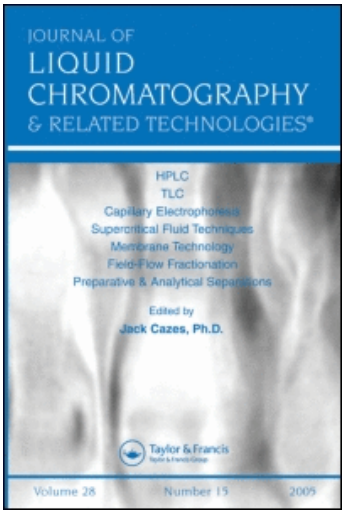

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HPLC ANALYSIS OF A NEW NEUROPROTECTIVE AGENT FOR ISCHEMIA-REPERFUSION DAMAGE, A BENZOPYRAN DERIVATIVE, IN PLASMA AND URINE

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method was developed for the determination of a neuroprotective agent for ischemia-reperfusion damage, KR-31543, in human plasma and urine. The method involved deproteinization of the biological samples with two volumes of acetonitrile. A 80 μL aliquot of the supernatant was injected onto a reversed-phase (C18) column.

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The mobile phase, 50 mM triethylamine acetate : acetonitrile (15 : 85, v/v), was run at a flow rate of 1.0 mL/min. The column effluent was monitored by an ultraviolet detector set at 310 nm. The retention time of KR-31543 was approximately 3.5 min. The detection limits of KR-31543 in human plasma and urine were 0.2 and 0.5 μg/mL, respectively. The coefficients of variation (within-day and between-day) were low (below 4.86%) for human plasma and urine. No interferences from endogenous substances were found.

INTRODUCTION

Both inhibition of lipid peroxidation induced by reactive oxygen species and stabilization of membranes, have been proposed as neuroprotective strategies in stroke. In this context, a novel benzopyran derivative, KR-31543, (2S, 3R, 4S)-6-amino-4-[4-chloro-(2-methyltetrazol-5-yl)methyl-phenylamino]-3,4-dihydro-3-hydroxy-2-dimethoxymethyl-2-methyl-2H-1-benzopyran (Fig. 1), was recently synthesized by Korea Research Institute of Chemical Technology (Taejeon, Korea) as a new therapeutic agent for neuroprotection. Possessing both antioxidant and potassium channel modulating activities, KR-31543 has been shown to protect cultured rat cortex neurons against iron-induced oxidative injury in vitro, and has also shown a significant reduction in infarct volume at 24 h, following occulusion in the rat model of transient cerebral-ischemia.

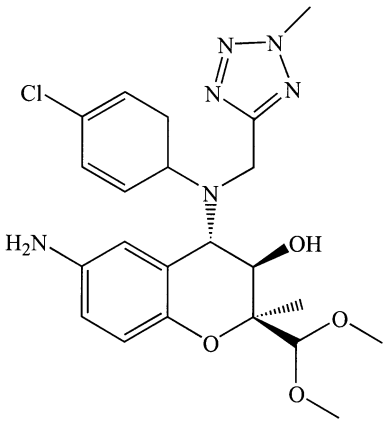

Figure 1. Chemical structure of KR-31543.

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The melting point, optical rotation ($[\alpha]_D^{20}$) and log partition coefficient of KR-31543 (a basic compound having a mol. wt. of 474.95 Da) were 153–154°C, +95.00 (in methanol) and 4.02, respectively. Solubilities of KR-31543 in ethanol, ethyl acetate, dimethylformamide, and dimethyl sulfoxide were 13.8, 144, >250, and >250 mg/mL, respectively. KR-31543 was highly safe; the highest non-lethal dose was 1200–1600 mg/kg after oral administration to mice. KR-31543 is being evaluated in preclinical study as a new neuroprotective agent for ischemia-reperfusion damage.

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-31543 in human plasma and urine.

EXPERIMENTAL

Materials

KR-31543 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-31543 was prepared in methanol (1 mg/mL). Appropriate dilutions of the stock solution were made with methanol. Standard solutions of KR-31543 in human plasma and urine 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 0.2, 0.5, 1, 2, and 10 μg/mL for human plasma and 0.5, 1, 2, and 10 μg/mL for human urine.

