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ELECTRON-CAPTURE—GAS CHROMATOGRAPHIC DETERMINATION OF ATENOLOL IN PLASMA AND URINE, USING A SIMPLIFIED PROCEDURE WITH IMPROVED SELECTIVITY

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SUMMARY

A sensitive gas chromatographic method for the quantitative analysis of atenolol in human plasma and urine is described. Atenolol is extracted with dichloromethane containing heptafluorobutanol to improve the extraction ability. Derivatization with trifluoroacetic anhydride in diethyl ether gives a bistrifluoroacetyl derivative which is more selectively detected by an electron-capture detector than is the corresponding heptafluorobutyryl derivative. The method allows determination down to 20 nmol/l (5 ng/ml) in 1 ml of sample with a relative standard deviation below 10%.

INTRODUCTION

Atenolol is a selective β_1-adrenergic receptor antagonist with an acetamide substituent. This hydrophilic group contributes to a low distribution ratio for atenolol between an aqueous phase and organic solvents, and extraction procedures using 45—70% of 1-butanol in the organic phase have been suggested [1—4]. Purification by pre-extraction followed by extraction with ethyl acetate has also been used [5]. However, such extraction solvents will dissolve a considerable amount of water and the co-extraction of interfering substances will be extensive. Assay methods for atenolol in biological fluids have been based on either gas chromatography (GC) with electron-capture detection [1, 2, 5] or liquid chromatography with fluorometric detection [3, 4, 6].

In the GC methods for atenolol either heptafluorobutyric anhydride [1, 2] or pentafluoropropionic anhydride [5] is used in the derivatization procedure. Without special precautions, such as back-extraction, these reagents will give high background signals, resulting in difficulties in obtaining accuracy and reproducibility at low atenolol levels. The method described includes a single efficient batch extraction with heptafluorobutanol in dichloromethane and a more selective acylation reaction with trifluoroacetic anhydride.

EXPERIMENTAL

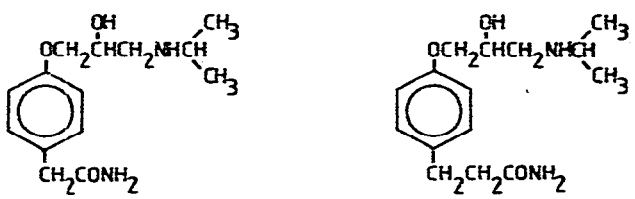
Apparatus

A Hewlett-Packard 5700 gas chromatograph equipped with a pulse-modulated ^{63}Ni electron-capture detector and an 1-mV recorder was used for the analysis. The glass column (2 m × 2 mm I.D.) was filled with 3% OV-1 on Gas-Chrom Q (100—120 mesh) (Applied Science Labs., State College, PA, U.S.A.) and operated at 180°C. An injector temperature of 200°C and a detector temperature of 300°C were chosen. The flow-rate of the carrier gas (argon with 5% methane) was 30 ml/min.

A Varian MAT 112 gas chromatograph—mass spectrometer was used to characterize the derivatives of atenolol and of the internal standard.

Reagents and chemicals

Dichloromethane, diethyl ether and toluene, obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.) were purified by distillation. 1H,1H-Heptafluorobutanol was purchased from Bristol Organics (Bristol, Great Britain). Trifluoroacetic anhydride (Fluka, Buchs, Switzerland) was purified by distillation and stored at −20°C. Sodium chloride (pro analysi from Merck, Darmstadt, G.F.R.) was baked at 500°C for 8 h. Atenolol and the internal standard, H 155/87 (Fig. 1), were supplied by the Department of Organic Chemistry, AB Hässle. Standard solutions of atenolol and the internal standard were prepared in dilute hydrochloric acid (0.01 mol/l) to produce working standard solutions with concentrations of 6 μmol/l.



ATENOLOL H 155/87

Fig. 1. Molecular structures of atenolol and the internal standard, H 155/87.

