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DETERMINATION OF METHIMAZOLE IN PLASMA USING GAS CHROMATOGRAPHY—MASS SPECTROMETRY AFTER EXTRACTIVE ALKYLATION

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SUMMARY

A gas chromatography—mass spectrometry method for quantitation of the thyreostatic agent methimazole in plasma is described. The drug was transferred from the plasma sample and derivatized in one step by extractive alkylation. Either of two alkylating agents benzyl-chloride or pentafluorobenzyl bromide were used. Deuterium-labelled methimazole was used as internal standard. The precision of the method at the level of 5 ng methimazole per ml plasma was 6%.

INTRODUCTION

Methimazole (1-methylimidazole-2-thiol) is a drug used in the treatment of hyperthyroidism. It is also generated in the body from carbimazole (ethyl-3-methyl-2-thioxo-4-imidazoline-1-carboxylate), another drug which is more widely used in the same indication. Their structures are shown in Fig. 1. Methods for the determination of methimazole in rat urine [1] and plasma [2—4] utilizing gas and liquid chromatographic techniques have been described. Only one of these methods described is sensitive enough to reach those levels of methimazole in plasma which result from standard oral therapy. But even this method lacks the sensitivity to monitor the lowest therapeutic levels.

The extraction of methimazole from plasma with organic solvents seems to be rather variable. Some authors claim that they have achieved about 70% recovery [4] when extracting into chloroform as organic solvent whereas others have achieved 54% recovery [3] using the same solvent. Extraction of methimazole from plasma with chloroform or ethyl acetate during the course of this work showed a variation in the extraction yield ranging from below 50%

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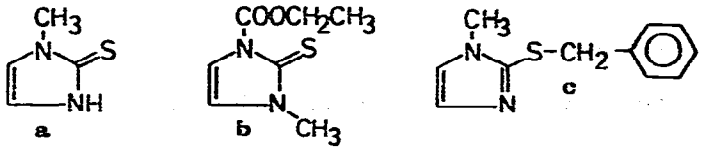


Fig. 1. Structural formulae of (a) methimazole; (b) carbimazole; (c) S-benzylated methimazole.

to about 70%. The plasma samples used for these extractions were collected from different persons on different occasions. These results indicate that it is unsatisfactory to rely on methods depending on this type of extraction when different plasmas are used.

In the method described in this paper, methimazole is extracted as an ion-pair into the organic phase where it is benzylated. The S-benzyl (or pentafluorobenzyl) derivative of methimazole (Fig. 1) is quantified on a gas chromatographic—mass spectrometric (GC—MS) system equipped with a glass capillary column. A deuterium-labelled analogue, 1-trideuteromethylimidazole-2-thiol, is used as internal standard (IS).

MATERIALS AND METHODS

Instrumental

The measurements were carried out in a Finnigan 4000 gas chromatograph—mass spectrometer equipped with a multiple ion monitoring device (Finnigan, Sunnyvale, Calif., U.S.A.). The injector of the gas chromatograph was of the Grob capillary type and operated at 200° in a splitless mode. The injector was equipped with valves which were programmed to vent the injector 60 sec after injection. The glass capillary column used was a 20-m UCON HB 5100 (Jaeggi, Trogen, Switzerland). A pressure of 20—25 kPa of helium was applied on the column which was directly interfaced to the ion source. The electron energy was 70 eV. The temperature of the GC oven was programmed to rise from 140 to 180° at a rate of 10°/min. The samples could be injected with 7—8 min intervals. For the injection, solid sample syringes (SGE, North Melbourne, Australia) were used. They were cleaned in a Hamilton syringe cleaner between the injections. A Jeol FX-100 NMR spectrometer was used for obtaining the NMR spectra.

Internal standard

Preparation of 1-trideuteromethylimidazole-2-thiol: Acetalylthiocarbimide [5] (5.45 g), prepared from aminoacetal, was mixed with trideuteromethyl amine HCl (2.86 g) and 5 ml ethanol in a screw-capped tube. The mixture was cooled in iced water and sodium hydroxide (1.64 g) was added. The tube was then slowly shaken until it reached room temperature (22°), where it was allowed to stand for a couple of hours. The sodium chloride that had formed was filtered off and the solvent evaporated. The residue was hydrolysed by refluxing it with 20 ml 30% sulphuric acid for 30 min. After cooling, it was neutralised with 4 *M* sodium hydoxide solution and extracted with ethyl acetate (4 × 50 ml). Evaporation of the solvent gave a crude residue which was purified on a silica column eluted with chloroform containing 4% ethanol.

