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Note

High-performance liquid chromatographic determination of glipizide and some other sulfonylurea drugs in serum

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Sulfonylurea drugs are widely used in the treatment of diabetes mellitus of the maturity-onset character. The first sulfonylurea generation, comprising compounds such as tolbutamide and chlorpropamide, is being gradually replaced by second-generation sulfonylureas, such as glibenclamide and glipizide, which offer a markedly enhanced potency without a corresponding increase in toxicity. Sensitive and selective chemical methods for the determination of blood concentrations of tolbutamide and chlorpropamide are available and have been applied in clinical studies [1—3]. For the second-generation drugs, however, no such methods have been developed so far.

The present report describes a high-performance liquid-chromatographic (HPLC) technique for the measurement of glipizide concentrations in blood. The method also permits the determination of the blood concentrations of tolbutamide, chlorpropamide and glibenclamide. Glibornuride is used as internal standard in the assay of glipizide and glibenclamide. Chlorpropamide serves as internal standard for tolbutamide and vice versa.

MATERIALS AND METHODS

The technique described below is the finally adopted method for the measurement of glipizide concentrations in serum. The influence of variations in extraction media, pH, internal standards, and UV absorption wavelengths are described under Results and discussion, and so are the techniques for measurement of glibenclamide, tolbutamide and chlorpropamide.

Apparatus

A Waters Model 6000 pump, equipped with a U6K injector and a Varian Vari-Chrom UV—Vis detector were used.

Column

μBondapak C_{18} columns (0.3 m × 3.9 mm I.D.; 10-μm particles) were obtained prepacked from Waters Associates, Göteborg, Sweden.

Chemicals

Mixtures of methanol and phosphate buffers were used for elution. Degassing was carried out by sonication for 15 min. All reagents employed were of analytical grade and were used without further purification. Glipizide (Mindiab®) and two glipizide metabolites, the 3-cishydroxycyclohexyl and the 4-transhydroxycyclohexyl derivatives were kindly supplied by Dr. Tosolini, Istituto Carlo Erba per Ricerche Terapeutiche, Milan, Italy. Glibenclamide (Euglucon®) and tolbutamide (Artosin®) were gifts from Dr. V. Hrtska, Boehringer-Mannheim, Mannheim, G.F.R. Glibornuride (Glutril®, Hoffman-LaRoche & Co., Basle, Switzerland) was used as internal standard. Chlorpropamide (Diabinese®) was obtained from Pfizer Corp., Groton, Conn., U.S.A. Standard solutions were made in methanol and were found to be stable for at least 3 months when kept refrigerated.

Extraction procedure

Eight hundred nanograms of glibornuride (internal standard) and 1 ml of HCl (0.05 mol/l) were added to 0.5 ml of serum, resulting in a pH of about 3. Extraction was made with 3 ml of benzene, during gentle automated shaking for 10 min. After centrifugation, the organic phase was transferred to a conical tube and evaporated to dryness in a water-bath at 45° under a stream of air. The extract was re-dissolved in 50 μl of methanol. An aliquot of 20 μl was injected into the chromatograph.

Blood samples

Venous blood samples were obtained from patients on medication with glipizide, glibenclamide, tolbutamide or chlorpropamide. Serum was prepared by centrifugation, and was stored at —20° until assayed. For the initial method development, and for making serum standards, known amounts of the different drugs were added to drug-free serum.

RESULTS AND DISCUSSION

Extraction media

The following extraction media were tried: hexane, diethyl ether, toluene, chloroform, benzene, methylene chloride, ethylene dichloride, butanol, and hexane—diethyl ether (1:1). Of these, only benzene was found suitable, as the others either extracted glipizide badly or yielded interfering peaks from their dried residues.

Influence of pH on glipizide yield

Glipizide is a weak acid with a pK_a of 5.94 [4]. As it was the aim to keep the drug undissociated to the largest possible degree, a pH of 3.0—3.5 seemed appropriate for the extraction. It was found that the yield was maintained at about 84% up to pH 5. At pH 6 and 7, the yield had fallen to 4%.

Influence of phosphate buffer on glipizide capacity factor

Changes in the amount of phosphate buffer in the mobile phase altered the capacity factor for glipizide as shown in Fig. 1. It appeared that a content of 40% phosphate buffer of 0.01 mol/l and a pH of 3.5 would be optimal.

