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# Gas-liquid chromatographic estimation of tolbutamide and chlorpropamide in plasma

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An improved method for the gas-liquid chromatographic estimation of tolbutamide and chlorpropamide in plasma is described. The drugs are extracted into toluene, back-extracted into alkali and methylated with dimethylsulphate. The methylated derivatives are extracted into n-hexane, concentrated by evaporation and chromatographed on 3·8% UC W98 or 1% Carbowax 20M columns. The standard deviation of replicate analyses of plasma samples containing 20–100 μg/ml of either drug was about 4%. There is no interference from known metabolites of tolbutamide or chlorpropamide.

Most of the previously described procedures for the estimation of tolbutamide and chlorpropamide in biological fluids have been colorimetric or spectrophotometric methods (Spingler & Kaiser, 1956; Forist, Miller & others, 1957; Spingler, 1957; Bladh & Norden, 1958; Toolan & Wagner, 1958; Popa & Voicu, 1962; Kalinowski & Korzybski, 1963). Without differential extractions or preliminary chromatographic separations, however, these methods lack specificity and are liable to interference from metabolites or other acidic drugs (Brook, Schrogie & Solomon, 1968; Brotherton, Grieveson & McMartin, 1969). Sabih & Sabih (1970) described a gas-liquid chromatographic method involving methylation which appears to be a direct application of the barbiturate assay of Martin & Driscoll (1966). Difficulties were encountered with this method because plasma extracts produced multiple interfering peaks on the chromatograms and no internal standard was used. Extensive modification of the procedure is now described.

## METHODS

### *Procedure*

To 1·0 ml of plasma in stoppered glass tubes add 1·0 ml of 1N HCl and 5·0 ml of toluene containing the appropriate internal standard (tolbutamide 5 μg/ml or chlorpropamide 20 μg/ml). The tubes are shaken mechanically for 10 min, centrifuged and the toluene is transferred to tubes containing 1·0 ml of 10% aqueous potassium carbonate. The tubes are shaken and centrifuged as above and the toluene is removed by aspiration and discarded. One ml of methanol and 100 μl of dimethylsulphate are added to each tube and the contents mixed. The loosely stoppered tubes are placed in a water bath at 60° for 10 min and after cooling, 1·0 ml of 1M acetate buffer (pH 5·2) is added. The methylated sulphonylureas are extracted by shaking with 5·0 ml of n-hexane and after centrifuging the hexane is transferred to tapered centrifuge tubes. The hexane is evaporated to dryness on a rotary vacuum evaporator at 35° and the residue dissolved in 25 μl of chloroform using a vortex mixer. 1–3 μl aliquots are injected into the gas chromatograph.

*Chromatography*

A Hewlett-Packard Model 402 gas chromatograph with a Model 7128A recorder and flame ionization detectors was used. The column was a glass U-tube 4 ft × ¼ in o.d., packed with 3·8% UC W98 on 80–100 mesh Diatoport S* and was run at 220°. The flash heater and detector temperatures were 235 and 260°. The nitrogen carrier gas flow rate was 25 ml/min, hydrogen and air flow rates were 20 and 200 ml/min respectively. Good separations were also obtained with a similar column packed with 1% Carbowax 20M on 80–100 mesh Gas Chrom Q** with a nitrogen flow rate of 60 ml/min at 220°.

## RESULTS

Under the described conditions the methylated derivatives of chlorpropamide and tolbutamide were completely separated and the retention times on the UC W98 column were 1·7 and 2·2 min respectively (Fig. 1). At the attentuation used for the

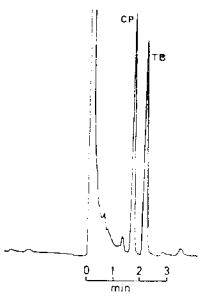


FIG. 1. Chromatogram of an extract of plasma obtained from a patient 7½ h after i.v. injection of 1·0 g of tolbutamide. The plasma tolbutamide concentration was 47 μg/ml. The peaks of methylated derivatives of chlorpropamide and tolbutamide are marked CP and TB respectively. For conditions see text.

assay of plasma containing therapeutic concentrations of the sulphonylureas, there was a small peak with a similar retention time to tolbutamide equivalent to about 1 μg/ml. No interference was observed when plasma extracts were chromatographed on the Carbowax 20M column.

The ratios of peak heights of the methylated sulphonylureas to internal standard plotted against concentrations gave straight lines with both drugs over the ranges 1–10 and 20–100 μg/ml. Replicate analyses of plasma samples containing 20–100 μg/ml of tolbutamide or chlorpropamide yielded a mean standard deviation of 3·8% (Table 1). The corresponding value for the range 1–10 μg/ml was 5·6%.