The amount of pure 1-trideuteromethylimidazole-2-thiol isolated was about 800 mg.

Extraction of methimazole and formation of derivatives

To 1 ml of plasma in a screw-capped tube were added 100 µl water containing internal standard (concentration 200 ng/ml) and 50 µl 0.1 *M* tetrabutylammonium solution followed by 1 ml 0.5 *M* carbonate buffer (pH 10). The tetrabutylammonium solution was prepared by adding an equimolar amount of 4 *M* sodium hydroxide to tetrabutylammonium hydrogen sulphate and then adding water until the desired concentration was reached. The sample mixture was extracted with 5 ml dichloromethane containing 2.5% (v/v) of benzyl chloride (or pentafluorobenzyl bromide when determining plasma concentrations below 20 ng/ml). The extraction was performed in a water-bath at 50° for 20 min. After cooling to room temperature the tube was centrifuged (500 *g*) and the organic phase was then transferred to a new tube and evaporated to dryness with a stream of nitrogen. The residue was dissolved in 20 µl of ethyl acetate and 1—2 µl was evaporated on the needle of the solid sample syringe.

RESULTS

Extraction and alkylation

Methimazole is extracted from plasma as an ion-pair with the tetrabutylammonium ion into dichloromethane. Since methimazole is converted to a more lipophilic compound in the organic phase through reaction with benzyl chloride (or pentafluorobenzyl bromide), it is efficiently extracted from the aqueous phase. Possible variations in the conditions are compensated for by the use of the labelled methimazole as internal standard.

The extraction and alkylation step is carried out at a temperature of 50°. The yield of S-benzyl methimazole decreased considerably when the extractive alkylation was performed at room temperature but this could be circumvented to some extent by an increase of the concentration of benzyl chloride in the organic phase. A comparison of the yields is shown in Table I, where the yield in the reaction at 50° has been set to 100. The reaction at 50° is completed within 20 min. The effect of elevated temperature has been reported previously

TABLE I

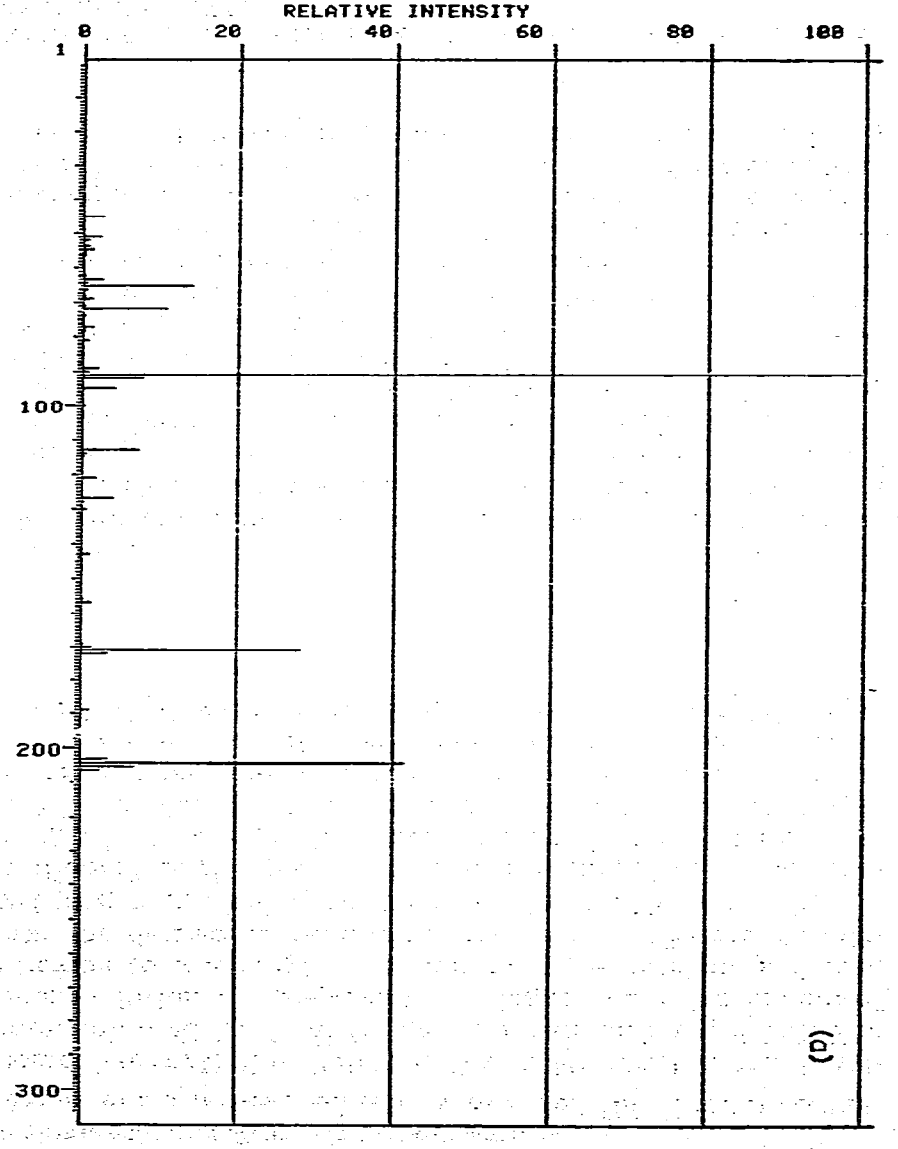
EXTRACTIVE BENZYLATION OF METHIMAZOLE IN CARBONATE BUFFER (pH 10)—DICHLOROMETHANE SYSTEM

