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Note

Measurement of ornidazole by high-performance liquid chromatography

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Ornidazole [α-(chloromethyl-2-methyl-5-nitroimidazole-1-ethanol] is a derivative of nitroimidazole, with antiprotozoal and antibacterial properties. It is used in the prevention and treatment of infections due to anaerobic germs, such as *Bacteroides fragilis*. The drug is especially useful in abdominal or gynecological surgery.

The measurement of plasma ornidazole allows the dosage to be adjusted in patients suffering from malabsorption or pathological conditions which modify excretion of the drug.

Nitroimidazole derivatives in general, and metronidazole in particular, can be measured by several methods: polarography [1], spectrophotometry [2], microbiology [3, 4], thin-layer chromatography [5, 6], gas—liquid chromatography [7, 8] or high-performance liquid chromatography [9—12]. Not all of these methods are capable of measuring the concentration of ornidazole.

Two types of pharmacokinetic ornidazole studies have been carried out: after administration of radiolabeled product, by separation of ornidazole and its metabolites using thin-layer chromatography [13, 14], and by microbiological assay [15]. Radioactive assay is not a feasible method in common medical practice, and the microbiological assay techniques are long and not very specific.

We have developed a method of measuring ornidazole by high-performance liquid chromatography. The method is rapid, selective and reproducible, by using an internal standard. Furthermore, the method allows metabolites to be separated and measured.

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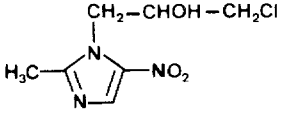
EXPERIMENTAL

Standards and solvents

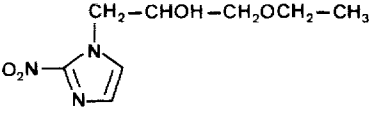
Ornidazole, the internal standard and the metabolites (Fig. 1) were kindly supplied to us by Roche, Neuilly/Seine, France. Ornidazole (Ro 07-0207) is α-(chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol. Internal standard (Ro 07-0913) is α-(ethoxymethyl)-2-nitroimidazole-1-ethanol. Metabolite 1 (Ro 11-4791) is α-(chloromethyl)-2-hydoxymethyl-5-nitroimidazole-1-ethanol. Metabolite 4 (Ro 11-2616) is 3-(2-methyl-5-nitroimidazole 1-yl)-1,2-propane diol. Metabolites M_1 and M_4 are quantitatively the most important metabolites [13, 14, 16].

The aqueous reagents, i.e. $HClO_4$ 0.7 mol/l (Prolabo, Paris, France), NaOH 1 mol/l (Prolabo), buffer Titrisol* citrate—HCl pH 4 (Merck, Darmstadt, G.F.R. Réf. 9884), are all prepared using distilled water.

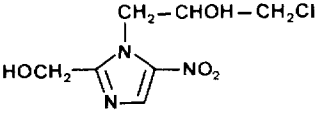
The mobile phase, water—ethanol (1:9, v/v), is filtered through a Whatman No. 2 filter and degassed by ultrasound (Bromsonic 52, Bioblock Apparatus, Strasbourg, France) before use. The flow-rate is 2 ml/min, corresponding to a pressure of 140 bars (2000 p.s.i.)



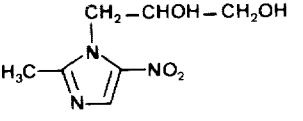
ORNIDAZOLE
Ro 07-0207



INTERNAL STANDARD
Ro 07-0913



METABOLITE M_1
Ro 11-4791



METABOLITE M_4
Ro 11-2616

Fig. 1. Chemical structures of compounds used.

Chromatographic conditions

The high-performance liquid chromatograph used is a Du Pont de Nemours (Paris, France) Model 870, coupled to a spectrophotometric detector, which is itself linked to a recording scope, both by Du Pont de Nemours.

Absorption is measured at 318 nm; the sensitivity is 0.02 absorbance units over the full scale. The products are injected with a 50-μl loop injector (Rheodyne 7125, Berkeley, CA, U.S.A.). The column used is a μBondapak C_{18} reversed-phase 30 cm × 4.6 mm column. The size of the particles is 10 μm (Waters, Paris, France). The peak areas are calculated using an integrating microprocessor ICAP 10 (Delsi, Suresnes, France).

