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Letter to the Editor

High-performance liquid chromatographic determination of gliclazide in human plasma

Sir,

Gliclazide (1-(4-methylbenzenesulphonyl)-3-(3-azabicyclo[3.3.0]octyl)urea) is one of the newer sulphonylurea drugs evaluated as a potential oral hypoglycaemic agent. Gliclazide, like most of the other hypoglycaemic sulphonylureas, is extensively metabolized by the liver, and less than 1% of the dose is excreted in urine. The main metabolite is present in plasma only to the extent of 1%, and is devoid of hypoglycaemic activity but possesses, as does the parent drug, effects on platelet function [1]. Gas chromatography [2,3] and high-performance liquid chromatography (HPLC) [4–6] have been used for the determination of gliclazide in plasma or serum. Most of these assays involve laborious extraction procedures [2–5], and an HPLC method for the quantitative measurement of gliclazide in plasma has, therefore, been developed. This procedure is simple and requires small volumes of plasma, and concentrations as low as 0.05 μg/ml can be detected.

EXPERIMENTAL

Reagents and standards

Gliclazide was supplied by Servier Laboratories (Orléans, France) and nadoxolol, used as internal standard, was obtained from Lafon Laboratories (Maisons-Alfort, France). All solvents and reagents were of analytical grade and were used without further purification. Stock solutions of gliclazide (3.20 mg/l) and nadoxolol (100 μg/ml) were prepared in methanol and were stable for at least one month when stored at 4 °C. From appropriate dilutions of an aqueous working solution of gliclazide, plasma standards were prepared in the concentration range 0.1–12.8 μg/ml.

Chromatography

The apparatus used was an HPLC unit consisting of a model M45 solvent-delivery system, a Model U6K manual injector and a Model M481 variable-wave-

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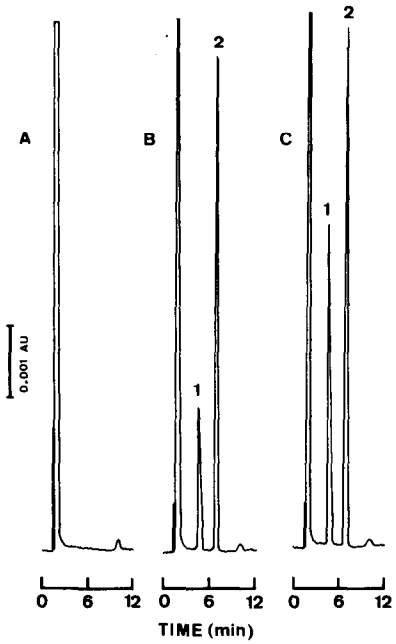

Fig. 1. Typical chromatograms of extracted plasma samples. (A) Blank plasma; (B) blank plasma spiked with 0.4 μg/ml gliclazide and 0.8 μg/ml nadoxolol; (C) plasma from a patient 24 h after administration of 80 mg of gliclazide. Peaks: 1 = gliclazide; 2 = nadoxolol (internal standard).

length absorbance detector set at 229 nm (Millipore-Waters, Saint-Quentin en Yvelines, France). Separations were performed on a LiChrosorb CN column (25 cm × 4 mm I.D., 10 μm particle size; E. Merck, Paris, France) with a mobile phase of methanol–2-propanol–water–1.16 *M* perchloric acid (70:29.5:0.5:0.3, v/v) operating at ambient temperature and at a flow-rate of 2.0 ml/min.

Sample preparation

To 250 μl of plasma sample in a glass hemolyse tube were added 50 μl (200 ng) of a 1:25 aqueous dilution of the stock solution of nadoxolol, 250 μl of 0.1 *M* citrate–phosphate buffer (pH 7.0) and 1 ml of toluene. The mixture was shaken vigorously for 30 s (Vortex), and after centrifugation the organic phase was transferred to a conical glass tube and evaporated to dryness at 55 °C under a stream of nitrogen. The residue was redissolved in 200 μl of methanol, and an aliquot was introduced into the HPLC unit.

RESULTS AND DISCUSSION

Representative chromatograms of extracted plasma samples are shown in Fig. 1. The retention times of gliclazide and internal standard were 4.7 and 6.9 min, respectively. Fig. 1A is the chromatographic profile of an extract of a blank human plasma free of interfering peaks at the retention times concerned. A small peak due to an endogenous component of plasma was eluted at 9.6 min, and the anal-

TABLE I

WITHIN-DAY AND DAY-TO-DAY PRECISION OF PLASMA GLICLAZIDE DETERMINATION ($n=5$)

Amount added (μg/ml)	Within-day		Day-to-day	
	Amount found (mean ± S.D.) (μg/ml)	C.V. (%)	Amount found (mean ± S.D.) (μg/ml)	C.V. (%)
0.100	0.107 ± 0.010	9.3	0.113 ± 0.015	13.3
0.200	0.197 ± 0.011	5.6	0.191 ± 0.017	8.9
0.400	0.400 ± 0.016	4.0	0.417 ± 0.027	6.5
1.600	1.605 ± 0.067	4.2	1.555 ± 0.067	4.3
3.200	3.148 ± 0.087	2.8	3.177 ± 0.088	2.8
6.400	6.459 ± 0.068	1.1	6.361 ± 0.108	1.7

ysis time was therefore extended to 10 min. Fig. 1B shows the chromatogram of an extract of a drug-free human plasma sample spiked with 0.4 μg/ml gliclazide and 0.8 μg/ml nadoxolol (internal standard). Fig. 1C is the chromatogram of an extracted plasma sample from a patient, obtained 24 h after administration of a 80-mg oral dose of gliclazide containing 0.76 μg/ml gliclazide.

Calibration curves were linear in the range 0.1–12.8 μg/ml ($r^2 \geqslant 0.999$). The daily fluctuation of the slope of these calibration curves was slight [coefficient of variation (C.V.) 3.7%; $n=5$] with an intercept of 3 ± 5 ng/ml (mean ± S.D.; $n=5$). The precision of the assay was determined by analyzing drug-free human plasma spiked with known amounts of gliclazide in the range 0.1–6.4 μg/ml. The results of five determinations, expressed as the mean ± S.D., are presented in Table I.

The limit of determination was estimated to be 0.1 μg/ml, and at this level the C.V. values were 9.3% and 13.3% when analyses were performed on same day or different days, respectively. The detection limit, based on a signal-to-noise ratio of 4:1, was 0.05 μg/ml.

In addition, it has been shown that phenobarbital, primidone, phenacetin, carbamazepine, clobazam, diazepam, caffeine, theophylline, theobromine, salicylic acid, tolbutamide, acebutolol, propranolol, sotalol, propafenone, lidocaine, amiodarone, verapamil, clomipramine and imipramine do not co-chromatograph with either gliclazide or internal standard. Among the assayed drugs only tocainide had a retention time similar to that of the internal standard, but it was not extracted under our experimental conditions.

In summary, an HPLC procedure for the determination of gliclazide in plasma has been developed. This method is sensitive, selective and could be particularly useful when numerous daily samples must be analysed.

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