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Note

Determination of metoprolol in plasma and urine by gas—liquid chromatography with electron-capture detection

C.D. KINNEY

Department of Therapeutics and Pharmacology, The Queen's University of Belfast, The Whitla Medical Building, 97 Lisburn Road, Belfast BT9 7BL (Northern Ireland)

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With the increasing use of β-adrenoreceptor blocking agents in the treatment of disease, there is a need for sensitive, trouble-free techniques for the measurement of plasma and urine concentrations of these drugs. Methods have been described for the determination of metoprolol but these have various problems and disadvantages. The method described by Ervik [1] required 2.0—4.0 ml of plasma, used benzene as an extraction solvent and also required 30 min per sample for chromatography, allowing only a very small number of samples to be analysed daily. It is here that the advantage of using a good extraction procedure prevails, and the method described here, in addition to allowing the analysis of 40 samples (manual injection) per day, requires only 250—500 μl of plasma and is trouble-free and sensitive with levels of metoprolol detectable down to 3.0 ng/ml, which is adequate for pharmacological studies with the drug in man. Although metoprolol is metabolized, it has been shown [2] that the four metabolites are of no pharmacodynamic significance in man and, as they do not interfere with the assay (possibly owing to the small sample size

TABLE I

MEAN PLASMA LEVELS OF METOPROLOL (ng/ml) IN TWO VOLUNTEER GROUPS RECEIVING A 50- AND A 400-mg ORAL DOSE

Dose (mg)	Time after dose (h)										
	1	1.5	2	3	4	6	8	10	24	33	48
50	56	73	55	51	28	25	20	92	4	3	0
400	538	521	479	351	291	232	152	86	10	5	0

and short derivatization time), the question of metabolites need not be considered. Table I gives some results of work carried out on metoprolol in this department using this method for the analysis of plasma samples [3].

EXPERIMENTAL AND RESULTS

Chemicals

Sodium hydroxide solution (10 *M*), hydrochloric acid (0.1 *M*), ethyl acetate (AnalaR, once distilled; Hopkin & Williams, Chadwick Heath, Great Britain), methanol (pro analysi; May & Baker, Dagenham, Great Britain) and trifluoroacetic anhydride (TFAA) (Phase Separations, Queensferry, Great Britain) were used. The extraction solvent was diethyl ether (anaesthetic grade; May & Baker)—dichloromethane (G.P.R. grade, once distilled) (2:1).

Methods

All extraction tubes should have tops that contain a paper-based liner (Searle Diagnostics, High Wycombe, Great Britain), as PTFE and rubber-based liners can produce interferences when used with electron-capture-sensitive derivatizing agents.

Derivatization was carried out in ground-glass-stoppered, conical-bottomed tubes that had beek soaked in methanol for at least 15 min to ensure cleanliness and dryness.

Plasma, urine, sodium hydroxide solution, ethyl acetate, standards and TFAA were all transferred using automatic pipettes that had been adapted to take glass Pasteur pipettes in preference to ordinary tips, which contain materials that should be avoided.

The extraction solvent and the hydrochloric acid were added using glass burettes, ensuring that the tap was made of glass and not PTFE.

Chromatography

A Perkin-Elmer F17 gas chromatograph fitted with a 10-mCi ^{63}Ni electron-capture detector and a glass column (0.5 m × 4 mm I.D.) packed with 3% OV-17 on Gas-Chrom Q (80—100 mesh) was used.

The instrument parameters were as follows: oven temperature, 200°C; injector and detector temperatures, 300°C; pulse, 5; range, 1; attenuation, 256; and carrier gas, argon at a flow-rate of 230 ml/min.

The reasons for using such a high carrier gas flow-rate are as follows: (1) better resolution of peaks can be achieved than with a higher column temperature and a lower flow-rate; (2) improved sensitivity (same reason as above); see Figs. 2 and 3); and (3) the detector is being continuously purged and is therefore less likely to become contaminated.

