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# ELECTRON-CAPTURE GAS CHROMATOGRAPHY OF PLASMA SULPHONYLUREAS AFTER EXTRACTIVE METHYLATION

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## SUMMARY

Conditions for the extractive alkylation of eight sulphonylurea hypoglycemic drugs have been evaluated. Extractive methylation of the compounds was achieved within 90 min using tetrabutylammonium as counter-ion (0.1 *M* at pH = 6.9) with 5% methyl iodide in dichloromethane as organic phase. Mass spectral analysis showed derivatives methylated at the sulphonamide nitrogen. A higher pH or use of tetrapentylammonium as counter-ion caused hydrolysis of the sulphonylureas.

The derivatives showed a high electron-capture response with minimum concentrations detectable in the range $1—4 \times 10^{-16}$ moles $sec^{-1}$.

Therapeutic plasma concentrations of glipizide and tolbutamide were determined by direct extractive methylation of the compounds from the plasma sample. The glipizide derivative was determined by electron-capture gas chromatography down to about 20 ng/ml in a 0.5-ml plasma sample. The relative standard deviation at the 0.2 μg/ml level of glipizide was 6% ($n$ = 6). The corresponding figure in the determination of tolbutamide at the 10 μg/ml level was 3% ($n$ = 10).

## INTRODUCTION

Oral hypoglycemic drugs of the sulphonylurea type have been in clinical use for about twenty years in the treatment of diabetes. Several analytical techniques have been employed in the analysis of the compounds from biological samples, the most frequently used being gas chromatography. Sulphonylureas have been determined by gas chromatography with flame ionization detection after methylation with dimethylsulphate [1—3] or diazomethane [4, 5]. After methylation, derivatives with good chromatographic properties and enhanced thermal stability were obtained. As well as a methyl derivative, some methyl enol ether was found [4, 6]. A low injection port temperature was claimed to be essential to minimize pyrolysis of the derivative.

Sulphonamide drugs have a high detectability in the electron-capture

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detector and tolbutamide and chlorpropamide have been determined by electron-capture gas chromatography after methylation with diazomethane [7]. Methylation followed by trifluoroacetylation has also been reported [8, 9]. In recent years, the low-dose hypoglycemic agents glibenclamide and glipizide have been introduced. Determination of therapeutic plasma concentrations of these drugs has been performed with radiolabelled material [10, 11] or by radioimmunological assays [12, 13]. The selectivity of these methods for metabolites has not been verified.

Extractive alkylation has found a widespread use in the analysis of acidic drugs and applications to the determination of sulphonamides in biological samples have appeared [14, 15]. The present paper discusses conditions for the extractive methylation of sulphonylurea drugs. The direct analysis of the compounds from plasma is demonstrated with tolbutamide and glipizide.

## EXPERIMENTAL

### *Apparatus*

A Pye GCV gas chromatograph was used and equipped with a flame ionization and an electron-capture detector operated in the constant-current mode. The glass column (210 cm × 0.2 cm) was filled with 3% OV-17 on Gas-Chrom Q (100—120 mesh) and operated at 300° for the analysis of the glipizide derivative and at 220° for the tolbutamide derivative. The flow-rate of nitrogen carrier gas was 40 ml/min. The injector and detector temperatures were 330°.

The derivatives were identified by mass spectral analysis in an LKB 2091 gas chromatograph—mass spectrometer. The glass column (90 cm × 0.2 cm) contained 3% OV-17 on Gas-Chrom Q (100—120 mesh). The ionization energy was 70 eV.

### *Reagents and chemicals*

Tetrabutylammonium, 0.1 *M*, was prepared by neutralization of the hydrogen sulphate salt (Labkemi, Stockholm, Sweden) and diluted to volume with buffer. Tetrapentylammonium ion solution, 0.2 *M*, was made from the iodide salt (Eastman—Kodak, Rochester, N.Y., U.S.A.) by shaking overnight with an equivalent amount of silver oxide in water and, after filtration, diluting to volume with water.

