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Note

Determination of ethacrynic and tienilic acid in plasma by gas—liquid chromatography—mass spectrometry

WOLFGANG STÜBER*, ERNST MUTSCHLER and DIETER STEINBACH

Pharmakologisches Institut für Naturwissenschaftler, Universität Frankfurt, and Zentrallaboratorium Deutscher Apotheker, Ginnheimerstrasse 20, D-6236 Eschborn (G.F.R.)*

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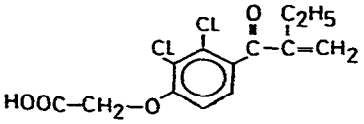
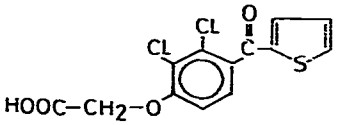
In 1962, Schultz et al. [1] reported that various unsaturated ketone derivatives of aryloxyacetic acids had diuretic activity. Today ethacrynic acid (Fig. 1) is one of the most potent diuretics next to furosemide [2]. Both



ethacrynic acid



tienilic acid

Fig. 1. Phenoxyacetic acids with diuretic activity.

have a very rapid onset of action, with diuresis beginning 20—60 min after oral administration. The duration of action is relatively short.

More recent investigations of Cragoe and Woltersdorf [3] have shown that the importance of the enone structure for SH-group binding capacity has been overestimated; dihydroethacrynic acid also possesses diuretic activity. Another phenoxyacetic acid derivative with diuretic effects is tienilic acid (2,3-dichloro-4-(2-thenoyl)phenoxyacetic acid (Fig. 1). Tienilic acid has a longer duration of action than ethacrynic acid but is less potent. The uricosuric effect of the mercurial diuretics absent in the case of ethacrynic acid is regained with tienilic acid [4, 5].

The major contra-indication of ethacrynic acid is hypertension, but in

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January 1980 the preparation Selacryn was withdrawn from the market on suspicion of causing liver damage. The Indanones MK-196 and MK-473 (Merck Sharp and Dohme) also possess diuretic and uricosuric effects and show very similar pharmacological properties.

Ethacrynic acid has a low rate of biotransformation and only limited amounts are excreted in the form of unstable glutathione, cysteine and acetylcysteine conjugates [6]. Tienilic acid has also a low rate of biotransformation. After oral administration the maximal plasma concentration of the unchanged drug is over twelve times higher than that of the major metabolite, the alcohol arising from reduction of the C=O group [7]. Oxidation of the thienylcarbonyl function to the corresponding acid, dichlorocarboxyphenoxyacetic acid, occurs to only a limited extent [8].

Except for a report using labelled drug no technique has been described for the quantitative estimation of ethacrynic acid in plasma [6]. Desager et al. [9] and Hwang et al. [7] measured tienilic acid by gas chromatography after extraction from plasma and reaction with diazomethane. A similar method for the quantitative determination of MK-196 has been described by Zacchei and Wishousky [10].

The aim of the present study was to develop a specific and sensitive technique for the estimation of phenoxyacetic acids using gas chromatography in conjunction with mass spectrometry.

MATERIALS AND METHODS

Extraction of ethacrynic acid and tienilic acid from plasma

A 1-ml volume of plasma was mixed with a methanolic solution of the sample containing 0.01—0.10 μg of substance, then the internal standard was added (1 μg of ethacrynic acid, or tienilic acid). After addition of 1 ml of 3 *N* hydrochloric acid and 5.0 ml of diethyl ether, the sample was subjected to a 3-min extraction (mechanical shaker, 250 l/min) and then centrifuged for 5 min at 1950 *g*. Four milliliters of the organic phase were evaporated to dryness. The residue was then subjected to derivatisation. For measurement of recovery, various concentrations of the substance were added to plasma (the internal standard was added immediately before derivatisation). The reproducibility of the technique was tested by repeated estimations in the concentration range 0.05—5 μg/ml of plasma.

Derivatisation with pentafluorobenzyl bromide

After evaporation of the ethereal plasma extract, the residue was mixed with 2 ml of a 2% solution of pentafluorobenzyl bromide (EGA Chemie, Steinheim, G.F.R.) in acetonitrile and approximately 10 mg of anhydrous potassium carbonate. After 45 min reaction at 70°C, the solution was evaporated to dryness in vacuo and the residue taken up in 50 μl of acetonitrile. After ultrasonic irradiation 5 μl of this solution were subjected to gas chromatography.

Gas chromatography—mass spectrometry

A Varian gas chromatograph Model 3700 coupled to a MAT 44 mass spec-

trometer was used. The column was glass (2 m × 2 mm) packed with 1% OV-17 on Chromosorb W, 80—100 mesh. The conditions of measurement were: programmed temperature operation from 200 to 300°C at 30°C/min; carrier gas, helium at a flow-rate of about 30 ml/min; injection port temperature, 300°C; line temperature, 300°C; ion source temperature, 250°C; Electron energy, 80 eV (electron-impact mode), 160 eV (chemical-ionisation mode); emission current, 0.8 mA. The investigations using chemical ionisation were carried out using isobutane as reaction gas (purity 99.5%; Messer, Griesheim, G.F.R.) at a pressure of between 330 and 340 μbar.