Extraction

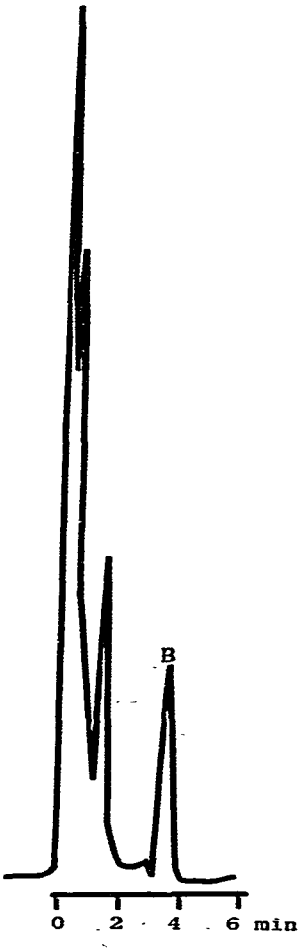
After allowing the deep-frozen plasma or urine sample to thaw at room temperature, 250—500 μl of sample are transferred into the extraction tube, then 100 μl of internal standard are added and the tube is vortexed for 3 sec (all subsequent vortexing is for 3 sec) prior to adding 100 μl of 10 *M* sodium hydroxide solution and vortexing. A 4.0-ml volume of extraction solvent is

added, the tube is vortexed, shaken for 10 min, centrifuged at 1500 *g* for 15 min, then 3.0 ml of supernatant are added to 3.0 ml of 0.1 *M* hydrochloric acid.

After vortexing, the tube is shaken for 10 min and centrifuged at 1500 *g* for 5.0 min, as phase separation is much easier than previously. After centrifugation, the supernatant is aspirated by vacuum and discarded, and the aqueous layer is made alkaline by addition of 100 µl of 10 *M* sodium hydroxide solution, followed by vortexing.

A 4.0-ml volume of extraction solvent is then added and the tube is vortexed and shaken for 10 min. Subsequently, the tube is centrifuged at 1500 *g* for 5.0 min, then 3.0 ml of supernatant are transferred into a ground-glass-stoppered conical-bottomed tube. This solvent is removed with a stream of nitrogen in a water-bath at 40°C.

After ensuring that all solvent has been removed, 100 µl of TFAA are added,



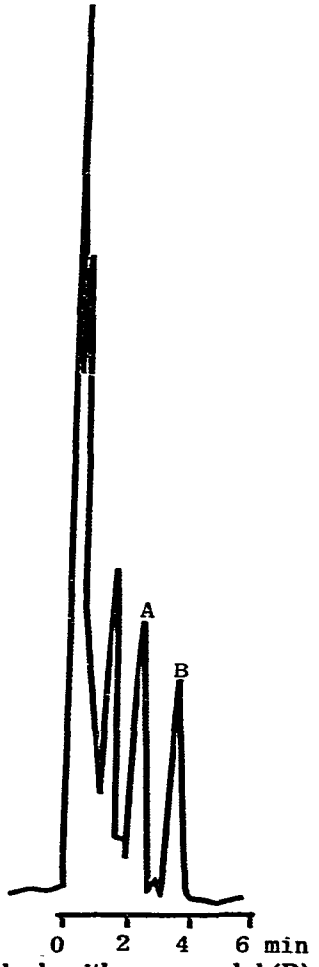


Fig. 1. Chromatogram obtained from a plasma blank with propranolol (B) added as internal standard.

Fig. 2. Chromatogram obtained from a patient plasma sample containing 295 ng/ml of metoprolol (A) and propranolol (B) added as internal standard. Conditions as in text.