Methyl iodide, dichloromethane and toluene were supplied by Merck (Darmstadt, G.F.R.). Toluene was distilled before use. Silver sulphate was prepared as a saturated solution in water.

A stock solution containing 100 μg of tolbutamide per ml was made up in water. Aliquots of 0.05, 0.10, 0.20 and 0.40 ml of this solution were diluted to 1.0 ml with plasma. Chlorpropamide, 10 μg/ml in water, was used as internal standard.

In the determination of glipizide, a stock solution was prepared containing 1.0 μg of the drug per ml. Aliquots of 0.05, 0.10, 0.20 and 0.40 ml of this solution were diluted to 1 ml with plasma. Glibenclamide dissolved and diluted to 5 μg/ml was used as internal standard.

*Identity of methylated sulphonylureas*

The following prominent peaks were seen in the mass spectra from the sulphonylureas after extractive methylation.

Methyl acetohexamide: *m/e* (percentage relative abundance) = 91 (28), 98 (80), 119 (71), 155 (43), 183 (100), 198 (95), 338 ($M^+$; *m/e* 8).

Methyl carbutamide: *m/e* = 92 (43), 109 (100), 156 (62), 285 ($M^+$; *m/e* 1).

Methyl chlorpropamide: *m/e* = 92 (41), 109 (100), 156 (62), 304 ($M^+$; *m/e* 2).

Methyl glibenclamide: *m/e* = 82 (38), 97 (37), 169 (100), 171 (33), 198 (16), 381 (2).

Methyl glipizide: *m/e* = 93 (60), 98 (25), 111 (64), 150 (100), 459 ($M^+$; *m/e* = 28).

Methyl tolbutamide: *m/e* = 91 (100), 113 (74), 121 (38), 155 (75), 284 ($M^+$, *m/e* approx. 0).

N-methyl-(*p*-methylbenzene)sulphonamide from glibornuride and tolazamide: *m/e* = 91 (100), 155 (21), 185 ($M^+$, *m/e* 24).

*Methods*

*Evaluation of methylation conditions.* The sulphonylurea ($10^{-3}$ *M*) was dissolved together with internal standard (hexadocosane or hexatriacontane, 0.2 mg/ml) in dichloromethane. To a 1-ml aliquot of this solution, 2.0 ml of tetrabutylammonium ion solution in 0.1 *M* buffer solution were added or 1 ml of 0.2 *M* tetrapentylammonium ion solution and 1 ml of 0.4 *M* buffer solution. Methyl iodide, 50 μl, was added and the mixture shaken at room temperature for the time given. The reaction was quenched and some microlitres were taken for analysis by gas chromatography with flame ionization detection. The ratio of the peak height of the methyl derivative to that of the internal standard was calculated.

*Minimum detectable concentration.* Methyl derivatives of the sulphonylureas were prepared in the mg/ml range. Dilutions in toluene were analyzed by electron-capture gas chromatography and the minimum detectable concentration was calculated from the amount giving a signal three times the background noise level.

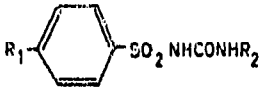
*Determination of glipizide in plasma.* To a plasma sample of glipizide (0.5 ml), 0.1 ml of internal standard solution and 2.0 ml of 0.1 *M* tetrabutylammonium ion solution in buffer (pH 6.9) were added. This solution was shaken for 1 h with 3 ml of dichloromethane containing 5% of methyl iodide. After centrifugation for 15 min at 500 *g* as much as possible of the organic phase was transferred to another tube and evaporated in a stream of nitrogen. A 0.5-ml volume of toluene was added and this solution was washed with saturated silver sulphate solution. A few microlitres of the organic phase were taken for analysis by electron-capture gas chromatography.

