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Determination of a new reversible proton pump inhibitor, DBM-819, in human plasma and urine, and rat tissue homogenates by high-performance liquid chromatography

Eun Jung Kim [a], Mi Hye Lee [a], So Hee Kim [b], Sun-Ok Kim [c], Dong Ha Lee [c], Hong Lim [c], Hye Suk Lee [d], Myung Gull Lee [a,*]

[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea*

[b] *College of Dentistry and Research Institute of Oral Science, Kangnung National University, 123, Jibyeon-Dong, Gangnung, Gangwon-Do 211-702, South Korea*

[c] *AgroPharm Research Institute, Dongbu Hannong Chemical Company, 103-2, Moonji-Dong, Daeduck Science Town, Taejeon 305-380, South Korea*

[d] *Bioanalysis Laboratory, College of Pharmacy and Medicinal Resources Research Center, Wonkwang University, Iksan 570-749, South Korea*

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Abstract

A high-performance liquid chromatographic (HPLC) method was developed for the determination of a new proton pump inhibitor, DBM-819, in human plasma and urine and rat tissue homogenates using KR-60461 as an internal standard. A 100-μl aliquot of acetonitrile (containing 0.5 μg/ml of the internal standard) and a 200-μl aliquot of 0.1 M Na_2HPO_4 (adjusted pH 11 with 1 N NaOH) were added to a 100-μl aliquot of biological sample. After vortex-mixing, the mixture was extracted with 1 ml of ethylacetate. After centrifugation at 12 000 × *g* for 3 min, the organic layer was collected and evaporated under nitrogen gas. The residue was then reconstituted with a 100-μl aliquot of mobile phase, and a 40-μl aliquot was injected onto the HPLC column. The mobile phase, 0.02 M phosphate buffer (pH 5): acetonitrile: methanol (46:44:10, v/v/v), was run at a flow rate of 0.5 ml/min and the column effluent was monitored by the fluorescence detector set at an excitation wavelenght of 340 nm and an emission wavelenght of 470 nm. The retention times for DBM-819 and the internal standard were approximately 10.5 and 12 min, respectively. The detection limits of DBM-819 in human plasma and urine, and rat tissue homogenates were 0.01, 0.02 and 0.02 (or 0.05) μg/ml. respectively. The coefficients of variation (CV) of the assay were below 11% for human plasma and urine, and rat tissue homogenates. No interferences from endogenous substances were found.

Keywords: DBM-819; HPLC; Pharmacokinetics

* Corresponding author. Tel.: +82-2-880-7855; fax: +82-2-889-8693
E-mail address: leemg@snu.ac.kr (M.G. Lee).

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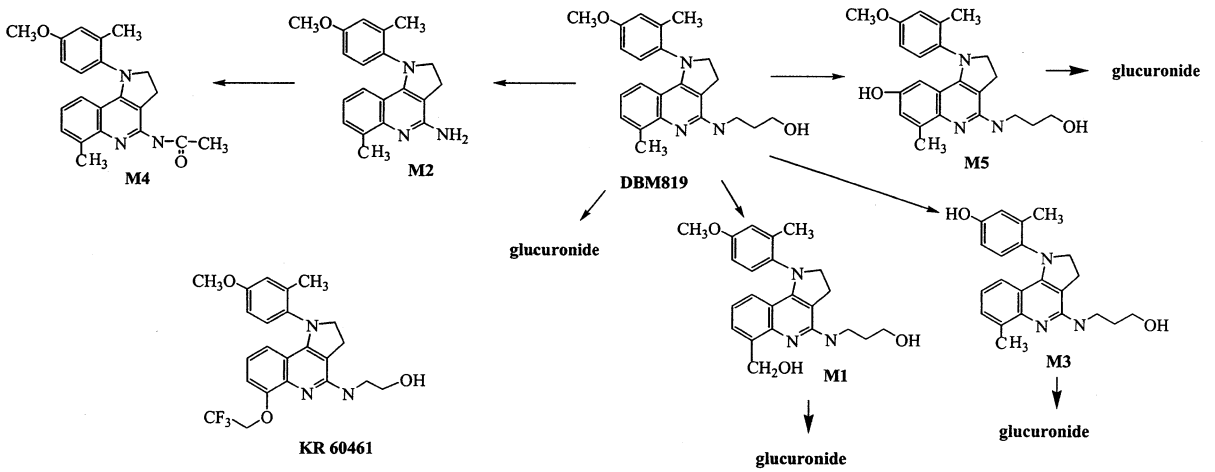

Fig. 1. Possible metabolic pathways of DBM-819 in rats, and chemical structure of KR60461 (the HPLC internal standard).