Glassware

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

Determination of distribution ratio

Distribution ratios (D) for atenolol between aqueous buffer solutions (pH 11—12, I = 0.10) and dichloromethane were determined by shaking in centrifuge tubes for 30 min at 25°C. The distribution constant was found to be 0.26. Solid sodium chloride was added to the aqueous phase in some of the studies and varying percentages of heptafluorobutanol to the dichloromethane phase

in others. The concentration of atenolol was determined photometrically in the aqueous phase before and after equilibration, the concentration of atenolol in the organic phase being calculated from the difference. Correction was made for the increase in volume of the aqueous phase after addition of sodium chloride. The results are shown in Figs. 2 and 3.

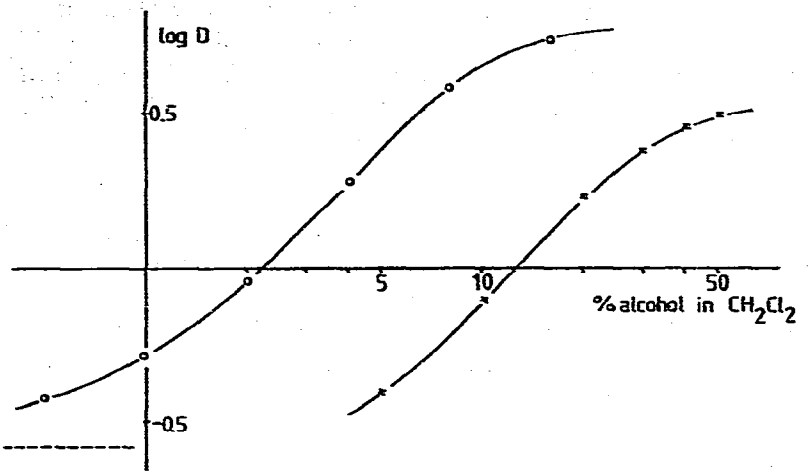


Fig. 2. Distribution ratio (D) of atenolol. Organic phase: dichloromethane containing varying amounts of 1-butanol (×) or heptafluorobutanol (○). Aqueous phase: carbonate buffer solution, pH = 11, I = 0.10. The dotted line represents the distribution ratio in the absence of added alcohol.

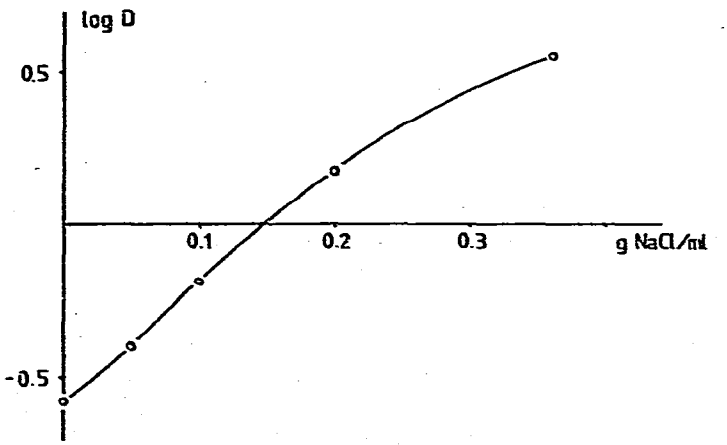


Fig. 3. Distribution ratio (D) of atenolol between dichloromethane and an aqueous phase (sodium hydroxide 0.1 mol/l) containing sodium chloride.