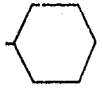
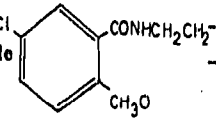
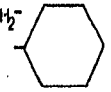
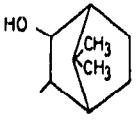
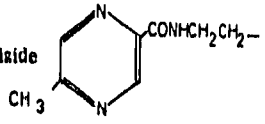
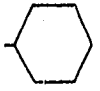
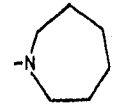
A standard curve was constructed by treating 0.5 ml of the standard samples according to the procedure above.

*Determination of tolbutamide in plasma.* A plasma sample of tolbutamide (0.1 ml) was mixed with 0.1 ml of internal standard solution and 2.0 ml of 0.1 *M* tetrabutylammonium ion solution in buffer (pH 6.9). This solution was shaken for 1 h with 1 ml of dichloromethane with 5% methyl iodide. The rest of the procedure was then essentially the same as for glipizide above.

TABLE I

EXTRACTIVE ALKYLATION OF SULPHONYLUREAS



| No. | Generic name | Structure | | Reaction time (min) using 0.1 *M* tetrabutylammonium at pH 6.9 and 5% methyl iodide in dichloromethane | Relative retention of monomethyl derivative compared to hexadocosane | Minimum detectable conc. ($\times 10^{-16}$ moles/sec) |
|---|---|---|---|---|---|---|
| | | $R_1$ | $R_2$ | | | |
| 1 | Acetohexamide | $CH_3CO$· |  | 30 | 3.3 | 2.8 |
| 2 | Carbutamide | $H_2N$· | ·$(CH_2)_3$·$CH_3$ | 90 | 3.5 | 4.2 |
| 3 | Chlorpropamide | Cl· | ·$(CH_2)_2$·$CH_3$ | 40 | 0.45 | 1.2 |
| 4 | Glibenclamide |  |  | 40 | 4.0* | 4.2 |
| 5 | Glibornuride | $CH_3$· |  | — | (0.17)** | — |
| 6 | Glipizide |  |  | 40 | 1.8* | 3.1 |
| 7 | Tolazamide | $CH_3$· |  | — | (0.17)** | — |
| 8 | Tolbutamide | $CH_3$· | ·$(CH_2)_3$·$CH_3$ | 20 | 0.50 | 1.8 |

*Relative retention compared to hexatriacontane.
**Formation of N-methylbenzenesulphonamide.

## RESULTS AND DISCUSSION

### *Extractive methylation of sulphonylureas*

The extractive alkylation of sulphonylurea drugs could be controlled to give either methylation of the sulphonamide nitrogen or hydrolysis to a sulphonamide and an amide. At a low pH in the aqueous phase methylation of the sulphonamide nitrogen took place, while complete hydrolysis was achieved using tetrapentylammonium as counter-ion at a pH > 10.

For selectivity towards metabolites, the derivatization was focused on the methylation of the sulphonamide nitrogen. The time for complete methylation using tetrabutylammonium ion as counter-ion at pH 6.9 and with 5% methyl iodide in dichloromethane is given in Table I. In all cases, except for tolazamide and glibornuride, the desired derivative was formed, as identified by mass spectrometry (see Experimental section).

*Tolbutamide*. Sulphonylureas are extremely sensitive to hydrolysis. Therefore conditions that yield a high concentration of hydroxide ion in the organic phase must be avoided. Use of tetrapentylammonium ion as counter-ion or a high pH in the aqueous phase, gave a low yield of derivative as can be seen from Table II. Tetrabutylammonium ion was therefore used as counter-ion. With this counter-ion a reaction time of 90 min was required for complete reaction at pH 5.3. In buffers with a pH exceeding 7.0 a maximum formation rate was seen with complete reaction within 20 min. In buffer pH 10, a 15% degradation of the derivative was observed after 4 h. The derivative, on the other hand, was stable for more than 3 h in the reaction mixture using buffer of pH 6.9.

