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Direct Assay of Tinidazole in Human Serum by Micellar Liquid Chromatography

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A liquid chromatographic procedure for the direct determination of tinidazole in human serum is presented. It includes the use of a micellar mobile phase consisting of SDS (5.10^{-2} M): propan-1-ol; (94:6; v/v) and a μBondapak CN column with UV detection at 320 nm. No solvent extraction or deproteinization are necessary. The linearity (0.1–10 mg L), the precision (3%), the reproducibility (1.3%), the recovery (99%), and the detection limit (0.1 mg L) in the tinidazole determination are comparable and sometimes greater than the corresponding tinidazole parameters when deproteinization and conventional reversed-phase HPLC are used. One hundred injections of serum samples do not affect the column life. The procedure is applied to ascertain the pharmacokinetics of 10 mg/kg of tinidazole.

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INTRODUCTION

Tinidazole (1-[2-ethylsulphonyl)-ethyl]-2-methyl-5-nitroimidazole) is an antiparasitary drug used against trichomonasis (Mettana *et al.*, 1993). To determine its serum concentration, a number of analytical methods have been described, including polarography (Crooper and Hodless 1967; De Silva *et al.*, 1970), spectrophotometry (Petrik *et al.*, 1994; Parimos and Umapath, 1994), spectrofluorimetry (Laue *et al.*, 1969; Laufen *et al.*, 1979), gas chromatography (Laufen *et al.*, 1979; Midha *et al.*, 1981; Wood, 1975; Bhatia and Shanbhag, 1984) and thin-layer chromatography with densitometry (Welling and Munro, 1972). A wide variety of revered-phase high-performance liquid chromatographic (RP-HPLC) procedures have been described using sample pre-treatments such as solvent extraction, protein precipitation or solid-phase extraction (Menouer *et al.*, 1987; Pyorala *et al.*, 1994; Tendolkar *et al.*, 1994).

Determination of drugs by direct injection of serum sample without extraction is a HPLC trend. For this purpose, a number of methods have been suggested in the literature such as the use of large-pore columns (Hagestam and Pinkerton, 1985), internal-surface reversed-phase chromatography (Nakagawa and Haginaka, 1987; Pinkerton *et al.*, 1986; Jussi and Eriksson, 1989) and micellar liquid chromatography (De Luccia *et al.*, 1985, 1986; Habel *et al.*, 1993).

In this study, we describe a simple and specific HPLC method for the direct determination (without extraction or sample pretreatment) of tinidazole in human serum using micellar mobile phase which contains sodium *n*-dodecylsulphate as a surfactant. The method was compared to a conventional reversed-phase HPLC procedure which includes serum extraction developed in our laboratory (Menouer *et al.*, 1987).

EXPERIMENTAL

Reagents. All solvents (spectroscopic grade) and sodium acetate used were from Fluka (Buchs, Switzerland). Tinidazole used was

Table 1 Chromatographic parameters of tinidazole using the direct and deproteinization method

	Direct method	Deproteinization method
Capacity factor of tinidazole	2.4	1.7
Capacity factor of internal standard	1.8	0.65
Plate numbers/m calculated on tinidazole peak	8000	15000
Asymmetry factor of tinidazole peak	1.1	1.2
Resolution between tinidazole and internal standard	2.3	8
Calibration Slope L/mg in the presence of serum	0.230	0.229
Calibration intercept mg/L in the presence of serum	$4.75.10^{-5}$	0.042
Calibration correlation coefficient in the presence of serum	0.997	0.996
Detection limit mg/L	0.1	0.5
Calibration slope L/mg in the absence of serum	0.231	0.248
Calibration intercept mg/L in the absence of serum	0.05	0.016
Calibration correlation coefficient in the absence of serum	0.990	0.994
Recovery (%) for tinidazole concentration of 5 mg/L	99	92
Precision (%)	3	5
Reproducibility (%)	1.3	5

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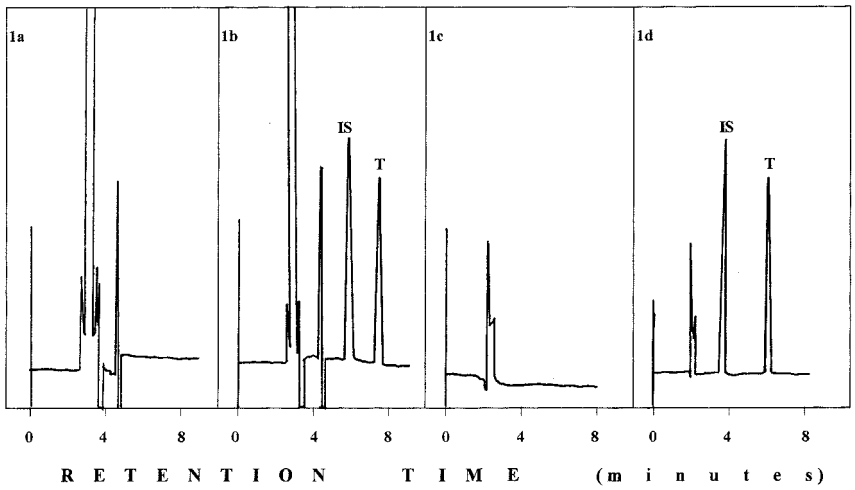

Figure 1. Typical chromatograms of blank serum (1a,1c) and patients' serum (1b,1d) obtained with the direct (1a,1b) and deproteinization (1c,1d) methods. IS : internal standard; T : tinidazole.

from Pfizer (Houston, Txs, USA). Sodium *n*-dodecylsuphate and propanol-1 were from Merck (Darmstadt, Germany) and Metronidazole was from Gedeon-Richter (Budapest, Hungary).

