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# ASSAY OF PRENALTEROL IN PLASMA AND URINE BY GAS CHROMATOGRAPHY—MASS SPECTROMETRY

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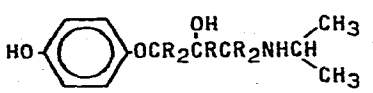
## SUMMARY

A sensitive and selective method for the quantitative analysis of prenalterol in plasma and urine is described. Prenalterol and the internal standard, deuterated prenalterol, are extracted with diethyl ether at pH 9.9 under salting-out conditions. Derivatization is performed by means of pentafluoropropionic anhydride in toluene before separation in the gas chromatograph. Detection and quantification of the triacyl derivatives are done by mass spectrometry in the selected ion monitoring mode. The method allows determination of concentrations down to 5 nmol/l (1 ng/ml) in 1 ml of sample, with a relative standard deviation below 10%.

## INTRODUCTION

Prenalterol (Fig. 1), the *laevo*-isomer of 4-hydroxyphenoxy-3-isopropylamino-2-propanol, is a selective $\beta_1$-adrenoceptor agonist [1]. It has been analysed in plasma by gas chromatography and electron-capture detection of the heptafluorobutyryl derivative after a single extraction step [2]. In low concentra-



Prenalterol R = H

Internal standard (deuterated prenalterol) R = $^2$H

**Fig. 1. Structural formulae of prenalterol and deuterated prenalterol.**

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tions interference from endogenous compounds prevents analysis of low concentrations of prenalterol in this way, and back- and re-extraction must be used in the work-up procedure [3].

The molecular structure of prenalterol resembles that of the 4-hydroxy metabolite of propranolol. This metabolite has been assayed in biological materials by gas chromatography—mass spectrometry [4—6]. The purpose of this paper is to describe a method for assaying prenalterol in plasma and urine that involves a single extraction step and determination by gas chromatography—mass spectrometry.

## EXPERIMENTAL

### *Apparatus*

A Finnigan MAT 44S mass spectrometer coupled to a Varian 3700 gas chromatograph interfaced by an open split coupling was used for both qualitative and quantitative work. The glass column (2 m × 2 mm I.D.) was filled with 3% OV-17 on Gas Chrom Q (120—140 mesh) (Applied Science Labs., State College, PA, U.S.A.) and operated at 185°C. The temperature of the injection port, the open split coupling and the connection line was kept at 200°C. The ion source temperature was 220°C. The flow-rate of the carrier gas (helium) was 15 ml/min.

Ions were generated by electron impact with an electron energy of 75 eV and an emission current of 0.8 mA, and detected with an electron multiplier voltage of 2000 V. Mass fragmentography was carried out at $m/z$ = 366 for the prenalterol derivative and at $m/z$ = 371 for the internal standard derivative.

### *Reagents and chemicals*

Diethyl ether and toluene, obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.), were purified by distillation. Sodium chloride (analytical grade from Merck, Darmstadt, G.F.R.) was baked at 500°C for 8 h. Pentafluoropropionic anhydride (PFPA) was purchased from Fluka (Buchs, Switzerland) and purified by distillation. Prenalterol was obtained from Ciba-Geigy (Basle, Switzerland) and the internal standard, deuterated prenalterol (H 80/62-$^2H_5$), was obtained from the Department of Organic Chemistry, Hässle, Mölndal, Sweden. Standard solutions of prenalterol and the internal standard were prepared in dilute hydrochloric acid (0.01 mol/l) to produce working standard solutions with concentrations of 1 μmol/l. The carbonate buffer solution, pH 10.8, was prepared by adjusting the pH of a sodium carbonate solution (2 mol/l) by addition of sodium hydrogen carbonate solution (1 mol/l).

### *Glassware*

All glassware was washed in a laboratory dishwasher with detergent at pH 12, rinsed with phosphoric acid solution (pH 2) and deionized water and dried at 60°C.

### *Determination of the distribution ratio*

Distribution ratios ($D$) for prenalterol between diethyl ether and aqueous buffer solutions (pH 8—12.5, $I$ = 0.10) were determined by shaking in centri-

fuge tubes for 30 min at 25°C. The distribution maximum occurred at pH 9.9, where a distribution ratio of 0.57 was obtained. The concentration of prenalterol was determined by spectrophotometry in the aqueous phase before and after equilibration, and the concentration of prenalterol in the organic phase was calculated from the difference.

