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## Note

# Improved method for the determination of methimazole in plasma by high-performance liquid chromatography

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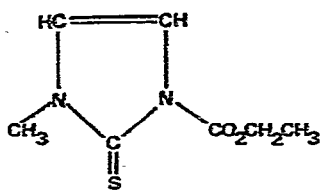
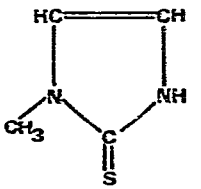
High-performance liquid chromatography (HPLC) would appear to be a clinically applicable method for the analysis of methimazole in biological fluids obtained from patients receiving therapeutic doses of either Carbimazole or methimazole. In an earlier communication we have shown that by using HPLC, methimazole, the active metabolite of Carbimazole, could be detected in the blood of patients receiving a single oral dose of Carbimazole[1]. Unfortunately, the method described was time consuming and lacked sensitivity due to low column efficiency and methimazole was not completely resolved from the solvent peak. In order to improve the method, we have looked at various column packings, solvent systems, and potential internal standards. In this report, a method for the quantitation of methimazole in plasma is described using either a column packed with 10 $\mu$m alumina or one packed with 10 $\mu$m silica, and benzamide as internal standard.




CARBIMAZOLE




METHIMAZOLE

## MATERIALS AND METHODS

### *Chemicals and reagents*

Methimazole (Aldrich, Gillingham, Great Britain) and benzamide (BDH, Poole, Great Britain) were reagent grade. All organic solvents were AnalaR grade, and the *n*-octanol was of reagent grade. Standard solutions of methimazole (0.2–1.4 $\mu$g/ml) and benzamide (18 $\mu$g/ml) were prepared in deionized water and methanol, respectively.

*Extraction procedure*

Plasma (1 ml) was placed in a stoppered test tube along with benzamide solution (0.5 ml) and water (0.5 ml). For the calibration standards the water was replaced by a methimazole standard solution. The mixture was extracted with chloroform (5 ml) containing 0.25% *n*-octanol, by mechanically shaking for 15 min. The resultant mixture was centrifuged and an aliquot of the chloroform phase (4 ml) transferred to a tapered test tube by pipette, and the chloroform evaporated off on a water-bath at 30°, under a stream of oxygen-free nitrogen. The extract was retained in a small volume of *n*-octanol, which was reconstituted with chloroform (50 $\mu$l). The test tube was vortex mixed and aliquots (15–20 $\mu$l) were injected onto the column using a stop flow injection technique.

*HPLC conditions*

The HPLC analyses were performed on a Spectra-Physics Model 3500B liquid chromatograph, with UV detection at 254 nm. Two columns were used, *viz.* (I) A commercial column (100 × 4.6 mm I.D., stainless steel) that had been dry packed with 10 $\mu$m alumina (Spherisorb A1; Phase Separations, Queensferry, Great Britain). Alternatively, when packing our own column, the deactivating procedure described by Snyder[2] was used. The mobile phase consisted of 2.0% methanol in chloroform (flow-rate, 1.2 ml/min). (II) A column (100 × 4.6 mm I.D., stainless steel) dry packed with 10 $\mu$m silica (Spherisorb Si; Phase Separations). The mobile phase consisted of 1.0% methanol in chloroform (flow-rate, 1.2 ml/min).

*Extraction yields and calibration curves*

For the quantitative determination of methimazole, standard curves were prepared by adding 0.2–1.4 $\mu$g of methimazole and benzamide (9 $\mu$g) to blank plasma samples (1 ml). The samples were extracted and chromatographed according to the method described above, and the peak height ratios plotted against the methimazole concentration. The recovery of methimazole and benzamide was obtained by adding constant amounts of each to blank plasma, and treating them in a similar manner to the calibration standards.

## RESULTS AND DISCUSSION

A method that is both simple and rapid has been developed for the quantitative determination of methimazole in plasma. Most of the work presented in this report was carried out using the prepacked alumina column. In order to reproduce the selectivity ratio for methimazole and benzamide from alumina columns we had packed ourselves, we found it necessary to pretreat the alumina by adding sufficient water to form a water monolayer on the alumina surface. The chromatograms (Figs. 1a and b) obtained from plasma extracts illustrate that methimazole and benzamide are well resolved from the solvent peak. The retention times for Carbimazole, methimazole, and benzamide are 0.5, 4.0, and 6.0 min, respectively. Octanol in the extracting solvent prevented loss of methimazole by adsorption on to the walls of the test tube, after the extract had been evaporated to dryness.

