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Ultraviolet spectral identification of ferulic acid in rabbit plasma by HPLC and its pharmacokinetic application

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Summary

A simple and sensitive high-performance liquid chromatography method for the analysis of ferulic acid in rabbit plasma has been developed. Up to 0.1 ml of plasma containing ferulic acid was deproteinized by acetonitrile, which contained an internal standard (coumarin). The supernatant was injected into a Nucleosil 7C18 column using acetonitrile-water-phosphoric acid (30:70:0.1, v/v; pH 2.5) as the mobile phase and UV detection at 322 nm, followed by UV spectral identification (between 200 and 380 nm) using a photodiode array detector. The method is rapid, easily reproduced, selective and sensitive. It was applied to pharmacokinetic studies. A biphasic phenomenon, i.e. a rapid distribution phase followed by a slower elimination phase, was observed from the plasma concentration-time curve. Compartmental analysis yielded a two-compartment open model.

Introduction

Ferulic acid is a phenolic compound contained in *Liguisticum wallichii Franch* and *Angelica sinensis (Oliv.) Diels (A. sinensis)*. Recent studies have indicated that ferulic acid is a nitrite scavenger (Kuenzig et al., 1984; Moller et al., 1988), uterine contraction inhibitor (Ozaki and Ma, 1990), glutathione *S*-transferase inhibitor (Sato et al., 1990) and antitumour agent (Huang et al., 1988; Nair and Panikkar, 1990).

Several HPLC methods for the determination of ferulic acid have been described in the literature for UV detection (Fujiwara et al., 1983; Pussayanawin and Wetzel, 1987), fluorescence detection (Nohta et al., 1989), electrochemical detection (Galletti et al., 1990), and mass fragmentographic determination (Fujiwara et al., 1982). However, none of the methods described permits the spectral identification of ferulic acid from plasma and application to pharmacokinetic study.

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Materials and Methods

Chemicals and reagents

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) and coumarin (internal standard) were purchased from Sigma (St. Louis, MO, U.S.A.).

HPLC grade acetonitrile, methanol, and phosphoric acid were obtained from Merck (Darmstadt, Germany). Stock solutions were prepared in methanol at a concentration of 1 mg/ml and stored at 4°C. The quality of solutions stored thus was found to remain dependable for at least 1 month. Stock solutions were diluted to 0.1, 0.01, and 0.001 mg/ml with methanol before use.

Chromatography conditions

The HPLC system consisted of a Waters U6K injector, a Waters M 990 photodiode array detector, which permits scanning of chromatographic and spectral data, and two Waters M510 chromatographic pumps (Milford, MA, U.S.A.). Separation was achieved on reversed-phase Nucleosil 7C18, 4 × 250 mm, i.d. (Dueren, Germany) fitted with a column inlet filter (0.5 μm × 3 mm, Rheodyne, U.S.A.). The mobile phase was acetonitrile-water-phosphoric acid (30 : 70 : 0.1, v/v; pH 2.5), at a flow rate of 1.0 ml/min. The detection was monitored at 322 nm and the wavelengths scanned between 200 and 380 nm for the photodiode array detector.

Animals

Male New Zealand white rabbits (2.5–3.0 kg) were obtained from the Laboratory Animal Center at the National Taiwan University. These animals were kept in our own environmentally controlled quarters, with the temperature maintained at 24 ± 1°C and light-dark cycle of 7 : 00-19 : 00 for at least 1 week before use. Water and standard laboratory chow were given ad libitum until 18 h before the experiments, at which time only food was withdrawn.

Sample preparation

A blood sample of 0.5 ml was directly withdrawn from the ear vein of conscious rabbits minimally restrained in a rabbit holder. Blood samples were collected at time intervals of 2.5, 5, 10, 15, 30, 45, 60, 90, and 120 min after intravenous administration of ferulic acid (5 mg/kg). The blood sample was then transferred to a heparinized microfuge tube and centrifuged at 8000 × *g* for 5 min (Eppendorf 5402). The resulting plasma (0.1 ml) was mixed with a 0.2 ml portion of acetonitrile, which contained 5 μg/ml of internal standard. The denatured protein precipitate was separated again by centrifugation at 8000 × *g* for 5 min. The supernatant (20 μl) was directly injected into the HPLC system for analysis.

Calculation

The ratio between the peak area of the drug analyzed and that of the internal standard was calculated using a calibration curve. The curve was plotted against the concentration of the drug tested after the analysis of blank samples respectively spiked with increasing concentrations (10 to 2000 ng/ml) of ferulic acid and a constant amount of internal standard (0.2 μg coumarin). The equation of the curve was $Y = 0.3069X - 0.0145$ for rabbit plasma. The value of the correlation coefficient was 0.999. For concentrations above 2000 ng/ml, the analyzed sample was diluted.

