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Note

Determination of serum tolbutamide and chlorpropamide by high-performance liquid chromatography

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Tolbutamide (1-butyl-3-(*p*-tolylsulphonyl)urea) and chlorpropamide (1-propyl-3-(*p*-chlorphenyl sulphonyl)urea) are two of the sulphonylurea derivatives used as oral hypoglycaemic agents for the treatment of diabetes mellitus [1, 2]. These compounds have been in use for a number of years and it is known that wide intra-individual variation is often present after similar therapeutic doses [3, 4] and that their therapeutic index is fairly low. Routine monitoring aimed at correlating serum levels with clinical effect or to estimate compliance is not widespread, however. One reason for this omission has been the absence of analytical techniques which meet the requirements of a routine monitoring service, i.e. that the assay involve the minimum number of operations on a small volume of sample, whilst producing accurate results with good long-term precision. Colorimetric [5, 6] and spectrophotometric [7, 8] procedures which are time-consuming, non-specific and lack sensitivity, clearly do not meet these standards. Gas chromatographic procedures have been reported [9—11]. However, tolbutamide and chlorpropamide are thermolabile and a derivatization step is required in the analysis. Moreover, the analysis depends upon on-column pyrolysis, followed by on-column methylation to the corresponding N,N-dimethylsulphonamide and it has been reported that reproducible chromatograms require a careful injection technique [11]. High-performance liquid chromatography (HPLC) of sulphonylureas in pharmaceutical preparations has been reported [12]. This present communication describes the application of this approach to the determination of therapeutic levels of tolbutamide and chlorpropamide in serum. The method meets the requirements of a routine assay outlined above. A single extraction step from a small volume of serum (200 μl) is followed by reversed-phase chromatography, without prior derivatization.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was an ALC Model 202, with Model 6000A pump, U6K injector and Model 440 absorbance detector (Waters Assoc., Milford, Mass., U.S.A.).

Chromatographic Conditions

A stainless-steel column (30 cm × 4 mm I.D.) was packed with a stable reversed-phase stationary phase, consisting of porous silica beads (mean diameter 10 μm) coated with a chemically-bonded monolayer of octadecylsilane (μBondapack C-18, Waters Assoc.). The mobile phase was 1% acetic acid (adjusted to pH 5.5 with NaOH (2 *N*)—acetonitrile (72:28, v/v). The operating temperature was ambient and the flow-rate 2.2 ml/min with an operating pressure of 17.25 MPa (2500 p.s.i.). The column effluent was monitored continuously at 254 nm, with a full scale deflection of 0.1 *A*. A short acetonitrile wash (20 min at 1 ml/min) at the end of each analytical day was included to remove strongly retained solutes.

Reagents

All chemicals were reagent grade. Tolbutamide and chlorpropamide were donated by Hoechst Pharmaceuticals, Willowdale, Canada. 1-Isopentyl-3-(*p*-tolylsulphonyl)urea was purchased from Aldrich (Milwaukee, Wisc., U.S.A.). Solvents are routinely filtered through 0.45-μm filters (Millipore Corp., Bedford, Mass., U.S.A.) prior to use in the liquid chromatograph.

Standards

Tolbutamide (200 mg) and chlorpropamide (200 mg) were dissolved in absolute ethanol (10 ml). 1 ml of this solution was made up to 100 ml with plasma. This standard (200 mg/l) was serially diluted with plasma to prepare standards containing 100, 50 and 25 mg/l respectively. These preparations were divided into 1-ml aliquots and frozen (—20°). The internal standard, 1-isopentyl-3-(*p*-tolylsulphonyl)urea (5 mg), was dissolved in chloroform (10 ml). A 5-ml volume of this solution was made up to 1 l with chloroform and this solution served as the extraction solvent.

Extraction

Serum or plasma (200 μl) is added to a 50-ml glass tube fitted with a PTFE-lined screw-cap. Chloroform (10 ml) containing the internal standard is added, followed by sodium chloride (ca. 1 g). Extraction is for 5 min (Buchler Omnishaker), followed by centrifugation at 500 *g* for 2 min. The aqueous phase is removed by aspiration, the chloroform layer decanted into a disposable tube and taken to dryness by warming under a stream of dry nitrogen. The residue is dissolved in acetonitrile (ca. 40 μl) and 25 μl is injected into the liquid chromatograph. This procedure is followed for patient and standard samples. Standard curves are constructed by plotting the peak height ratios of each drug to the internal standard against the drug concentration in each standard. The level of drug in an unknown sample is derived from this curve.

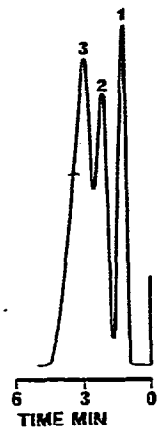

Fig. 1. Chromatogram of chlorpropamide (1), tolbutamide (2) and 1-isopentyl-3-(*p*-tolylsulphonyl)urea (3). Solvent system, acetonitrile—water (28:72, v/v).