Extraction yields and distribution ratios are shown in Table I. Although a single extraction technique has the disadvantage of decreasing the overall sensitivity of the

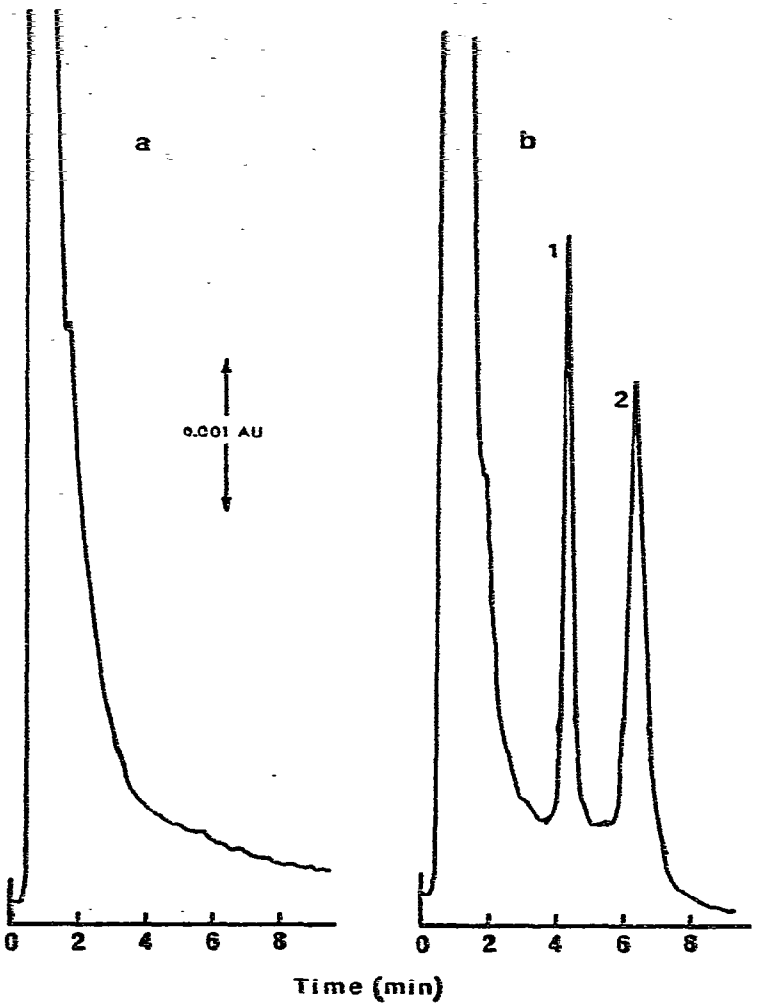



Fig. 1. Chromatograms of (a) a plasma blank; (b) methimazole (1) and benzamide (2) internal standard recovered from plasma. Conditions: column (100 × 4.6 mm I.D.) dry packed with 10 μm alumina; mobile phase, 2% methanol in chloroform; flow-rate, 0.17 cm $sec^{-1}$; pressure, 160–180 p.s.i.; ambient temperature.

TABLE I

DISTRIBUTION RATIOS AND RECOVERIES OF METHIMAZOLE (MEAN ± S.D., $n = 6$)

| | *Methimazole* | | *Benzamide* | |
|---|---|---|---|---|
| | *Distribution ratio* | *Extraction yield (%)* | *Distribution ratio* | *Extraction yield (%)* |
| Chloroform/water | 0.57 ± 0.06 | 58.0 | 0.60 ± 0.08 | 56.0 |
| Chloroform/plasma | 0.49 ± 0.05 | 54.0 | 0.71 ± 0.06 | 60.0 |

