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Letter to the Editor

Simple liquid chromatographic method for the determination of ornidazole and metronidazole in human serum

Sir,

Ornidazole (ORN) and metronidazole (MET) are nitroimidazole derivatives used to treat intestinal and liver amoebiasis, lambliasis and vaginal trichomoniasis. Nitroimidazole derivatives can be measured by various methods: microbiological techniques [1], spectrophotometry [2] and thin-layer chromatography, gas chromatography or high-performance liquid chromatography (HPLC) [3–11]. At our clinic, both drugs are applied in the therapy, of the above-mentioned infections, although not simultaneously, and we have elaborated a very simple HPLC method for the determination of both compounds in one system, in which MET is used as the internal standard for the determination of ORN and vice versa. The method is rapid, selective, reproducible and owing to the variable internal standard, flexible.

Human serum was prepared by centrifugation and kept frozen at $-20\,^{\circ}C$ until analysis. To 1.0 ml of serum, 10 μg of internal standard in 100 μl of methanol–water (1:1) solution and 1.0 ml of 1.0 *M* disodium hydrogenphosphate were added. After shaking for 5 s in a shaker (Kutesz, Hungary), the samples were poured onto a chromatographic column (250 × 5 mm I.D.) filled with 1.5 g Extrelut (E. Merck, F.R.G.) and were eluted with 10 ml of methylene chloride after 15 min. The eluate was evaporated to dryness in a nitrogen stream, the dry residue was dissolved in 100 μl of methanol–water (1:1) solution by shaking for 30 s, and 5-μl aliquots were injected onto the analytical column.

HPLC was performed on a Hewlett-Packard 1090 chromatograph equipped with a diode-array detector and an HP 85B system master (with an HP 9121 dual disc drive). Area integrations, calculations and plotting of the chromatogram were carried out by a Hewlett-Packard 3390A integrator. A MOS-Hypersil RP-8 prepacked column (100 × 2.1 mm I.D.; 5 μm particle size) (Hewlett-Packard) at room temperature was used for the separation of the compounds. The flow-rate

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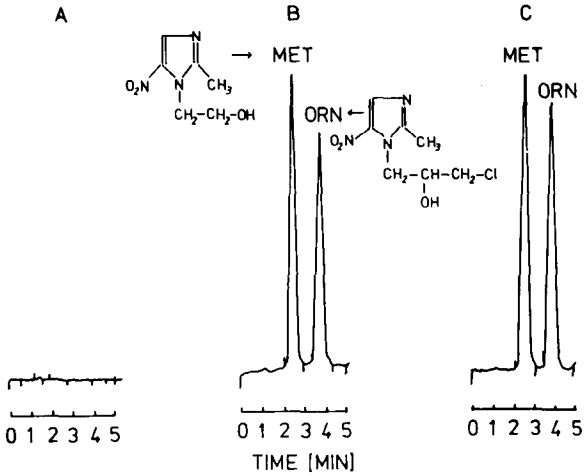

Fig. 1. Chromatograms of human serum extracts. (A) Blank serum extract; (B) serum spiked with 100 ng/ml ORN and 100 ng/ml MET; (C) volunteer serum extract 0.5 h after the infusion of 0.5 g of ORN (Tiberal®) (internal standard 10 μg MET per ml serum, ORN serum concentration 9.2 μg/ml).

was 0.2 ml/min. The absorbance of the effluent was monitored at 318 nm. The mobile phase was methanol–0.01 *M* potassium dihydrogenphosphate (3:7, v/v).

Typical chromatograms obtained by this procedure are shown in Fig. 1. Under the conditions used no interference by endogenous compounds has been observed, and ORN and MET showed two symmetric, well resolved peaks. The retention times of MET and ORN were 2.45 and 3.36 min, respectively. The relation of the detector response ratio to the serum concentrations of ORN and MET was linear. The correlation coefficient for MET was 0.997 and for ORN 0.996. The day-to-day coefficient of variation of the slope of the calibration curves was 3.61% ($n=5$) for ORN and 3.59% ($n=5$) for MET. The detection limit was found to be 30 ng of ORN and MET per ml of serum. Table I shows the accuracy and the coefficients of variation for identical samples in the concentration range 0.1–20 μg/ml of serum.

TABLE I

ACCURACY AND REPRODUCIBILITY OF THE ASSAY

$n=5$ at each concentration.

Amount added (μg/ml of serum)	Amount found (μg/ml of serum)		Coefficient of variation (%)	
	ORN	MET	ORN	MET
0.1	0.086	0.089	6.2	6.0
0.5	0.43	0.45	4.3	4.2
1.0	0.90	0.93	3.6	3.5
5.0	4.55	4.55	1.8	1.8
10.0	9.50	9.41	1.7	1.5
20.0	19.20	19.00	0.9	1.2

This assay has the advantages of giving clean extracts from serum, good resolution and high sensitivity. It provides a very rapid method for the routine analysis of ORN and MET levels in hospitalized patients in the therapeutic concentration range.

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