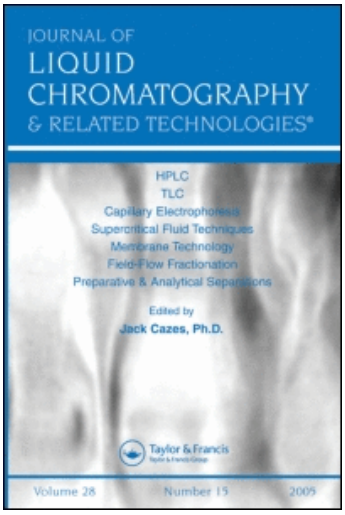

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DETERMINATION OF CAPSAZEPINE IN RAT PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Capsazepine is a competitive capsaicin receptor antagonist. Assay method of capsazepine in biological fluid has not been reported yet. We developed a high performance liquid chromatographic (HPLC) method for the determination of capsazepine in rat plasma. A deproteinated serum sample was subjected to an organic phase extraction procedure. The residue was reconstituted in acetonitrile and then an aliquot was directly injected onto an octadecylsilica column. The mobile phase employed was acetonitrile-water (60% acetonitrile in water, v/v). The flow rate was 1.2 mL/min, and capsazepine elution from the HPLC column was monitored by UV absorption at 234 nm. The retention time of capsazepine and YH439 (internal standard) were 4.6 min and 10.8 min, respectively. The detection limit in rat plasma was 0.05 μg/mL. The mean percentage recovery of the drug in the

concentration range of 0.05-5 μg/mL was 97.05%, while the inter-day coefficient of variation of the same concentration range was less than 10%. The method for quantitation of capsazepine in rat plasma was accurate and sensitive for in vivo studies.

INTRODUCTION

Capsaicin excites sensory neurons, thus causing severe pain, by opening a capsaicin receptor present exclusively in small sensory neurons. Capsazepine (2- [2- (4-chlorophenyl) ethylamino-thiocarbonyl] -7,8-dihydroxy-2,3,4,5-tetrahydro-1H-2-benzazepine) in Figure 1A is a synthetic analogue of the sensory neurone excitotoxin,[1-2] capsaicin. Capsazepine has been described as a competitive capsaicin receptor antagonist because capsazepine specifically blocks responses of sensory neurons to capsaicin.[3-9] Capsaicin receptors are largely implicated by nociception but the precise physiological role is not yet known. The difficulty to assess the physiological role is partly because of the lack of physicochemical analytical data of capsazepine in vivo. The novel competitive antagonist of capsaicin receptor, capsazepine, prevented the capsaicin-induced pain or hyperalgesia when administered systemically. But, there is no proper analytical description referring to the determination of concentration of capsazepine in vivo. Therefore, it is necessary to develop an analytical method to evaluate the relationship between plasma concentration and efficiency of capsazepine. In doing so, pharmacokinetic paramaters are also evaluated in order to determine the effective route of administration or dosage forms of capsazepine.

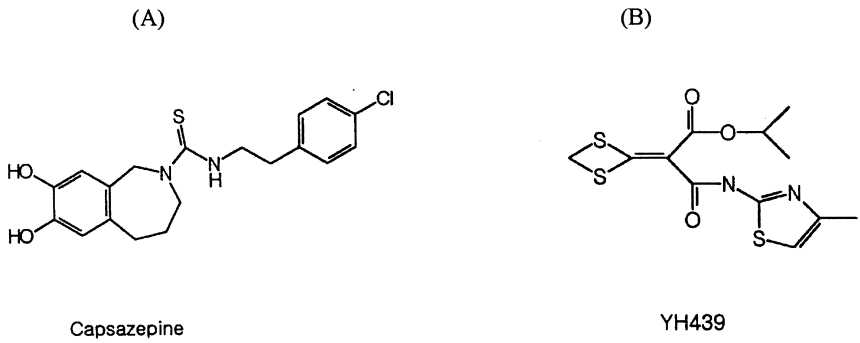

Figure 1. Chemical structure of capsazepine and YH439 (internal standard).