The reaction time was 1 h.

Benzyl chloride concentration (%)	Temperature (°C)	Yield (%)
2.5	50	100
2.5	22	32
5	22	60
10	22	75

RUN IDENTIFICATION BENSYLMETIMAZOL
SPECTRA FILE NAME 000
FILE POSITION 24
BACKGROUND 10
MAXIMUM INTENSITY 11129 % OF TOTAL ION 33.0
OUTPUT MASS RANGE 1 TO 492
SCAN SPEED 5

MASS	91	204	171	65	72	92	113	285	93	127
INTENSITY	10000	5261	2831	1430	1105	824	774	735	456	444



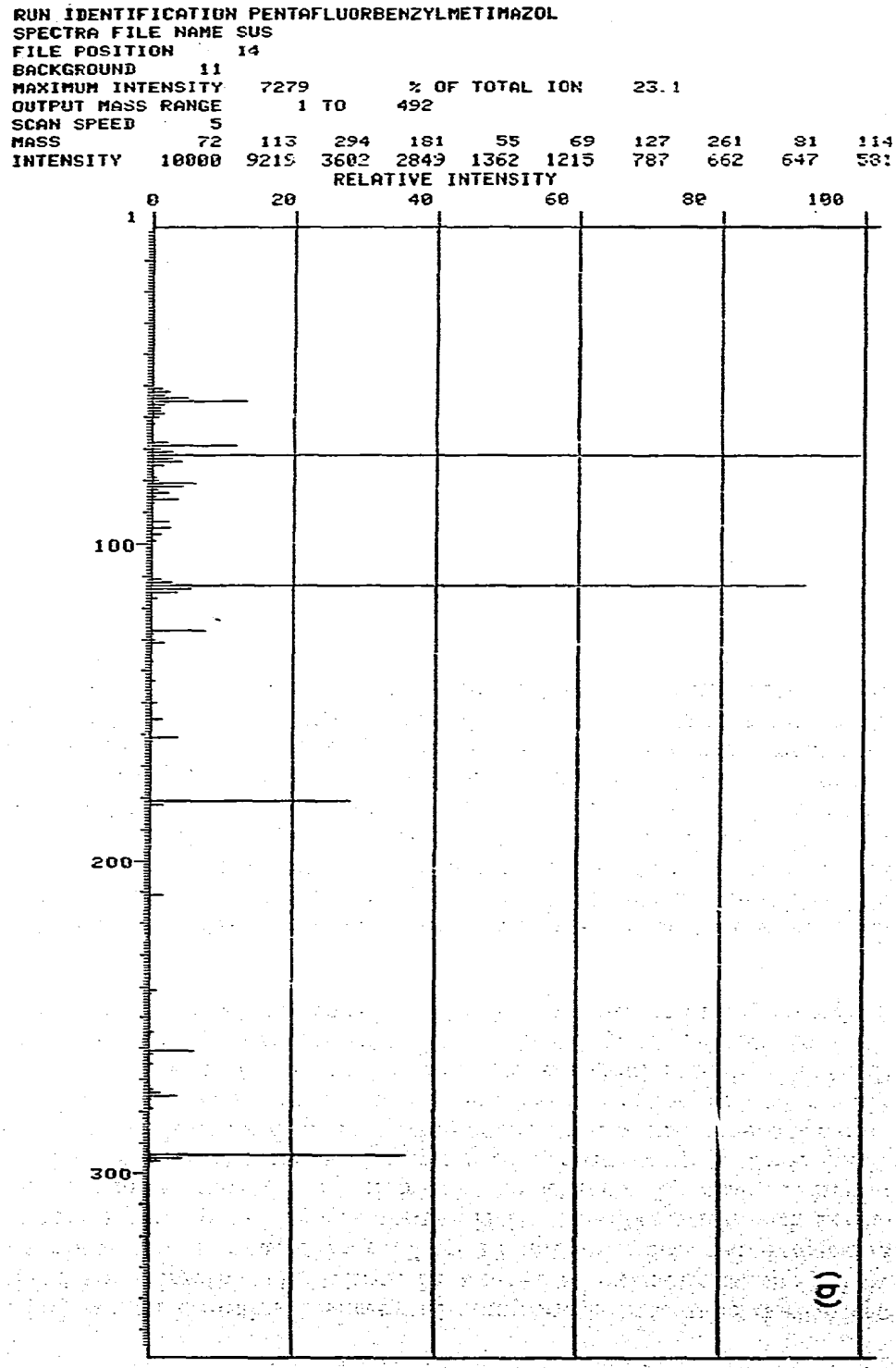


Fig. 2. Mass spectra of (a) S-benzylated methimazole and (b) S-pentafluorobenzylated methimazole.

in a report [6] which contains a survey of compounds that yield derivatives when subjected to extractive alkylation. In a series of extractions, the pH of the aqueous phase was increased from 7 to 12 and an improved extraction could be demonstrated up to but not above pH 10. The alkylation with pentafluorobenzyl bromide resulted in increased sensitivity because a higher molecular weight moves the molecular ion into a range with a lower background. Benzyl chloride is used as alkylating agent for samples where the concentration is likely to be above 20 ng/ml because it is cheaper and readily available. The structure of the benzyl