium dodecyl sulphate, 0.01 M sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 5.1 at a flow rate of 1.5 ml/min. The calibration curve was linear over the concentration range 0.2–2.5 μg/ml. The coefficients of variation for inter-day and intra-day assay were within the range of clinical usefulness.

Keywords: Metformin; Plasma; HPLC

1. Introduction

Metformin is an antidiabetic agent which used in the treatment of non-insulin-dependent diabetes [1]. Metformin is slowly and incompletely absorbed from the gastro–intestinal tract and the absolute bioavailability of a single 500-mg dose is reported to be about 50–60%. Following absorption plasma protein binding is negligible, and it is excreted unchanged in the urine [2]. For pharmacokinetic studies, a sensitive method that allows an accurate measurement of low concentrations of metformin in plasma is required. Methods for the quantitation of this drug in biological fluids include electron-capture GLC [3,4] and high-performance liquid chromatography [5–9]. However, the GLC methods used a complex derivatization procedure and are time-consuming. HPLC methods differ with respect to the mode of HPLC (ion-exchange, ion-pair or reversed-phase) and sample preparation. Most of HPLC methods required liquid–liquid extraction with evaporation

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of the extract or on-line solid-phase extraction and, therefore, sample preparation is time-consuming, complex or both. In particular, metformin is highly polar and is, therefore, extremely difficult to extract from biological fluids. This paper describes a rapid and sensitive HPLC method, which enables the determination of metformin with good accuracy at low drug concentrations in plasma using single-step extraction procedure. The sample preparation only involves protein precipitation and no evaporation step is required. We also demonstrate the applicability of this method for pharmacokinetic studies in humans.

2. Experimental

2.1. Chemicals

Metformin and phenytoin were supplied by Osveh Pharmaceuticals (Tehran, Iran). Metformin is available as oral tablet containing 500 mg of metformin and the following inactive ingredients: Povidone, magnesium stearate and hydroxypropyl methyl cellulose coating. HPLC-grade acetonitrile and all other chemicals were obtained from Merck (Darmstadt, Germany).

Water was obtained by double distillation and purified additionally with a Milli-Q system.

2.2. Instruments and chromatographic conditions

A Knauer HPLC system (Germany) employed consisted of a model Wellchrom K-1001 pump, a model Rheodyne 7125 injector and a model K 2501 UV detector connected to a model Eurochrom 2000 integrator.

The separation was performed on an analytical 150×4.6 mm i.d. μbondapak C_{18} (4 μm, particle size) column. The wavelength was set at 235 nm. The mobile phase was 40% acetonitrile, 0.01 M sodium dodecyl sulphate, 0.01 M sodium dihydrogen phosphate, and distilled water to 100%, adjusted to pH 5.1 at a flow rate of 1.5 ml/min. The mobile phase was prepared daily and degassed by ultrasonication before use. The mobile phase was not allowed to recirculate during the analysis.

2.3. Standard solutions

Stock solutions (1 mg/ml) and appropriate dilutions of metformin and were prepared in methanol and stored at +4 °C. No change in stability over the period of 2 weeks was observed.

2.4. Sample preparation

To 200 μl of plasma in a glass-stoppered 15 ml centrifuge tube were added 20 μl of pheytoin as internal standard (50 μg/ml) and 300 μl of acetonitrile. After mixing (30 s), the mixture centrifuged for 15 min at 6000 rpm. Then 100 μl of supernatant was injected into liquid chromatograph.

2.5. Biological samples

Metformin was administered in a single dose of 1 g to healthy volunteers after over night fasting. Plasma samples were collected at several intervals after dosing and then frozen immediately at −20 °C until assayed.

2.6. Stability

The stability of metformin was assessed during all the storage steps and during all steps of the analytical method.

2.7. Plasma standard curve

Blank plasma was prepared from heparinized whole-blood samples collected from healthy volunteers and stored at −20 °C. After thawing, stock solution of metformin was added to yield final concentrations ranging from 0.1 to 4 μg/ml. Internal standard solution was added to each of these samples to yield a concentration of 2 μg/ml. The samples were then prepared for analysis as described above.

3. Results and discussion

Under the chromatographic conditions described, metformin and the internal standard