EXPERIMENTAL

Materials

Capsazepine, (2- [2- (4-chlorophenyl) ethylamino - thiocarbonyl] -7,8-dihydroxy-2,3,4, 5-tetrahydro-1H-2-benzazepine) was kindly supplied by Dong-A Pharma. Co. (Seoul, Korea). YH439, [isopropyl 2-(1,3-dithietane-2-ylidene)-2-[N-(4-methyl-2-thiazolyl) carbamoyl]acetate] in Figure 1B, an internal standard of the HPLC assay, was obtained from Research Center of Yuhan Corporation (Kunpo, South Korea). Egg phosphatidylcholine (EPC) and distearoyl phosphoethanolamine polyethylene glycol 2000 (DSPC-PEG) were the products of Avanti Polar Lipids Inc. Other chemicals were of reagent grade or HPLC grade and used without further purification. Water was deionized, and distilled in house.

HPLC System

The HPLC system consisted of a Hitachi LC system (Tokyo, Japan); L-6200 pump, L-4200 UV detector and D-2500 integrator. Samples were injected via a Hitachi L-7200 autoinjector equipped with a 20 μL loop. Separations were performed on a octadecylsilica column (250 × 4.6 mm, 4 μm particle size, YMC, Kyoto, Japan) with a guard column (3.2 × 15 mm, 7 μm particle size, P. J. Cobert Associates, Inc. St. Louis, MO, USA) at ambient temperature. The mobile phase used in the study was a mixture of acetonitrile and water in a volume ratio of 60:40. The mixture was degassed prior to use and delivered at a flow rate of 1.2 mL/min. Capsazepine in the eluent was monitored spectrophotometrically at 234 nm. A preliminary study showed that the UV absorption maximum of capsazepine was approximately 234 nm.

Extraction of Capsazepine from Standard Samples

A stock solution was prepared by dissolving the drug in methanol (1 mg/mL).[10] Standard solutions of capsazepine in rat plama were prepared by spiking the appropriate volume (less than 10 μL per mL) of variously diluted stock solutions giving final concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, and 5.0 μg/mL. Three hundred microliters of solution for extraction (3/1, ethyl acetate/hexane) containing internal standard (YH439 5 μg/mL) were added to 100 μL of each standard solution with internal standard (5 μg/mL). The mixture was vortexed for 5 min and then centrifuged at 2500 rpm for 10 min. An aliquot (250 μL) of the supernatant was placed in the eppendorf tube. The organic phase was evaporated under nitrogen gas. The residue was then reconstituted in 50 μL of acetonitrile and then an aliquot (20 μL) was injected into the HPLC system. The ratio of peak height of capsazepine and the internal standard was used as an assay parameter for this study. The peak height ratio was plotted

