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High-Performance Liquid Chromatographic Determination of Ferulic Acid in Plasma

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A simple, rapid and sensitive reversed-phase high performance liquid chromatographic (HPLC) method for the quantitative determination of ferulic acid in plasma was developed. Ferulic acid in plasma was extracted with ethyl ether, separated on a reversed-phase C_{18} column with a mixture of acetonitrile and 0.1 M acetic acid (1: 3, v/v) as a mobile phase, and quantitated by ultraviolet (UV) absorbance measurement at 313 nm. 3,4-Dimethoxycinnamic acid was used as an internal standard. The detection limit (signal-to-noise ratio = 3) and the mean recovery from plasma were 1 ng (as amount injected) and 99.5 ± 3.3%, respectively.

The plasma levels of ferulic acid following oral administration of γ-oryzanol were determined by the present method and the mass fragmentographic (MF) method. A close correlation between the results of HPLC and those of MF was observed ($r = 0.988$).

The present HPLC method was found to be applicable to the determination of ferulic acid in plasma in place of the MF method.

Keywords——ferulic acid; γ-oryzanol; plasma; HPLC; mass fragmentography

In the previous paper[1] we reported a mass fragmentographic (MF) method for the determination of ferulic acid in plasma after oral administration of γ-oryzanol, and also suggested that the area under the concentration curve (AUC) of ferulic acid might be a good indicator for estimating the absorption of γ-oryzanol. However, the MF method is technically complex and time-consuming because it requires clean-up of the plasma and derivatization of ferulic acid. We therefore tried to utilize high-performance liquid chromatography (HPLC) for the determination of ferulic acid in plasma because of its rapidity, simplicity and sensitivity. Though some reports[2] on the separation of phenolic compounds, including ferulic acid, in plant tissues by HPLC have been published, there are few reports[3] concerning the determination of very small amounts of ferulic acid in biological samples. This paper describes a simple and sensitive HPLC method for the determination of ferulic acid in plasma.

Experimental

Materials——Ferulic acid and 3,4-dimethoxycinnamic acid were obtained from Tokyo Kasei Kogyo Co., Ltd. and purified by recrystallization from water. Acetonitrile (special grade for liquid chromatography) was obtained from Wako Pure Chemical Industries Ltd. All other chemicals were of analytical-reagent grade.

Apparatus and Chromatographic Conditions——A Waters Assoc. high-performance liquid chromatography, equipped with a Model 6000A pump, a Model U6K universal injector and a Model 440 absorbance detector, was used. The column was a μ-Bondapak C_{18} column (30 cm × 4 mm i.d.) obtained from Waters Assoc. The mobile phase consisted of 0.1 M acetic acid–acetonitrile (3: 1, v/v) and the flow rate was 1.0 ml/min. The solvent was ultrasonicated before use. The effluent from the column was monitored at 313 nm with a ultraviolet (UV) absorbance detector (AUFS; 0.005).

Assay Procedure——To 1 ml of plasma were added 25 ng of the internal standard (3,4-dimethoxycinnamic acid) and 1 ml of 0.2 N HCl, and the sample was extracted with 6 ml of ethyl ether with vigorous shaking for 10 min. After centrifugation (3000 rpm, 5 min) of the mixture, the organic layer was transferred into another test tube and evaporated to dryness at room temperature under a stream of nitrogen. The residue was dissolved in 50 to 100 μl of methyl alcohol, an aliquot of the solution was injected with a microsyringe into the HPLC column, and the peak height ratio of ferulic acid to the internal standard was measured.

Calibration Curve——To 1 ml of plasma were added 20 to 200 ng of ferulic acid and 100 ng of the internal standard, and the plasma samples were analyzed by the procedure described above.

Results and Discussion

Several mobile phases were examined to obtain the best sensitivity. Acetonitrile gave a sharper peak than methanol or ethanol. By using a mixture of acetonitrile and 0.1 M acetic acid (1 : 3, v/v), ferulic acid could be separated from biological components of dog and rabbit plasma. The concentration of acetic acid, in the range from 0.05 to 0.4 M, did not affect the peak height or the retention time of ferulic acid.

We chose 3,4-dimethoxycinnamic acid as an internal standard because this compound had a suitable retention time and no interfering peak was observed at the same retention time as the peak of this compound in the blank plasma sample. Further, in order to confirm that 3,4-dimethoxycinnamic acid does not exist in plasma as a metabolite of γ-oryzanol, HPLC analysis was performed with plasma samples after oral administration of γ-oryzanol to dogs and rabbits. No peak corresponding to 3,4-dimethoxycinnamic acid was observed, showing that this compound was suitable for use as the internal standard.

