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Short communication

Determination of a chemoprotective agent, 2-(allylthio)pyrazine, in plasma, urine and tissue homogenates by high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic method was developed for the determination of a chemoprotective agent, 2-(allylthio)pyrazine (I), in human plasma and urine, and in rat blood and tissue homogenate using diazepam as an internal standard. The sample preparation was simple; 2.5 volumes of acetonitrile were added to the biological sample to deproteinize it. A 50–100 μl aliquot of the supernatant was injected onto a C_{18} reversed-phase column. The mobile phase employed was acetonitrile–water (55:45, v/v), and it was run at a flow-rate of 1.5 ml/min. The column effluent was monitored using an ultraviolet detector at 330 nm. The retention times for I and the internal standard were 4.0 and 5.1 min, respectively. The detection limits of I in human plasma and urine, and in rat tissue homogenate (including blood) were 20, 20 and 50 ng/ml, respectively. The coefficients of variation of the assay (within-day and between-day) were generally low (below 6.1%) in a concentration range from 0.02 to 10 μg/ml for human plasma and urine, and for rat tissue homogenate. No interferences from endogenous substances were found.

Keywords: 2-(Allylthio)pyrazine

1. Introduction

Diallylsulfide (a component of *Allium sativum*) has a chemoprotective activity, and pyrazine has a binding affinity to cytochrome P450 2E1 (inhibits CYP2E1 activity). Therefore, pyrazine was attached to the allylsulfide radical of diallylsulfide to form 2-(allylthio)pyrazine (I, Fig. 1) to increase the binding affinity of diallylsulfide to CYP2E1. The hepatoprotective effects of I have been reported previously [1]. Compound I is now being evaluated in a preclinical trial as a chemoprotective agent.

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

2. Experimental

2.1. Chemicals

Compound I was supplied by the Central Research Institute of Bukwang Pharmaceutical Company (Seoul, South Korea) and diazepam, the internal

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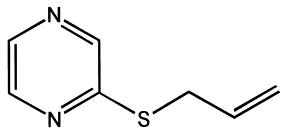
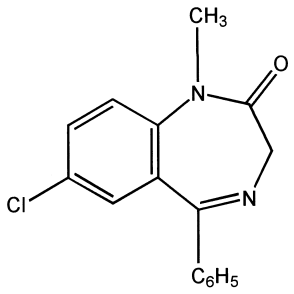


Compound I



Diazepam (the internal standard)

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

standard for the HPLC assay (Fig. 1), was donated by Hwan In Pharmaceutical Company (Seoul, South Korea). The other chemicals were of reagent grade or HPLC grade and were used without further purification.

2.2. Preparation of stock and standard solutions

A stock solution of I was prepared in methanol (1 mg/ml). Appropriate dilutions of 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 0.02, 0.05, 0.1, 1 and 10 μg/ml. Only the HPLC assay results on 0.02, 0.05 and 10 μg/ml for human plasma and urine are listed in Table 1. The internal standard solution was prepared by dissolving diazepam in acetonitrile to give a final concentration of 1 μg/ml. An internal standard that had a similar chemical structure, and similar chemical and physical properties to I was not found, therefore, diazepam was used as an internal standard in the present study.

2.3. Sample preparation

A 125-μl volume of acetonitrile containing the internal standard (1 μg/ml of diazepam) was added to 50 μl of human plasma or urine samples [2,3]. After vortex-mixing and centrifugation at 9000 *g* for 10 min, 50–100 μl of the supernatant were injected

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

Theoretical concentration (μg/ml)	Response factor[a]		Accuracy[b] (%)	
	Within-day (n=5)	Between-day (n=3)	Within-day (n=5)	Between-day (n=3)
Human plasma				
10	2.27 (2.5)	2.33 (3.2)	105 (2.5)	102 (4.2)
0.05	2.14 (3.3)	2.15 (5.9)	99.0 (3.3)	98.3 (5.1)
0.02	2.08 (4.9)	2.17 (3.8)	96.3 (4.9)	99.6 (3.4)
Human urine				
10	2.41 (3.2)	2.33 (6.1)	99.1 (3.2)	98.2 (5.8)
0.05	2.40 (5.5)	2.46 (2.6)	98.9 (5.5)	104 (3.0)
0.02	2.48 (6.0)	2.37 (4.6)	102 (6.0)	100 (4.4)