Apparatus. HPLC was performed with a Waters Liquid Chromatograph (ALC/GPC 244) consisting of 6000A pump, an U6K universal injector and a Pye Unicam PU 4020 UV detector operating at 320 nm. For the direct method, a μ Bondapack CN

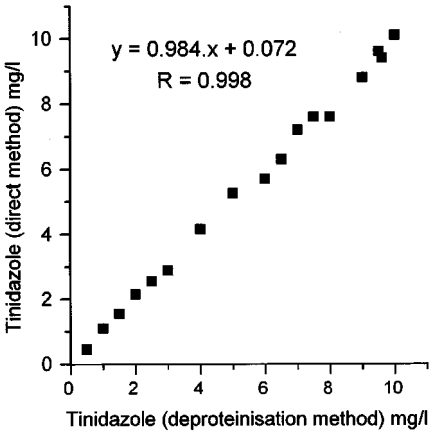

Figure 2. Correlation between results of the direct (*y*) and deproteinization (*x*) methods in the determination of tinidazole in serum.

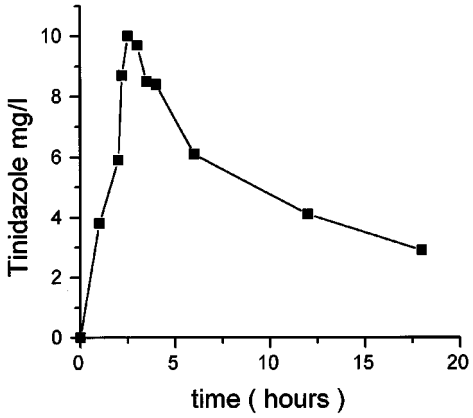

Figure 3. Serum concentration–time profile after the administration of 10 mg/kg tinidazole.

(3.9 × 300 mm) column was used; it was preceeded by a guard column (4 × 0.4 cm) laboratory-packed with Corasil II C18 (Waters). Between the pump and injector, a precolumn (5 × 0.4 cm) packed with silica was used to saturate the solvent. Mobile phase was : SDS (5.10^{-2} M) and 6% (v/v) of propan-1-ol.

For the method including protein precipitation called indirect method, the column was μ Bondapak C18 (Waters) preceeded by an identical guard column. Mobile phase was acetate buffer (0.05 M) pH = 4.7 containing 22% (v/v) of acetonitrile.

Sample preparation. *Direct method*. A 100 mg/L solution of internal standard (metronidazole) was made. To 490 μL of patient serum, 10 μL of internal standard solution were added. The solution was filtering using a 0.45 μm millex filter (millipore, USA) and 20 μL were injected into the chromatograph.

Deproteinization method. This was done by the method of Menouer *et al*. (1987) to 0.1 mL of serum, 0.1 mL of metronidazole (internal standard; 10 mg/L) were added. Proteins were precipitated by 0.8 mL of acetonitrile. After centrifugation (2500 G, 10 min), the supernatant was filtered on a Millex 0.45 μm filter (millipore). The aliquot was evaporated to approximately 0.1 mL and 25 μL were injected into the chromatograph.

Calibration, recovery, precision and reproducibility. The same procedure was used for direct and deproteinization methods. Calibration was accomplished using the following procedure: Ten 0.5 mL aliquots of blank serum were used to which various Tinidazole concentrations (0.1–10 mg/L[9]) and a constant internal standard concentration (2 mg/L) were added. The samples were treated and analysed as described above. Reproducibility was determined in the same way but tinidazole had a constant concentration of 10 mg/L.

Recovery was established with the calibration procedure using standard solutions without serum. The within-run precision was estimated by analysing ten 20 μL aliquots of blank serum containing tinidazole and internal standard, both added at a concentration of 2 mg/L.

RESULTS AND DISCUSSION

The main results obtained with direct and deproteinization methods are listed in Table 1. The following remarks can be made: (I) with the direct method, capacity factors of

tinidazole and internal standard were lower; (ii) the chromatographic system with micellar mobile phase (direct method) showed a lower efficiency, a more symmetrical tinidazole peak, a lower detection limit and a better recovery, precision and reproducibility.

Several patients' sera were analysed by direct and deproteinization methods. Typical chromatograms are shown in Fig. 1. The data presented in Fig. 2 were indicative of satisfactory correlation between the results obtained from the direct and deproteinization methods over the concentration range studied. The equation of the straight lines is given in Fig. 2.

Serum samples were obtained prior to dosing at several subsequent time points. Using micellar mobile phase (direct method), the highest tinidazole concentration was found in the 2 h sample (Fig. 3) after the administration of 10 mg/kg. The last sample in which tinidazole was detected was 18 h after administration.

CONCLUSION

The method described in this report has proved useful for the analysis of tinidazole in the concentration range 0.1 to 10 mg/L. It includes direct injection of the serum without solvent extraction or deproteinization and the use of micellar mobile phase. The precision and accuracy of the method are better compared with conventional reversed-phase HPLC using protein precipitation. The assay described is more attractive because of its speed and the simplicity of sample treatment. It can be used for routine analysis in the clinical laboratory.

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