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Note

Determination of metoprolol in plasma and urine using high-resolution gas chromatography and electron-capture detection

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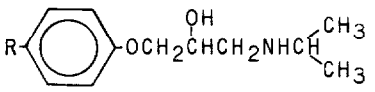
Metoprolol (Fig. 1), a selective β_1-adrenergic receptor antagonist, has been determined in biological samples by gas chromatography [1—9] and by liquid chromatography [10—21]. When aiming at the lower limit of determination without access to a mass spectrometric detector, selectivity has to be achieved by other means to increase the accuracy in the determination. Improved accuracy and sensitivity in the determination of β-adrenoceptor antagonists using a capillary column have been demonstrated by DeBruyne et al. [22] in the determination of oxprenolol. The selectivity improvement by using a capillary column for the determination of metoprolol has been shown by Gyllenhaal and Hoffmann [8].



Metoprolol R= $CH_2CH_2OCH_3$

Int.st.(H 93/47) R= $CH_2CH_2OCH_2CH_3$

Fig. 1. Structure of metoprolol and internal standard.

The purpose of this work was to adapt the method used in our laboratory [1] to the more sensitive and selective capillary gas chromatography technique with electron-capture detection.

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EXPERIMENTAL

Apparatus

A Hewlett-Packard 5700 gas chromatograph equipped with a pulse-modulated ^{63}Ni electron-capture detector and a split—splitless injector for capillary columns was used. The injector was operated in the split mode with a preset split ratio of 1:10 at 250°C. Detector temperature: 300°C.

The column was made of fused-silica tubing (25 m × 0.32 mm I.D.), persilylated at 400°C and coated with methyl—phenyl polysiloxane (10% phenyl). Helium was used as carrier gas with an inlet pressure of 1.4 bar, giving a linear velocity of 35 cm/s. Make-up gas was a mixture of argon—methane (95:5) with a flow-rate of 20 ml/min. Column temperature was held isothermally at 170°C until the sample and the internal standard were eluted, and then raised to 250°C to force out late-eluting components. Injection was performed by a Hewlett-Packard 7671A autosampler.

Reagents and chemicals

Hexane, dichloromethane and toluene, obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.), were purified by distillation. The extraction solution was prepared by mixing four volumes of hexane with one volume of dichloromethane. Trifluoroacetic anhydride (TFAA) was purchased from Fluka (Buchs, Switzerland) and purified by distillation. Metoprolol and the internal standard (H 93/47, Fig. 1) were both obtained from the Department of Organic Chemistry, AB Hässle, Mölndal, Sweden. Standard solutions of metoprolol and the internal standard were prepared in dilute hydrochloric acid (0.01 mol/l) to produce working standard solutions with concentrations of 7 μmol/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 120°C.

Analytical procedure

Urine (0.1 ml) or plasma (2.0 ml) samples were transferred to a 15-ml centrifuge tube (fitted with a PTFE-lined screw cap). Sample volumes of < 2 ml were adjusted by adding water. A 100-μl volume of the internal standard solution was added. The mixture was made alkaline by adding 0.3 ml of a sodium hydroxide solution (1 mol/l) and extracted with 5.0 ml of the hexane—dichloromethane mixture (4:1). After shaking for 10 min and centrifuging, the organic layer was transferred to a second screw-capped tube and 100 μl of TFAA were added. The reaction mixture was held at 40°C for 45 min and then evaporated to dryness under a gentle stream of dry nitrogen at room temperature. The residue was dissolved in 200 μl of toluene and 3 μl were injected into the gas chromatograph.

Quantitation

Five reference samples were prepared by adding 100 μl of the metoprolol standard solution (7 μmol/l) to 2.0 ml of blank plasma (or 0.1 ml of urine).

These samples were then analysed according to the analytical procedure. The peak-height ratio of the metoprolol derivative over the internal standard derivative was calculated for each chromatogram. The median value of the peak-height ratios for these five reference samples was used for the quantitative evaluation of the authentic samples.