Sample Preparation

A 200 μL aliquot of acetonitrile[1,2] was added to deproteinize a 100 μL aliquot of the biological sample. After vortex-mixing and centrifugation at 9000 *g* for 10 min, a 80 μL aliquot of the supernatant was injected directly onto the HPLC column. The mobile phase, 50 mM triethylamine acetate : acetonitrile (15 : 85, v/v), was run at a flow rate of 1.0 mL/min. The column effluent was monitored by a UV detector set at 310 nm.

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HPLC Apparatus

The HPLC system consisted of a model 7120 injector (Rheodyne, Cotati, CA, USA), a model PU-980 pump (Jasco, Tokyo, Japan), a reversed-phase (C_{18}) column (150 mm, *l.* × 4.6 mm, i.d.; particle size, 5 μm; Alltech Altima, Deerfield, IL, USA), a model UV975 detector (Jasco) and a model 1200 recorder (Linear, Reno, NV, USA).

RESULTS AND DISCUSSION

The UV absorption maximum of KR-31543 occurred at 310 nm and this was, therefore, used for the HPLC analysis. Figure 2 shows typical chromatograms of drug-free human plasma, drug standard in human plasma, plasma collected at 30 min after intravenous administration of KR-31543, 10 mg/kg, to a Sprague–Dawley rat, drug-free human urine, drug standard in human urine, and rat urine collected between 0 and 24 h after intravenous

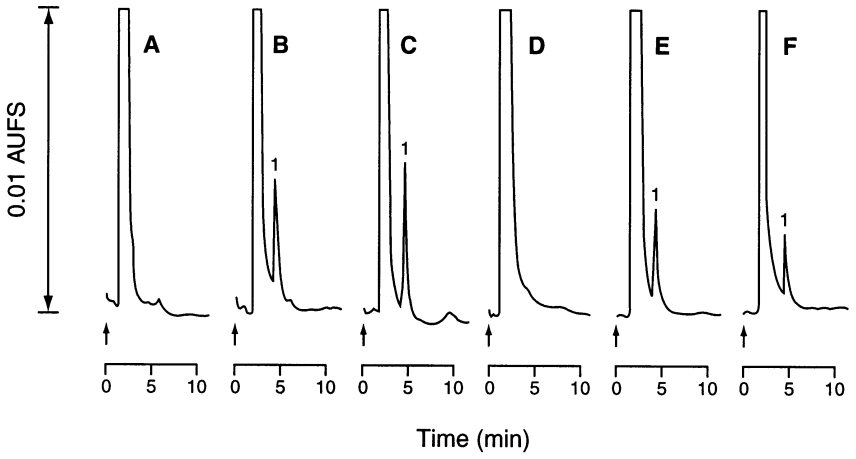

Figure 2. Chromatograms of drug-free human plasma (A), human plasma spiked with 1 μg/mL of KR-31543 (B), plasma collected from a male Sprague–Dawley rat at 30 min after intravenous infusion of 10 mg/kg of KR-31543 (C), drug-free human urine (D), human urine spiked with 1 μg/mL of KR-31543 (E) and rat urine collected from a male Sprague–Dawley rat between 0 and 24 h after intravenous infusion of 10 mg/kg of KR-31543 (F). Peak: 1 = KR-31543 (3.5 min). The arrow marks the point of injection. The detector's sensitivity was set at 0.005 AUFS (absorption unit full scale) and recorder's sensitivity was set at 10 mV. The chart speed was 10 cm/h.

administration of KR-31543, 10 mg/kg, to a Sprague–Dawley rat. No interferences from endogenous substances were observed in any of the biological samples (Fig. 2). The peak of KR-31543 was symmetrical and eluted at approximately 3.5 min (Fig. 2).

