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Short Communication

Determination of 18β-glycyrrhetic acid in human plasma by high-performance liquid chromatography

Ken-ichi Abe*, Akira Suzuki, Hiroshi Katayama, Yoshio Tatsumi, Eizaburo Yumioka

Traditional Chinese Medicine Research Labs., Kanebo Ltd, 1-5-90, Tomobuchi-cho, Miyakojimaku, Osaka 534, Japan

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Abstract

A sensitive high-performance liquid chromatographic method has been developed for the determination of 18β-Glycyrrhetic acid in human plasma. 18α-Glycyrrhetic acid was used as an internal standard. The procedure includes pretreatment by liquid–liquid extraction and chromatographic separation on a reversed-phase column with ultraviolet detection. The detection limit of the drug was 5 ng/ml. The method was applied to the determination of 18β-glycyrrhetic acid in plasma after oral administration of a herbal medicine containing 18β-glycyrrhitic acid in human volunteers.

1. Introduction

18β-Glycyrrhitic acid (G), one of the principal constituents of *Glycyrrhizae radix*, is widely used as an antiinflammatory [1,2] and antihepatotoxic [3,4] agent in clinical practice and is known to be metabolized mainly to 18β-glycyrrhetic acid (β-GA) after oral administration in human [5,6]. In recent years, several HPLC methods have been developed for the determination of β-GA in biological specimens [6–10]. In this paper we describe a sensitive HPLC method newly developed for the quantification of β-GA in human plasma, which involves the use of 18α-glycyrrhetic acid (α-GA) as an internal standard (I.S.) and pretreatment by liquid–liquid extraction of the plasma sample. Since our method was sensitive enough to detect β-GA at a concentration as low as 5 ng/ml, it was applied to the study of plasma disposition of β-GA in human subjects after oral administration of Shosaiko-to (SS), a G-containing herbal medicine.

2. Experimental

2.1. Chemicals and reagents

α-GA and β-GA were purchased from Sigma (St. Louis, MO, USA) and Alps Pharm. Ind. (Gifu, Japan), respectively. SS was prepared in

* Corresponding author.

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our laboratory by spray-drying a hot water extract of the mixture of seven herbal drugs [11]. Other reagents were of HPLC grade or reagent grade.

2.2. Apparatus and liquid chromatographic conditions

The HPLC apparatus consisted of a liquid chromatograph (Waters Model 600 E System controller) and a spectrophotometer (Waters Model 484 tunable absorbance detector). Separation was achieved on a reversed-phase column (Nucleosil $5C_{18}$, 200 mm × 4.6 mm I.D., 5 μm particle size, Nihon Chromato Tec., Tokyo, Japan). The mobile phase was 0.1 *M* phosphate buffer (pH 5.8)–CH_3CN (9:11, v/v) and the flow-rate was 0.9 ml/min. The chromatogram was monitored at a wavelength of 254 nm. The system was maintained at 40°C.

2.3. Sample preparation

To 0.5 ml of plasma, 0.5 ml of 0.3 *M* phosphate buffer (pH 2.1) and 10 μl of I.S. solution (methanol containing α-GA at 5 μg/ml) were added. The mixture was extracted with 4 ml and 3 ml of benzene, successively. The combined benzene layers were shaken with 0.5 ml of 0.5 *M* NaOH. The alkaline aqueous layer was washed with 4 ml of benzene and then acidified with 0.5 ml of 1 *M* phosphoric acid. The acidic aqueous layer was extracted with 4 ml of diethyl ether. The ether layer was washed with 0.5 ml of 0.2 *M* phosphate buffer (pH 7.0) and evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of a mixture of 0.1 *M* phosphate buffer (pH 5.8)–CH_3CN (2:1, v/v). An aliquot (20 μl) of the solution was injected onto the HPLC apparatus.

2.4. Calibration curve

The blank plasma samples were spiked with β-GA at concentrations of 10, 30, 90, and 150 ng/ml, respectively. Each spiked plasma sample (0.5 ml) was put through the complete sample preparation procedure including the addition of 10 μl of I.S. solution, as described above. The resulting sample solution was subjected to HPLC analysis. The peak-height ratio of β-GA relative to I.S. was plotted against the concentration of β-GA in the sample. The linear regression line was determined by the least-squares method.

2.5. Drug administration study

Six healthy male volunteers weighing 55–65 kg participated in the study. Each subject, after a 12-h fast, received 5.4 g of SS containing 23.2 mg of G. Blood samples taken at 0, 2, 4, 6, 8, 10, and 24 h after drug administration were analyzed by the method described above.