1. Introduction

The area of antiulcer research 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, l-(2-methyl-4-methoxyphenyl)-4-[3-hydroxypropyl]amino-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline (DBM-819, Fig. 1) was synthesized as a conformationally constraint structure by forming of an additional ring.

DBM-819 has been identified as a reversible inhibitor of the gastric (H^+/K^+)-ATPase in gastric membrane vesicle preparations enriched in the (H^+/K^+)-ATPase with IC_{50} of 5 μM, which is more potent than SK&F 96067 with IC_{50} of 22 μM. Also, DBM-819 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: DBM-819 has greater antisecretory activity and anti-ulcer activity than those of SK&F 96067 and could be developed as a new therapeutic agent for peptic ulcer disease.

DBM-819 and its metabolites after incubation in liver microsomes were analyzed by high-performance liquid chromatography (HPLC) and electrospray mass spectrometry [3]. The HPLC analysis of pyrroloquinoline quinone derivatives [4] and quinoline derivatives [5,6] have also been reported. This paper describes an HPLC analysis with extraction procedure for the determination of DBM-819 in human plasma and urine, and rat tissue homogenates. The pharmacokinetics and tissue distribution of DBM-819 in a male Sprague–Dawley rat were also reported.

2. Experimental

2.1. Chemicals

DBM-819 (as an HCl salt) and KR60461 (an internal standard of HPLC assay, Fig. 1) were supplied by AgroPharma Research Institute, Dongbu Hannong Chemical Company (Taejon, South Korea). Various pH solutions ranging from 1 to 2 (HCl–KCl buffer), 3 ($KHC_8H_4O_4$–HCl buffer), 4 to 5 ($KHC_8H_4O_8$–NaOH buffer), 6 to 7 (KH_2PO_4–NaOH buffer), 8 to 9 (H_3BO_3–KCl–NaOH buffer), 10 to 11 ($NaHCO_3$–NaOH buffer), 12 (Na_2HPO_4–NaOH buffer), to 13 (KCl–NaOH buffer) were purchased from Shinyo Pure Chemi-

Table 1
Response factors and accuracies of DBM-819 at various concentrations in human plasma and urine using the extraction method ($n = 5$)

Theoretical concentration (μg/ml)	Response factor[a]	Accuracy[b] (%)
Human plasma		
10	2.39(1.3)	99.0
0.1	2.30(0.46)	95.3
0.01	2.47(0.21)	102
Human urine		
10	1.56(0.27)	105
0.1	1.53 (0.53)	103
0.02	1.41 (0.94)	94.8

Values in parenthesis are coefficients of variation (%).
[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 observed concentration/theoretical concentration) × 100; mean.

cals (Osaka, Japan). Other chemicals were of reagent grade or HPLC grade and, therefore, were used without further purification. Full names of metabolites of DBM-819 (M1–M5), and the internal standard (KR60461) are as follows; M1, 1-(2-methyl-4-methoxyphenyl)-4-[(3-hydroxypropyl)amino]-5-oxy-6-hydroxymethy1-2,3-dihydropyrrolo[3,2-c]quinoline; M2, 1-(2-methyl-4-methoxyphenyl)-4-amino-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline; M3, 1-(2-methyl-4-hydroxyphenyl)-4[(3-hydroxypropyl)amino]-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline; M4, 1-(2-methyl-4-methoxyphenyl)-4-acetamido-6-methyl-2,3-dihydropyrrolo[3,2-c]quinoline; M5, 1-(2-methyl-4-methoxyphenyl)-4-[(3-hydroxypropyl)amino] - 6-methyl-8-hydroxy-2,3-dihydropyrrolo[3,2-c]quinoline; KR60461, 1-(2-methyl-4-methoxyphenyl)-4-[(2-hydroxyethyl)amino]-6-*O*-(2,2,2-trifluoroethyl)-2,3-dihydropyrrolo[3,2-c]quinoline.