The recovery of tolbutamide from aqueous solutions was only 88–91% of that from human plasma, whereas the opposite effect was seen with chlorpropamide (104–114% recovery). Similar results were obtained with plasma from different individuals. For the estimation of sulphonylureas in unknown plasma samples, a plasma standard containing 100 μg/ml of the appropriate drug is also run through the procedure. The concentration of sulphonylurea in the unknown sample (U) is calculated as follows: $U = (Y \times S)/W$, where Y is the peak height ratio of drug

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Table 1. *Results of replicate analyses of tolbutamide and chlorpropamide in human plasma.**

| Drug | Plasma concentration (μg/ml) | Peak height ratio Mean ± s.d. (%) |
|---|---|---|
| Tolbutamide .. .. .. | 20 | 0·37 3·8 |
| | 40 | 0·72 3·3 |
| | 60 | 1·08 2·2 |
| | 80 | 1·43 2·8 |
| | 100 | 1·78 4·4 |
| Chlorpropamide .. .. | 20 | 0·39 5·1 |
| | 40 | 0·79 5·3 |
| | 60 | 1·22 5·2 |
| | 80 | 1·62 2·5 |
| | 100 | 1·99 3·4 |

* For each drug, 8 samples were assayed at each concentration. Extractions were made using toluene containing 20 μg/ml of chlorpropamide (tolbutamide assay) or 5 μg/ml of tolbutamide (chlorpropamide assay).

to internal standard, S is the drug concentration in the standard sample and W is its peak height ratio.

*Metabolites*

Small amounts of carboxytolbutamide, hydroxymethyltolbutamide, *p*-chlorobenzenesulphonamide and *p*-chlorobenzenesulphonylurea were dissolved in 10% potassium carbonate solution, methylated, extracted with hexane and chromatographed. None of these metabolites interfered with the assay and peaks were obtained on both columns with the derivatives of *p*-chlorobenzenesulphonamide, carboxytolbutamide and hydroxymethyltolbutamide. With the latter compound, the peak was small and derivative formation was presumably incomplete. The relative retention times are shown in Table 2.

## DISCUSSION

The method has a number of advantages over the procedure of Sabih & Sabih (1970). Reproducibility is improved by the use of an internal standard and there is no serious interference from plasma extracts. Therapeutic plasma concentrations of tolbutamide and chlorpropamide are probably in the range 50–200 μg/ml (Baird & Duncan, 1957; Knauff, Fajans & others, 1959) and the small peak coinciding with methylated tolbutamide on the UC W98 column is therefore of little significance.

Table 2. *Relative retention times of tolbutamide and chlorpropamide and their metabolites.*

| Compound | Retention time relative to tolbutamide 3·8% UC W98 (220°) | 1% Carbowax 20M (220°) |
|---|---|---|
| *p*-Chlorobenzenesulphonamide .. .. | 0·3 | 0·4 |
| Tolbutamide .. .. .. .. .. | 1·0 | 1·0 |
| Chlorpropamide .. .. .. .. | 0·8 | 1·4 |
| Carboxytolbutamide .. .. .. .. | 2·2 | 4·5 |
| Hydroxymethyltolbutamide .. .. .. | 1·9 | 2·8 |

If necessary, this interference can be avoided by use of the Carbowax 20M column, but the analysis takes longer.

The chromatographic step takes less than 3 min on the UC W98 column and there is no interference from slowly eluting peaks or from the known metabolites of tolbutamide and chlorpropamide. Gas liquid chromatography of these metabolites has not been reported previously, and *p*-chlorobenzenesulphonamide and carboxytolbutamide could perhaps be estimated by the same method using appropriate solvents for extraction. With colorimetric and spectrophotometric methods, inactive metabolites and other drugs may be extracted and measured together with the parent drug, giving rise to errors. For example, probenicid may interfere with the spectrophotometric assay of tolbutamide (Brook & others, 1968), and metabolites can cause serious errors in the estimation of chlorpropamide in plasma (Brotherton & others, 1969).

Tolbutamide and chlorpropamide undergo thermal decomposition and cannot be chromatographed directly. Although the *N*-methyl derivatives are more stable, decomposition may still occur since poor reproducibility with variable peak-widening was observed with a number of other column packings. Wickramasinghe & Shaw (1971) recently described a gas-liquid chromatographic method for tolazamide based on the thermal fragmentation to *p*-toluenesulphonamide. This method could possibly be applied to tolbutamide and chlorpropamide, but is time-consuming since a preliminary thin-layer chromatographic step is necessary.

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