The effect of adding sodium chloride to the aqueous phase was also studied. Correction was made for the increase in volume of the aqueous phase after addition of sodium chloride. The pH of the buffer was adjusted to 9.9 as the addition of various amounts of sodium chloride had considerable influence on the pH. The results are shown in Fig. 2.

*Analytical procedure*

Plasma or urine samples were mixed and centrifuged and 0.1—1.0 ml was transferred to a 15-ml centrifuge tube (fitted with a PTFE-lined screw-cap) containing 100 μl (100 pmol) of the internal standard solution and 0.4—0.5 g of sodium chloride. Sample volumes of less than 1.0 ml were adjusted by adding water. The aqueous phase was buffered to pH 9.9 by adding 100 μl of the carbonate buffer solution, and extracted with 10 ml of diethyl ether. After shaking for 10 min and centrifuging, the organic layer was transferred to a second screw-capped tube and evaporated to dryness at 35°C under a gentle stream of dry nitrogen. The residue was dissolved in 200 μl of toluene and 20 μl of pentafluoropropionic anhydride were added. The reaction mixture was allowed to stand for 60 min at 60°C (or for 90 min at 40°C) and then evaporated to dryness under a gentle stream of dry nitrogen at 35°C. The residue was dissolved in 100 μl of toluene and 4 μl of this solution were injected into the gas chromatograph.

*Quantitation*

Four reference samples were prepared by adding 100 μl of the prenalterol standard solution (1 μmol/l) to 1.0 ml of blank plasma (or urine). These samples were then analysed according to the analytical procedure. The peak area ratio of the prenalterol derivative over the internal standard derivative was calculated for each chromatogram. The average of the peak area ratios for the reference samples was used for the quantitative evaluation of the authentic samples.

## RESULTS AND DISCUSSION

*Extraction*

Prenalterol is an aminophenol and is preferably extracted at its isoelectric point, calculated to be 10.0 by use of the acid dissociation constants $pK_{H_2A}$ = 9.6 and $pK_{HA}$ = 10.5 (both constants determined by spectrophotometry). This calculated pH optimum is in good agreement with the experimentally found pH optimum. At the isoelectric point the distribution ratio was determined with a weak proton-donating solvent (dichloromethane) and two proton acceptors (ethyl acetate and diethyl ether). The results are summarized in Table I. The highest extraction yield was obtained with ethyl acetate, but, because of excessive interference from coextracted substances with this solvent, diethyl ether was chosen for the extraction procedure. The theoretical recovery of the

TABLE I

DISTRIBUTION OF PRENALTEROL BETWEEN DIFFERENT ORGANIC SOLVENTS AND AQUEOUS BUFFER ($I$ = 0.10) AT pH 9.9.

| Solvent | $D$ | log $D$ | Percentage prenalterol at $V_{org}/V_{aq}$ = 8 |
|---|---|---|---|
| Diethyl ether | 0.5 | −0.30 | 80 |
| Dichloromethane | 0.14 | −0.84 | 53 |
| Ethyl acetate | 5.6 | +0.75 | 98 |

extraction using a phase volume ratio ($V_{org}/V_{aq}$) of 8, as proposed in the method, is 80%. This can be improved by the addition of sodium chloride to the aqueous phase (Fig. 2). Thus, after saturation of the aqueous phase with sodium chloride (0.36 g/ml) and with a phase volume ratio of 8, 98.5% of the prenalterol will be extracted into the organic phase.

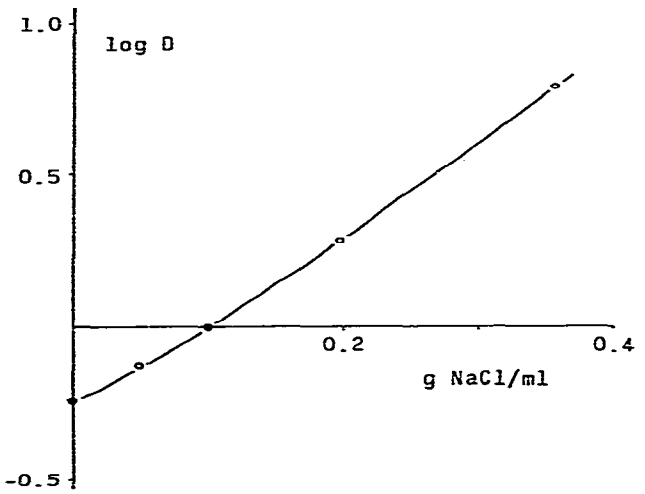


Fig. 2. Distribution ratio ($D$) of prenalterol between diethyl ether and an aqueous phase (buffer solution pH 9.9) containing sodium chloride.