RESULTS AND DISCUSSION

Extraction

Benzene was found suitable as extracting solvent for metoprolol [1], but is not appropriate in routine laboratory work for reasons of health protection. The use of dichloromethane, ethyl acetate or diethyl ether as solvent necessitates the use of a highly selective mass spectrometric detector [5] or a back-extraction step [3, 4]. The use of a mass spectrometer as a gas chromatographic detector for routine analysis is an expensive technique and not as widely available as the electron-capture detector.

When the number of samples to be analysed exceed about 5000 annually, every step in the analytical procedure has to be thoroughly scrutinized. We have found that the back-extraction procedure is too laborious in this respect and that a careful selection of extraction solvent and derivatization reagent could give chromatograms sufficiently clean for determinations in the low nanogram region. For this reason we used a mixture of hexane—dichloromethane (4:1), giving a distribution ratio of 6.5 and, according to the proposed analytical procedure, a theoretical extraction recovery of 96%. The actual recovery from the plasma sample was determined to be 91% using tritiated metoprolol.

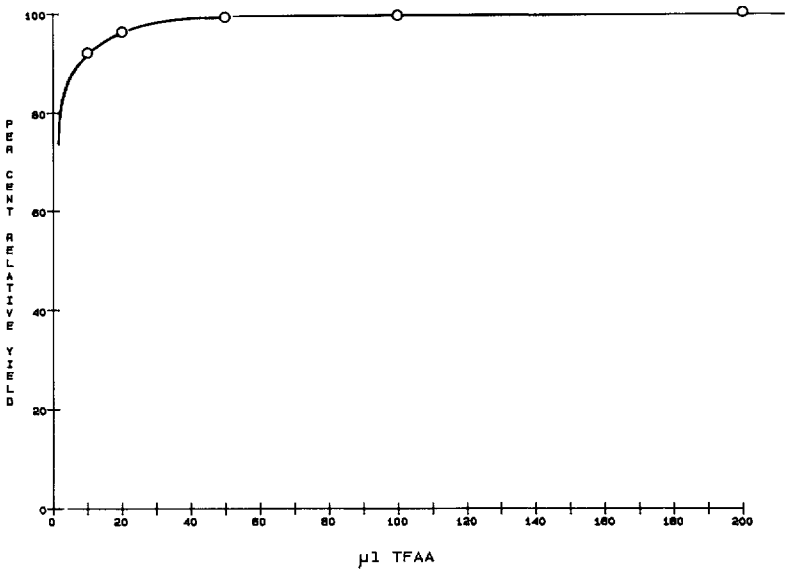

Fig. 2. Influence of the amount of TFAA added to the separated organic layer on the formation of the trifluoroacetyl derivative. Reaction conditions: 45 min at 40° C. The concentration of metoprolol in the organic layer was 73 nmol/l.

Derivatization

As pointed out above, it is of great value to choose a selective derivatization reagent in order to decrease interference in the chromatograms. The advantage of using TFAA as compared to heptafluorobutyric anhydride has been discussed previously for the determination of atenolol [23]. Those arguments are also valid for metoprolol; monoderivatives of TFAA give between 100 and 1000 times less response with electron-capture detection than monoderivatives of heptafluorobutyric anhydride. The diderivatives, on the other hand, provide almost equal sensitivity irrespective of the character of the perfluoroanhydride used.

Derivatization is performed directly in the organic phase by adding TFAA. The relation between trifluoroacetylation and the concentration of the reagent is shown in Fig. 2. The influence of the reaction temperature and the reaction time on the acylation is shown in Fig. 3.

