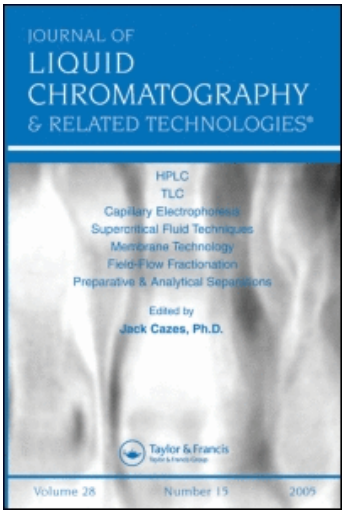

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HPLC ANALYSIS OF A NEW REVERSIBLE PROTON PUMP INHIBITOR, A DIHYDROPYRROLOQUINOLINE DERIVATIVE, IN PLASMA, URINE, AND TISSUE HOMOGENATES

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method was developed for the determination of a new reversible proton pump inhibitor, KR-60436, in human plasma and urine and in rat tissue homogenates. The method involved deproteinization of the biological samples with three volumes of acetonitrile. A 100 μL aliquot of the supernatant was injected onto a

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reversed-phase (C_{18}) column. The mobile phase, 0.02 M phosphate buffer (pH 5) : acetonitrile : methanol (30 : 60 : 10, v/v/v), was run at a flow rate of 0.7 mL/min and the column effluent was monitored by a fluorescence detector set at an excitation wavelength of 340 nm and an emission wavelength of 484 nm. The retention time of KR-60436 was approximately 7.5 min. The coefficients of variation (within-day and between-day) were low (below 10.5%) for human plasma and urine and rat tissue homogenates. No interferences from endogenous substances were found.

INTRODUCTION

The strategy for the development of an antiulcer drug has changed dramatically since the introduction of reversible proton pump inhibitors, such as SK&F 96067.[1] It has been speculated that the carbonyl group in SK&F 96067 is responsible for restricting the conformation of the arylamino group, both by forming a hydrogen bond and by increasing the conjugation between nitrogen and quinolone ring.[2] Therefore, a novel dihydropyrroloquinoline derivative, KR-60436 (1-(2-methyl-4-methoxyphenyl)-4-[(2-hydroxyethyl)amino]-6-b,b,b-trifluoromethoxy-2,3-dihydropyrrolo[3,2-c]quinoline, Fig. 1), was designed and synthesized by Korea Research Institute of Chemical Technology (Taejeon, Korea) as a conformationally constraint structure by forming of an additional ring.

KR-60436 has been identified as a reversible inhibitor of the gastric (H^+/K^+)-ATPase in gastric membrane vescicle preparations enriched in the (H^+/K^+)-ATPase with IC_{50} of 7.3 μM, which is more potent than SK&F 96067 with IC_{50} of 22 μM. Also, KR-60436 was shown to be a potent inhibitor of basal and histamine-stimulated gastric acid secretion in rats, and to have good protective activity against various ulcer models. KR-60436 has greater antisecretory activity

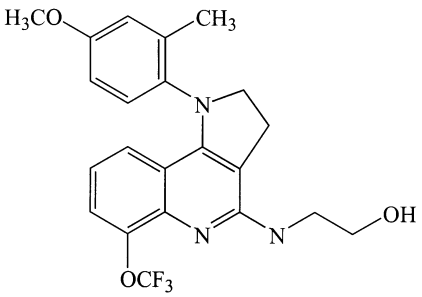

Figure 1. Chemical structure of KR-60436.

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and antiulcer activity than those of SK&F 96067, and could be developed as a new therapeutic agent for peptic ulcer disease. KR-60436 is being evaluated in preclinical study as a new reversible proton pump inhibitor.

The purpose of this paper is to report the high-performance liquid chromatographic (HPLC) method with a simple sample preparation (deproteinization with acetonitrile) for the determination of KR-60436 in human plasma and urine and in rat tissue homogenates.

EXPERIMENTAL

Materials

KR-60436 was supplied by AgroPharma Research Institute, Dongbu Hannong Chemical Company (Taejeon, Korea). Other chemicals were of reagent grade or HPLC grade and, therefore, were used without further purification.