TABLE II

METHYLATION TIMES OF TOLBUTAMIDE

Organic phase: dichloromethane with 5% methyl iodide, 1 ml.

| Aqueous phase (2 ml) counter-ion | pH | Time for complete reaction (min) |
|---|---|---|
| Tetrabutylammonium (0.05 *M*) | 5.3 | 90 |
| Tetrabutylammonium (0.05 *M*) | 6.3 | 45 |
| Tetrabutylammonium (0.05 *M*) | 6.9 | 20 |
| Tetrabutylammonium (0.05 *M*) | 10.0 | 20 |
| Tetrapentylammonium (0.05 *M*) | 6.9 | (20)* |
| Tetrapentylammonium (0.05 *M*) | 10.0 | (30)** |

*Maximum yield: 30%.
**Maximum yield: 15%.

*Glipizide*. Three products could be detected after extractive methylation of glipizide. The formation and degradation of the products with time using 0.05 *M* tetrabutylammonium ion as counter-ion in 0.1 *M* sodium hydroxide is shown in Fig. 1. Peak I corresponds to the methylated derivative of glipizide and was rapidly degraded. Peak II was an unidentified product and peak III was the sulphonamide hydrolysis product. The reaction conditions were chosen to give the methylated product and a low pH in the aqueous phase was used. A complete derivatization was achieved in 40 min using buffer of pH 6.9.

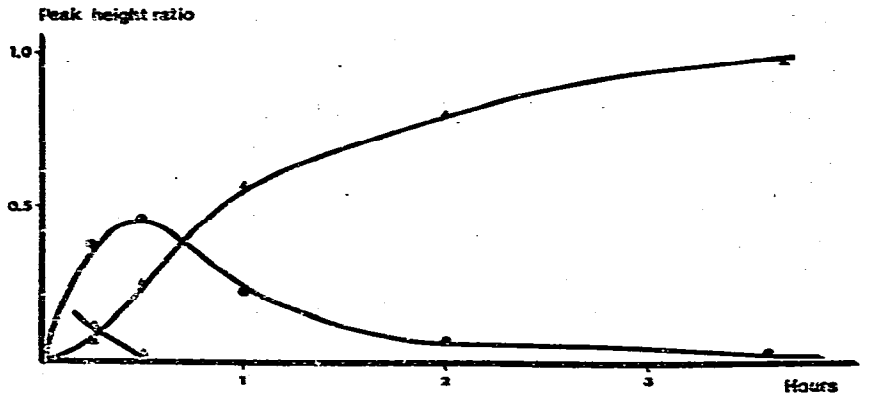



Fig. 1. Time course of the extractive methylation of glipizide. Conditions: 0.05 *M* tetrabutylammonium ion solution in 0.1 *M* sodium hydroxide (2 ml) and 5% methyl iodide in dichloromethane (1 ml). (△—△) product I = methylated derivative: (●—●) product II = unidentified product; (▲—▲) product III = hydrolysis product.

*Acetohexamide.* The derivative from acetohexamide was very sensitive to hydrolysis and it was necessary to perform the reaction at pH 6.0 with 0.05 *M* tetrabutylammonium ion as counter-ion.

*Carbutamide.* Only the sulphonamide nitrogen was methylated in the case of carbutamide, which means that the aniline moiety remains intact. For complete derivatization 90 min were required.

*Chlorpropamide and glibenclamide.* With the reaction conditions used, 40 min were required for complete methylation of the sulphonamide nitrogen of chlorpropamide and glibenclamide. Hydrolysis was pronounced at pH >10.

*Tolazamide and glibornuride.* Apart from the hydrolysis product, N-methyl-(*p*-methylbenzene)sulphonamide, no other derivatives could be detected from either tolazamide or glibornuride even in buffers of low pH.

