GLC Analysis of Metronidazole in Human Plasma

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Abstract □ A sensitive procedure was developed for metronidazole in human plasma. The metronidazole is extracted from the plasma with chloroform and determined as the trimethylsilyl derivative by GLC using a flame-ionization detector. Myristyl alcohol is used as the internal standard for quantitation by relative peak height.

Keyphrases □ Metronidazole—GLC analysis in human plasma □ GLC—analysis, metronidazole in human plasma

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) (I) is an antiprotozoal agent that has been widely used for many years in the oral treatment of trichomoniasis. The methods most frequently used for determining metronidazole in biological fluids have been polarography (1, 2) and colorimetry involving reduction of the nitro moiety (2, 3). A comparative study (2) found that these methods gave inconsistent results and that even a biological method was more reliable. Recently, more specific chromatographic methods were reported: a TLC method for metronidazole in serum (4) and a GLC method for metronidazole in plasma (5).

The GLC method described in the present work was developed before publication of the method of Midha *et al.* (5); although similar in approach, it has some advantages over the Midha *et al.* method.

EXPERIMENTAL

GLC—A gas chromatograph[1] equipped with a flame-ionization detector was used. A 183 × 0.04-cm column packed with OV-1 (3%) on Gas Chrom Q was operated at 160° with a carrier of nitrogen at 70 ml/min. Detector flow rates were: hydrogen, 45 ml/min, and air, 300 ml/min. Electrometer attenuations were 10 × 16 or 10 × 32. Injection size was 2 μl. Retention times of trimethylsilyl derivatives were as follows: metronidazole, 4.1 min; 2-methyl-5-nitroimidazole-1-ylacetic acid, 5.1 min; and myristyl alcohol, 8.1 min. After each plasma analysis, it was found preferable to raise the column temperature to 200° for about 10 min to eliminate the possibility of strongly retained substances interfering with subsequent analyses.

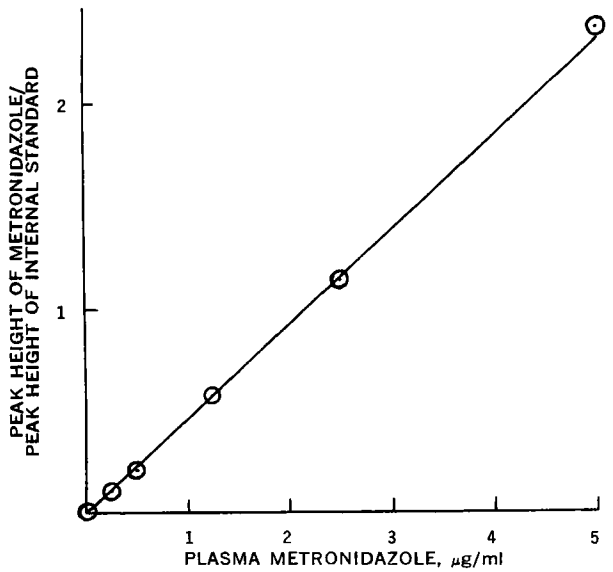


Figure 1—*Calibration chart for metronidazole.*

[1] Tracor MT-220.

Table I—Recovery of Metronidazole Added to Human Plasma

Metronidazole, μg/ml	Number of Experiments	Range of Results, μg/ml	Average Result, μg/ml	*RSD*, % ±	*RE*, %
0.5	2	0.48–0.60[a]	0.54	15.37	+8.00
1.5	3	1.51–1.65[a]	1.60	4.74	+6.67
2.5	4	2.37–2.53	2.49	3.09	−0.40
3.75	3	3.80–4.12	3.98	4.11	+6.13
5.0	2	4.62–4.80	4.71	2.70	−5.80
Average recovered		102.9%			

[a] Corrected for blank of 0.05 μg/ml.

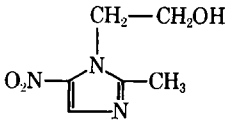
Method—Plasma (2 ml) adjusted to pH 8 was extracted with chloroform (1 × 20, 1 × 10, and 1 × 5 ml), and 100 μl of chloroform containing 5 mg/100 ml of myristyl alcohol was added to the extract. The extract was dried over anhydrous sodium sulfate for a few minutes and then decanted into an evaporation flask. After removal of chloroform using a rotary evaporator, the residue was transferred to a 1-ml vial with 0.5 ml of methanol. The methanol was removed in a stream of nitrogen, 50 μl of bis(trimethylsilyl)trifluoroacetamide was added, and the vial was immediately capped with a Teflon-coated rubber septum.

After standing overnight, the solution was examined by GLC and the ratio of the peak heights of the metronidazole and the myristyl alcohol derivatives was determined. Finally, the metronidazole was found by reference to a calibration chart relating the peak height ratio to plasma concentration. The calibration chart was prepared from standard mixtures of metronidazole with myristyl alcohol in bis(trimethylsilyl)trifluoroacetamide.

RESULTS AND DISCUSSION

The generally recommended dosage of metronidazole for women is one oral tablet of 250 mg three times daily. According to McFadzean (6), a single oral dose of 200 mg leads to a maximum serum level of 5 μg/ml after 1 hr; the serum level then decreases to 1 μg/ml after 24 hr. On this basis, the presently described method was developed so as to give good quantitation for metronidazole in plasma from 0.5 to 5 μg/ml.