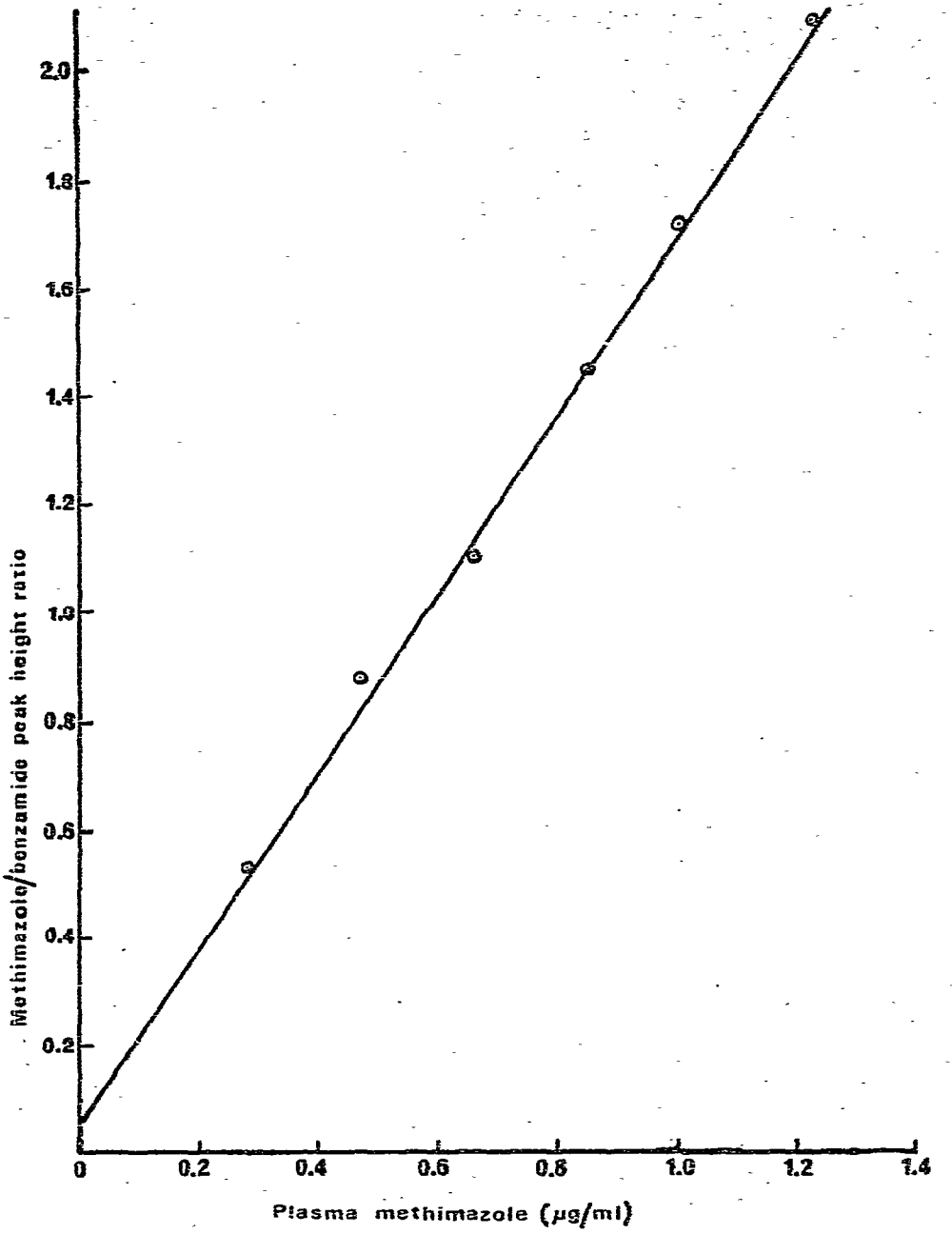



Fig. 2. Calibration curve for the determination of methimazole in plasma. Each point is the mean of three methimazole/benzamide peak ratios. S.E. = 0.04 μg/ml.

method, the on-column detection limit for methimazole is sufficiently low (0.6 ng) to permit this procedure for the determination of methimazole in plasma above a concentration of 0.1 μg/ml.