*Gas chromatography and electron-capture detection of methylated sulphonylureas*

The gas chromatographic stability of methylated sulphonylureas has been reported to be poor [2, 4]. Therefore, small amounts (0.8 ng) of the tolbutamide derivative were repeatedly chromatographed on the stationary phase OV-17 together with an inert internal standard using injector temperatures in the range 200—350° and detector temperatures in the range 250—350°. In all cases, the relative standard deviation was in the range 1.4—3.0% ($n$ = 10), indicating that stability problems were of small importance.

For some derivatives (for example, glibenclamide and glipizide) a slight tailing of the peaks was observed, most probably caused by remaining acidic hydrogens in the molecules. The relative retentions of the derivatives are given in Table I and two gas chromatograms are shown in Fig. 2.

The sulphonamide grouping has a high electron-capture response after methylation [14—16]. The response was particularly good with the sulphonamide moiety in close conjugation with an aromatic ring. Conjugation of the sulphonamide grouping to a carbonyl group as in the sulphonylureas would

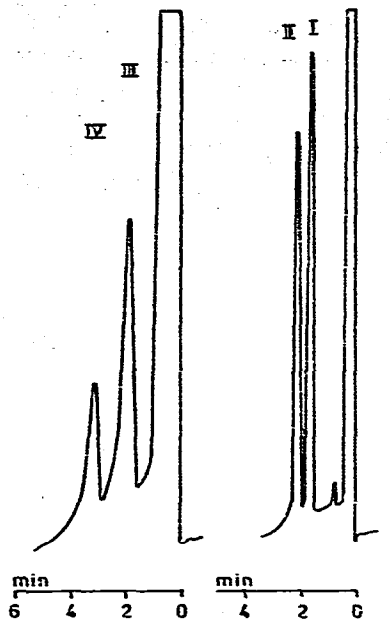



Fig. 2. Gas chromatograms after extractive alkylation of plasma sulphonylureas. Left panel: analysis of glipizide (III), 0.4 μg/ml. Internal standard: glibenclamide (IV), 0.5 μg added. Right panel: analysis of tolbutamide (II), 10 μg/ml. Internal standard: chlorpropamide (I), 1.0 μg added. For gas chromatographic conditions see Experimental section.

further increase the response. Minimum detectable concentrations of the methylated sulphonylureas were in the range 1—4 × $10^{-16}$ moles/sec (see Table I). The electron-capture response of N-methyltolbutamide was found to be independent of the detector temperature in the range 200—350°.

*Determination of sulphonylureas from plasma samples*

Antidiabetic sulphonylurea drugs are extensively metabolized in man. For some sulphonylureas, the metabolites are also reported to exhibit pharmacological activity [11, 17]. The possibility of co-determination of metabolites could not be neglected after hydrolysis of the sulphonylureas and methylation of the sulphonamide nitrogen was carried out.

The plasma concentrations after therapeutic doses of sulphonylurea drugs are usually in the lower μg/ml range. Direct extractive methylation from a 0.1-ml plasma sample of the drugs was possible, as has recently been demonstrated for other sulphonamide drugs [14, 15]. A chromatogram from an analysis of plasma tolbutamide can be seen in Fig. 2. The relative standard deviation in the analysis of 10 μg of tolbutamide per ml of plasma was 3.0% ($n$ = 10).

Although the plasma concentrations of glipizide or glibenclamide are in the submicrogram/ml range, direct extractive methylation of the plasma sample could also be used in these cases owing to the high detection selectivity of the method. Use of tetrabutylammonium ion as counter-ion at pH 6.9 gave no interfering components from the biological sample and glipizide could be detected down to 20 ng/ml in a 0.5-ml plasma sample. Before gas chromato-

graphic analysis, methyl iodide was removed by evaporation, and tetrabutylammonium iodide formed in the reaction by washing with saturated silver sulphate solution. Rectilinear standard curves through the origin were obtained in the range 50—400 ng of glipizide per ml. The relative standard deviation in the determination of glipizide at the 200 ng/ml level was 6.0% ($n$ = 6). A typical gas chromatogram is seen in Fig. 2. The procedure for glipizide is at present in use for pharmacokinetic studies of the drug in man.

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