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Note

Determination of metformin in plasma at therapeutic levels by gas—liquid chromatography using a nitrogen detector

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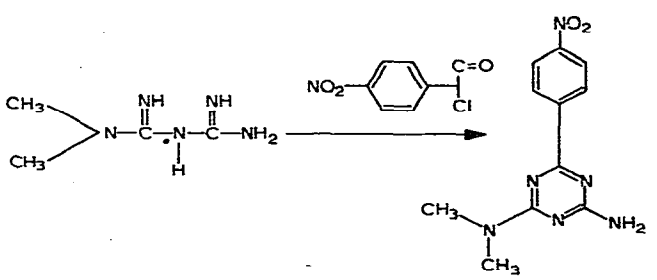
In the literature dealing with the identification and quantification of the hypoglycaemic biguanides in biological fluids there is little information concerning specific methods for metformin (N,N-dimethylbiguanide) [1—7]. Unfortunately, even the most sensitive of these do not demonstrate sufficient reproducibility; this is due principally to difficulties in extraction, derivatization and linearity of the internal standardisation. In order to overcome these extraction difficulties Ross [8] described a technique for derivatization in aqueous medium using high-performance liquid chromatography for the determination of urinary levels of metformin. However, this has limited application to other biological fluids, due to the sensitivity of the detector. We have adapted this useful technique of derivatization to gas—liquid chromatography (GLC). By using a nitrogen detector and a linear internal standard, a reproducible, specific and quantitative analysis of metformin has been possible with a sensitivity suitable for plasma and tissue levels of the drug after the administration of therapeutic doses.

PRINCIPLE

Metformin is derivatized directly in the biological medium with *p*-nitrobenzoyl chloride to form the corresponding substituted triazine derivative: 2-amino-6-dimethylamino-4-(4′-nitrophenyl)-1-3-5-triazine, thus:



The reaction is carried out in the presence of an excess of acetonitrile and as soon as the triazine is formed it passes into the acetonitrile phase. The extract is analysed by GLC. A thermionic detector sensitive to nitrogen assures specific detection of the molecule and its quantitative analysis with good sensitivity. The calculation of the metformin level is performed using propylbiguanide hydrochloride as an internal standard.

EXPERIMENTAL

Reagents

Metformin and propylbiguanide hydrochloride were kindly supplied by S.N.E.L. Aron (Suresnes, France).

The preparation of derivatives of metformin: the 2-amino-6-dimethylamino-4-(4′-nitrophenyl)-1-3-5-triazine and of propylbiguanide: the 2-amino-6-propylamino-4-(4′-nitrophenyl)-1-3-5-triazine were carried out using the technique described by Ross [8].

Gas—liquid chromatography

The analyses were performed on a Carlo Erba Chromatograph, Fractovap series 2350, equipped with a nitrogen detector (KCl salt). The glass columns (200 cm × 3 mm I.D.) were packed with 3% OV-17 on silanised Chromosorb W (80—100 mesh). Nitrogen was used as the carrier gas at a flow-rate of 40 ml/min. The oven temperature was maintained at 250°.

Extraction and derivatization of metformin

Samples of 2 ml of plasma were placed into 10-ml stoppered test tubes. After addition of 2.5 μg propylbiguanide hydrochloride (in 100 μl of water) as the internal standard, sodium chloride was added to saturation and 1 ml of acetonitrile. The tubes were agitated for 15 min on a roto-reciprocating stirrer. After centrifugation the acetonitrile layer was removed using a Pasteur pipette and rejected. 1 ml of 5 *N* sodium hydroxide, sodium chloride to saturation point, 1 ml of acetonitrile and about 10 mg of *p*-nitrobenzoyl chloride were added. The stoppered tubes were shaken for 15 min. A further 10 mg of *p*-nitrobenzoyl chloride was added and the tubes were shaken again for 15 min. After centrifugation the acetonitrile layer was transferred to a "minivial" and evaporated to dryness in a water bath at 60°. The residue was taken up in tetrahydrofuran (usually 100 μl) and the solution was injected into the chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows the trace obtained from 2 ml of control dog plasma. Fig. 2 shows the trace obtained from 2 ml of treated dog plasma taken 1 h after the intravenous administration of metformin at a dose of 10 mg/kg body weight with the addition of 2.5 μg of propylbiguanide hydrochloride. The derivatives of metformin and propylbiguanide hydrochloride were characterized by their Kováts retention indices obtained from an injection containing the two syn-