Values in parentheses are coefficients of variation (%).
[a] [Drug peak height (in cm) divided by its concentration (μg/ml)]/[internal standard peak height (in cm) divided by its concentration (μg/ml)]; mean.
[b] (Mean observed concentration/theoretical concentration)×100; mean.

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, Staufen, Germany) and immediately centrifuged at 9000 *g* for 10 min. A 50-μl volume of the 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 increase the reproducibility of I [4,5]. After vortex-mixing and centrifugation at 9000 *g* for 10 min, a 50–100 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 7120 injector (Rheodyne, Cotati, CA, USA), a Model PU-980 pump (Jasco, Tokyo, Japan), a reversed-phase C_{18} column (30 cm×3.9 mm, I.D.; particle size, 10 μm; Waters, Milford, MA, USA), a Model UV-975 UV detector (Jasco) and a Model 807-IT integrator (Jasco). The mobile phase, acetonitrile–water (55:45, v/v), was run at a flow-rate of 1.5 ml/min and the column effluent was monitored by UV detection at 330 nm.

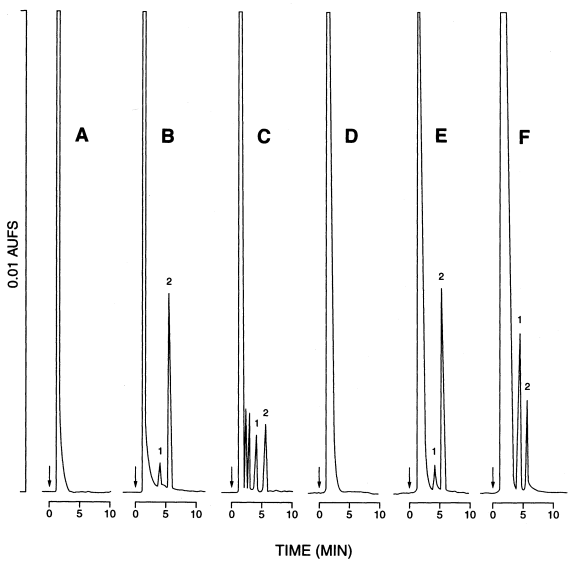


Fig. 2. Chromatograms (after deproteinization) of drug-free human plasma (A), human plasma spiked with 50 ng/ml of I and 1 μg/ml of internal standard (B), plasma collected from a male Sprague-Dawley rat at 2 h (0.350 μg/ml) after intravenous administration (over 1 min) of 20 mg/kg of I (C), drug-free human urine (D), human urine spiked with 50 ng/ml of I and 1 μg/ml of the internal standard (E) and urine collected from a male Sprague-Dawley rat between 0 and 24 h (0.704 μg/ml) after intravenous administration (over 1 min) of 20 mg/kg of I (F). Peaks: 1=I (4.0 min); 2=internal standard (5.1 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 2 mV (A, B, D and E) and 4 mV (C and F).

3. Results and discussion

Fig. 2 shows typical chromatograms of drug-free human plasma, drug standards in human plasma, plasma collected at 2 h after intravenous administration of 20 mg/kg of I to a rat, drug-free human urine, drug standards in human urine and in urine collected between 0 and 24 h after intravenous administration of 20 mg/kg of I to a rat using the deproteinization method; the corresponding chromatograms for rat kidney homogenate are shown in Fig. 3. No interferences from endogenous substances were observed in any of the biological samples. The retention times for I and the internal standard were 4.0 and 5.1 min, respectively. It should be noted that the biological sample was diluted with 2.5 volumes of acetonitrile, therefore, the concentration of acetonitrile was approximately 71% (v/v). The concentration of acetonitrile in the mobile phase was 55% (v/v). The injection volume after deproteinization was 50–100 μl and the flow-rate of the mobile phase was 1.5 ml/min. Although the elution strength of the injected fluid after deproteinization was higher than that of the mobile phase, the retention time of I in all of the biological fluids was fairly constant.