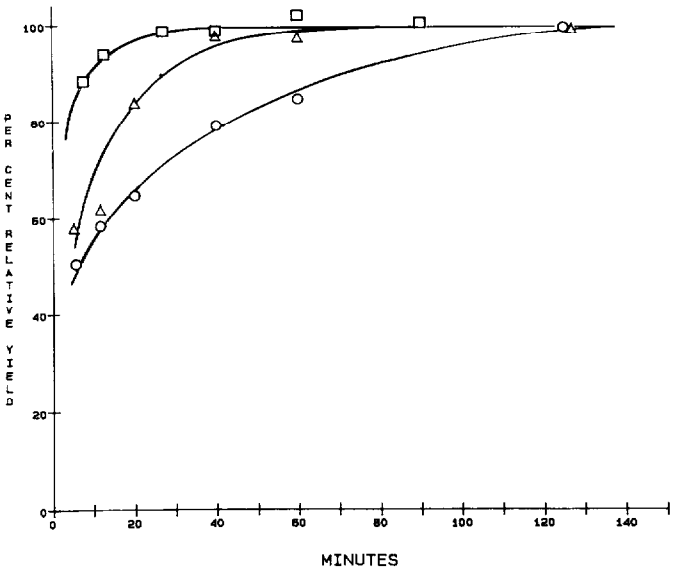

Fig. 3. Formation of the trifluoroacetyl derivative of metoprolol at different reaction temperatures versus time. A 50-μl volume of TFAA was added to the organic layer. The concentration of metoprolol in the organic layer was 73 nmol/l. (○) 22° C; (△) 32° C; (□) 42° C.

Gas chromatograms from analysed authentic plasma samples (Figs. 4 and 5) demonstrate the absence of interfering peaks.

As a rule, concentrations of metoprolol in urine are much higher than in plasma samples drawn at the same time; only 100 μl of the urine sample are used in this assay. Gas chromatograms from such samples are also free from interfering peaks, as demonstrated in Fig. 6.

Chromatography

Two of the main metabolites, O-desmethylmetoprolol and α-hydroxymetoprolol, have a relative retention to metoprolol of 0.96 and 1.20, respec-