Results and Discussion

Under the conditions described above, the retention times of ferulic acid and coumarin were found to be 4.63 and 8.86 min, respectively (Fig. 1). Fig. 2 shows the chromatograms and spectra from blank plasma spiked with ferulic acid, with coumarin as the internal standard. The main characteristic spectral data obtained in the mobile phase were the absorption maxima at 322 and 218 nm for ferulic acid, and at 278 and 320 nm for coumarin.

Recovery

For recovery studies, 1 ml portions of blank plasma spiked with 0.01, 0.1, or 1 μg of ferulic acid ($n = 4$) were extracted as described above. The internal standard was added to the eluate. Peak area ratios of the extracts were compared with those determined from direct injection of the residue of the methanolic standard solutions (mixture of 0.1, 1, or 10 μg of ferulic acid with the same amount of internal standard). The recoveries were between 92 and 96% (coefficient of variation: % CV = 3.9–6.7) for the plasma.

Reproducibility

Reproducibility was examined using plasma which contained three levels (0.01, 0.1 and 1

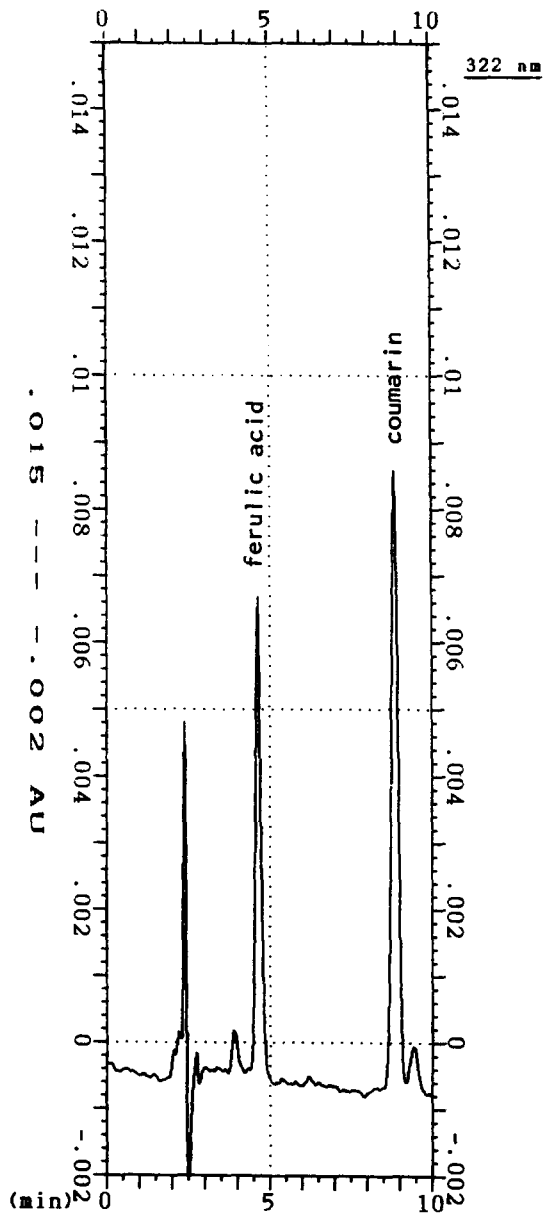

Fig. 1. Chromatogram spiked ferulic acid with coumarin, as internal standard in rabbit plasma.

μg/ml) of ferulic acid. Within-day coefficients of variation were 7.56, 5.19, and 2.23%. Between-day coefficients of variation were 8.01, 7.90, and 3.34% over a period of 6 days.

Sensitivity

With the M990 photodiode array detector, the detection limit established for plasma ferulic acid was 10 ng/ml (signal-to-noise ratio = 3). Thus, UV detection was considered as a suitable procedure for pharmacokinetic quantitative studies of ferulic acid.

Selectivity

The complementary use of the photodiode array detector for identifying the analyzed compound by its retention time/wavelength absorbance is more reliable than the UV detector which only provides the value of the retention time. A chromatogram of a blank plasma sample showed no background interference from endogenous constituents.

Pharmacokinetics

A statistical nonlinear regression program was accessed through the JANA program (SCI Software Inc., Lexington, KY, U.S.A.) for the kinetic analysis. A two-compartment open model with elimination from the central compartment was proposed and validated through the program to explain the apparent biphasic distribution of ferulic acid in plasma after i.v. injection as demonstrated in Fig. 3. The plasma concentration as a function of time can be described by the following equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t}$$

where C_p is the plasma concentration of ferulic acid at time t, A and B denote two pre-exponential constants consisting of the first-order distribution between central and peripheral compartments, α and β are two exponents, representing the distribution and elimination phases, k_{12} and k_{21} represent the rate constant between the central and peripheral compartments, and k_{10} is the elimination rate constant.