2.2. Preparation of stock and standard solutions

A stock solution of DBM-819 (as an HCl salt) was prepared in distilled water (1 mg/ml as free DBM-819). Appropriate dilutions of the stock solution were made with distilled water. Standard solutions of DBM-819 in human plasma and urine, and rat tissue homogenates [1 g of each tissue or organ was homogenized (Ultra-Turrax, T25, Janke and Kunkel, 1KA-Labortechnik, Staufen, Germany) with 4 vol. of 0.9% NaCl injectable solution, centrifuged for 10 min at 9000 g and the supernatant was collected] were prepared by spiking appropriate volume (less than 10 μl/ml biological sample) of the variously diluted stock solutions to give the final concentrations of 10, 2, 1, 0.2, 0.1, 0.05, 0.02 and 0.01 μg/ml for human plasma, 10, 2, 1, 0.1, 0.05 and 0.02 μg/ml for human urine, and 10, 1, 0.5, 0.1, 0.05 and 0.02 μg/ml for rat tissue homogenates. The HPLC assay results on human plasma and urine, and rat tissue homogenates with three representative concentrations are listed in Tables 1 and 2, respectively.

2.3. Sample preparation

A 100-μl aliquot of acetonitrile (containing 0.5 μg/ml of the internal standard) and a 200-μl aliquot of 0.1 M Na_2HPO_4 (adjusted pH 11 with 1 N NaOH) were added to 100-μl aliquot of human plasma or urine, or the supernatant of rat tissue homogenates. After vortex-mixing, the mixture was extracted with 1 ml of ethylacetate. After centrifugation at $12\,000 \times g$ of 3 min, the organic layer was collected and evaporated under nitrogen. The residue was then reconstituted with a 100-μl aliquot of mobile phase, and a 40-μl aliquot was injected directly onto the HPLC column.

DBM-819 (HCl salt of DBM-819 was dissolved in distilled water and filtered through a 0.45 μm filter), 10 mg/kg as free DBM-819, was intravenously administered for 30 min (total injection volume was 1.2 ml) via the jugular vein of a male Sprague–Dawley rat (Charles River Company, Atsugi, Japan) and 10 mg/kg, was also administered orally to an additional rat. Blood samples (0.22 ml) were collected at designated times via the carotid artery. After centrifugation for 2 min at $12\,000 \times g$, plasma samples (100 μl) were stored in the −20 °C freezer prior to HPLC analysis of DBM-819. Urine sample was collected for 24 h. For tissue studies, each rat tissue (or organ) was removed at 30 min postinfusion of the drug (10 mg/kg), and homogenized with 4 vol. of 0.9%

Table 2
Response factors and accuracies of DBM-819 at various concentration in rat tissues homogenates using the extraction method ($n = 4$)

Tissues	Theoretical concentration (μg/ml)	Response factor[a]	Accuracy[b] (%)	Tissues	Theoretical concentration (μg/ml)	Response factor[a]	Accuracy[b] (%)
Brain	10	2.22 (1.5)	114	Liver	10	2.04(1.4)	110
	1	1.91 (2.8)	96.3		1.88	(2.4)	102
	0.05	1.84 (1.2)	95.1		0.02	1.76 (3.6)	94.9
Fat	10	1.22 (1.6)	100	Lung	10	2.07(2.0)	109
	0.05	1.18 (3.9)	96.8		1	1.84 (1.2)	97.0
	0.02	1.25 (4.3)	102.5		0.02	1.83 (7.7)	96.8
Heart	10	2.03 (11)	107	Mesentery	10	1.66 (0.98)	93.6
	0.5	1.80 (2.5)	95.1		0.05	1.69 (4.0)	104
	0.02	1.85 (0.20)	97.7		0.02	1.52 (6.3)	102
Small intestine	10	1.79 (1.4)	98.8	Muscle	1	1.80 (1.6)	106
	0.05	1.83 (2.2)	101		0.05	1.74 (6.4)	102
	0.02	1.81 (5.9)	99.8		0.02	1.63(4.0)	96.2
Large intestine	10	2.04 (2.0)	106	Spleen	10	2.21 (0.97)	111
	0.05	1.83 (2.8)	95.1		1	1.92(1.5)	96.6
	0.02	1.95(1.9)	101		0.05	1.94(1.4)	97.9
Kidney	10	1.99 (0.97)	107	Stomach	10	2.13 (3.5)	106
	1	1.89 (2.1)	102		1	1.92(1.8)	95.9
	0.02	1.80 (0.14)	95.3		0.02	1.96 (2.4)	98.0

Values in parentheses are coefficients of variation (%).
[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 observed concentration/theoretical concentration) × 100; mean.