RESULTS AND DISCUSSION

Acceptable and rapid separation of the sulphonylureas in this reversed-phase system is not only a function of the amount of organic modifier in the solvent, but also depends on the effective pH and ionic strength of the aqueous component. A simple acetonitrile—water system (28:72, v/v) with an effective pH of 5.5 does not resolve the solutes [k'(chlorpropamide) = ca. 0; k'(tolbutamide) = 0.8; and k'(internal standard) = 1.4] (Fig. 1). Since the sulphonylureas are weak acids (pk_a(tolbutamide) = 5.4 [13]), their capacity factors are increased by lowering the effective pH of this solvent [12], and the system acetonitrile—1% acetic acid (28:72, v/v) (effective pH = 2.9) resolves the three solute peaks [k'(chlorpropamide) = 3.3; k'(tolbutamide) = 4.8; and k'(internal standard) = 8.5]. However, the increase in overall elution time is inappropriate for a routine assay procedure. A return to pH 5.5 by titration with NaOH (see Experimental) produces a solvent with the same effective pH as the acetonitrile—water but with an effective ionic strength (ca. 0.14) which is infinitely larger. In reversed-phase chromatography this solvent is weaker than the acetonitrile—water and unlike this system effects excellent separation of the sulphonylureas in the minimum time.

A chromatogram of a plasma standard (100 mg/l) is shown in Fig. 2. The chromatography is complete within 10 min, with baseline separation between the three solutes [k'(chlorpropamide) = 0.8; k'(tolbutamide) = 2.6; and k'(internal standard) = 5.0]; other than unretained material no endogenous peaks are present. Fig. 3 shows the chromatogram from a patient on tolbutamide therapy with a found plasma level of 79 ± 5 (1 S.D.) mg/l. Analysis of the standards and plasma blank showed the relationship between the plasma concentration of both drugs and the peak height ratios of each drug to the internal standard to be linear between 0 and 200 mg/l. This range encom-

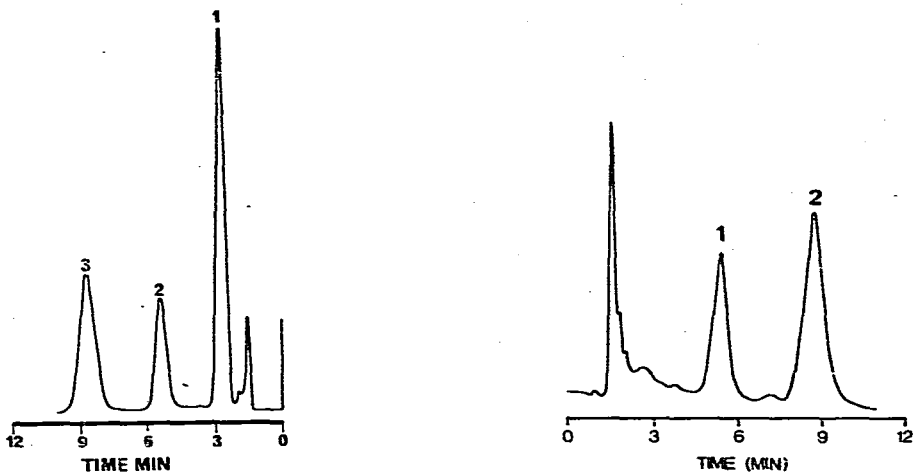

Fig. 2. Chromatogram of a plasma extract: chlorpropamide (1), tolbutamide (2) and 1-isopentyl-3-(*p*-tolylsulphonyl)urea (3). Solvent system, acetonitrile—1% acetic acid (28:72, v/v, adjusted to pH 5.5 as described in the text).

Fig. 3. Chromatogram of a patient plasma extract: tolbutamide (1), 1-isopentyl-3-(*p*-tolylsulphonyl)urea (2). Solvent system, acetonitrile—1% acetic acid (28:72, v/v, adjusted to pH 5.5 as described in the text).

passes the therapeutic ranges for tolbutamide (53—96 mg/l) [14] and chlorpropamide (30—140 mg/l) [14]. The regression equations are $y = 0.004 + 0.01\ x$, $r = 0.9993$ for tolbutamide and $y = -0.04 + 0.03\ x$, $r = 0.9994$ for chlorpropamide (y = peak height ratio drug/internal standard and x = drug concentration). The limits of sensitivity are 6 mg/l for tolbutamide and 7 mg/l for chlorpropamide. The extraction procedure yields greater than 95% recovery for each drug. A pool sample containing each drug (100 mg/l) was processed to determine the accuracy and precision of the method. The between batch variations are 5.4%, mean = 98.0 ± 5.3 (1 S.D.) (n = 30) for chlorpropamide and 6.6%, mean = 102.5 ± 6.4 (1 S.D.) (n = 30) for tolbutamide.

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