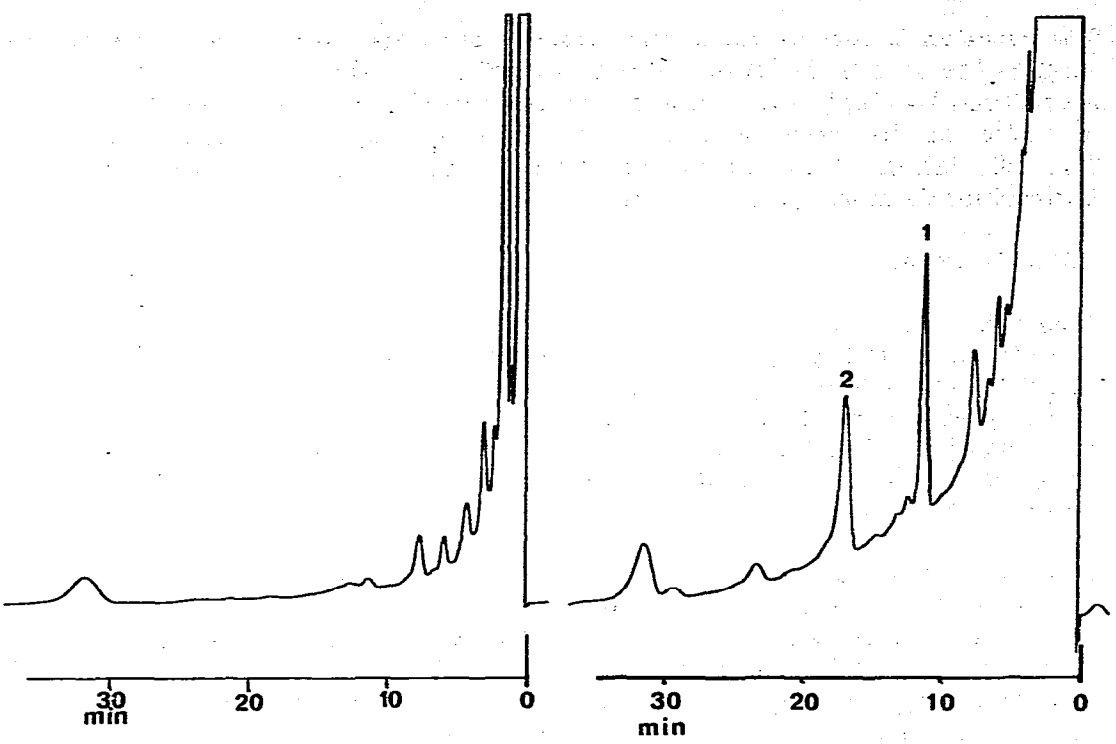


Fig. 1. Gas chromatogram of an extract of 2 ml of dog plasma containing no drug.

Fig. 2. Gas chromatogram of 2 ml of dog plasma previously treated with 10 mg/kg metformin i.v. sampled after 1 h. 2.5 μg propylbiguanide hydrochloride added as internal standard. 1 = Metformin derivative; 2 = propylbiguanide derivative.

thesised *p*-nitrobenzoyl derivatives and selected normal saturated hydrocarbons (C28 and C32):

p-nitrobenzoyl derivative of metformin: IRK = 2975
p-nitrobenzoyl derivative of propylbiguanide: IRK = 3120

Considering the large variations in recovery observed between the two preparations it was necessary to use an internal standard. The area under the peaks corresponding to each of these derivatives is measured on the chromatogram. The concentration of metformin is directly proportional to the areas under the peaks. Experimental points obtained by adding known quantities of metformin and propylbiguanide hydrochloride to rat plasma fall on a straight line, the equation of which has been mathematically calculated, ($y = 1.35\,x - 0.04$) with an excellent correlation ($r = 0.994$).

The derivatization techniques for metformin previously described [1, 2] necessitated a preliminary extraction procedure. This extraction from blood plasma has a poor recovery, of the order of 40% under the best conditions

giving a very poor overall recovery of about 10%. In this new derivatization technique, performed directly in aqueous solution without previous extraction, it is possible to obtain a total recovery of 88% (10 determinations) with a standard variation of 15%.

In order to use GLC for this determination it is important that the extract from plasma should be as pure as possible while consistent with minimal loss of metformin. For this reason, an initial washing of the plasma sample with acetonitrile was carried out. This solvent is, however, incompatible with a nitrogen detector and the dried extract was dissolved in tetrahydrofuran for injection into the chromatograph. Using this procedure levels of metformin below 25 ng/ml could be easily determined, providing adequate sensitivity for the determination of therapeutic blood levels. It should be noted that the procedure is also applicable to other biological fluids such as urine and tissue homogenates.

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