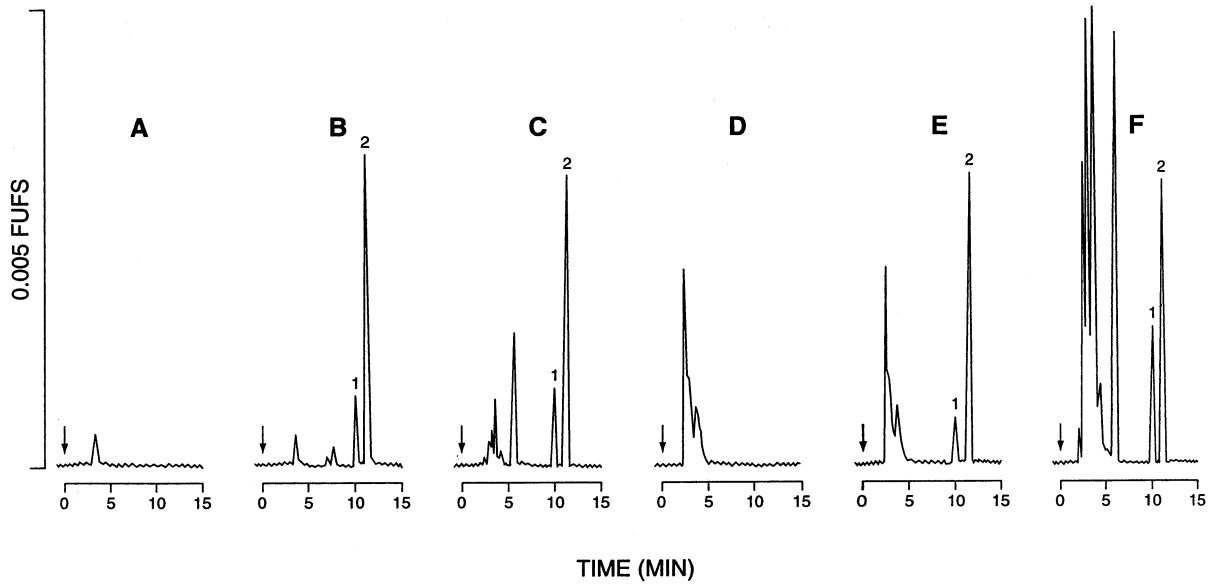

Fig. 2. Chromatograms after extraction of drug-free human plasma (A), human plasma spiked with 0.1 μg/ml of DBM-819 and 0.5 μg/ml of internal standard (B), plasma collected from a male Sprague–Dawley rat at 6 h (0.124 μg/ml) after 30-min intravenous infusion of 10 mg/kg of DBM-819 (C), drug-free human urine (D), rat urine spiked with 0.1 μg/ml of DBM-819 and 0.5 μg/ml of internal standard (E), and urine collected from a male Sprague–Dawley rat between 0 and 24 h (0.270 μg/ml) after 30-min intravenous infusion of 10 mg/kg of DBM-819 (F), Peak: (1) DBM-819 (10.5 min); (2) internal standard (12 min). The arrows mark the points of injection. The detector's sensitivity was set at 0.005 FUFS and the recorder's sensitivity was set at 20 mV.

NaCl injectable solution using a tissue homogenizer (Ultra-Turrax, T25, Janke & Kunkel) and immediately centrifuged at $9000 \times g$ for 10 min. A 100-μl aliquot of the supernatant was collected and treated as described for human plasma and urine samples. Pretreatment of a rat, cannulation method and handling of plasma and urine samples were the same as reported previously [7].

2.4. HPLC apparatus

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, $l \times 4.6$ 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). The mobile phase, 0.02 M phosphate buffer (pH 5): acetonitrile: methanol (46:44:10, v/v/v), was run at a flow rate of 0.5 ml/min and the column effluent was monitored by the fluorescence detector set at an excitation wavelenght of 340 nm, and an emission wavelength of 470 nm.