The detection limits of KR-31543 in human plasma and urine were 0.2 and 0.5 μg/mL, respectively, 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 2.37% (ranging from 0.843 to 3.56%) and 3.06% (ranging from 0.666 to 4.86%), respectively, within concentration ranges from 0.2 (0.5 for urine sample) to 10 μg/mL (Table 1). The between-day C.V.s of the analysis of the same samples on three consecutive days for human plasma and urine were lower than 3.97 and 3.09%, respectively, within concentration ranges from 0.2 (0.5 for urine sample) to 10 μg/mL. The mean accuracies [(mean observed concentration/theoretical concentration) × 100] from human plasma and urine spiked with standards for KR-31543 were 96.7–102% and 98.0–102%, respectively, within concentration ranges from 0.2 (0.5 for urine sample) to 10 μg/mL (Table 1). Note, that the response factors (peak height of KR-31543, mm/concentration of KR-31543, μg/ml) in human urine samples were lower (37.1% decrease) than those in human plasma samples (Table 1). This could be the result of binding or adsorption of KR-31543 to the endogenous compounds in urine. Similar results were also

Table 1. Response Factors and Accuracies of KR-31543 at Various Concentrations in Human Plasma and Urine

Added Amount (μg/mL)	Response Factor[a]	Accuracy[b] (%)
	Human Plasma	
0.2	10.2 ± 0.293 (2.87)	100
0.5	11.7 ± 0.416 (3.56)	102
1	12.6 ± 0.242 (1.92)	97.4
2	13.5 ± 0.360 (2.67)	97.1
10	12.1 ± 0.102 (0.843)	96.7
	Human Urine	
0.5	8.71 ± 0.413 (4.74)	98.0
1	9.18 ± 0.446 (4.86)	102
2	8.02 ± 0.158 (1.97)	98.1
10	9.17 ± 0.0611 (0.666)	100

Values in parentheses are within-day C.V.s (%), $n = 3$.
[a]KR-31543 peak height (mm) divided by its concentration (μg/ml); mean ± standard deviation.
[b](Mean observed concentration)/(theoretical concentration) × 100.

found with azosemide,[2] methotrexate,[3] YH-1885,[4] a new proton pump inhibitor, and 7-anilino-5,8-isoquinolinedione, a new isoquinolinedione derivative (our unpublished data).

The present HPLC analysis was also successful for the pharmacokinetic studies of KR-31543 in rats. Figure 3 shows plasma concentration–time profiles of KR-31543 after intravenous (10, 20, and 50 mg/kg) and oral (10, 20, and 50 mg/kg) administration to rats ($n = 1$ for each dose). After intravenous administration, the plasma concentrations of KR-31543 declined in a polyexponential fashion (Fig. 3A) with terminal half-lives of 272–327 min for three doses. The absorption of KR-31543 from a rat gastrointestinal tract was fast; the

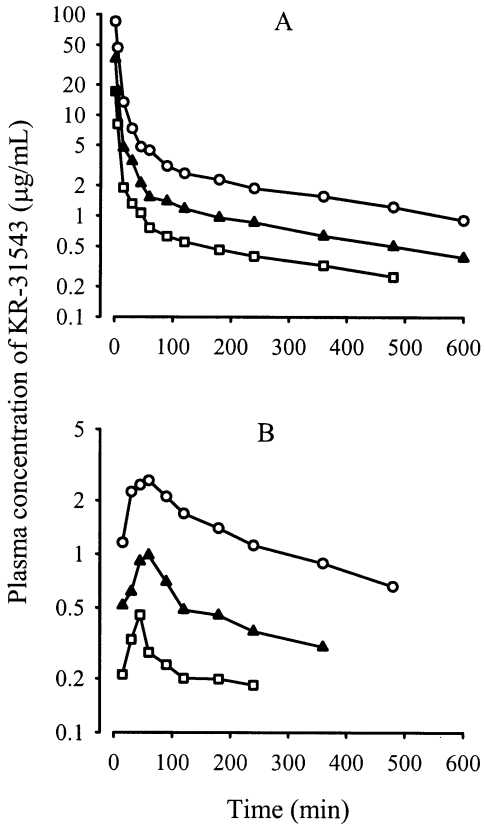

Figure 3. Arterial plasma concentration–time curves of KR-31543 after 1 min intravenous infusion, 10 (□), 20 (▲) and 50 (○) mg/kg (A) and oral administration, 10 (□), 20 (▲) and 50 (○) mg/kg (B) of the drug to rats ($n = 1$ for each dose).

KR-31543 was detected in plasma from the first blood sampling time (15 min) (Fig. 3B). After oral administration, the plasma concentrations of KR-31543 reached respective peak at 45–60 min and declined in a polyexponential fashion for three doses (Fig. 3B).

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