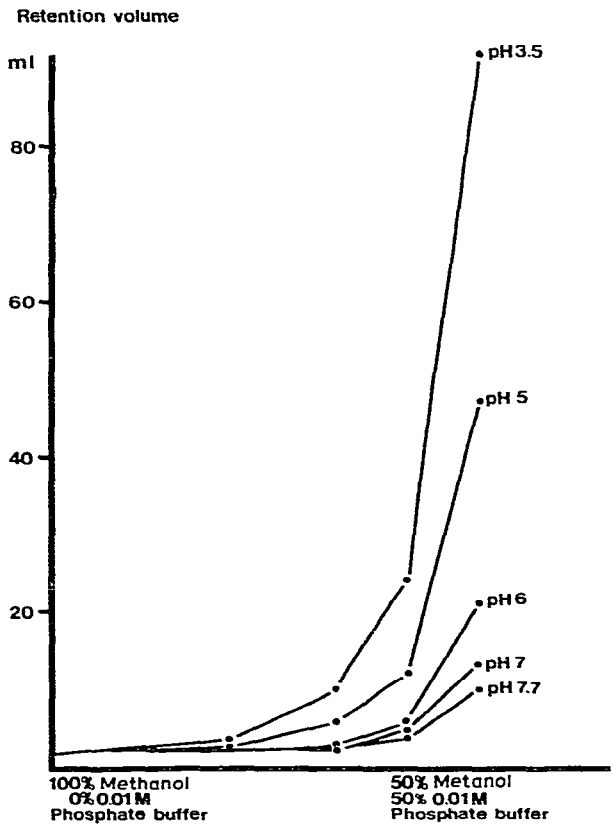

Fig. 1. Influence on the capacity factor for glipizide of amount of phosphate buffer and of pH in the mobile phase.

Detection wavelength and limit

Absorption maxima were 225 nm for glipizide and 275 nm for glibornuride. The molar absorption at the chosen detection wavelength, 225 nm, was found to be 24,000 for glipizide and 16,000 for glibornuride. The detection limit for glipizide was 10 ng/ml, using an injection volume of 20 μl. The sensitivity of the spectrophotometer was kept on 0.02 a.u.f.s. throughout. A typical chromatogram is shown in Fig. 2.