*Derivatization*

Trifluoroacylation has been applied to the determination of 4-hydroxypropranolol in a method which involves extraction with buffer to remove the excess of reagent before the gas chromatographic procedure [4]. In order to obtain a derivative with increased stability and avoid the treatment with aqueous buffer, which might be hazardous, we have chosen pentafluoropropionic anhydride as reagent. The pentafluoropropionyl derivative of prenalterol is stable during evaporation of the excess of reagent. The long-term stability of this derivative is good, and a solution in toluene stored in a refrigerator for six days shows no degradation. Gas chromatographic—mass spectrometric determinations during this period gave results with a relative standard deviation of 1.5% ($n$=6). A corresponding procedure for the trifluoroacyl derivative gave a significantly higher standard deviation.

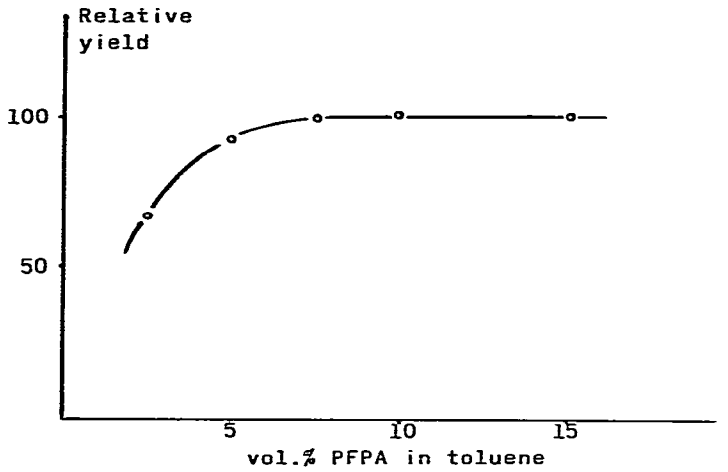


**Fig. 3. Influence of the pentafluoropropionic anhydride (PFPA) concentration on the formation of the pentafluoropropionyl derivative of prenalterol. Reaction conditions: 60 min at 60°C.**

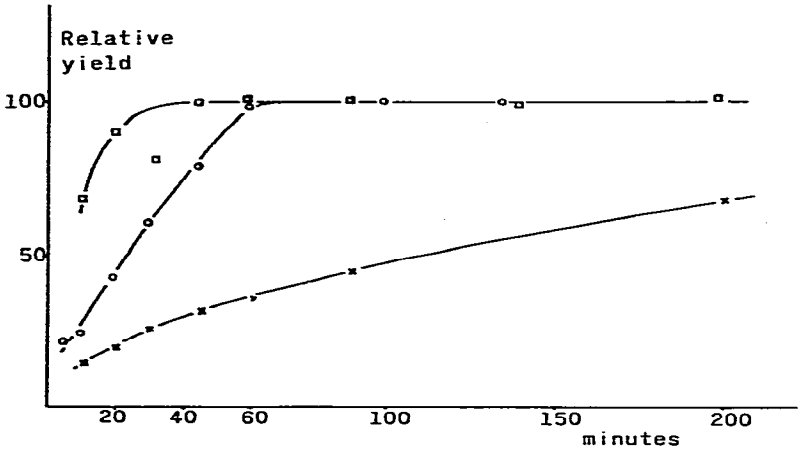


**Fig. 4. The formation of the pentafluoropropionyl derivative of prenalterol at different reaction temperatures: ×, 22°C; ○, 40°C; □, 60°C.**

The influence of reagent concentration and reaction temperature on the pentafluoroacylation of prenalterol in toluene was studied (Figs. 3 and 4) and the structure of the formed derivative was confirmed by mass spectrometry (Fig. 5).