Preparation of Stock and Standard Solutions

A stock solution of KR-60436 (1 mg/mL) was prepared in dimethyl sulfoxide (DMSO). Appropriate dilutions of the stock solution were made with DMSO. Standard solutions of KR-60436 in human plasma and urine and in rat tissue homogenates [approximately 1 g of each rat tissue or organ was homogenized (Ultra-Turrax, T25, Janke and Kunkel, TKA-Labortechnik, Staufen, Germany) with four volumes of distillated water, 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 fluids) of the variously diluted stock solutions, giving final concentrations of 0.05, 0.1, 0.5, 1, 10, and 20 μg/mL for human plasma and urine and 0.1, 1, and 10 μg/mL for rat tissue homogenates.

Preparation of Sample for HPLC Analysis

A 150 μL aliquot of acetonitrile[3,4] was added to deproteinize a 50 μL aliquot of the biological sample. After vortex-mixing and centrifugation at 9000 *g* for 10 min, a 100 μL aliquot of the supernatant was injected directly onto the HPLC column. The mobile phase, 0.02 M phosphate buffer (pH 5) : Acetonitrile : methanol (30 : 60 : 10, v/v/v), was run at a flow rate of 0.7 mL/min. The column effluent was monitored by a fluorescence detector set at an excitation wavelength of 340 nm and an emission wavelength of 484 nm.

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HPLC System

The HPLC system consisted of a model 7125 injector (Rheodyne, Cotati, CA, USA), a model 2250 pump (Bischoff, Leonberg, Germany), a reversed-phase column (RP-18; 15 cm, *l.* × 4.6 mm, i.d.; particle size, 3.5 μm; Hichrom, Berkshire, England), a model FL 3000 fluorescence detector (Thermo Separation Products, Riviera Beach, FL, USA) and a model 1200 recorder (Linear, Reno, NV, USA).

RESULTS AND DISCUSSION

Figure 2 shows typical chromatograms of drug-free human plasma, drug standard in human plasma, plasma collected at 60 min after 30 min intravenous administration of KR-60436, 20 mg/kg, to a rat, drug-free human urine, drug

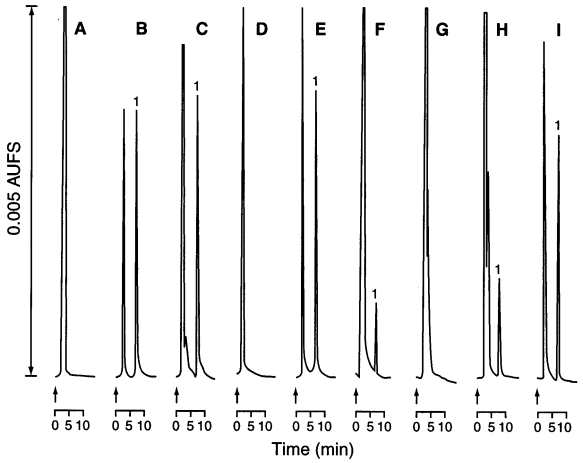

Figure 2. Chromatograms of drug-free human plasma (A), human plasma spiked with 1 μg/mL of KR-60436 (B), plasma collected from a male Sprague–Dawley rat at 60 min after 30 min intravenous infusion of 20 mg/kg of KR-60436 (C), drug-free human urine (D), human urine spiked with 1 μg/mL of KR-60436 (E), urine collected from a male Sprague–Dawley rat between 0 and 24 h after 30 min intravenous infusion of 20 mg/kg of KR-60436 (F), drug-free rat liver homogenate (G), rat liver homogenate spiked with 1 μg/mL of KR-60436 (H), and rat liver homogenate collected from a male Sprague–Dawley rat at 120 min after 30 min intravenous infusion of 20 mg/kg of KR-60436 (I). Peak: 1 = KR-60436 (7.5 min). The arrow marks the point of injection. The detector's sensitivity was set at 0.01 AUFS (absorption unit full scale) and recorder's sensitivity was set at 20 mV. The chart speed was 10 cm/h.

standard in human urine, urine collected between 0 and 24 h after 30 min intravenous administration of KR-60436, 20 mg/kg, to a rat, drug-free rat liver homogenate, drug standard in rat liver homogenate, and rat liver homogenate collected at 2 h after 30 min intravenous administration of KR-60436, 20 mg/kg, to a rat. No interferences from endogenous substances were observed in any of the biological samples (Fig. 2). The peak of KR-60436 was symmetrical and eluted at approximately 7.5 min (Fig. 2).

