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Journal of Chromatography B, 691 (1997) 208–211

JOURNAL OF CHROMATOGRAPHY B

Short communication

Rapid and selective analysis of secnidazole in human plasma using high-performance liquid chromatography with ultraviolet detection

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Received 22 January 1996; revised 15 August 1996; accepted 9 September 1996

Abstract

A rapid, reproducible high-performance liquid chromatographic method for the determination of secnidazole, 5-nitroimidazole class of antiprotozoals from blood is described. Metronidazole was used as an internal standard. A simple extraction step with dichloromethane was done before chromatography on a C_{18} column with the wavelength fixed at 276 nm on the UV detector. Blood levels up to 500 ng/ml have been measured with good precision in the healthy volunteers after 1 g of secnidazole was administered. The present described method can readily be utilized for routine pharmacokinetic studies.

Keywords: Secnidazole

1. Introduction

Secnidazole, a nitroimidazole derivative [(hydroxy-2-propyl)-1-methyl-2-nitro-5-imidazole] and metronidazole [(hydroxy-2-ethyl)-1-methyl-2-nitro-5-imidazole] are two chemically related drugs used in the chemotherapy of protozoal and anaerobic bacterial infections [1]. Barring their broad similarity, the compounds differ markedly with respect to their pharmacokinetic characteristics and, hence, in dosage regimens. Recommended dose of 2 g of secnidazole single administration produced peak concentrations ranging from 35.7 to 46.3 mg/l and were achieved between 1.2–3.0 h [2–4]. Different analytical methods have been employed for comparative studies of N-1-substituted 5-nitroimidazoles like ornidazole, tinidazole and metronidazole. These include bioassay [5], absorptiometry [6], polarography [7], "flying-spot" thin-layer chromatographic (TLC) densitometry [8], high-performance liquid chromatography [9–12] and gas chromatography with flame ionisation detection [13–15], and with electron-capture detection [16,17].

In this paper, we report another sensitive and selective high-performance liquid chromatographic procedure for the analysis of secnidazole. Compared to many above methods described, the present method is easy, reproducible and suitable for routine pharmacokinetic studies following therapeutic doses of secnidazole.

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PII S0378-4347(96)00419-7

2. Experimental

2.1. Chemicals and equipment

Secnidazole and metronidazole (I.S.) were obtained from Biological E. (Hyderabad, India). Reagent-grade sodium acetate, sodium hydroxide and acetic acid and all HPLC-grade solvents like methanol, acetonitrile, water etc., were obtained from Qualigens (India). A vortex mixer (Model 2MLH), a 16-tube centrifuge (R87C) from Remi Instruments (India) and a metabolic shaker (NSW 133) from NSW (Delhi, India) were used for sample preparation.

2.2. Stock and standard solutions

Stock solutions of secnidazole and metronidazole equivalent to 1 mg/ml were prepared in methanol. Different working standard solutions giving 1.0, 5.0, 10.0, 20.0 and 40.0 μg/ml were prepared from stock solution. Working internal standard (metronidazole) solution having a concentration of 1 μg/25 μl was also prepared.

2.3. Chromatographic systems and conditions

HPLC separations were performed using a Shimadzu (Shimadzu corporation, Kyoto, Japan) Model LC-6A reciprocating pump at a flow-rate of 1.5 ml/min for solvent delivery. A Shimadzu SPD-6AV variable-wavelength detector, operated at 276 nm, was used throughout the determination. The column (250×4.6 mm I.D.) packed with 5 μm ODS material (Shimpack of Shimadzu, Kyoto, Japan) was used to separate the peaks. The eluent was composed of 10% (v/v) acetonitrile in 0.02 *M* sodium acetate, pH 4.0 (adjusted with acetic acid). The samples were injected into the column by a SIL-6B auto-injector (Shimadzu). The column temperature was maintained at 40°C with the help of a column oven CTO-6A (Shimadzu).

2.4. Sample preparation procedure

2.4.1. Extraction

To 0.5 ml of plasma, 1 μg of metronidazole (I.S.) and 100 μl of 2.5 *M* sodium hydroxide was added in a screw-type centrifuge tube and mixed well. Eight milliliters of dichloromethane was then added to each tube, the tubes were stoppered tightly with a teflon seal and extraction was carried out on a reciprocal shaker horizontally for 10 min. Following centrifugation, 6–7 ml of organic phase was transferred to a 10-ml conical glass tube and evaporated to dryness in a water bath at 40–50°C under a gentle stream of nitrogen. The sides of the tubes were rinsed with 200 μl of mobile phase and a 20-μl aliquot was injected into the chromatographic system.

2.5. Calibration curves

Sets of standard were prepared by the addition of secnidazole ranging from 1–40 μg to 1 ml of blank plasma. The chromatographic peak-height ratios of secnidazole/metronidazole (I.S.) vs. the corresponding secnidazole concentrations were subjected to linear regression.

3. Results and discussion

Fig. 1 shows typical chromatograms of extracted samples of blank human plasma (A), plasma spiked with secnidazole equivalent to 1 μg/ml and 1 μg/ml metronidazole, the internal standard (B) and plasma from a human volunteer who received 1 g of secnidazole with 1 μg/ml metronidazole (I.S.) added (C). Total run time of the chromatogram was within 10 min.

