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Note

Determination of deacetylmetipranolol in body fluids by gas chromatography—chemical-ionization mass spectrometry

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Metipranolol [1-4(acetoxy-2,3,5-trimethyl-phenoxy)-3-isopropyl-amino-propan-2-ol, Fig. 1a] is a β-adrenoreceptor blocking drug, known under the name Disorat® or Torrat® (Boehringer-Mannheim, Mannheim, G.F.R.) and is used as an antihypertensive agent. For therapeutic purposes it is usually administered twice a day in 10- or 20-mg oral doses. For pharmacokinetic studies a single dose of 40 mg is given. Metipranolol is very rapidly metabolized to the pharmacologically active metabolite deacetylmetipranolol (DMP). A few minutes after the oral application the metabolite is detected in serum and urine. Metipranolol could not be identified in biological fluids.

A detailed study concerning the pharmacokinetics and pharmacology of metipranolol (MP) in animals and men has been published [1—3]. β-Blocking drugs can be determined specifically with high sensitivity after derivatization with trifluoroacetic anhydride (TFAA) or heptafluorobutyric anhydride by gas chromatography, using an electron-capture detector [4—6]. A very recent publication [7] discusses the determination of biological levels of β-blocking agents using high-performance liquid chromatography.

For the determination of DMP in our laboratory, first a gas chromatographic method was developed on the basis of methods described in the literature [4—6]. This procedure worked well with pure substances. The analysis of DMP in biological materials, however, showed interfering peaks on the chromatogram which made a reliable quantitative measurement of the metipranolol metabolite impossible. Due to the high biological background a gas chromatographic methane chemical-ionization mass spectrometric method was developed in our laboratory. Chemical-ionization mass spectrometry was chosen for its ability to produce stable high-mass molecular ions of greater relative intensities

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than did electron-impact mass spectrometry, allowing the highly specific measurement of DMP with excellent sensitivity.

PRINCIPLE OF ASSAY

To serum, or urine, containing DMP a certain amount of 1,4(acetoxy-2,3,5-trimethylphenoxy)-3-D_6-isopropyl-amino-propan-2-ol (Fig. 1b) as internal standard was added. After extraction under alkaline conditions (pH $\geqslant$ 10) the organic phase is evaporated to dryness. The residue is derivatized with TFAA to give tris-trifluoroacetyl-D_0-DMP [$(TFA)_3$-D_0-DMP] and its hexadeutero analogue [$(TFA)_3$-D_6-DMP], respectively. DMP reacts in positions 1, 2 and 3.

After injection into the gas chromatograph, the mass spectrometer monitors the quasimolecular ions of $(TFA)_3$-D_0-DMP at *m/z* 556 and $(TFA)_3$-D_6-DMP at *m/z* 562 in the multiple ion detection mode. Methane is used both as the carrier gas and as the chemical ionization reactant gas.

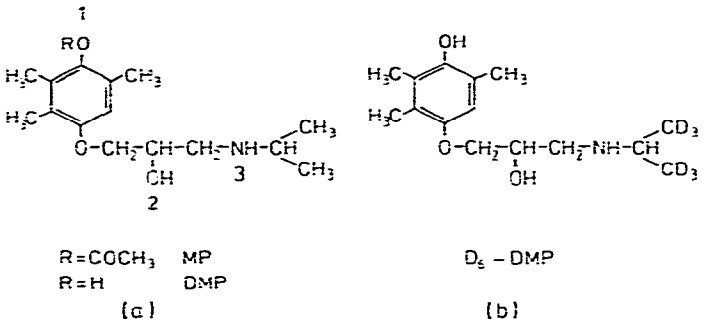

Fig. 1. Structural formulae of (a) metipranolol (MP) and deacetylmetipranolol (DMP) and (b) the internal standard.

EXPERIMENTAL

Materials

DMP and the internal standard D_6-DMP were synthesized in the research laboratories of Boehringer Mannheim and were of analytical grade. The content of D_0-DMP in D_6-DMP was $< 0.01\%$ and did not influence the assay. The solvents (Merck, Darmstadt, G.F.R.) were of analytical grade and were used without further purification. TFAA was purchased from Macherey, Nagel & Co. (Düren, G.F.R.). A solution of the ethyl ester of gallic acid in methanol (100 mg/l) was applied prophylactically to prevent autoxidation during the work-up procedure.