The detection limits of KR-60436 in human plasma and urine were both 0.05 μg/mL, based on a signal-to-noise ratio of 3.0 (Table 1). The mean within-day coefficients of variation (C.V.s) in human plasma and urine were 3.59% (range 1.24–5.32%) and 2.55% (range 0.898–6.87%), respectively, within concentration ranges from 0.05 to 20 μg/mL (Table 1). The between-day C.V.s of the analysis of the same samples on consecutive three days in human plasma and urine were lower than 3.94 and 6.37%, respectively, within concentration ranges from 0.05 to 20 μg/mL. The mean accuracies [(mean observed concentration/theoretical concentration) × 100] from human plasma and urine spiked with standards for KR-60436 were 93.0–104% and 92.0–105%, respectively, within concentration ranges from 0.05 to 20 μg/mL (Table 1). Note, that the mean response factor (peak height of KR-60436, mm/

Table 1. Response Factors and Accuracies of KR-60436 at Various Concentrations in Human Plasma and Urine

Added Amount (μg/mL)	Response Factor[a]	Accuracy[b] (%)
	Human Plasma	
0.05	112 ± 5.94 (5.32)	99.5
0.1	114 ± 4.99 (4.38)	102
0.5	114 ± 1.42 (1.24)	102
1	112 ± 4.61 (4.11)	100
10	117 ± 4.31 (3.69)	104
20	104 ± 2.93 (2.81)	93.0
	Human Urine	
0.05	123 ± 8.44 (6.87)	98.1
0.1	132 ± 4.20 (3.19)	105
0.5	124 ± 1.44 (1.16)	98.8
1	129 ± 1.43 (1.10)	103
10	129 ± 1.15 (0.898)	103
20	115 ± 2.43 (2.11)	92.0

Values in parentheses are within-day C.V.s (%), $n = 3$.
[a]KR-60436 peak height (mm) divided by its concentration (μg/mL); mean ± standard deviation.
[b](Mean observed concentration)/(theoretical concentration) × 100.

Table 2. Response Factors and Accuracies of KR-60436 at Various Concentrations in Rat Tissue Homogenates

Tissue	Added Amount (μg/mL)	Response Factor[a]	Accuracy[b] (%)	Tissue	Added Amount (μg/mL)	Response Factor[a]	Accuracy[b] (%)
Liver	0.1	36.6 ± 1.20 (3.28)	97.7	Stomach	0.1	88.0 ± 6.93 (7.87)	106
	1	37.2 ± 1.69 (4.53)	99.4		1	81.9 ± 2.40 (2.93)	98.2
	10	38.5 ± 2.27 (5.89)	103		10	80.3 ± 83.8 (1.04)	96.3
Lung	0.1	54.8 ± 3.61 (6.60)	103	Small intestine	0.1	55.0 ± 4.33 (7.87)	101
	1	53.7 ± 0.552 (1.03)	101		1	55.3 ± 0.973 (1.76)	101
	10	51.8 ± 1.45 (2.81)	97.0		10	53.9 ± 2.09 (3.88)	98.5
Heart	0.1	75.4 ± 2.00 (2.65)	99.9	Large intestine	0.1	87.3 ± 1.27 (1.46)	105
	1	77.9 ± 2.52 (3.24)	103		1	79.5 ± 6.61 (8.32)	96.0
	10	73.0 ± 4.15 (5.68)	96.8		10	81.7 ± 4.45 (5.45)	98.6
Brain	0.1	88.3 ± 2.46 (2.78)	106	Spleen	0.1	73.9 ± 1.81 (2.46)	109
	1	83.2 ± 8.73 (10.5)	99.9		1	67.0 ± 3.16 (4.72)	99.1
	10	78.4 ± 1.38 (1.77)	94.1		10	62.0 ± 0.331 (0.533)	91.6
Kidney	0.1	61.7 ± 1.80 (2.92)	106	Mesentery	0.1	67.1 ± 3.68 (5.49)	103
	1	61.6 ± 2.70 (4.38)	105		1	65.0 ± 2.74 (4.22)	99.4
	10	52.3 ± 2.44 (4.66)	89.3		10	64.1 ± 0.206 (0.321)	98.0
Muscle	0.1	53.7 ± 4.08 (7.60)	106	Fat	0.1	70.5 ± 3.94 (5.59)	103
	1	48.4 ± 3.00 (6.20)	95.3		1	68.4 ± 4.91 (7.18)	99.5
	10	50.2 ± 1.11 (2.21)	98.9		10	67.3 ± 0.678 (1.00)	97.9

Values in parentheses are within-day C.V.s (%), $n = 3$.
[a]KR-60436 peak height (mm) divided by its concentration (μg/mL); mean ± standard deviation.
[b](Mean observed concentration)/(theoretical concentration) × 100.