2.5. Stability of DBM-819 in various pH solutions and human gastric juices

DBM-819 stock solution was spiked (less than 10 μl/ml) into each glass test tube containing 10 ml of various pH solutions ranging from 1 to 13 to make a final concentration of 0.5 μg/ml. After vortex-mixing, each test tube was placed in a water-bath shaker kept at 37 °C and at a rate of 50 oscillations per min (opm.). At 0, 1, 2, 4, 6, 8 and 24 h, a 50-μl aliquot was sampled from each test tube and immediately injected (without extraction procedure) onto the HPLC column. Similar studies were also performed with 5 ml of human gastric juices obtained from patients before surgery at Seoul National University Hospital (Seoul, South Korea) having pHs of 2.86, 3.24, 4.97, 2.69 and 4.85, respectively. Each human

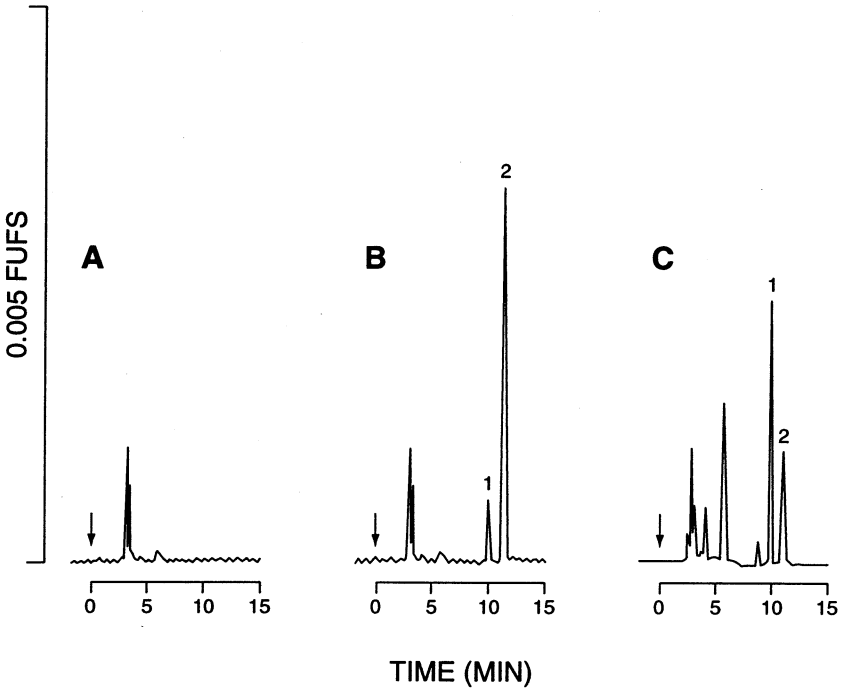

Fig. 3. Chromatograms after extraction of drug-free rat liver homogenate (A), rat liver homogenate spiked with 0.1 μg/ml of DBM-819 and 0.5 μg/ml of internal standard (B) and liver homogenate collected from a male Sprague–Dawley rat at 30 min (11.2 μg/g tissue) after 30-min intravenous infusion of 10 mg/kg of DBM-819 (C). Peaks: (1) DBM-819 (10.5 min); (2) internal standard (12 min). The arrows mark the points of injection. The detector's sensitivity was set at 0.005 FUFS and the recorder's sensitivity was set at 20 mV (A and B) and 100 mV (C).

gastric juice containing DBM-819 (1 μg/ml) was incubated for up to 3 h at 37 °C and at a rate of 50 opm.

3. Results and discussion

DBM-819 was stable for up to 48 h incubation in various pH solutions ranging from 1 to 13 at 0.5 μg/ml of DBM-819 and for up to 3 h incubation in 5 human gastric juices at 1 μg/ml of DBM-819; more than 97.7% of the spiked amount of DBM-819 was recovered.

We measured the maximum excitation (340 nm) and emission (470 nm) wavelength for DBM-819 using the spectrofluorometer and the wave numbers were chosen in the present fluorescence detector. The increase in pH of mobile phase from 2 to 7 increased the retention time of DBM-819. To eliminate endogenous interferences from biological sample and to increase assay sensitivity, the liquid–liquid extraction method was employed for sample preparation.

Fig. 2 shows typical chromatograms of drug-free human plasma, drug standard in human plasma, plasma collected at 6 h after intravenous administration of DBM-819, 10 mg/kg, to a rat, drug-free human urine, drug standards in human urine and in a urine collected between 0 and 24 h after intravenous administration of DBM-819, 10 mg/kg, to a rat using the extraction method; the corresponding chromatograms for rat liver homogenates are shown in Fig. 3. No interferences from endogenous substances were observed in any of the biological samples. The retention times for DBM-819 and the internal standard were approximately 10.5 and 12 min, respectively.