Gas chromatography

A Hewlett-Packard Model 5710A gas chromatograph in conjunction with a Hewlett-Packard autosampler, Model 7671 (36 samples), was used. The autosampler was controlled by a Finnigan autosequencer box and a home-built interface, which enables communication between the two devices. The Finnigan autosequencer box switches the vacuum diverter valve, the filament and the electron multiplier. The total run time for one sample was 6 min.

A glass column (180 cm × 2 mm I.D.) was packed with 3% SE-30 on Chromosorb W HP. The column was conditioned at 320°C overnight. The flow-rate of the carrier gas was adjusted to 20 ml/min. The temperature settings were 250°C for the injector and 210°C for the oven. Under these conditions $(TFA)_3$-DMP eluted with a retention time of approximately 3 min.

Interface

The gas chromatographic column was coupled via a stainless-steel needle valve to the mass spectrometer. The transfer line (glass-lined tubing) was held at a temperature of 250°C.

Mass spectrometer

A Finnigan 3300 quadrupole mass spectrometer in combination with a Finnigan Model 6100 data system was used. The instrument was run in the chemical-ionization mode with methane as reagent gas. The source pressure was maintained at 1 Torr, the electron energy at 100 eV. The emission current was 30 μA.

Extraction conditions

In order to obtain a high extraction efficiency of DMP from serum or urine a series of solvents under various pH conditons was investigated. It was found that a pH value of approximately 10 and rather polar solvents were necessary to extract DMP to an acceptable extent. In addition to that, a saturated sodium chloride solution was added to the biological fluid to enhance the recovery.

Work-up procedure

Into a 50-ml separation funnel (separation funnels were used instead of centrifuge tubes to prevent formation of emulsions) are pipetted 0.5 ml of 0.2 *M* sodium hydroxide, 1 ml of a saturated solution of sodium chloride, 15 μl of antioxidant solution, 4 ml of water, 10 ml of diethyl ether, 0.2 ml of an aqueous solution containing 0.1 mg/l D_6-DMP, 1 ml of serum (urine is diluted 1:5), and 2.5 ml of dichloromethane. The funnel is manually shaken for 1 min. After phase separation the aqueous phase is discarded. The organic phase is collected in a 10-ml centrifuge tube; 8 ml of the organic phase are transferred into another tube and evaporated to dryness under a stream of nitrogen. The residue is dissolved in 150 μl of diethyl ether, transferred into a 300-μl capacity conical vial and evaporated to dryness. After adding 25 μl of acetonitrile and 50 μl of TFAA the bottle is capped and heated for 30 min at 50°C in a metal block. After complete evaporation the sample is taken up in 50 μl of acetonitrile; 5 μl are injected into the chromatograph. The sample solutions in acetonitrile proved to be stable for at least one week.

Evaluation and calculation

Evaluation of the peak areas by electronic integration was either carried out by the Finnigan 6100 data system or by the Hewlett-Packard Lab Data System HP 3354. For calculation of the drug concentration a calibration curve was established together with each series of biological samples, using the following concentrations: serum, 100, 50, and 10 ng/ml; urine, 500, 100, 50, and 10

ng/ml. The plot of area ratios D_0/D_6-DMP against concentrations is linear in the range 2—500 ng/ml.

As an example the linear regression of a typical calibration is given below:

$$C_{D_0\text{-DMP}} = 10{,}164 \cdot A_{rel} - 0.145$$

with $r = 0.99998$.

Equations of this type were used to calculate the serum concentration of DMP (D_0-DMP) as a function of the measured relative area

$$A_{rel} = \frac{A_{D_0\text{-DMP}}}{A_{D_6\text{-DMP}}}$$

RESULTS

The chemical-ionization mass spectrum of $(TFA)_3$-D_0-DMP is shown in Fig. 2. The base peak at *m/z* 442 formed after the elimination of one molecule of trifluoroacetic acid from the quasimolecular ion is shifted in the spectrum of the internal standard by six mass units to higher mass. The ions chosen for the quantitative determination of DMP were the quasimolecular ions at *m/z* 556 and *m/z* 562 for the internal standard.