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concentration of KR-60436, μg/mL) in human plasma samples was lower, 11.6% decrease, than that in human urine samples (Table 1). This could be the result of binding or adsorption of KR-60436 to the endogenous compounds in plasma. The detection limit of KR-60436 in rat tissue homogenates was approximately 0.1 μg/mL, based on a signal-to-noise ratio of 3.0 (Table 2). The mean within-day coefficients of variation (C.V.s) in rat tissue homogenates ranged from 0.321% (mesentery at 10 μg/mL) to 10.5% (brain at 1 μg/mL) within concentration ranges from 0.1 to 10 μg/mL (Table 2). The mean accuracies [(mean observed concentration/theoretical concentration) × 100] from rat tissue homogenates spiked with standards for KR-60436 ranged from 89.3% (kidney at 10 μg/mL) to 109% (spleen at 0.1 μg/mL), within concentration ranges from 0.1 to 10 μg/mL (Table 2).

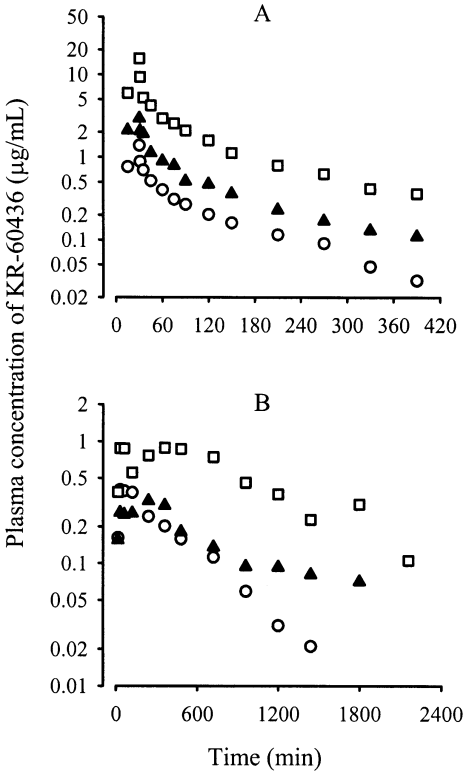

Figure 3. Arterial plasma concentration–time profiles of KR-60436 after 30 min intravenous infusion, 5 (○), 10 (▲) and 20 (□) mg/kg (A) and oral administration, 20 (○), 50 (▲) and 100 (□) mg/kg (B), of the drug to rats ($n = 1$ for each dose).

The present HPLC analysis was also successful for the pharmacokinetic studies of KR-60436 in rats. Figure 3 shows plasma concentration–time curves of KR-60436 after 30 min intravenous infusion, 5, 10, and 20 mg/kg (Fig. 3A) and oral administration, 20, 50, and 100 mg/kg (Fig. 3B), of the drug to rats ($n = 1$ for each dose). After intravenous administration, the plasma concentrations of KR-60436 declined in a polyexponential fashion (Fig. 3A) with terminal half-lives of 77.6–84.3 min for three doses. The absorption of KR-60436 from rat gastrointestinal tract was fast; the KR-60436 was detected in plasma from the first blood sampling time (15 min) (Fig. 3B). After reaching respective peak plasma concentration of KR-60436, the plasma concentrations of KR-60436 declined in a polyexponential fashion for three oral doses (Fig. 3B). The amounts of KR-60436 recovered from 30 min after 30 min intravenous administration of the drug, 20 mg/kg, to a rat were 49.6, 17.4, 62.1, 33.3, 40.1, 6.4, 21.9, 17.2, 6.27, 12.2, 37.8, 28.3, and 2.49 μg/g tissue (or μg/mL plasma) for liver, kidney, lung, spleen, heart, muscle, mesentery, small intestine, large intestine, fat, stomach, brain, and plasma, respectively.

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