3. Results and discussion

3.1. Chromatogram

Typical chromatograms of a blank plasma sample, a plasma sample spiked with β-GA and a plasma sample taken 10 h after drug administration are shown in Fig. 1. Both peaks of β-GA and I.S. were well separated from interfering peaks under the analytical conditions employed.

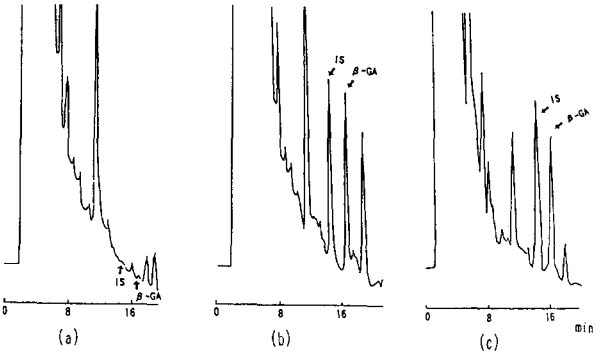

Fig. 1. Chromatograms obtained from (a) blank plasma sample, (b) plasma sample spiked with β-GA (90 ng/ml), (c) plasma sample at 10 h after oral adiministration of SS (23.2 mg as G). β-GA:18 β-glycyrrhetic acid; I.S.:18 α-glycyrrhetic acid; SS:Shosaiko-to (= Hsiao-Chai-Hu-Tang).

Table 1
Intra-day precision for β-GA determination in human plasma

Spiked concentration (ng/ml)	n	Measured concentration (mean ± S.D.) (ng/ml)	Precision (C.V., %)	Accuracy (%)
10	5	10.2 ± 0.6	5.9	101.4
50	5	48.3 ± 2.4	5.0	96.4
150	5	158.0 ± 9.2	5.8	105.2

3.2. Linearity, reproducibility and detection limit

The peak-height ratios (β-GA to I.S.) were linearly related to the concentrations of β-GA over the range of 10–150 ng/ml. The regression equation for β-GA was y (peak-height ratio) = $0.0104x$ (ng/ml) + 0.0196 ($r = 1.000$). The intra- and inter-assay precisions were determined at three different concentrations of the analyte (10, 50, 150 ng/ml). The coefficients of variation (C.V.) were all less than 7% at the concentrations studied (Tables 1 and 2). The detection limit of β-GA in 0.5 ml of plasma was estimated to be 5 ng/ml at a signal-to-noise ratio of 3:1.

3.3. Application

Fig. 2 shows the concentration–time profile of β-GA in plasma after oral administration of SS to human volunteers. The appearance of β-GA in plasma was rather fast, which agrees with the results of the pharmacokinetic study on orally administered G reported by Yamamura *et al.* [5]. The elimination pattern of β-GA was in accordance with that observed with another G-containing herbal medicine [10]. Since the present method developed for plasma was shown to be

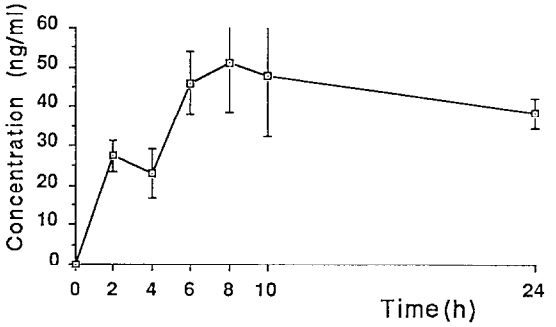

Fig. 2. Plasma concentration–time profile of β-GA after oral administration of SS (23.2 mg as G) to human volunteers. Each point and vertical bar represents the mean ± S.E. of six subjects.

Table 2
Inter-day precision for β-GA determination in human plasma

Spiked concentration (ng/ml)	n	Measured concentration (mean ± S.D.) (ng/ml)	Precision (C.V., %)	Accuracy (%)
10	3	10.6 ± 0.7	6.3	106.3
50	3	47.5 ± 2.8	6.0	94.9
150	3	149.5 ± 9.5	6.4	99.6

applicable to urine with the same sensitivity, urine samples obtained at appropriate intervals in the above study were subjected to the analysis. The analytical results demonstrated that the excretion of β-GA over a 10-h period was quite low, constituting less than 0.1% of the dose. In conclusion, this sensitive HPLC method for the determination of β-GA in biological samples proved to be useful for detailed pharmacokinetic study of G.

4. References

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