A mass chromatogram obtained from human plasma containing the internal standard in the amount of 20 ng/ml is shown in Fig. 3a. In none of the investigated blank serum samples were interfering peaks observed. An ion-

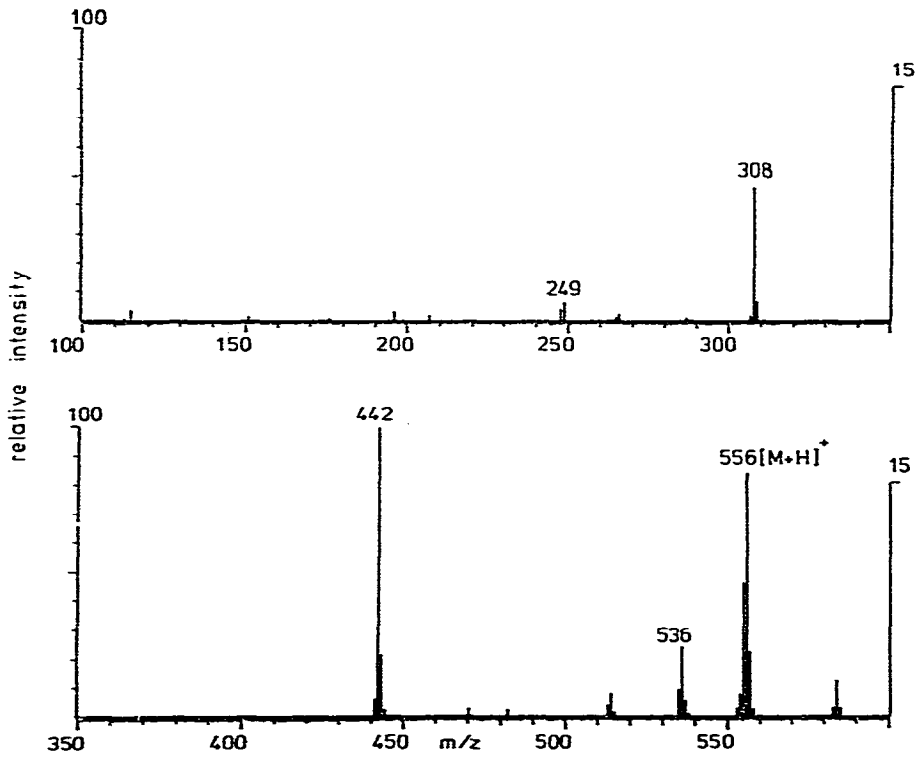

Fig. 2. Chemical-ionization mass spectrum of the TFA derivative of D_0-DMP.