*Quantitation*

From the electron-impact mass spectrum (Fig. 5) two ions can be selected for quantitative work, $m/z$ = 366 and $m/z$ = 408, both originating from the derivatized aminoalcohol chain, as shown by Garteiz and Walle [7]. The most abundant ion, $m/z$ = 366, was chosen. Consequently, the internal standard, which has an identical fragmentation pattern and incorporates five deuterium atoms in the aminoalcohol chain, will give a corresponding ion with $m/z$ = 371.

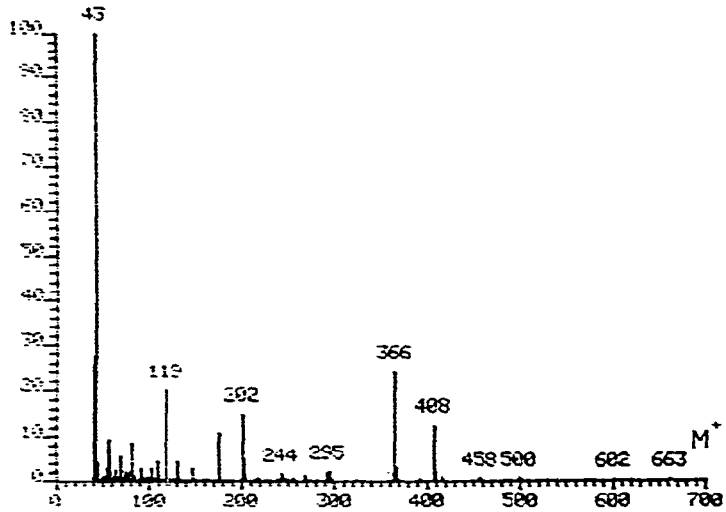


Fig. 5. Mass spectrum of the pentafluoropropionyl derivative of prenalterol. Finnigan MAT 44S, gas chromatography inlet, electron impact, 75 eV.

A chromatogram obtained from analysing an authentic plasma sample is shown in Fig. 6.

Efforts were made to adapt the chemical ionization technique to the quantitation but, although the fragmentation favoured the formation of heavier ions (Fig. 7), these ions were produced in too low yields to give any advantage over the electron-impact ionization technique. Table II shows the relative yield with three of the most commonly used gases in chemical ionization (methane, isobutane and ammonia) compared to electron impact. The mass spectrometer was focused on the most abundant ion in each of the mass spectra (Fig. 7).

Using the electron-impact technique and focusing on $m/z$ = 366, standard

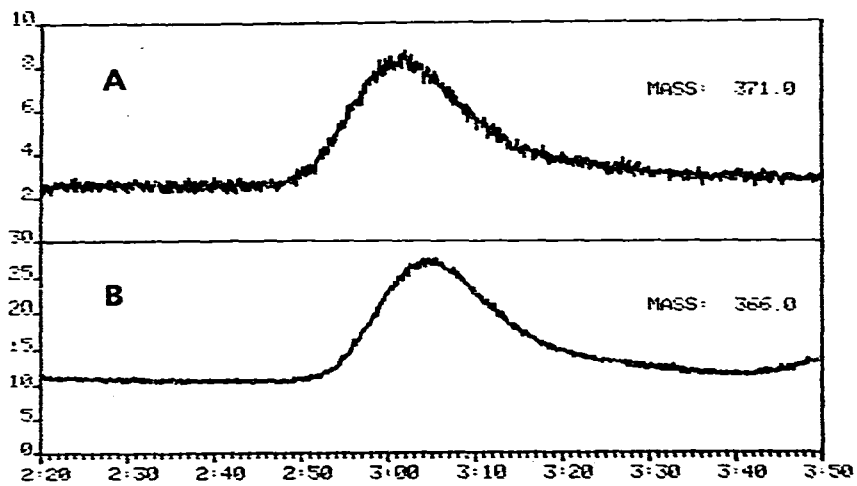


Fig. 6. Selected ion monitoring of the pentafluoropropionyl derivatives of prenalterol (B) and deuterated prenalterol (A). The amount injected on the column corresponds to 4.2 pmol of prenalterol and 2.0 pmol of deuterated prenalterol.