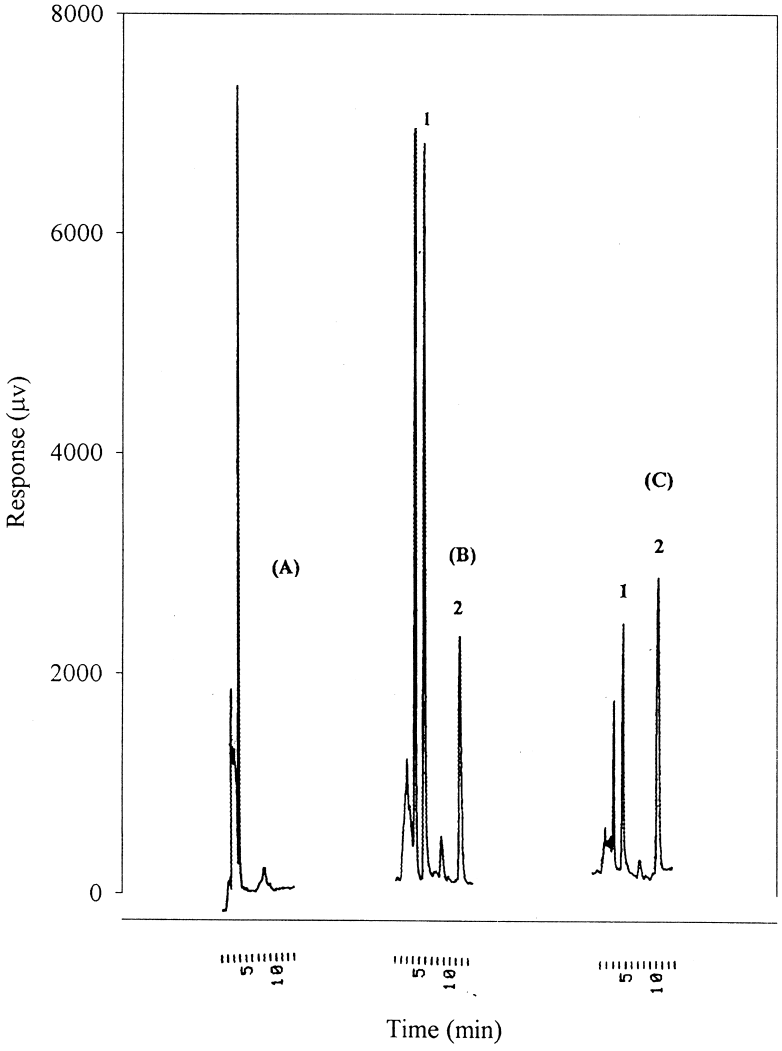

Figure 2. Chromatograms of drug-free rat plasma (A); Rat plasma spiked with capsazepine (5.0 μg/mL) and YH439 (5.0 μg/mL) (B); Plasma obtained from a rat at 5 min after intravenous administration of capsazepine (1 mg/kg) loaded in microemulsion (C); Peaks: 1=capsazepine (4.6 min); 2=YH439 (internal standard, 10.8 min).

against the corresponding concentration of capsazepine in serum sample. Standard calibration curves were obtained from linear regression analysis.

In Vivo Study

Capsazepine-loaded microemulsion was administered intravenously to rats at the dos of 1 mg/kg. The formulation of capsazepine microemulsion is composed of triglyceride, normal saline, EPC and DSPC-PEG. Blood samples were collected from the femoral artery at designated time intervals. One hundred microliters of plasma sample were stored in a freezer prior to the HPLC assay.

RESULTS AND DISCUSSION

Figure 2 depicts the typical chromatograms of blank serum, standard sample, and serum taken from rat at 5 min after intravenous administration of 1 mg of capsazepine per kg of body weight. There were no apparent interfering peaks from serum components, either capsazepine or internal standard (Figure 2). The retention time for capsazepine and YH439 were 4.6 and 10.8 min, respectively. The detection limit for capsazepine in rat plasma was 0.05 μg/mL, based on a signal-to-noise ratio of 3.0. The interday coefficient of variation (C.V.) of capsazepine in rat plasma was less than 9.61% (Table 1). The intraday C.V. for the analysis of the same samples on three days was less than 8.47% (Table 1). The recovery from plasma was obtained by dividing the peak height of the drug in rat plasma by that in water (Table 2). Response factors were calculated by

Table 1

Intra- and Inter-Day C.V.s at Various Concentrations of Capsazepine in Rat Plasma

	Intra-Day[a]		Inter-Day[b]	
Expected Conc. (μg/mL)	Calculated Conc. (μg/mL, mean ± SD)	CV (%)	Calculated Conc. (μg/mLl, mean ± SD)	CV (%)
0.05	0.058 ± 0.005	8.62	0.052 ± 0.005	9.61
0.1	0.098 ± 0.009	9.18	0.110 ± 0.005	4.51
0.5	0.585 ± 0.023	3.95	0.480 ± 0.021	4.31
1	0.965 ± 0.007	0.72	1.002 ± 0.053	4.16
2	2.031 ± 0.034	1.66	2.062 ± 0.085	4.16
5	4.990 ± 0.012	0.24	4.983 ± 0.018	0.45

[a] n = 4. [b] n = 3.