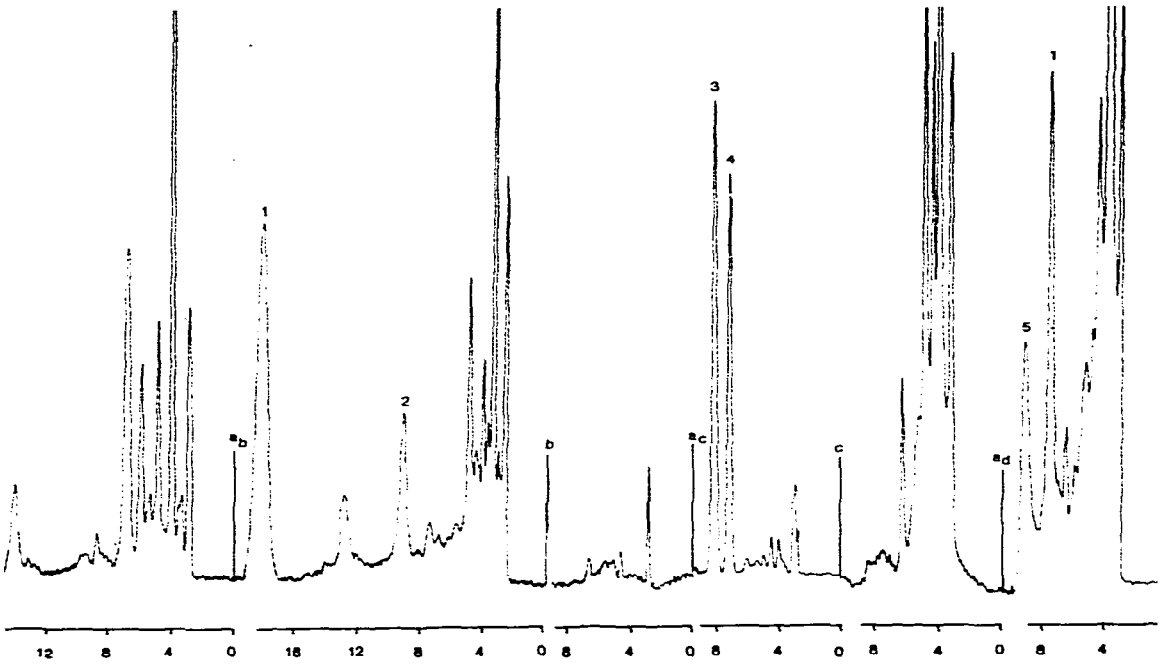

Fig. 2. Chromatograms of serum blanks (a_b, a_c, a_d), and serum from patients being treated with glipizide (b), chlorpropamide (c) and glibenclamide (d). Peaks: 1 = glibornuride, 2 = glipizide, 3 = tolbutamide, 4 = chlorpropamide, 5 = glibenclamide.

Quantitation

The standard curve was linear over a range of 20—1500 ng of glipizide. Analyses of the same sample ten times in the same run yielded a standard deviation of 1.8%. Inter-assay was found to be 6.2% (S.D.) as judged from seven consecutive assays.

Internal standards

The following compounds were tested as internal standards: tolbutamide, chlorpropamide, glibenclamide, methylglipizide and glibornuride. Of these, only glibornuride and glibenclamide had a retention volume that was not equal to that of any endogenous compound. Efforts to clean up serum were unsuccessful. Under conditions optimal for glipizide detection, both glibornuride and glibenclamide eluted later than glipizide. Glibornuride eluted closer to glipizide than did glibenclamide and was hence chosen as internal standard. The total retention volume was 18 ml.

Metabolites

Neither of the two glipizide metabolites found in human plasma — the *cis*-hydroxycyclohexyl derivative and the 4-*trans*-hydroxycyclohexyl de-

rivative — interfered with the parent drug in the chromatogram; both eluted with the serum front.

Glibenclamide

Glibenclamide was extracted with benzene after acidification to pH 3. The mobile phase was 30% 0.01 *M* phosphate buffer (pH 3.5) in 70% methanol. Glibornuride served as internal standard.

Tolbutamide and chlorpropamide

The therapeutic serum concentrations of these two sulfonylureas are in the range of several μg/ml, while those of glipizide apparently are in the ng/ml range, i.e. 1000-fold less. This made it possible to use the glipizide extraction procedure also in the determination of tolbutamide and chlorpropamide in serum, even though the two compounds could not be used as internal standards. Indeed, this method was found to be very sensitive and rapid and to employ as little as 50 μl of serum. Toluene could be used as extraction medium instead of benzene; however, more serum was then needed, as the dried residue of toluene yielded some slowly eluting peaks that interfered significantly when detector sensitivity was high. Tolbutamide was used as internal standard for chlorpropamide and vice versa.

Interference of biguanides

Sulfonylureas are often used in combination with biguanides, such as metformin or phenformin. It was of particular importance, therefore, to assess whether biguanides would interfere with the determination of glipizide, glibenclamide, tolbutamide or chlorpropamide. No such interference was seen with either metformin or phenformin.

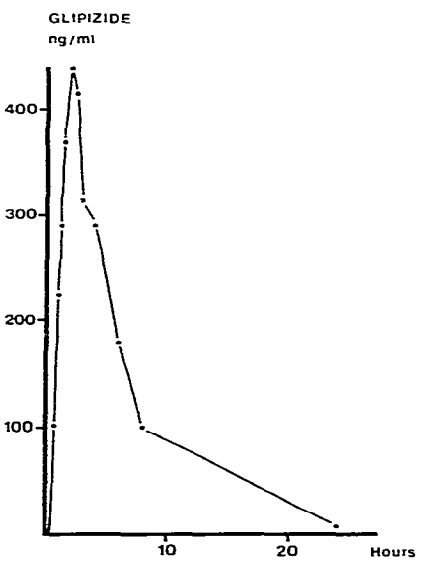

Fig. 3. Serum concentrations of glipizide during a 24-h period after ingestion of a single dose of 5 mg by a subject previously unexposed to the drug.

Glipizide in serum

Fig. 3. shows a glipizide concentration curve in serum from a subject who ingested 5 mg of glipizide. It is seen that the method allows detection of the drug over the whole 24-h period examined.

CONCLUSION

The presented method appears to be sufficiently selective, sensitive and rapid to allow accurate and precise measurements of the serum concentrations of glipizide and some other sulfonylureas during therapeutic conditions.

ACKNOWLEDGEMENTS

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