Determination of Tinidazole in Human Plasma by RP-HPLC

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Key words Tinidazole; Metronidazole; HPLC

1 Introduction

Tinidazole, ethyl-2-(2-methyl-5-nitro-1-imidazolyl ethyl) sulfone, one of the most active compounds in a series of nitroimidazole derivatives active against *Trichomonas vaginalis*, has been used as an antiprotozoal drug for many years. The compound also possesses excellent inhibitory activity against anaerobic bacteria and has become a mainstay as an effective therapeutic and prophylactic agent in treatment of infections caused by these microorganisms, notably by *Bacteroides fragilis*[1].

In an attempt to measure tinidazole or other *N*-1-substituted nitroimidazoles in plasma samples, here we report a simple and selective RP-HPLC method using an internal standard which was different from previous reports[2~6].

2 Experimental

2.1 *Material*

Tinidazole standard was obtained by courtesy of China Pharmaceutical University in Nanjing China. Metronidazole (China Pharmaceutical University, Nanjing, China) was used as internal standard. Acetonitrile was HPLC grade. Other solvents and regents were of analytical grade.

2.2 *Analytical instruments*

The HPLC system used consisted of a pump (Shimadzu LC-6A, Shimadzu, Kyoto, Japan), a reversed-phase colum packed with Spherisorb ODS (10μm, 300 mm× 4.6 mm, I.D.), a column oven (Shimadzu CTO-6A) operated at 25℃, a spectrophotometric detector (Shimadzu SPD-6AV) operated at 0.005 a.u.f.s. and a wavelength of 318 nm and an integrator (Shimadzu C-R4A Chromatopac).

2.3 *Mobile phase*

The mobile phase consisted of acetonitrile and water (22∶78, v/v), and was pumped at a constant flow-rate of 1.0 ml/min.

2.4 *Extraction and assay procedures*

The sample for analysis was prepared as follows. An aliquot of 2 ml of plasma sample was placed in a 10-ml tube, centrifuged at 2000 g for 10 min. A 0.5 ml of the supernatant was transferred to a new 5-ml tube, followed by 2.5μl metronidazole solution (1 mg/ml) as an internal standard and 0.5 ml acetonitrile. After Vortex mixing for 1 min, the mixture was centrifuged at 3000 g for 10 min, and a 20μl aliquot of the supernatant was directly injected into the HPLC system.

2.5 *Calibration standard*

Standard solution of tinidazole was p-

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repared by weighing 10 mg tinidazole accurately, transferring it to a volumetric flask, and adding water to 100 ml to produce a stock solution of 100 μg/ml. A 100 mg amount of metronidazole was also accurately weighed, transferred to a 100-ml volumetric flask, and water added to 100 ml to produce a stock solution of internal standard of 1000 μg/ml. Both stock solutions were stable for at least three months at 4℃. Control blank plasma samples were spiked with tinidazole in the concentration (range of 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 μg/ml) and subjected to the treatment described above. Peak-area ratios of tinidazole/metronidazole(A_s/A_{in}) versus the concentration of tinidazole(μg/ml) were subjected to linear regression analysis. The concentration of tinidazole in samples was then obtained from the regression equation.

2.6 *Samples from healthy volunteers*

Blood (2 ml) samples obtained from eight healthy male volunteers by oral administration of 400 mg capsules of tinidazole were drawn into heparnized dry tubes. The samples were centrifuged for 10 min at 2000 g and treated as described above.

3 Results and Discussion

Standard calibration curve was constructed by plotting the peak-area ratios of tinidazole/metronidazole (A_s/A_{in}) against the concentration of tinidazole. A linear regression analysis revealed a standard curve of $C = 3.226\ A_s/A_{in} - 0.0295$, $r = 0.9998$.

The absolute recovery of tinidazole was calculated by comparing the peak area obtained after direct injecton of standard solutions with that obtained after the plasma extraction procedure. Table 1 shows that the average recovery of tinidazole was (94.2± 5.1)%.