GLC analysis of metronidazole as the trimethylsilyl derivative rather than as the parent compound itself was chosen because of superior peak shape and detector response. Moreover, it was anticipated that trimethylsilylation would improve the chances for detection of metronidazole metabolites, which would be expected to be more polar than metronidazole itself. Trimethylsilyl derivatives are usually sensitive to moisture and frequently unstable. Actually,



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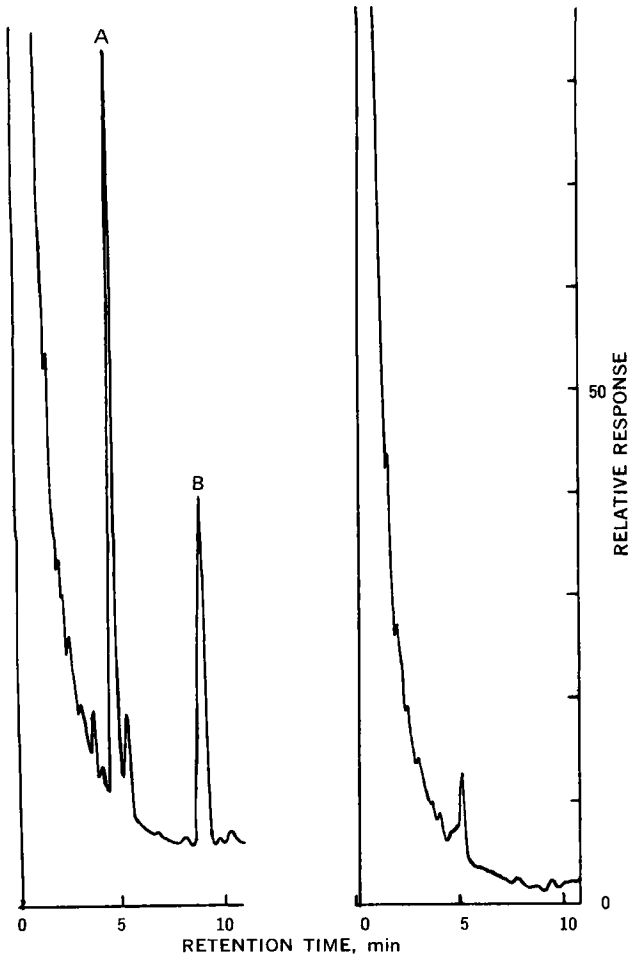
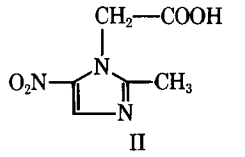


Figure 2—*Recovery of metronidazole from plasma. Chromatogram on left was obtained from a plasma sample with added metronidazole (5 μg/ml) and internal standard (5 μg/ml). Chromatogram on right was obtained from a control sample. Key: A, metronidazole; and B, internal standard.*

the derivatives of metronidazole and the chosen internal standard of myristyl alcohol are probably simple derivatives of primary alcohols (5) and, as such, do not suffer from these disadvantages. Indeed, the derivatives may be recovered from dimethylformamide solution by addition of water and extraction into hexane or chloroform. No peculiar behavior during GLC was noted.

A brief study was made of the conditions necessary for quantitative trimethylsilylation of a mixture of metronidazole and myristyl alcohol. In dimethylformamide–bis(trimethylsilyl)trifluoroacetamide (1:1), reaction was complete in less than 30 min; but it was obvious that at low levels of metronidazole, the tail of the dimethylformamide peak would interfere with quantitation. In bis(trimethylsilyl)trifluoroacetamide alone, reaction was complete in less than 1 hr at 50° and almost complete in 2 hr at room temperature. It was decided to trimethylsilylate the plasma extract by allowing it to stand in bis(trimethylsilyl)trifluoroacetamide at room temperature overnight.

Determinations of metronidazole were based on the ratio of the peak height of the metronidazole derivative to that of the internal



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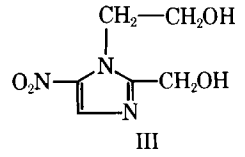

III

standard. Calibration charts relating this ratio to the concentration of metronidazole in plasma were prepared from standard mixtures of metronidazole with the internal standard in bis(trimethylsilyl)trifluoroacetamide. Such a chart is shown in Fig. 1, and it can be seen that a linear relationship exists in the 0–5-μg/ml range.

The results of some recovery experiments for standards of metronidazole added to human plasma are given in Table I. Typical chromatograms from a 5-μg/ml plasma sample and a control plasma sample are shown in Fig. 2.

Although 2-methyl-5-nitroimidazole-1-ylacetic acid (II) and 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (III) have been reported as metabolites of metronidazole in urine (7, 8), no clear evidence for metabolites of metronidazole in plasma was found in the present work during the analysis of several clinical samples. Compound III ought to lend itself to analysis since it is structurally related to metronidazole simply by having an additional primary alcohol moiety. Unfortunately, no sample of III was available. However, a submilligram sample of II was available, and some preliminary recovery experiments with plasma were performed. Results showed good recovery of II, giving a sharp chromatographic peak eluting immediately behind, and sharply resolved from, that of metronidazole.

The method described here is similar to that of Midha *et al.* (5). Midha *et al.* also carried out trimethylsilylation prior to analysis, but they used a reagent consisting of a mixture of hexamethyldisilazane and trimethylchlorosilane in acetonitrile rather than bis(trimethylsilyl)trifluoroacetamide. They were not able to analyze the resulting metronidazole derivative satisfactorily by GLC using a single liquid as the stationary phase as described here, but instead they had to resort to a mixture of three liquids. One practical difficulty in the application of the method of Midha *et al.* is that the internal standard, 1-(2-hydroxyethyl)-2-methoxy-5-nitroimidazole, may be difficult to obtain since it is quite a rare chemical and unavailable commercially.

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