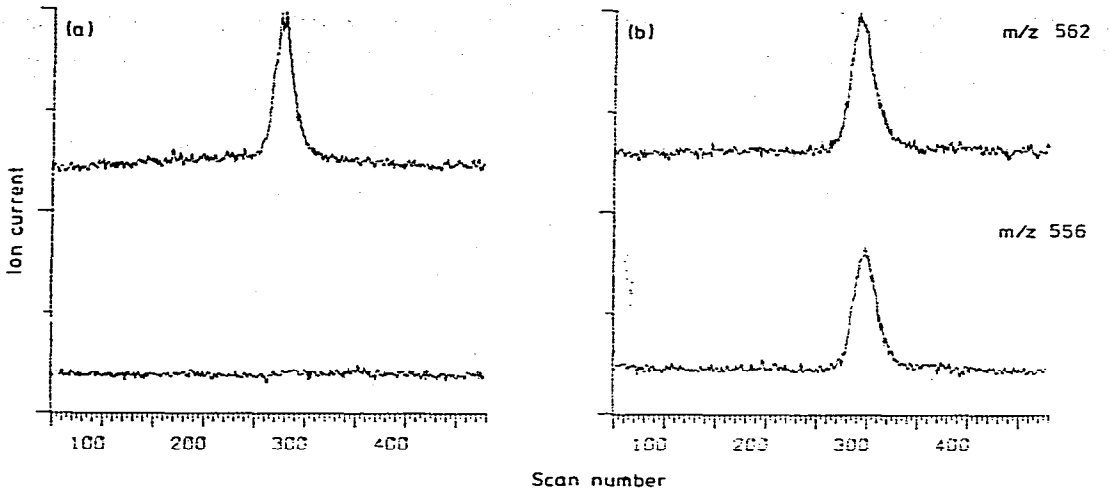

Fig. 3. Selected ion monitoring of derivatized serum extract, recording D_0-DMP (m/z = 556) and D_6-DMP (m/z = 562). (a) Blank spiked with 20 ng/ml D_6-DMP; (b) containing 10 ng/ml D_0-DMP and 20 ng ml D_6-DMP.

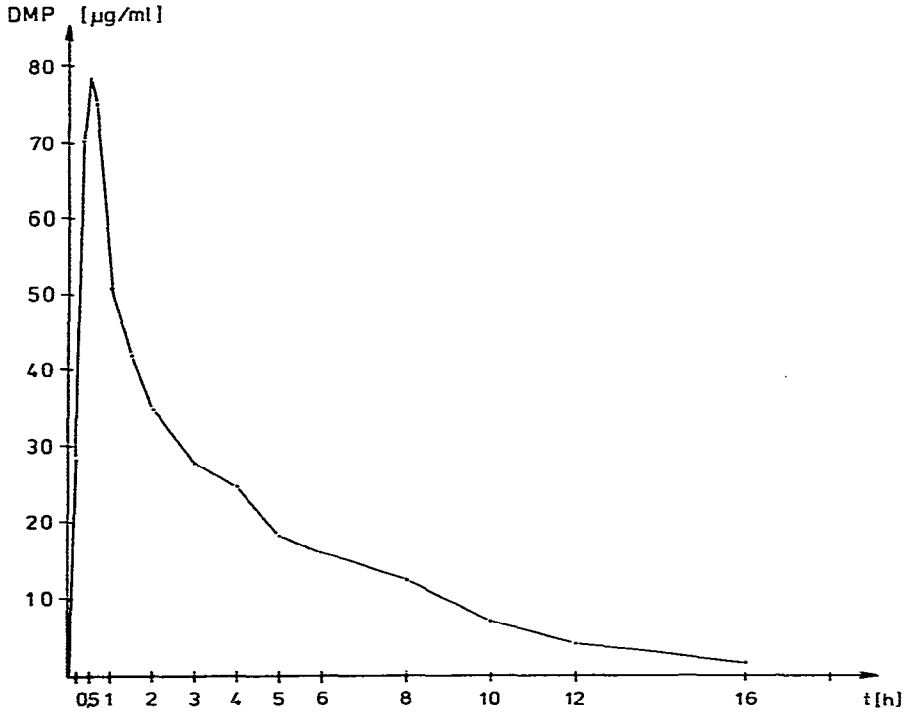

Fig. 4. Serum concentration—time curve of DMP after an oral dose of 40 ng of MP to a healthy volunteer.

monitor recording of a 10 ng/ml DMP human plasma sample spiked with 20 ng/ml D_6-DMP is shown in Fig. 3b.

The detection limit was found to be in the 1—2 ng range using 1-ml sample volumes. In this range the quantitative measurements were still reliable enough

for pharmacokinetic studies. The development of a method to measure concentrations in the range below 1 ng/ml, which seems to be necessary for further kinetic studies applying different mass spectrometric techniques, is proceeding in our laboratories.

An example of a pharmacokinetic profile of DMP after application of an oral dose of 40 mg of metipranolol to a healthy volunteer is presented in Fig. 4. The peak plasma concentration was 78 ng/ml 0.6 h after application.

The assay was routinely applied for more than two years and some thousands of biological samples were analyzed with good results. Under these conditions the average life-time of a column is between 4 and 6 weeks. Cleaning of the ion source was necessary in the same time interval.

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