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Gas chromatographic determination of 4-allyloxy-3-chlorophenylacetic acid (alclofenac*) and its metabolites

The metabolism of Alclofenac (A), a new anti-inflammatory and analgesic drug[1], has been investigated in man and in seven different animal species[2-4]. Three major metabolites in plasma and urine were identified: A itself, 3-chloro-4-hydroxyphenylacetic acid (4-HCPA) and racemic 3-chloro-4-(γ-β,dihydroxy)-propyloxyphenylacetic acid (DHA). The assessment of interspecies and individual variations observed in the excretion rate of these metabolites was carried out by gas-liquid chromatography (GLC). The procedures used are described in this paper.

Experimental

A Packard (Series 7400) gas chromatograph is used. Glass columns, 2 m × 2 mm I.D., packed with Supelcoport 80/100 mesh (Supelco. Inc.) coated with XE-60 (3 %) are employed. The carrier gas is nitrogen (60 ml/min). The inlet temperature is 230°. The flame ionisation detector is operated at 220°. The three silylated metabolites can be easily chromatographed either separately under isothermal conditions (*e.g.* 4-HCPA, 140°; A, 160°; DHA, 190°) or, simultaneously, using a "curvilinear" temperature programme (see Fig. 1) increasing from 170° to 210°. Quantification is achieved using calibration curves based on peak area determinations.

Since at least two of the above-mentioned metabolites are partially excreted in urine as conjugates[4], HCl-hydrolysis is required to free these compounds prior to the extraction and the assay. Analytical grade methyl isobutyl ketone (MIBK; UCB, Brussels) is used for the extraction of the metabolites from plasma and urine (yield: about 100 % for the three free acid compounds as determined using ^{14}C-labelled metabolites). Samples to be analysed are prepared as follows:

Plasma. 4 ml of plasma is taken and diluted with 6 ml of 1 *N* HCl (final normality, 0.6). The 10-ml specimen is hydrolysed at 100° for 20 min and thereafter extracted twice with MIBK (1:3) using an automatic shaker (Gerhardt, G.F.R.). The combined extract is dried with anhydrous Na_2SO_4 and evaporated under reduced pressure (Rotavapor, Büchi) at 35°. The residue is dissolved in 100 µl of 1,4-dioxane (Merck, Darmstadt; analytical grade). Silylation is performed by adding 100 µl of hexamethyldisilazane (HMDS; Pierce Chemical Co.) and 50 µl of trimethylchlorosilane (TMCS; Pierce Chemical Co.). After standing overnight at room temperature, the mixture is centrifuged and 1 or 2 µl of the clear supernatant are injected into the gas chromatograph.

Urine. 1 ml of 12 *N* HCl is added to 20 ml of clear urine. The sample is then hydrolysed, extracted twice (1:3) and evaporated as described above. The residue is dissolved in 1 ml of 1,4-dioxane; 0.2 ml is silylated by adding 200 µl of HMDS and 100 µl of TMCS. 1 or 2 µl are injected into the gas chromatograph.

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* Mervan®, Continental Pharma S.A., Brussels, Belgium.

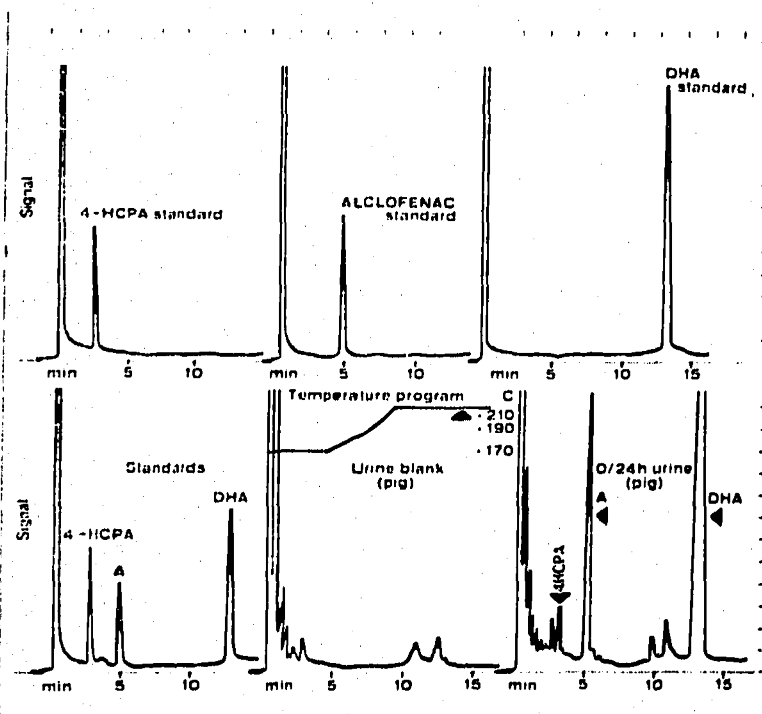

Fig. 1. Chromatograms showing the simultaneous determination of Alclofenac metabolites by GLC. The upper chromatograms show (from left to right) the separation of the three synthetic metabolites as silylated derivatives: 4-HCPA (1 μg), A (2 μg), and DHA (4 μg). The lower chromatograms show (from left to right): the simultaneous determination of the three silylated standards (1, 1.2 and 2.3 μg, respectively), a urine blank, and the separation of actual metabolites in pig urine after the administration of a 100 mg/kg oral dose of Alclofenac. The temperature programme used is also shown (see text for further details). Mass spectrometric analysis of the derivatives formed shows that A is monosilylated (mol. wt. 208, ester), 4-HCPA disilylated (mol. wt. 330, ester and ether) and DHA trisilylated (mol. wt. 476, ester and diether).

Results

Using synthetic 4-HCPA, A and DHA it has been shown that under the experimental conditions described, neither hydrolysis nor extraction alter, to any significant extent, the native structure of these compounds.

Isothermal chromatography is generally used for metabolite determinations in plasma because better resolution is obtained. Under these conditions the retention times are 7.6 (140°), 6.0 (160°) and 7.8 (190°) min for 4-HCPA, A and DHA, respectively. In the case of urine no interfering peaks are observed when temperature programming is used; therefore this method is preferred as it speeds the analysis. Fig. 1 shows that the separation is quite satisfactory. The methods described above have been employed with comparable efficacy for the determination of Alclofenac metabolites in plasma and urine of man and different animal species. According to the availability, initial smaller volumes of sample can, however, be used. In this case, the volume of solvents and reagents is proportionally reduced.

The sensitivity of the assays under isothermal as well as under programmed conditions is better than 0.25 μg/injection for all the three compounds tested.

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