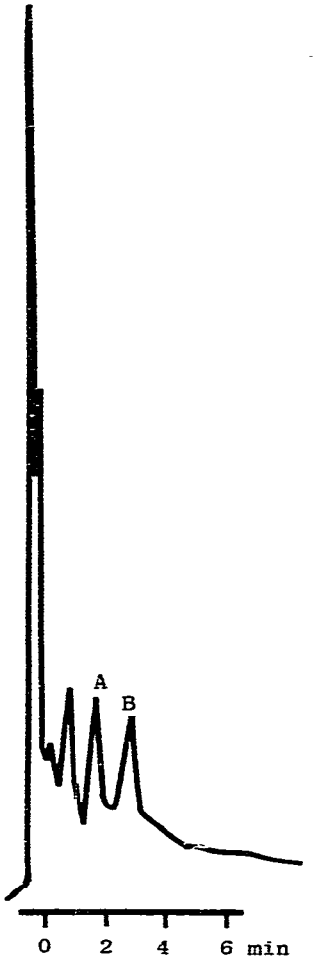


Fig. 3. Same injection as in Fig. 2, except oven temperature 230°C and carrier gas flow-rate 80 ml/min.

followed by 100 μl of ethyl acetate. The tube is then tightly stoppered, vortexed and allowed to react at 40°C for 20 min. After derivatization is complete, the tube contents are evaporated to dryness with a stream of nitrogen in a water-bath at 40°C and the residue is dissolved in 50 μl of ethyl acetate. Finally, the tube is vortexed and 1 μl of solution is injected onto the gas chromatographic column.

Typical chromatograms for plasma extracts are shown in Figs. 1 and 2 (similar chromatograms are obtained for urinary extracts).

Standards

Metoprolol. A stock solution of 1 mg/ml of metoprolol in methanol was prepared. Included in each run of patient plasma samples was a set of pooled plasma samples, to each of which was added a known amount of metoprolol (0, 50, 100, 200 and 400 ng/ml). These samples were treated in the same way as those in the test run.

Internal standard. A stock solution of 1 mg/ml of propranolol in methanol was prepared, and this was added to give a concentration of 100 ng/ml in plasma.

Calculation

To calculate the level of metoprolol in samples from patients, a calibration graph of peak-height ratio versus concentration of known standard was drawn. The graph was linear in the range 0—400 ng/ml ($r = 0.99$).

Precision

To test for fluctuation of the detector response, a single sample (230 ng/ml) was injected at five different times over a working day and the results obtained showed a mean peak-height ratio of 0.87 (standard deviation 0.03) with a coefficient of variation of 3.4%.

In order to test the reproducibility of the method, 5.0 ml of pooled plasma were spiked with 200 ng (unknown to the analyst) of metoprolol and the sample was divided into five 1.0-ml fractions and re-frozen. These fractions were analysed on different occasions over 9 days and the results obtained showed a mean level of 40.6 ng/ml (standard deviation 3.36) with a coefficient of variation of 8.2%.

DISCUSSION

Extraction

Several methods have been described for the determination of metoprolol and other β-blocking drugs and each uses a different extraction solvent. Degen and Riess [4] used diethyl ether—dichloromethane (4:1) but by increasing the polarity by doubling the proportion of dichloromethane it was found to be unnecessary to use a final clean-up stage as advocated in their method. Ervik [1] used a one-step benzene extraction, but the time saved at this stage is subsequently lost by having a very long chromatography time. Zak et al. [5] also described a method for the determination of metoprolol but it involves an exceptionally complicated and time-consuming extraction procedure.

Derivatization

Metoprolol was derivatized for 20, 40 and 60 min and it was found that there was no improvement in yield on extending the reaction time beyond 20 min.

TFAA storage

Our experience with TFAA has shown that on opening a new vial the contents are best transferred to a ground-glass-stoppered tube and stored in subdued light surrounded by a desiccating agent. These precautions have ensured consistent results and an increased shelf-life of TFAA.

Column stabilization

Before commencing a sample run, it was found that by injecting a plasma blank extract three or four times, until the peaks produced were symmetrical

(Figs. 1 and 2), the reproducibility of the method was maintained. A similar observation was also made by Kangas [6] when using an OV-17 column.

This method has been used for the analysis of over 400 samples and has proved to be straightforward and trouble-free, with good reproducibility and sensitivity, and is useful in pharmacokinetic studies on metoprolol.

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