The detection limits for I in human plasma and urine were both 20 ng/ml (Table 1), based on a signal-to-noise ratio of 3.0. At 20 ng/ml, the within-day and between-day (three consecutive days) coefficients of variations (C.V.s) for response factor were lower than 4.9 and 6.0% for human plasma and urine, respectively, and the corresponding values for accuracy were lower than 4.9 and 6.0% (Table 1). The mean within-day C.V.s of I in human plasma and urine were 3.6% (range 2.5–4.9%) and 4.9% (range 3.2–6.0%), respectively, within a concentration range of 0.02–10 μg/ml (Table 1). The between-day

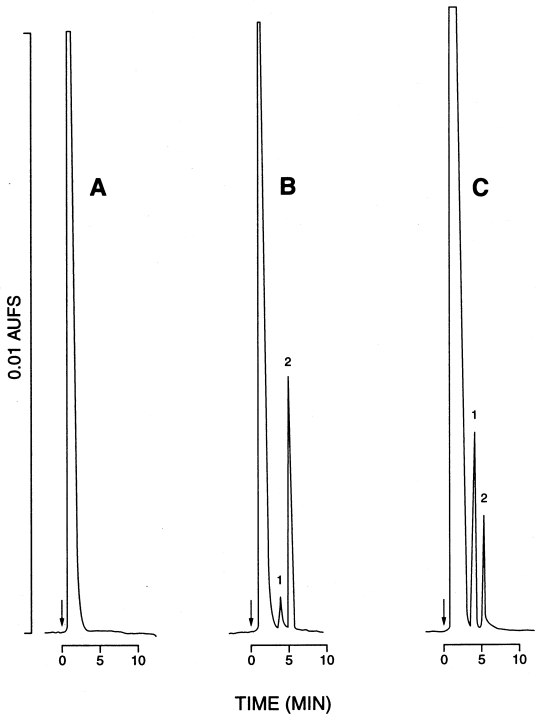


Fig. 3. Chromatograms (after deproteinization) of drug-free rat kidney homogenate (A), rat kidney homogenate spiked with 0.1 μg/ml of I and 1 μg/ml of the internal standard (B) and kidney homogenate collected from a male Sprague-Dawley rat at 30 min (1.15 μg/ml) after the intravenous administration (over 1 min) of 10 mg/kg of I (C). Peaks: 1=I (4.0 min); 2=internal standard (5.1 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 2 mV (A and B) and 4 mV (C).

C.V.s of I in human plasma and urine for the analysis of the samples on three consecutive days were 4.3% (range 3.2–5.9) and 4.4% (range 2.6–6.1%), respectively, within the concentration range 0.02–10 μg/ml (Table 1). The within-day accuracy [(mean observed concentration/theoretical concentration)× 100] of I was 96.3–105% and 98.9–102% for human plasma and urine, respectively, within the concentration range of 0.02–10 μg/ml (Table 1). The between-day accuracy of I was 98.3–102 and 98.2–104% for human plasma and urine, respectively, within the concentration range of 0.02–10 μg/ml (Table 1).

This HPLC method was also successful for the determination of I in rat tissues (liver, lung, heart, brain, kidney, muscle, stomach, intestine and spleen) and in blood. The detection limit for I was 50 ng/ml for rat blood and the tissues (or organs) studied. The mean within-day C.V.s for I in rat tissues ranged from 1.6 to 4.2% within the concentration range of 0.05–10 μg/ml. The accuracy of I in rat tissues ranged from 95.6 to 106% within the concentration range of 0.05–10 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 this study.

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