The calibration curve, obtained by plotting the ratios of the peak height of ferulic acid to that of the internal standard against the amounts of ferulic acid added, was linear ($r=0.999$) up to 200 ng/ml and passed through the origin. The detection limit of ferulic acid was 1 ng (amount injected) at a signal-to-noise ratio of three. Typical chromatograms of an extract from dog plasma containing ferulic acid and the internal standard, and an extract from blank plasma are shown in Fig. 1.

In order to check the recovery, 25.0 to 150.0 ng of ferulic acid and the internal standard (50 or 100 ng) were added to 1 ml of dog plasma, and the samples were treated according to the assay procedure described in "Experimental." The results are shown in Table I. Ferulic acid was recovered quantitatively from plasma, independently of the amount added, and the standard deviation was 3.3%, which is smaller than that of the MF method (4.5%).[1]

γ-Oryzanol dissolved in sesame oil was administered orally (50 mg/kg) to dogs, and the plasma levels of ferulic acid analyzed by the present HPLC method were compared with those

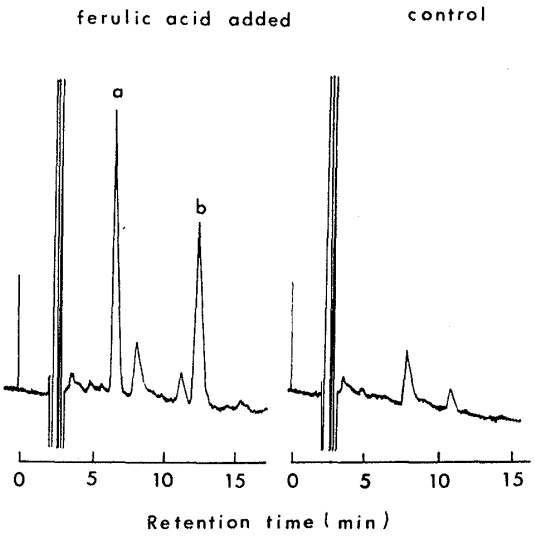

Fig. 1. HPLC Chromatograms of Extracts from Dog Plasma

Dog plasma samples (1 ml) supplemented with 50 ng of ferulic acid or without ferulic acid were extracted. a, ferulic acid; b, internal standard.

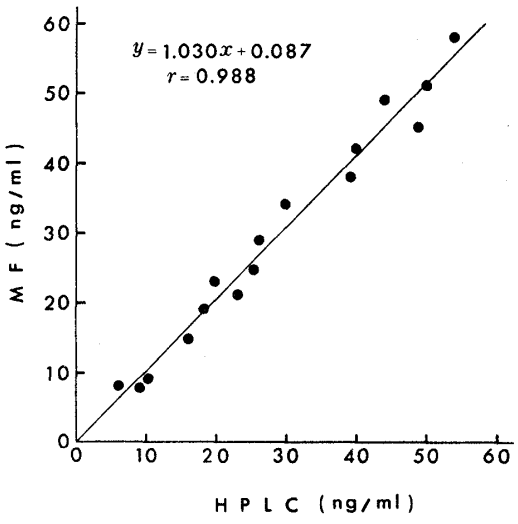

Fig. 2. Correlation of Ferulic Acid Levels in Dog Plasma as Determined by HPLC and by MF following Oral Administration of γ-Oryzanol

Dose of γ-oryzanol: 50mg/kg animal.

TABLE I. Recovery of Ferulic Acid added to Dog Plasma

Amount added (ng/ml)	Amount found (ng/ml)	Recovery (%)	Mean±S.D.
	23.6	94.2	
25.0	25.4	101.6	99.0±2.8
	24.7	98.8	
	25.1	100.4	
	46.5	93.0	
50.0	48.3	96.6	97.4±3.4
	49.8	99.6	
	50.2	100.4	
	102.5	102.5	
100.0	98.2	98.2	101.7±2.6
	104.3	104.3	
	101.9	101.9	
	152.3	101.5	
150.0	146.7	97.8	100.2±3.7
	145.1	96.7	
	157.2	104.8	
			99.5±3.3 (n=16)

found by the MF method. The two results agreed well, as shown in Fig. 2, indicating that the present method is suitable for the quantitative determination of ferulic acid in plasma.

In conclusion, the proposed method is very satisfactory for the determination of ferulic acid in plasma, being rapid, simple and sensitive.

References and Notes

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