RESULTS

Derivatisation with pentafluorobenzyl bromide

For derivatisation with pentafluorobenzyl bromide, a reaction temperature of 70°C and reaction time of 45 min were found to give the best results. This confirms the data given by other workers from similar studies [11]. The derivatives were stable for at least 24 h. Using a 2% solution of reagent in acetonitrile, evaporation of the poorly volatile excess pentafluorobenzyl bromide in vacuo was possible.

Gas chromatography

One per cent OV-17 as stationary phase was found to be the most suitable with regard to shape of the peaks and sharpness of separation. Hwang et al. [7] and Desager et al. [9] used OV-17 or OV-225 as stationary phase for the measurement of the methyl derivative of tienilic acid.

Mass spectrometry

In the electron-impact mode, detection of the molecular ion peak was possible for both substances; the relative intensity with the ethacrynic acid derivative was under 1%. A high intensity of *m/e* 180 is due to the reagent. With quantitative measurement in the lower mass regions, interference is likely, especially after plasma extraction. However, using chemical ionisation, the molecular ion peaks became the base peaks. Little, if any, fragmentation occurred (see Figs. 2 and 3). Therefore quantitative measurement could be carried out in the mass region > 480.

Adjustment to constant reaction gas pressure is particularly important with chemical ionisation, since the decrease or increase in sensitivity brought about by a change in reaction gas pressure is not the same for all substances tested. Fig. 4 illustrates the relatively higher sensitivity to variations in pressure shown by ethacrynic acid compared to tienilic acid.

Quantitative estimation of ethacrynic acid and tienilic acid in plasma

After ether extraction the rate of recovery of ethacrynic acid and tienilic acid was 94.5 ± 2.9% and 95 ± 4.2%, respectively. A 3-min extraction achieved an almost total recovery with separation of the ether-extractable impurities. Both Hwang et al. [7] and Desager et al. [9] used the same solvent, but their respective extraction times of 15 min and 3 sec differed considerably. Recovery rates of 98% and 95% were reported.

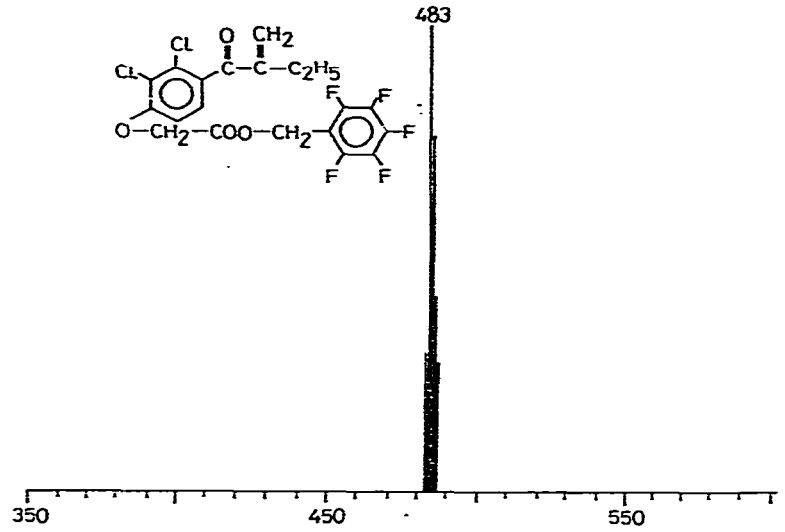

Fig. 2. Mass spectrum of ethacrynic acid after derivatisation with pentafluorobenzyl bromide and gas chromatography (chemical-ionisation mode).

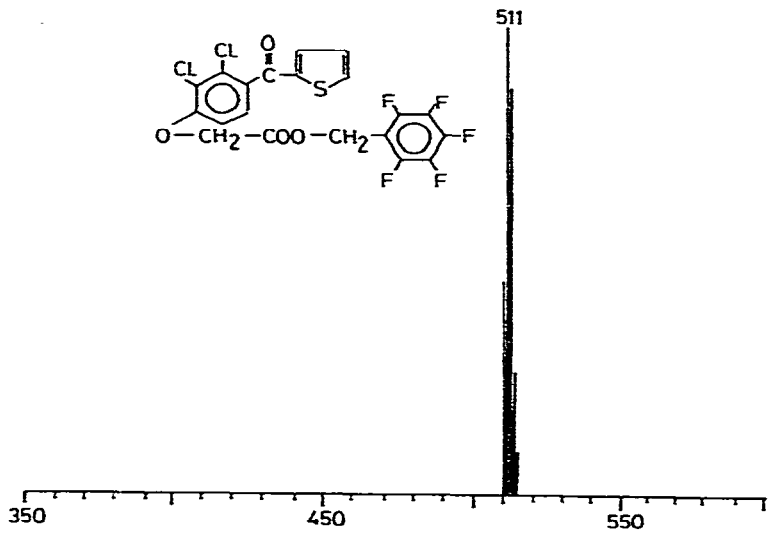

Fig. 3. Mass spectrum of tienilic acid after derivatisation with pentafluorobenzyl bromide and gas chromatography (chemical-ionisation mode).