Extraction

A 1-ml volume of plasma (sample to be assayed or standard) is added to 50 μl of an aqueous solution of 100 μg/ml internal standard and 1 ml of 0.7 mol/l perchloric acid. The mixture is agitated for 5 min using an alternating agitator (Realis, Villejuif, France) and then the solution is centrifuged for 10 min at 1000 *g*. Then 1 ml of the supernatant is taken off, neutralized with 0.3 ml of 1 mol/l sodium hydroxide and buffered with 0.2 ml of citrate—HCl buffer. After shaking, 50 μl are injected into the chromatograph.

Calibration

A standard concentration curve is obtained by adding ornidazole at concentrations of 0.2, 0.5, 1, 2, 5, 10, 15 and 20 μg/ml to control plasma samples under the same experimental conditions. The ratios of the peak areas of ornidazole and internal standard are plotted.

The precision of the method is estimated by determining the coefficient of variation over three concentrations.

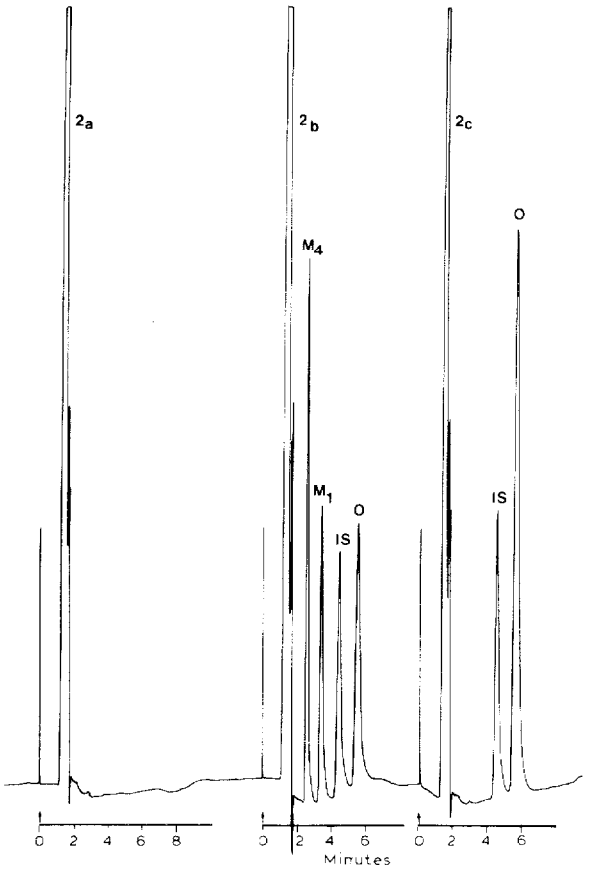


Fig. 2. Chromatograms of extracts from blank human plasma (a), standard plasma containing 5 μg/ml ornidazole (O), metabolites M_1 and M_4 and 5 μg internal standard (b), and patient plasma (c). The retention times are 2.6 (M_4), 3.4 (M_1), 4.5 (internal standard, IS) and 5.6 (O) min.

RESULTS

Fig. 2 shows the chromatograms obtained with 1 ml of control plasma, 1 ml of standard plasma (containing 5 μg/ml ornidazole, metabolites M_1 and M_4 and 5 μg of internal standard), and 1 ml of plasma obtained from a patient 1 h after the slow intravenous injection of 500 mg of ornidazole. The different substances are well separated and there is no interference between the peaks.

The retention times of metabolites M_4 and M_1, internal standard and ornidazole are, respectively: 2.6, 3.4, 4.5 and 5.6 min, (capacity coefficient k': 0.9, 1.4, 2.2 and 3.0 respectively).

Fig. 3 shows the standard concentration curve from 0.2 to 20 μg/ml. Each point repesents the mean ± 2 S.E.M. over five measurements. Under the present experimental conditions, the limits of detection of M_4, M_1 and ornidazole are 0.1, 0.2 and 0.2 μg/ml, respectively.

Reproducibility

The reproducibility of the method was checked for three plasma concentrations (1, 10 and 20 μg/ml). Twelve measurements were made at each concentration. The coefficients of variation ($\sigma/m \times 100$) were 4.7, 1.7, 1.8%, respectively, for the three concentrations.