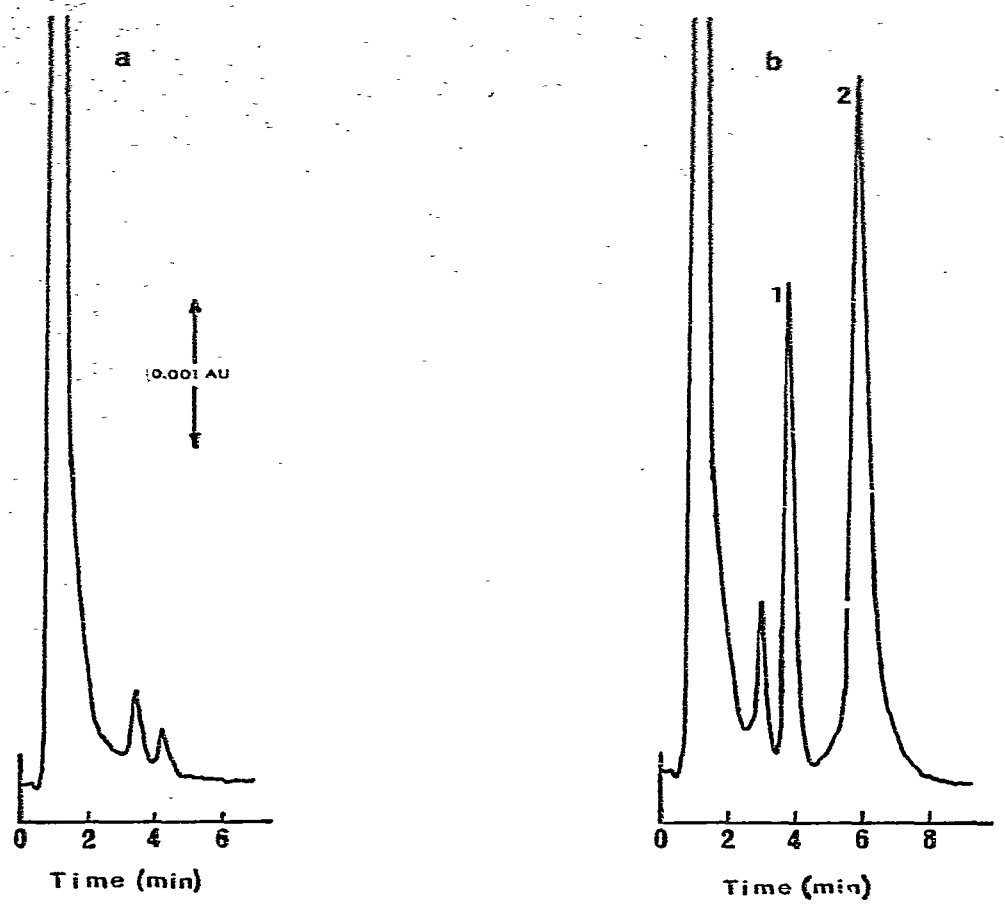



Fig. 3. Chromatograms of plasma samples from thyrotoxic patients receiving methimazole (10 mg) intravenously. (a) Control extract; (b) Test extract. Peaks: 1 = methimazole; 2 = benzamide. Conditions, as in Fig. 1.

Examination of the reproducibility of the method showed that the relative standard deviation of the peak height ratios is 14% ($n = 7$). The plots of peak height ratio against methimazole concentration proved linear up to 1.4 $\mu$g/ml of methimazole (Fig. 2). For routine analysis quantitation was achieved by adding methimazole (0.6 $\mu$g) to three samples of blank plasma from the patient under study. The median value of the peak height ratios was then used to calculate the methimazole concentration in plasma samples from thyrotoxic patients receiving either methimazole or Carbimazole. Fig. 3 shows typical chromatograms from patients receiving therapeutic doses of methimazole.

Methimazole and benzamide are well resolved on the silica column (Fig. 4), which has the advantage over the alumina column in not requiring any pre-treatment. However, we have found that with the silica column a peak from endogenous plasma material appears at a retention time similar to that of methimazole and would interfere with quantitative determination if not completely resolved. We have found that by packing the top 1.5 cm of the column with 10 $\mu$m alumina the interfering peak ap-

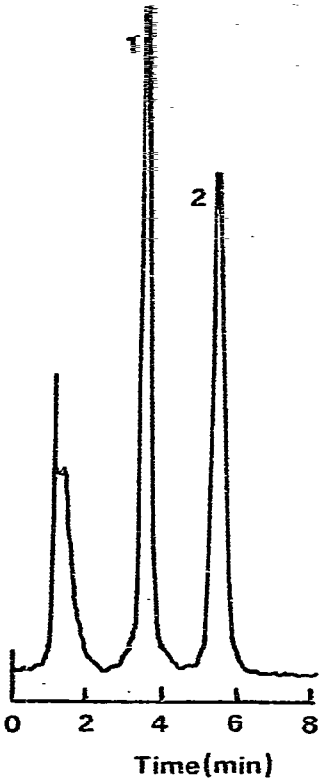



Fig. 4. Chromatogram of methimazole (1) and benzamide (2). Conditions: column (100 × 4.6 mm I.D.) dry packed with 10 $\mu$m silica: mobile phase, 1% methanol in chloroform; flow-velocity, 0.17 cm/sec; pressure, 160–180 p.s.i.; ambient temperature.

pears at a shorter retention time and is completely resolved from the methimazole peak without any appreciable loss of column efficiency. The two phases are separated with a 400-mesh stainless-steel disc.

This improved method makes it possible to analyse at least twenty biological samples in a day.

ACKNOWLEDGEMENTS

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