Analytical procedure

Urine or plasma (0.1—1.0 ml) was transferred to a 15-ml centrifuge tube (fitted with a PTFE-lined screw cap) containing 100 µl (0.6 nmol) of the internal standard solution and 0.5 g of sodium chloride. Sample volumes of less

than 1.0 ml were corrected by adding water. The aqueous phase was made alkaline by adding 50 μl of a sodium hydroxide solution (2 mol/l) followed by 50 μl of phosphate buffer (pH = 12, I = 2) and extracted with 10 ml of dichloromethane containing 3% (v/v) of heptafluorobutanol. After shaking for 10 min and centrifuging, the organic layer was transferred to a second screw-capped tube and evaporated to dryness at 40°C under a gentle stream of dry nitrogen. The residue was dissolved in 500 μl of diethyl ether and 40 μl of trifluoroacetic anhydride was added. The reaction mixture was allowed to stand for 15 min at room temperature and then evaporated to dryness under a gentle stream of dry nitrogen at room temperature. The residue was dissolved in 300 μl of toluene and 2 μl were injected into the gas chromatograph.

Quantitation

Three reference samples were prepared by adding 100 μl of the atenolol working standard solution (6 μmol/l) to 1 ml of blank plasma or urine. These samples were analysed as described above. The peak height ratio of the atenolol derivative to the internal standard derivative was calculated for each chromatogram. The average of the peak height ratios for the reference samples was used for the quantitative evaluation of the authentic samples.

RESULTS AND DISCUSSION

Extraction

As an alternative to 1-butanol, Hartvig et al. [7] have suggested the addition of heptafluorobutanol to the extraction solvent. Heptafluorobutanol added to dichloromethane improved the distribution ratio of atenolol significantly, as shown in Fig. 2. Heptafluorobutanol, 6% (v/v), in dichloromethane gave about the same distribution ratio as 50% 1-butanol. Using our method, 3% heptafluorobutanol in dichloromethane (D = 1.35) will give an extraction degree of about 92% with a phase volume ratio (V_{org}/V_{aq}) of 8.

The extraction of atenolol into dichloromethane can also be improved by the addition of sodium chloride to the aqueous phase (Fig. 3). By saturation of the aqueous phase with sodium chloride (0.36 g/ml) and with a phase volume ratio of 8, 96.5% of atenolol will be extracted into the organic phase.

Combining the addition of sodium chloride with the presence of heptafluorobutanol in the dichloromethane phase as proposed in the method will give a quantitative extraction of atenolol (> 99%). The shaking procedure should be performed with care as the presence of heptafluorobutanol in the organic phase in combination with sodium chloride in the aqueous phase may otherwise cause formation of an emulsion that makes extraction more difficult. Since the internal standard H 155/87 contains one methylene group more than atenolol (Fig. 1), the extraction of this substance will always be better.

Derivatization

Amines extracted from the biological sample will undergo acylation when treated with perfluorated anhydrides. The electron-capture response of such derivatives is very dependent on the type of anhydride used. Mono-derivatives of trifluoroacetic anhydride are between 100 and 1000 times less sensitive to

electron-capture detection than derivatives of heptafluorobutyric anhydride, as reported by Ervik et al. [8]. However, di-derivatives of the same type as acylated atenolol are of almost equal sensitivity irrespective of the character of the perfluoroanhydride used (Walle and Ehrsson [9]). The use of trifluoroacetic anhydride as acylating agent will thus increase the selectivity of the method for atenolol and related compounds compared to most co-extracted interfering amines.

Gas chromatograms from analysed plasma samples (Fig. 4) demonstrate the advantage of trifluoroacetic anhydride over heptafluorobutyric anhydride with regard to the extent of interfering peaks. The derivatization reaction is performed according to the method by Scales and Copsey [1]. No increase in the relative formation of atenolol derivative was achieved by using another solvent or by adding a catalyst such as triethylamine. In the studies of the reaction conditions, a known amount of 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane

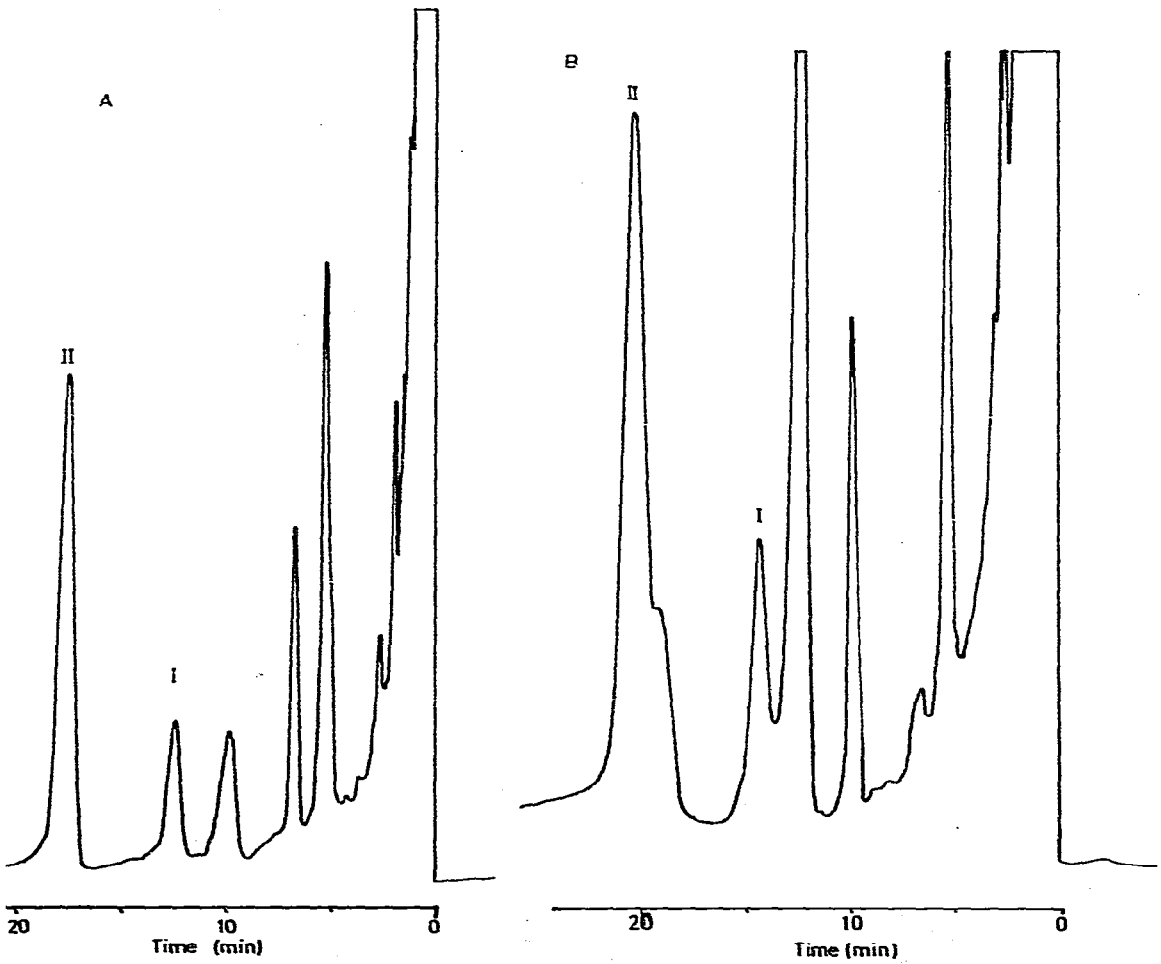


Fig. 4. Gas chromatograms obtained by analysing the same plasma sample by using (A) trifluoroacetic anhydride and (B) heptafluorobutyric anhydride as the derivatizing reagent. Peaks: I = atenolol (0.3 μmol/l) and II = internal standard.