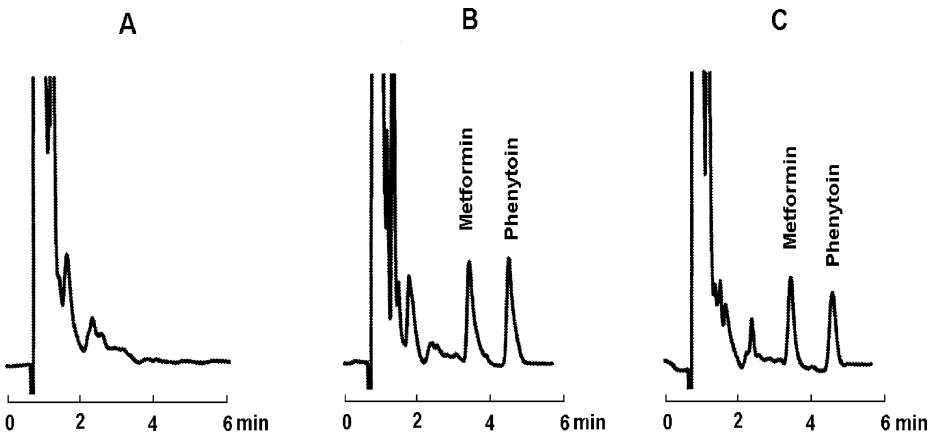

Fig. 1. Chromatograms of (A) blank plasma; (B) blank plasma spiked with 1.5 μg/ml metformin and 2 μg/ml phenytoin (internal standard); (C) plasma sample from a healthy volunteer 1 h after oral administration 1 g of metformin.

peaks were well resolved. Endogenous plasma components did not give any interfering peaks. Fig. 1 shows typical chromatograms of blank plasma in comparison to spiked samples analyzed for a pharmacokinetic study. The average retention times of metformin and phenytoin were 3.4 and 4.5 min, respectively. The calibration curve for the determination of metformin in plasma was linear over the range 0.1–4 μg/ml. The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. The correlation coefficients (r) for calibration curves were equal to or better than 0.999. The relative standard deviation (R.S.D.) values of the slope were equal to or better than 6%. For each point of calibration standards, the concentrations were recalculated from the equation of the linear regression curves (Table 1). The relative analytical recovery for plasma at three different concentrations of metformin was determined. Known amounts of metformin were added to drug-free plasma in concentrations ranging from 0.2 to 2.5 μg/ml. The internal standard was added and the relative recovery of metformin was calculated by comparing the peak areas for extracted metformin from spiked plasma and a standard solution of metformin in methanol containing internal standard with the same initial concentration. The average recovery was 100.1 ± 1.1% ($n = 6$). The limit of detection was defined as the metformin concentration that produced a

Table 2
Reproducibility of the analysis of metformin in human plasma ($n = 5$)

Concentration added (μg/ml)	Concentration measured (mean ±S.E.)	
	Intra-day	Inter-day
0.2	0.21 ± 0.01 (5.2)	0.22 ± 0.01 (4.5)
1	0.98 ± 0.02 (2.0)	0.98 ± 0.03 (3.1)
2.5	2.46 ± 0.05 (2.3)	2.45 ± 0.06 (2.4)

Values in parentheses are coefficients of variation (%).

Table 1
Assay linearity

Coefficient of the linear regression analysis (r ±S.D.)		Slope ±S.D.	Intercept ±S.D.
Intra-assay	$0.9990 \pm 5.54 \times 10^{-4}$	0.800 ± 0.0317	0.0180 ± 0.0036
$N = 6$	R.S.D. = 0.0554%	R.S.D. = 3.96%	–
Inter-assay	$0.9993 \pm 6.81 \times 10^{-4}$	0.791 ± 0.0291	0.0215 ± 0.0043
$N = 9$	R.S.D. = 0.0680%	R.S.D. = 3.68%	–

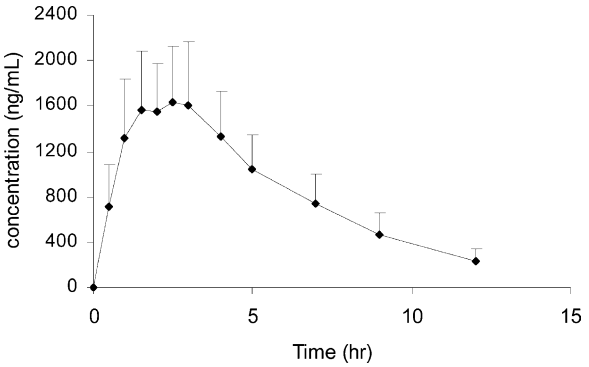

Fig. 2. Mean plasma concentration–time profile of metformin in healthy volunteers ($n = 12$) after a single 1 g metformin.

signal-to-noise ratio greater than 3. The limit of detection in plasma was 20 ng/ml based upon this criterion. At this level, the R.S.D. was lower than 10%. This is sensitive enough for drug monitoring and other purposes such as pharmacokinetic studies. We assessed the precision of the method by repeated analysis of plasma specimens containing known concentrations of metformin. As shown in Table 2, coefficients of variation were less than 6%, which is acceptable for the routine measurement of metformin. The aim of our study was to develop a rapid and sensitive method for the routine analysis of biological samples in pharmacokinetic metformin research. This method is well suited for routine application in the clinical laboratory because of the simple extraction procedure and good sensitivity. Over 350 plasma samples were analyzed by this method without problems, thus proving its suitability. In this study plasma concentrations were determined in twelve healthy volunteers, who received 1 g of metformin each. Fig. 2 shows the mean plasma concentration–time curve of metformin: plasma concentration reached a maximum 2.25 ± 0.72 h after dosing with a level of 1.95 ± 0.59 μg/ml.

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