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Short communication

Determination of a new proton pump inhibitor, YH1885, in human plasma and urine by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method was developed for the determination of a new proton pump inhibitor, YH1885 (I), in human plasma and urine, and rat blood and tissue homogenate using fenticonazole as an internal standard. The sample preparation was simple: a 2.5 volume of acetonitrile was added to the biological sample to deproteinize it. A 50-μl aliquot of the supernatant was injected onto a C_8 reversed-phase column. The mobile phase employed was methanol–0.005 *M* tetrabutylammonium dihydrogenphosphate (77:23, v/v), and it was run at a flow-rate of 1.0 ml/min. The column effluent was monitored using an ultraviolet detector at 270 nm. The retention times for I and the internal standard were 9.0 and 10.3 min, respectively. The detection limits for I in human plasma and urine, and in rat tissue homogenate (including blood) were 50, 100 and 100 ng/ml, respectively. The coefficients of variation of the assay (within- day and between-day) were generally low (below 8.84%) for human plasma and urine, and for rat tissue homogenate. No interferences from endogenous substances were found. © 1997 Elsevier Science B.V.

Keywords: YH1885; [5,6-Dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl pyrimidine]

1. Introduction

The Yuhan Research Center of Yuhan Corporation (Kunpo, South Korea) has recently developed a new proton pump inhibitor, YH1885, [5,6-dimethyl-2-(4-fluorophenylamino)-4-(1-methyl-1,2,3,4-tetrahydroisoquinolin-2-yl pyrimidine] (I, Fig. 1). Compound I is now being considered for evaluation in Phase I clinical trials.

This paper describes a high-performance liquid chromatographic (HPLC) method with a simple sample preparation procedure (deproteinization with acetonitrile) for the determination of I in human plasma and urine, and in rat blood and tissue homogenate.

2. Experimental

2.1. Chemicals

Compound I and fenticonazole (as a nitrate salt), the internal standard for the HPLC assay (Fig. 1), were donated by the Yuhan Research Center of

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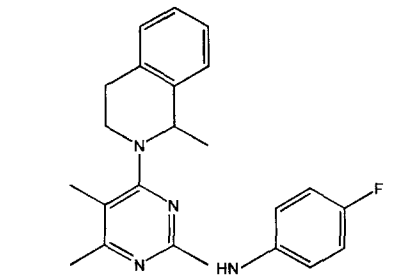
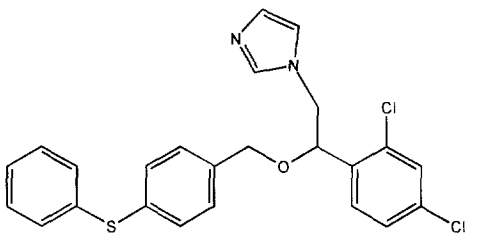


Compound I



Fenticonazole nitrate (the internal standard)

Fig. 1. Chemical structures of I and fenticonazole (the HPLC internal standard).

Yuhan Corporation. The other chemicals were of reagent grade or HPLC grade and were used without further purification.

2.2. Preparation of standard solutions

A stock solution of I was prepared in methanol (1 mg/ml). Appropriate dilutions of the stock solution were made with methanol. Standard solutions of I in human plasma and urine, and in rat blood and tissue (or organ) homogenate (1 g of each tissue or organ was homogenized with four volumes of a 0.9% NaCl injectable solution, centrifuged for 10 min at 9000 *g* and the supernatant was collected) were prepared by spiking with an appropriate volume (less than 10 μl per ml of biological sample) of the diluted stock solution, giving final concentrations of 0.05, 0.1, 0.2, 1 and 5 μg/ml. The internal standard solution was prepared by dissolving fenticonazole nitrate in acetonitrile to give a final concentration of 1 μg/ml as free fenticonazole. Only the HPLC assay results

Table 1
Response factors and accuracies of I at various concentrations in human plasma and urine using the deproteinization method

Spiked concentration (μg/ml)	Response factor[a]	Accuracy[b] (%)
Human plasma		
5	0.403 (6.25)	98.3
0.1	0.401 (2.56)	97.8
0.05	0.427 (3.47)	104
Human urine		
5	0.285 (3.76)	96.9
1	0.279 (4.43)	94.9
0.1	0.317 (2.61)	108

Values in parentheses are within-day coefficients of variation (%); $n=5$.

[a] (Drug peak height (cm) divided by its concentration, μg/ml)/(internal standard peak height (cm) divided by its concentration, μg/ml); mean.

[b] (Mean measured concentration/spiked concentration)×100; mean.

obtained using 0.05, 0.1 and 5 μg/ml for human plasma, and 0.1, 1 and 5 μg/ml for human urine are listed in Table 1; those found using 0.1 and 1 μg/ml for rat tissue (or organ) homogenate are not listed. Response factors were calculated by dividing the peak height (cm) of I by its concentration (μg/ml)/dividing the peak height (cm) of internal standard by its concentration (μg/ml). Accuracy was calculated by dividing the mean measured concentration by the spiked concentration.