(DDT) was added to the solution before injection as an internal marker. In instances where the catalyst was present, the resulting reaction mixture was purified according to the method of Walle and Ehrsson [10]. The influence of the anhydride concentration in diethyl ether was also examined and the results are shown in Fig. 5. The highest relative recovery is obtained when the concentration of the anhydride is about 8% (v/v). The formation of the derivative is completed within 15 min at room temperature. The derivative is stable in toluene for several days. Acylation of the amide group results in the formation of a nitrile, as shown by Scales and Copsey [1]. The structure of the derivative was confirmed by mass spectrometry (Fig. 6).

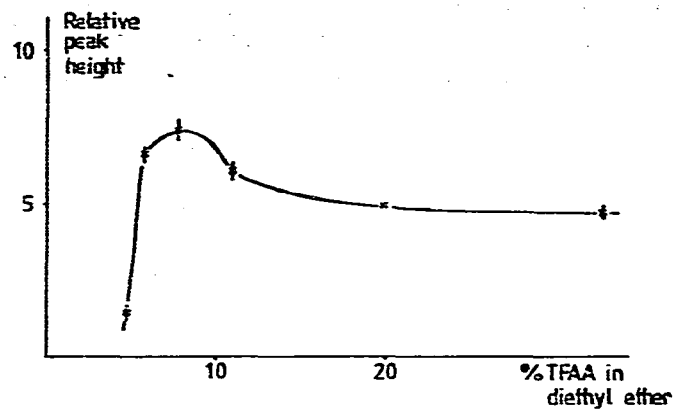


Fig. 5. Influence of trifluoroacetic anhydride (TFAA) concentration on the formation of the trifluoroacetyl derivative of atenolol. Reaction conditions: time, 15 min and room temperature.

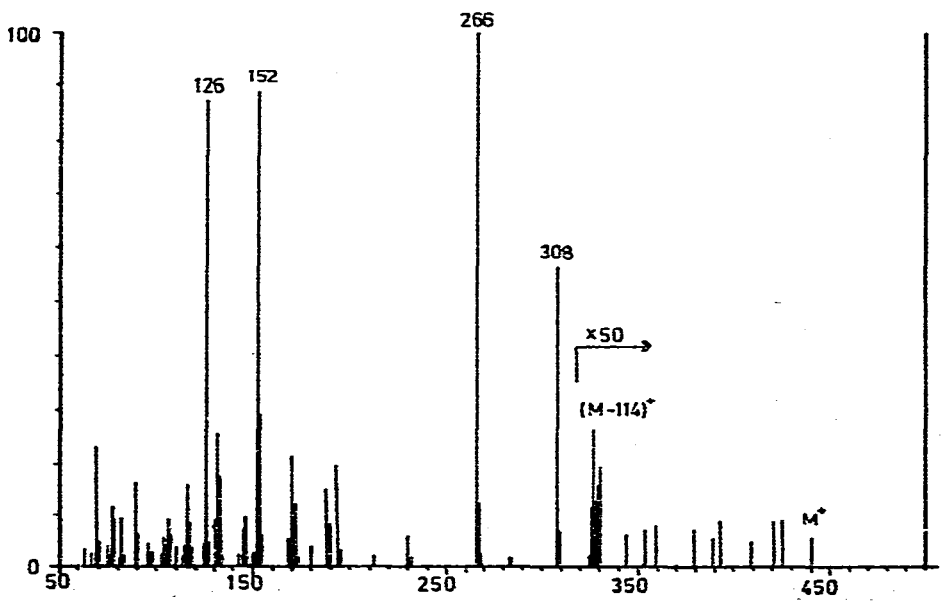


Fig. 6. Mass spectrum of the trifluoroacetyl derivative of atenolol. Varian MAT 112, GC inlet, electron impact, 60 eV.

Quantitative evaluation

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

The precision of the method was studied within the concentration range of 1.3 μmol/l to zero. The relative standard deviation was below 10% down to a concentration of 20 nmol/l of sample, and this level was defined as the minimum determinable concentration when using 1 ml of sample.

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