derivatives was indicated by ^{13}C-NMR spectra. The thiocarbonyl carbon situated at 159.9 ppm relative to TMS disappeared and a new peak appeared at 138.9 ppm corresponding to =C—S.

Gas chromatography

The UCON HB 5100 glass capillary column was used to achieve good peak symmetry when chromatographing the methimazole derivatives and the low background produced by this column was also beneficial to the sensitivity of the system. The benzyl and pentafluorobenzyl derivatives eluted after 4.2 and 6.2 min respectively. The mass spectrometer was focused on the molecular ions of the two types of methimazole/IS derivatives which had *m/e* values of 204/207 and 294/297 respectively. Fig. 2a and b shows mass spectra of benzyl and pentafluorobenzyl methimazole. Figs. 3 and 4 illustrate chromatograms recorded when the molecular ions of the different methimazole derivatives were monitored.

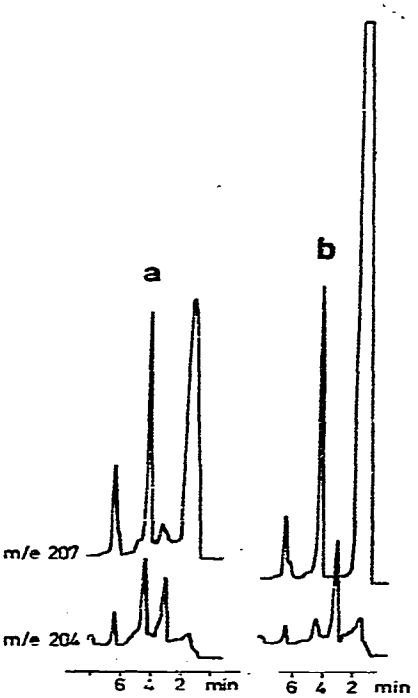


Fig. 3. (a) Chromatogram of plasma containing 20 ng/ml of methimazole obtained after benzylation; benzyl methimazole corresponds to the peak eluting after 4.2 min. (b) Chromatogram of blank plasma sample, internal standard, *m/e* = 207.