Linear regression of the calibration graph gave a value of 0.998; the mean standard deviation of the method for measurement in the range 0.05—1 μg/ml plasma amounted to ± 5%. The limit of detection was between 10 and 20 ng/ml of plasma.

Fig. 5 shows a typical gas chromatogram of a spiked plasma sample after derivatisation with pentafluorobenzyl bromide (1 μg/ml tienilic acid and 0.5 μg/ml ethacrynic acid). The quantitative estimation was made by the molecular ions and base peaks, *m/e* 511 and *m/e* 483.

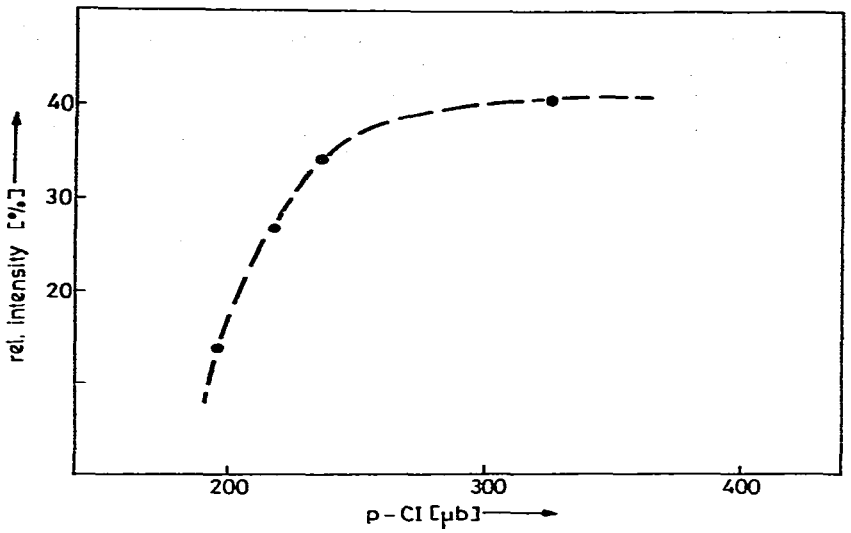

Fig. 4. Influence of reaction gas pressure (in µbar) on the relative intensity of the ethacrynic acid peak (1 µg of tienilic acid vs. 500 ng of ethacrynic acid).

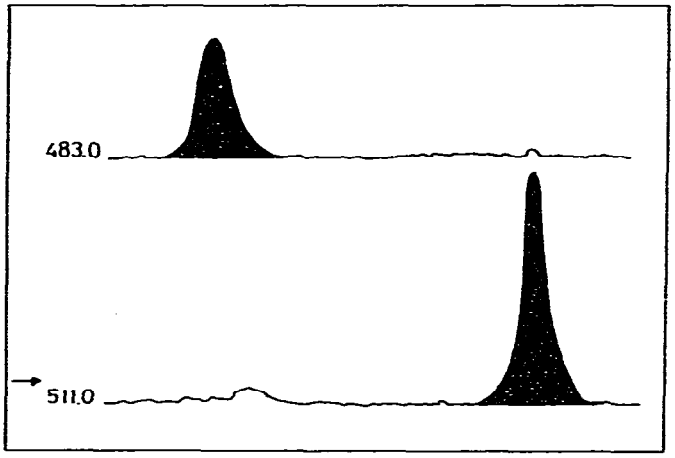

Fig. 5. Quantitative estimation of ethacrynic acid and tienilic acid in plasma.

DISCUSSION

A method has been developed for the gas chromatographic—mass spectrometric measurement of ethacrynic acid and tienilic acid, after derivatisation with pentafluorobenzyl bromide, which can be applied to further phenoxyacetic acids such as MK-196 and MK-473 (Merck Sharp and Dohme). Various reports have appeared in the literature concerning the use of pentafluorobenzyl bromide for derivatisation of organic acids [11—15]. Due to the high reactivity of pentafluorobenzyl bromide, interference can occur through acid impurities, especially from biological material [15]. Greving et al. [14] reported interference in the gas chromatographic measurement by fatty acids, especially palmitic acid. Using chemical ionisation with isobutane, fragmentation of substances during mass spectrometry can be avoided and an inter-

ference-free evaluation of the molecular ion peaks in the mass region > 480 is possible. The practical importance of the technique was demonstrated by the quantitative estimation of ethacrynic acid and tienilic acid from plasma. Measurements of plasma concentrations in the ng/ml and μg/ml range were possible.

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