2.3. Sample preparation

A 125-μl volume of acetonitrile containing the internal standard (1 μg/ml) was added to 50-μl of human plasma or urine samples. After vortex mixing and centrifugation at 9000 *g* for 10 min, a 50-μl volume of the supernatant was injected directly onto the HPLC column. Each rat tissue (or organ) was homogenized with four volumes of a 0.9% NaCl injectable solution using a tissue homogenizer (Ultra-Turrax, T25, Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) and was immediately centrifuged at 9000 *g* for 10 min. A 50-μl volume of supernatant was collected and treated as described for human plasma and urine samples. Two volumes of distilled water were added to rat blood to facilitate

the hemolysis of blood cells and to increase the reproducibility of I [1,2]. After vortex mixing and centrifugation at 9000 *g* for 10 min, a 50-μl aliquot of the supernatant was collected and processed as described for human plasma and urine samples.

2.4. HPLC apparatus

The HPLC system consisted of a Model 7125 injector (Rheodyne, Cotati, CA, USA), a Model 1330 pump (Bio-Rad, Japan Servo, Tokyo, Japan), a reversed-phase C_8 column (15 cm×3.9 mm I.D.; particle size, 4 μm; Waters, Milford, MA, USA), a Model 1306 UV detector (Bio-Rad) and a Model 1200 recorder (Linear, Reno, NV, USA). The mobile phase, methanol–0.005 *M* tetrabutylammonium dihydrogenphosphate (77:23, v/v), was run at a flow-rate of 1.0 ml/min and the column effluent was monitored by UV detection at 270 nm.

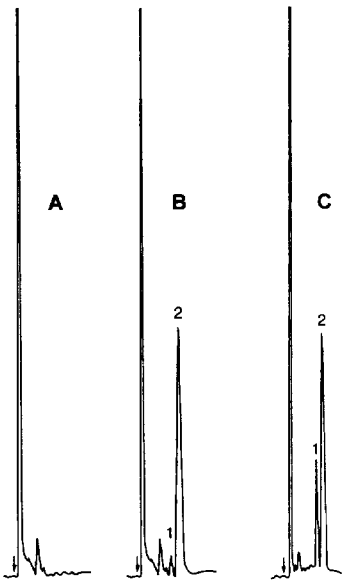


Fig. 2. Chromatograms after deproteinization of drug-free human plasma (A), human plasma spiked with 0.2 μg/ml of I and 1 μg/ml of internal standard (B), and of plasma collected from a male Sprague-Dawley rat 5 min (1.13 μg/ml) after the intravenous administration (over 1 min) of 20 mg/kg of I (C). Peaks: 1=I (9.0 min); 2=internal standard (10.3 min). The arrows mark the point of injection. The detector's sensitivity was set at 0.01 AUFS and the recorder's sensitivity was set at 10 mV.

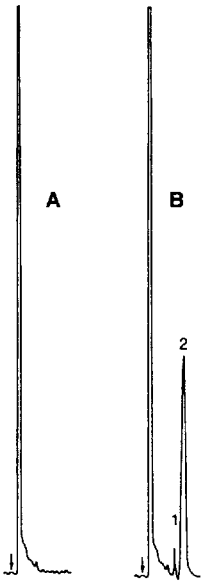


Fig. 3. Chromatograms after deproteinization of drug-free human urine (A) and human urine spiked with 0.5 μg/ml of I and 1 μg/ml of internal standard (B). Peaks: 1=I (9.0 min); 2=internal standard (10.3 min). The arrows mark the point of injection. The detector's sensitivity was set at 0.01 AUFS and the recorder's sensitivity was set at 10 mV.

3. Results and discussion

Fig. 2. shows typical chromatograms of drug-free human plasma, drug standards in human plasma and of plasma collected 5 min after the intravenous administration of 20 mg/kg of I to a rat using the deproteinization method; the corresponding chromatograms for human urine and rat liver homogenate are shown in Figs. 3 and 4, respectively. No interferences from endogenous substances were observed in any of the biological samples. The retention times for I and the internal standard were 9.0 and 10.3 min, respectively. The detection limits for I in human plasma and urine were 50 and 100 ng/ml, respectively (Table 1), based on a signal-to-noise ratio of 3.0. The mean within-day coefficients of variation (C.V.s) of I in human plasma and urine were 4.09% (range 2.56–6.25%) and 3.60% (range 2.61–4.43%), respectively (Table 1). The between-day C.V.s for the analysis of the same samples on three consecutive days for I were 3.96 and 6.08% in human plasma and