Table 2

Recoveries at Various Concentration of Capsazepine in Rat Plasma

Concentration (μg/mL)	Response Factor* Mean ± S.D.	Recovery (%)
0.05	1.555 ± 0.044	98.75 ± 3.28
0.1	1.557 ± 0.019	96.85 ± 6.71
0.5	1.447 ± 0.057	95.49 ± 8.61
1	1.398 ± 0.042	97.26 ± 4.52
2	1.469 ± 0.086	94.84 ± 2.72
5	1.248 ± 0.046	99.16 ± 3.57
Mean ± SD	1.445 ± 0.118	97.05 ± 4.90

* Peak height (10^{-1} mV)/concentration (μg/mL), mean ± S.D. (n=4).

dividing the peak heights of drug by their concentrations. The mean percent recoveries and response factors of spiked capsazepine from plasma were 97.05% and 1.445 (Table 2), respectively. In all standard samples studied, a good linear relationship was observed (Table 3). It is indicated that the assay parameter (peak height ratio) was proportional to the capsazepine concentration in the range of 0.05 to 5 μg/mL. The concentration-time profile of capsazepine after being intravenously administered to rats is shown in Figure 3. The mean terminal half-life, total body clearance, apparent volume of distribution at

Table 3

Summary of Linearity for Capsazepine Assay

Day	Slope ± SD	Intercept ± SD	Regression Coefficient (R^2 ± SD)
Inter-day (n=4)	0.5567 ± 0.0134	0.0022 ± 0.00809	0.999 ± 0.028
Intra-day (n=3)	0.5408 ± 0.0093	0.0087 ± 0.0074	0.999 ± 0.005

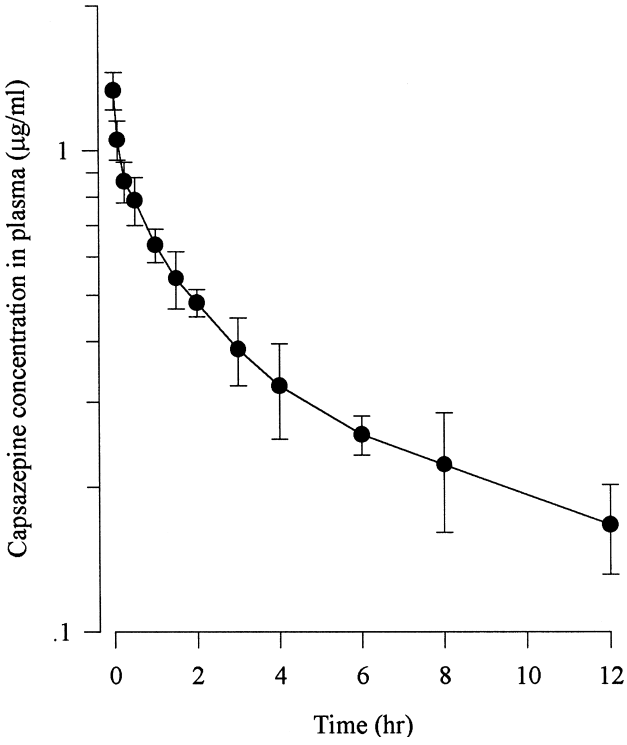

Figure 3. Plasma concentration-time profile of capsazepine after intravenous administration of capsazepine (1 mg/kg) loaded in microemulsion to Sprague-Dawley rats. Bars represent standard deviations. Each point represents the mean ± S.D. (n=3).

the steady state, and mean residence time of capsazepine was 9.91 hr, 156.29 mL/hr/kg, 1982.16 mL/kg, and 12.68 hr, respectively. In conclusion, this HPLC method for measuring capsazepine concentration in rat plasma was accurate, reproducible, and sensitive to evaluate the pharmacokinetic parameters of capsazepine in whole body.

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