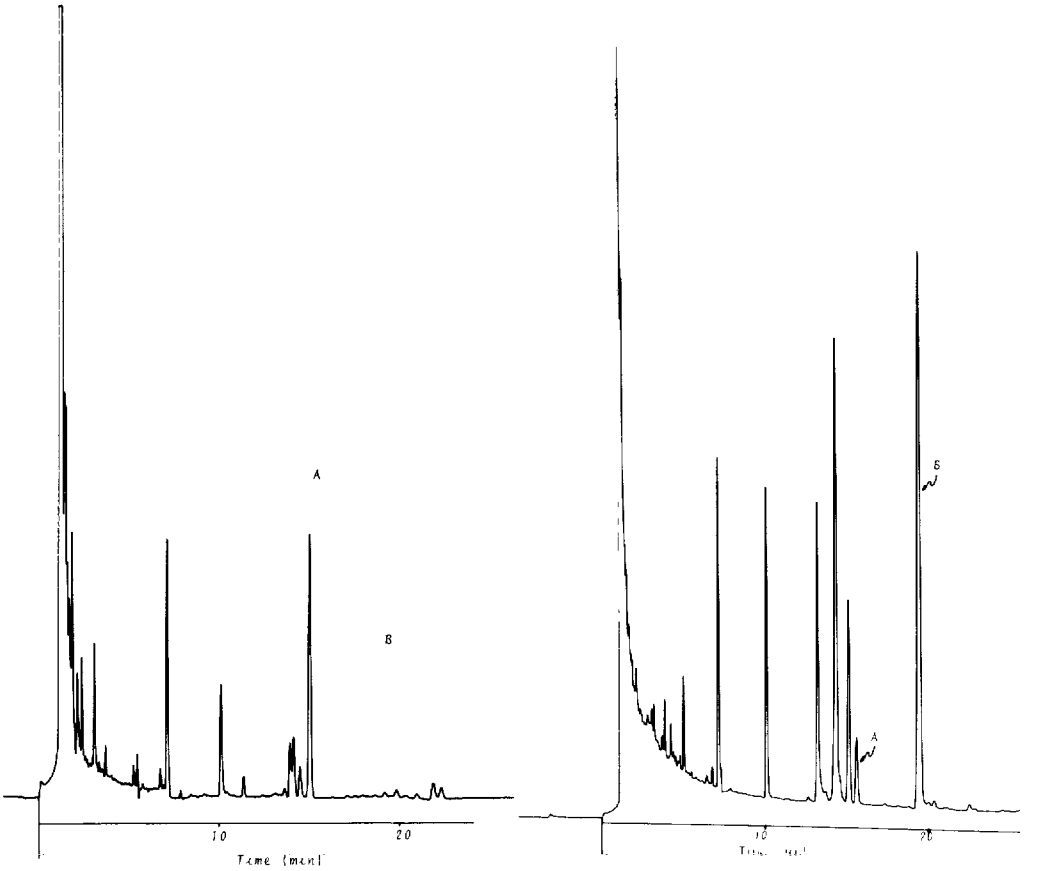

Fig. 4. Gas chromatogram obtained by analysing blank plasma. Conditions according to text. The elution positions for metoprolol and internal standard derivatives are marked A and B, respectively.

Fig. 5. Gas chromatogram obtained by analysing a plasma sample with a found concentration of 50 nmol/l metoprolol (A). Conditions according to text

tively, and do not interfere with metoprolol or with the internal standard (relative retention = 1.25). Acidic major metabolites are not co-extracted and are not present in the injected sample.

Perfluoroacyl derivatives of metoprolol and related substances have been found to be catalytically decomposed on certain glass capillary and fused-silica columns [22]. This calls for a careful selection of the capillary column. The way we select our column is to run the test procedure proposed by Ahnoff et al. [24]. Only columns deactivated by siloxane treatment or by persilylation were found to be useful.

Quantitative evaluation

Standard curves were constructed by analysing plasma and urine samples, to which known amounts of metoprolol had been added. The concentration range was 0—800 nmol/l. The precision of the method was studied within this range. The standard curves were straight and passed through the origin, which

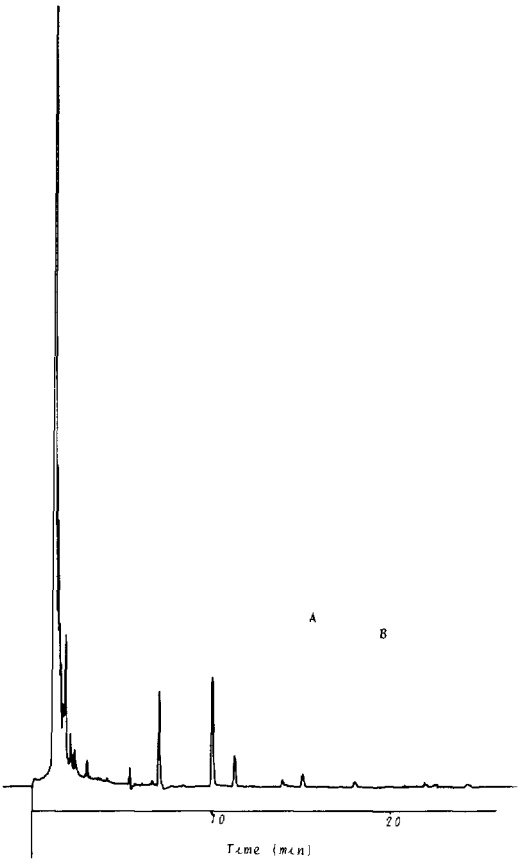

Fig. 6. Gas chromatogram obtained by analysing a urine sample (blank) Conditions according to text.

indicates no losses and no interferences. The relative standard deviation was < 10% down to a concentration of 10 nmol/l of sample when 2 g of sample were used. This concentration level was defined as the minimum determinable concentration.

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 a known concentration of metoprolol (296 nmol/l) were kept at −18°C until the day of analysis. Over a period of 4 months, 59 separate assays were carried out. The results gave a standard deviation of 4.1% at a constant level throughout the period. Regression analysis gave a slope of 0.014 nmol/l per day. The mean recovery was 104.2%.

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