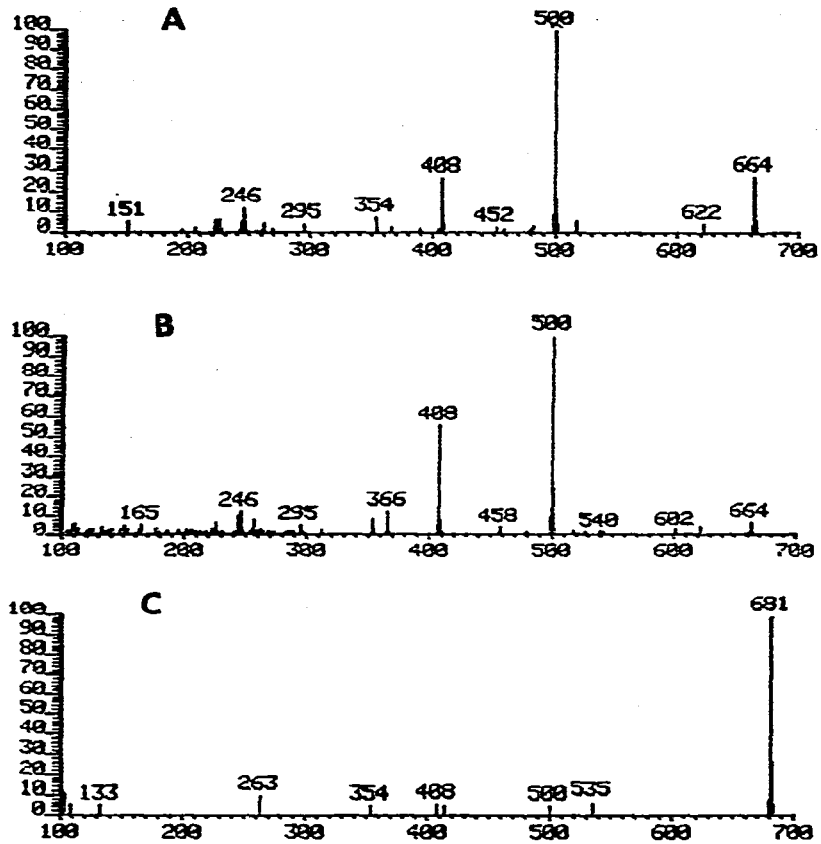


Fig. 7. Chemical-ionization mass spectra of the pentafluoropropionyl derivative of prenalterol with methane (A), isobutane (B) and ammonia (C) as ionization gas.

TABLE II

COMPARISON BETWEEN THE INTENSITY OF THE MOST ABUNDANT ION IN THE MASS SPECTRUM OF THE PFPA DERIVATIVE OF PRENALTEROL USING ELECTRON IMPACT AND CHEMICAL IONIZATION

| Ionization mode* | Relative intensity | *m/z* |
|---|---|---|
| EI | 1.00 | 366 |
| CI—methane | 1.00 | 500 |
| CI—isobutane | 0.25 | 500 |
| CI—ammonia | 0.03 | 681 |

*EI = electron impact; CI = chemical ionization.

curves were constructed by analysing plasma and urine samples to which known amounts of prenalterol had been added. The standard curves constructed were straight and passed through the origin, indicating no interferences.

The precision of the method was studied in the concentration range 0—400 nmol/l. The relative standard deviation was 2% in the concentration range

400—100 nmol/l and below 10% down to a concentration of 5 nmol/l of sample. This level (5 nmol/l) was defined as the minimum determinable concentration when 1 ml of sample was used. Long-term inter-assay variation of the method was studied in combination with a stability test for the storage of plasma samples at —18°C. Plasma samples with an added amount of prenalterol to give a concentration of 72 nmol/l were kept at —18°C until the day of analysis. Over a period of six months 60 separate analyses were carried out. The results gave a relative standard deviation of 3.2% at a constant level throughout the period. Regression analysis gave a slope of —0.0028 nmol per l per day.

The method has also been compared with a liquid chromatographic method with electrochemical detection [8]. Thirty-eight authentic plasma samples with concentrations of prenalterol ranging from 5 to 120 nmol/l were assayed by both methods, giving a quotient of 0.99 between the results, and a relative standard deviation of 9%.

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