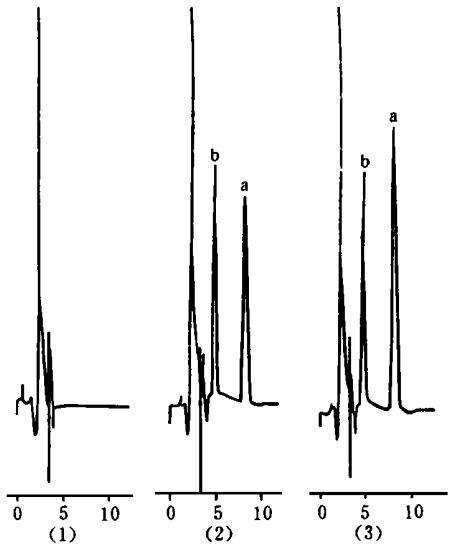

Fig 1. RP-HPLC of tinidczole

(1) blank plasma; (2) plasma containing tinidazole(a) and metronidazole(b); (3) sample plasma

Fig. 1 shows a chromatogram of tinidazole and metronidazole. Fig. 1-(1), (2) shows that the plasma components did not interfere with tinidazole and metronidazole (internal standard), and Fig. 1-(3) shows a chromatogram of a plasma extract from a healthy volunteer treated with tinidazole. The retention time of tinidazole and metronidazole was 8.35 min and 4.97 min, respectively.

Tab 1. Extraction recovery of tinidazole

Added (μg/ml)	Found (μg/ml)	Recovery (%)
0.5	0.424	84.7
1	0.928	92.8
2	1.924	96.2
4	3.921	98.0
8	7.589	94.9
16	15.781	98.6

Table 2 shows the good reproducibility of the assay. The coefficient of variation (RSD) was less than 7% for both inter-and intra-assay of tinidazole. Limit of detection was defined as the lowest detectable concentration with a signal-to-noise ratio higher than 3∶1. The low limit of detection of tinidazole was 2 ng.

Tab 2. Intra-day and Inter-day reproducibility of the assay(n= 5)

Concentration (μg/ml)	RSD (%)	
	Intra-day	Inter-day
0.5	3.92	5.06
1	5.57	5.57
8	5.49	6.14

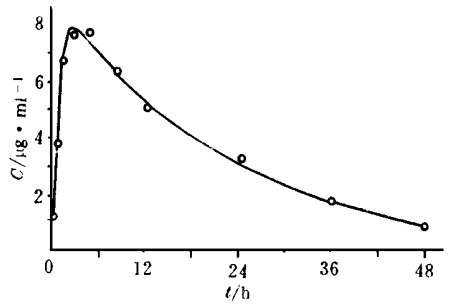

Fig 2. Concentration of tinidazole in human plasma after oral administration of 400 mg tinidazole capsules(n= 8) ○ observed; — calculated

The method described was applied to the determination of tinidazole in plasma samples of healthy volunteers following oral administration of 400 mg of tinidazole (Fig. 2): $t_{1/2}$= 16.52 h, C_{max}= 7.89μg/ml, t_{max}= 2.30 h.

Determination of tinidazole in plasma by HPLC has been reported[2-6]. However quantitations were accomplished using an external standard and some of the extraction techniques were complicated. This report describes a simple, accurate and efficient RP-HPLC method. We adopted a rapid extraction procedure of tinidazole, imple mobile phase, and metronidazole as an internal standard. Tinidazole and metronidazole(internal standard) were well seperated from each other and from plasma components, and their chromatographic peaks were sharp and the retention time was short.

This method can be automated to handle a large number of plasma samples and suitable for the rapid estimation of tinidazole in human plasma at concentrations for pharmacokinetic studies.

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反相高效液相色谱法测定人血替硝唑浓度

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摘 要 采用反相高效液相色谱法测定人血浆替硝唑浓度。色谱柱为 Shperisorb ODS,紫外吸收检测波长为 318 nm 以甲硝唑为内标物。本法检测日内和日间相对标准偏差均小于 7%。从血浆提取替硝唑的平均回收率为 94.2%。

关键词 替硝唑; 甲硝唑; HPLC