Selectivity

The quantitatively more important metabolites of ornidazole do not interfere with the measurement of plasma concentration. Firstly, their retention time is different from that of ornidazole and the internal standard, and secondly, their plasma concentrations are low, much lower than ornidazole, especially when the drug is given by a single intravenous injection.

Neither was there any interference with the other derivatives of nitroimidazole (tinidazole, metronidazole, misonidazole). The different peaks were well apart.

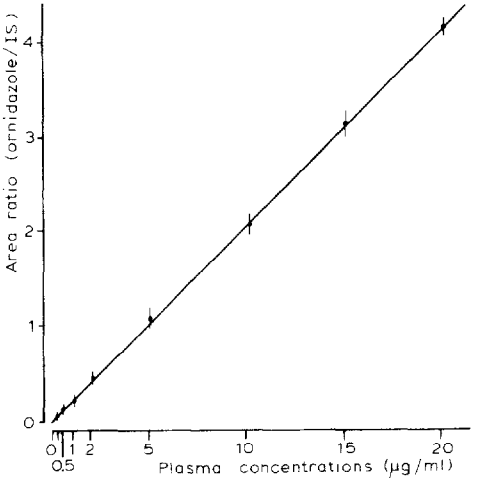


Fig. 3. Area ratio (ornidazole/IS) versus plasma concentrations of added ornidazole. Each point corresponds to the mean ± 2 S.E.M. of five analytical determinations.

Recovery

The absolute percentage of extraction could not be calculated because we were not able to obtain radioactive ornidazole. But we were able to make an estimation by comparing the peak areas obtained after the direct injection of a pure aqueous solution and after the injection of plasma containing the same concentration of ornidazole (5 μg/ml). By this method, the percentage extraction was greater than 98%.

DISCUSSION AND CONCLUSION

Several proportions of the ethanol—water mixture were tried at different input rates. The ratio 1:9 (v/v) at an input rate of 2 ml/min gave the best results with an acceptable analysis time.

Similarly, we also tested several columns (LiChrosorb RP-2 15 cm, LiChrosorb RP-8 15 and 20 cm, LiChrosorb RP-18 15 and 25 cm; Merck, France). The best separations were obtained with the column we suggested above. The technique is easy. However, one step must be conducted with extreme care: this is the neutralization and then buffering of the acid phase, because at pH above 5.5—6.0, ornidazole is degraded and a double peak is obtained. So the addition of sodium hydroxide and buffer should be very exact and the proportions properly respected.

Although metabolites M_4 and M_1 are quantitatively the most important metabolites of ornidazole (they account for 70% of total urinary metabolites [17]), they do not appear on the chromatogram presented on Fig. 2c. The reason is that they are slowly produced and rapidly eliminated from the blood stream. This leads to very low plasma concentrations after a single dose. They should become detectable after repeated administrations [16].

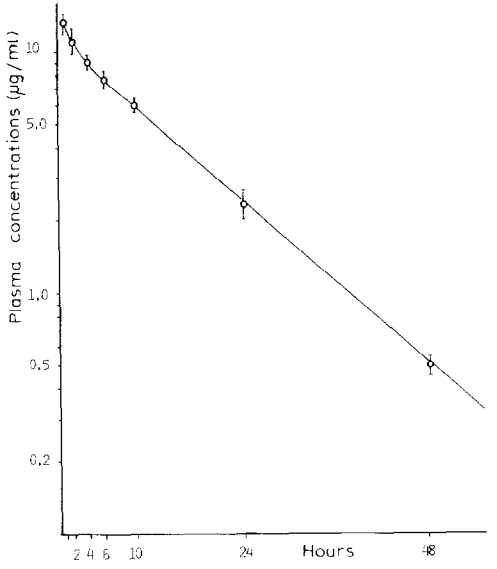


Fig. 4. Mean plasma levels ± S.E.M. of ornidazole after intravenous injection of 500 mg in eight patients.

To demonstrate the usefulness of this method, Fig. 4 represents the mean pharmacokinetic profile of ornidazole in plasma after an intravenous injection of 500 mg.

This method is rapid and reproducible. The selectivity is good and its sensitivity is sufficient for pharmacokinetic studies and therapeutic drug monitoring.

ACKNOWLEDGEMENTS

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