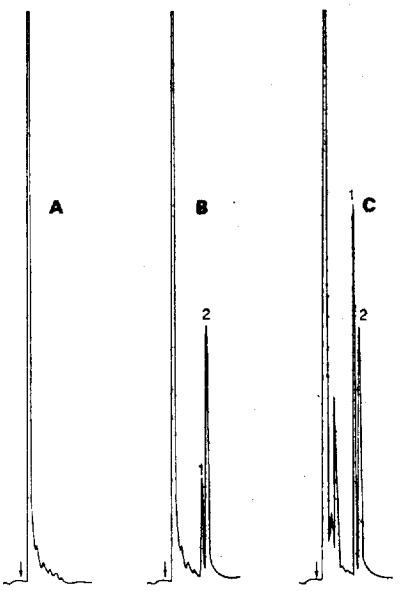


Fig. 4. Chromatograms after deproteinization of drug-free rat liver homogenate (A), rat liver homogenate spiked with 1 μg/ml of both I and the internal standard (B), and of liver homogenate collected from a male Sprague-Dawley rat 30 min (3.90 μg/ml) after the intravenous administration (over 1 min) of 10 mg/kg of I (C). Peaks: 1=I (9.0 min); 2=internal standard (10.3 min). The arrows mark the point of injection. The detector's sensitivity was set at 0.01 AUFS and the recorder's sensitivity was set at 10 mV.

urine, respectively. The accuracy [(mean measured concentration/spiked concentration)×100] of I was 97.8–104% and 94.9–108% for human plasma and urine, respectively (Table 1). The peak heights of fenticonazole were comparable for the plasma (Fig. 2) and urine (Fig. 3) samples. However, the peak height of I was less in the urine sample than in the plasma sample. Therefore, the response factor of I in the urine sample was lower than in the plasma sample (Table 1). This could be the result of binding and/or adsorption of I to the endogenous compounds in urine. Similar results were also found with azosemide [3] and methotrexate [4].

This HPLC method was also successful for the determination of I in rat blood and tissues (liver, lung, heart, brain, kidney, muscle, stomach, intestine and spleen). The detection limit for I was 100 ng/ml for rat blood and for the tissues (or organs) studied. The mean within-day C.V.s for I ranged from 0.554% (spleen at 1 μg/ml) to 8.84% (intestine at 0.1 μg/ml). The accuracy ranged from 92.3% (liver at 0.1 μg/ml) to 108% (liver at 1 μg/ml). It should be noted that because of the poor water solubility of I, the relative recovery of I compared with water could not be measured in our biological samples.

Compound I, 10 mg/kg, was administered intravenously over 1 min via the jugular vein of a male Sprague-Dawley rat. The arterial plasma concentration–time profile of I is shown in Fig. 5. The terminal half-life, total body clearance, apparent volume of distribution at the steady state and the mean residence time of I in a rat were 215 min, 86.6 ml/min/kg, 19 200 ml/kg and 221 min, respectively. Compound I was not detected in rat urine samples.

The amount of I remaining per g of tissue (or organ) was also measured 30 min after intravenous administration (over 1 min) of 10 mg/kg of I to a male Sprague-Dawley rat; the values were 0.986 μg/ml for plasma, and 12.6, 21.6, 2.86, 5.48, 16.9, 4.26, 9.03, 1.42 and 5.94 μg/g for liver, lung, heart, brain, kidney, stomach, small intestine, large intestine and spleen, respectively.

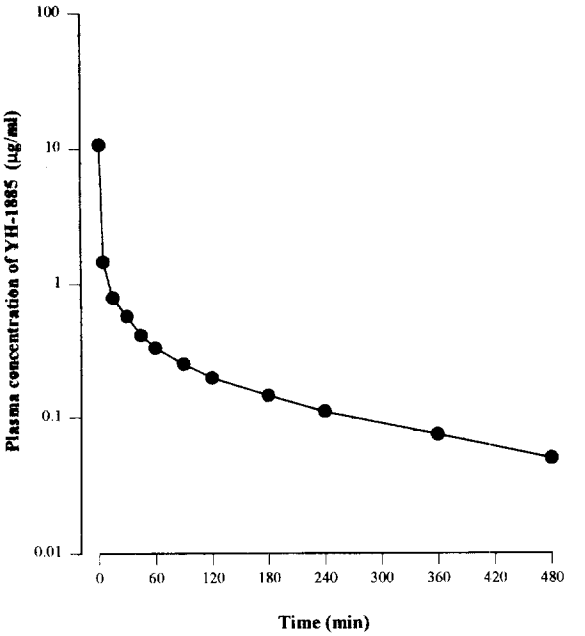


Fig. 5. Arterial plasma concentration–time profile of I after intravenous administration (over 1 min) of 10 mg/kg of I, to a male Sprague-Dawley rat.

Acknowledgments

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References

[1] M.G. Lee, C.Y. Lui, M.-L. Chen, W.L. Chiou, Int. J. Clin. Pharmacol. Ther. Toxicol. 22 (1984) 530.
[2] W.G. Shin, M.G. Lee, M.H. Lee, N.D. Kim, Biopharm. Drug Dispos. 13 (1992) 305.
[3] S.H. Lee, M.G. Lee, J. Chromatogr. B 656 (1994) 367.
[4] M.-L. Chen, W.L. Chiou, J. Chromatogr. 226 (1981) 125.