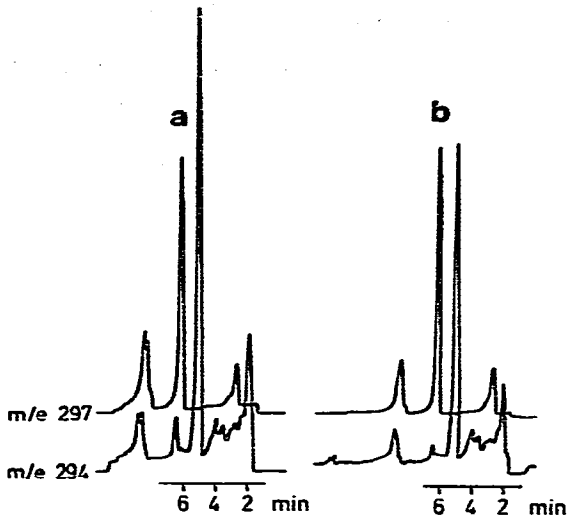


Fig. 4. (a) Chromatogram of plasma sample containing 5 ng/ml of methimazole obtained after pentafluorobenzylation; pentafluorobenzyl methimazole corresponds to the peak eluting after 6.2 min. (b) Chromatogram of blank plasma sample, internal standard, *m/e* = 297.

In the chromatograms of the blank samples a background peak can be observed which interferes with the methimazole derivatives. In the case when benzyl derivatives were used this background peak corresponds to about 7 ng/ml of methimazole in a plasma sample. The use of the pentafluorobenzyl derivative gave a background peak corresponding to about 1 ng/ml. The background peak originates from the plasma samples since no interfering peak could be detected when a sample of pure water was analysed according to the method. Due to the blank peak the detection limits of the method have been set to 15 and 2 ng/ml of methimazole for the benzyl and pentafluorobenzyl derivatives respectively. Blank plasma samples have been analysed both from healthy volunteers and from thyreotoxic patients. In these samples no significant variation in the interfering background peaks could be detected.

Calibration graph

The two calibration graphs in Fig. 5a and b were prepared by adding different amounts of methimazole to plasma samples and analysing them according to the described method. In Fig. 5a, the ratio of the peak heights resulting from the molecular ions 204 and 207 of the benzyl derivatives were plotted versus the concentration of methimazole. The amounts of methimazole that had been added to the plasma samples ranged from 15 to 150 ng/ml. In Fig. 5b, a similar plot is shown using the molecular ions 294 and 297 obtained from the pentafluorobenzyl derivatives. In this case the amounts that had been added to the plasma samples ranged from 2—50 ng/ml. The calibration graphs were both linear in the range investigated. The two graphs were constructed in the lowest range possible for each derivative. The precision of the method was determined by adding 50 ng/ml of methimazole to plasma samples, which were analysed according to the method. The precision was 2.5 (n = 10) and 1.5% (n = 10) for the benzyl and pentafluorobenzyl derivatives

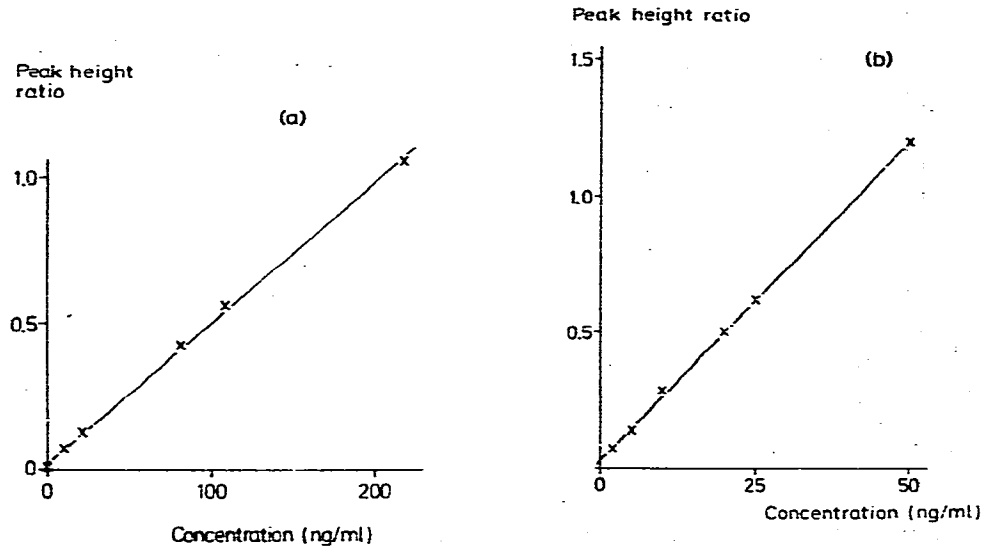


Fig. 5. Typical standard curves constructed by (a) plotting the 204/207 peak height ratios versus concentration of methimazole and (b) plotting the 294/297 peak height ratios versus concentration of methimazole.

respectively. The precision at the 5 ng/ml level of methimazole determined as pentafluorobenzyl derivatives was found to be about 6% ($n = 9$).

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