3.1. Recovery and detection limit

Recovery of secnidazole from the extraction procedure was determined by comparing the peak height ratios of aqueous chemical samples to blank plasma samples spiked with known concentration of secnidazole and subjected to a similar extraction procedure. Based on a signal-to-noise ratio of 4:1 (three times to basal line), the lower limit of detection was 500 ng/ml. Blood levels as low as 50 ng/ml for 5-nitroimidazoles have been measured with good precision [17], however, this method requires gas chromatography with an electron-capture detector. A similar method for ornidazole requires an elaborate

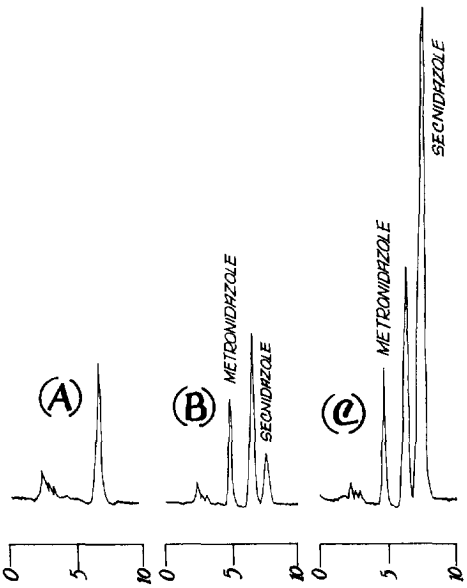

Fig. 1. Chromatograms of human plasma extracts: (A) tracing of human blank plasma; (B) human plasma spiked with 1 μg/ml secnidazole and 1 μg/ml metronidazole (I.S.); (C) plasma from human volunteer who received orally 1 g of secnidazole. Lower panel shows the total run time of chromatogram.

extraction procedure followed by a TLC clean-up step [6].

3.2. Linearity and reproducibility

The calibration curve was linear in the range tested, 1–40 μg/ml with a correlation coefficient >0.998. The accuracy (difference between the amount added to blank plasma and the amount found) and the reproducibility (represented by the relative standard deviation to the mean of replicate analyses) of the method were determined by processing spiked plasma samples. Six replicate samples were run for each concentration. The results are given in Table 1.

3.3. Accuracy and precision

The method was applied to determine concentrations of spiked blood samples in the range 1–40 μg/ml for assessing the accuracy and precision of the procedure. Table 1 shows the mean values and C.V. values.

Determination of secnidazole in the range 0.5–50 ng/μl in microsamples of gingival crevicular fluid and plasma by HPLC yielded C.V. values ranging from 1.4 to 11% [17]. Although the method has the advantage of no extraction step and requires a low sample volume, it requires microfiltration and gradient elution.

Table 1
Recovery, accuracy and reproducibility derived from spiked blood samples

Added known concentration of secnidazole (μg/ml)	Observed mean peak-height ratio	Calculated concentration of secnidazole (percentage of theory) (μg/ml)	Relative standard deviation (%)
1.00	0.47	1.02 (102)	8.5
5.00	2.23	4.85 (97)	4.0
10.00	4.74	10.30 (103)	2.7
20.00	9.38	20.40 (102)	2.4
40.00	18.98	40.04 (100)	8.4
Mean			5.2

Results are based upon six within-day replicate injections.

3.4. Application

The described procedure has been used to measure the blood levels of secnidazole following a single oral dose of 1 g secnidazole to six healthy volunteers. A mean C_{max} of 25.68±3.44 μg/ml with range (21.9–31.1 μg/ml) was achieved at a mean t_{max} of 2.67±1.21 h with range (1.0–4.0 h), and a mean level of 2.88±1.09 μg/ml with a range (1.0–4.0 μg/ml) was detected at 72 h (Fig. 2). Under the present assay conditions, there were no interferences with the analysis of the secnidazole. A comparison of various weighting factors showed that 1/response-squared ($1/R^2$) weighting was most similar to 1/variance weighting for the plasma standard curve (Table 1). Using $1/R^2$ weighting (Table 1), the mean calculated plasma concentrations ranged from 97 to 103% of the added concentrations. The relative standard deviation (R.S.D.) values of the peak-height ratios ranged from 2.4 to 8.5% (mean 5.2%). Mean recoveries of secnidazole from this extraction procedure were 97 and 100% at 1 and 40 μg/ml, respectively.

During the method development, we tried using

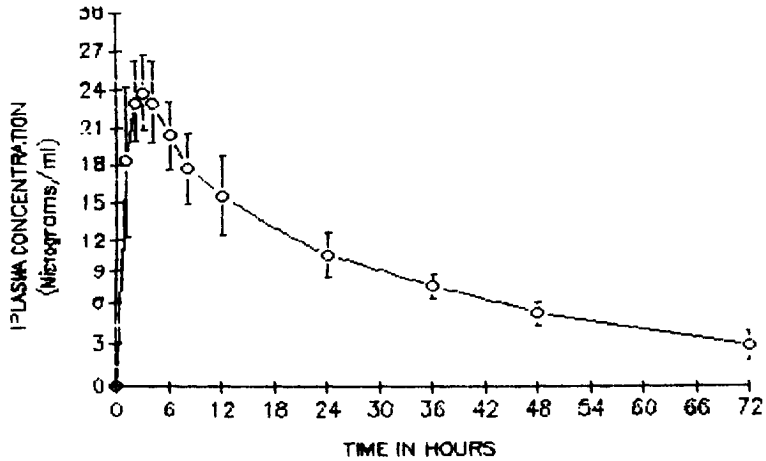

Fig. 2. Mean plasma secnidazole concentrations–time profile in 6 healthy human volunteers, after oral administration of single dose of 1 g secnidazole.

less toxic organic solvents like methanol and acetonitrile for the extraction, but did not achieve a good recovery of secnidazole, and there were a lot of interfering peaks with rising column pressure. This paper describes a rapid and reproducible procedure for the estimation of secnidazole in human plasma using a related compound, metronidazole, as an internal standard. The method is less expensive, involving a simple single step extraction procedure and can readily be adopted for routine pharmacokinetic studies following therapeutic doses.

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