The detection limits for DBM-819 in human plasma and urine were 10 and 20 ng/ml, respectively, based on a signal-to-noise ratio of 3.0

(Table 1). The ranges of coefficients of variation (CVs) of DBM-819 in human plasma and urine were 0.21–1.3% and 0.27–2.5%, respectively, within concentration ranges from 0.01 (0.02 for urine sample) to 10 μg/ml (Table 1). The mean accuracies [(mean observed concentration/theoretical concentration) × 100] from human plasma and urine spiked with standards for DBM-819 were 90.9–110 and 89.3–107.4%, respectively, within concentration ranges from 0.01 (0.02 for urine sample) to 10 μg/ml (Table 1). Note that the response factors [(peak height of DBM-819, (cm)/concentration of DBM-819, (μg/ml))/(peak height of internal standard, (cm)/concentration of internal standard, (μg/ml))] in human urine samples were lower than those in human plasma samples (Table 1). This could be the result of binding or adsorption of DBM-819 to the endogenous compounds in urine. Similar results were also found with azosemide [8], methotrexate [9], YH-1885, a new proton pump inhibitor [10], and IQO4, a new isoquinolinedione derivative [11].

This HPLC method was also successful for the determination of DBM-819 in rat tissues. The detection limit for DBM-819 was 20 ng/ml (50 ng/ml for brain and spleen) for rat tissues (or organs) studied (Table 2). The ranges of CVs for DBM-819 in rat tissues were 0.14% (kidney at 0.02 μg/ml) to 11% (heart at 10 μg/ml) within the concentration ranges from 0.02 to 10 μg/ml (Table 2). The accuracy of DBM-819 in rat tissues ranged from 93.6 (mesentery at 10 μg/ml) to 114% (brain at 10 μg/ml) within the concentration ranges from 0.02 to 10 μg/ml (Table 2). The response factor of DBM-819 in rat tissues ranged from 1.18 (fat at 0.05 μg/ml) to 2.22 (brain at 10 μg/ml) within the concentration ranges from 0.02 to 10 μg/ml (Table 2). Note that the response factor of DBM-819 in fat, 1.22, was the lowest among the tissues studied (Table 2). Again, this could be a result of binding or adsorption of DBM-819 to the endogenous compounds in fat. The amount of DBM-819 recovered 30 min after intravenous administration of DBM-819 to a rat were 17.9, 3.83, 17.3, 16.6, 9.05, 26.6, 11.2, 95.3, 17.7, 16.5, 39.9, and 29.9 μg/g tissue for brain, fat, heart, small intestine, large intestine, kidney, liver, lung, mesentery, muscle, spleen, and stomach, respectively.

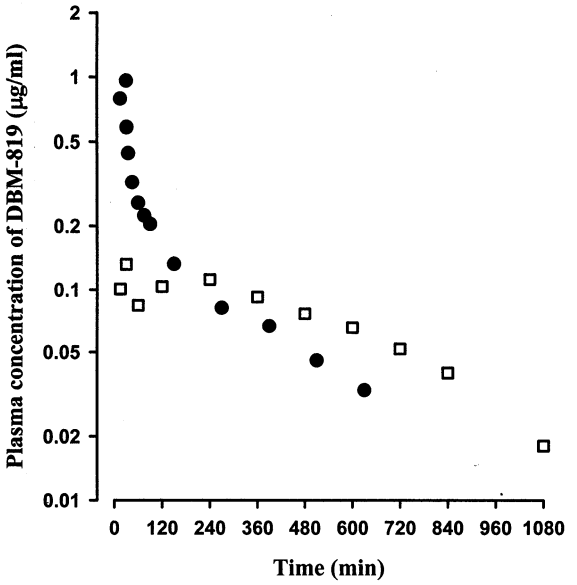

Fig. 4. Arterial plasma concentration-time profiles of DBM-819 after 30-min intravenous (●) and oral (□) administration of 10 mg/kg of DBM-819 to a male Sprague–Drawley rat.

In small animals, it is not easy to collect a large volume of blood samples. This HPLC method was successful for the pharmacokinetic studies of DBM-819 in small animals; less than 100-μl aliquot of plasma sample was needed for the analysis of DBM-819. After 30-min intravenous infusion of DBM-819, 10 mg/kg, to a rat, the arterial plasma concentrations of DBM-819 declined in a polyexponential fashion (Fig. 4). With terminal half-life, total body clearance and apparent volume of distribution at steady state of 269 min, 108 ml/min per kg and 46 792 ml/kg, respectively. After oral administration of DBM-819, 10 mg/kg, to a rat, the absorption of DBM-819 was rapid; the arterial plasma concentration of the drug was detected from the first blood sampling time (15 min) and reached its peak at 30 min.

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