The elimination half-life ($T_{1/2,\beta}$) of ferulic acid from the central compartment as indicated in the terminal phase of the plasma concentration-time curve was determined from the equation:

$$T_{1/2,\beta} = 0.693/\beta$$

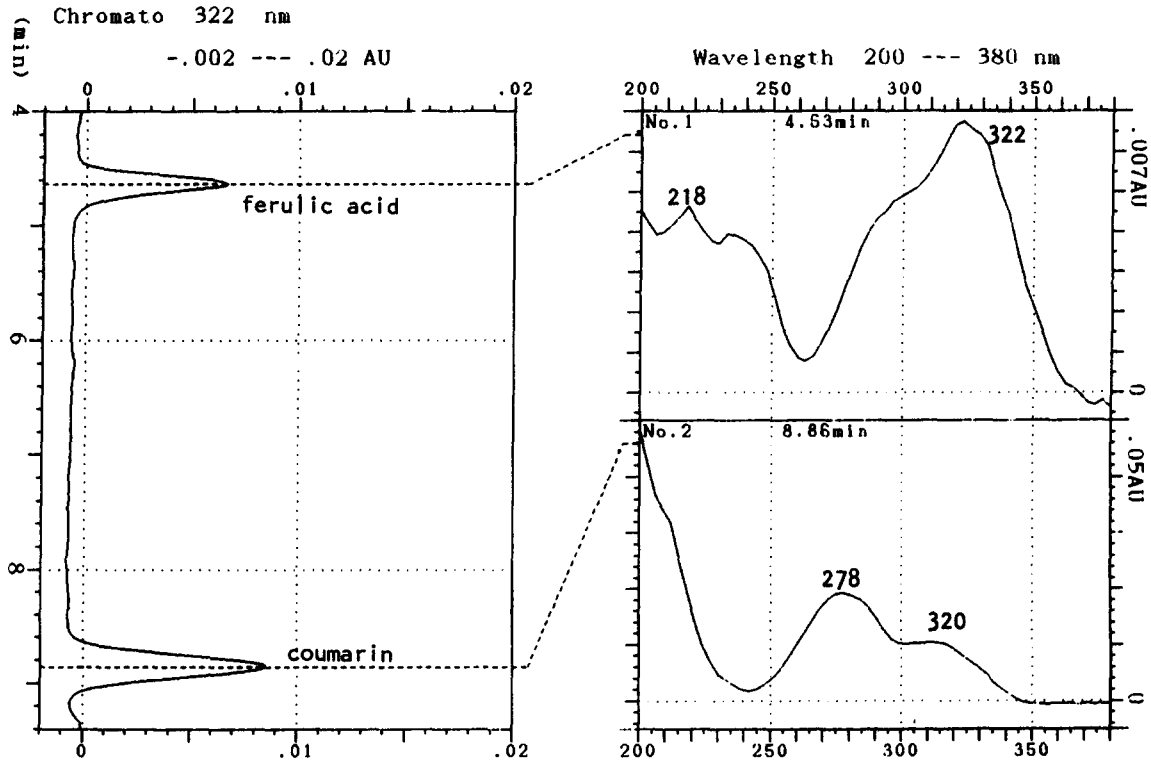

Fig. 2. Chromatogram and spectrum of ferulic acid in rabbit plasma sample 30 min after i.v. administration of a 5 mg/kg dose.

The parameters AUC and V_c, AUC being the area under the concentration-time curve and V_c the apparent volume of distribution in the central

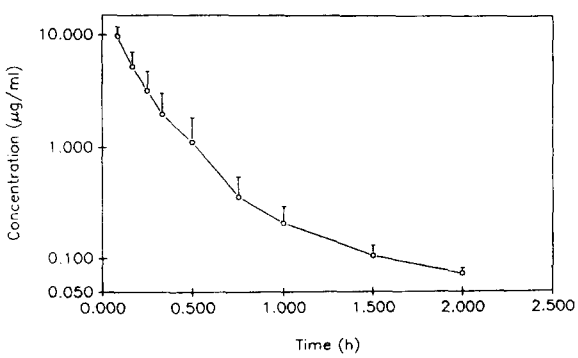

Fig. 3. Plasma concentration-time curve for ferulic acid following i.v. administration of 5 mg/kg to rabbits.

TABLE 1

Estimates of pharmacokinetic parameters according to a two-compartment open model with elimination from the central compartment for the case of i.v. administration of a dose of 5 mg / kg ferulic acid to male rabbits (n = 6)

Parameter (units)	Estimate
A (μg ml^{-1})	18.65 ± 2.15
B (μg ml^{-1})	1.51 ± 0.22
α (h^{-1})	9.45 ± 1.40
β (h^{-1})	1.91 ± 0.18
k_{10} (h^{-1})	7.49 ± 0.97
k_{12} (h^{-1})	1.99 ± 0.55
k_{21} (h^{-1})	4.12 ± 0.69
$T_{1/2,\beta}$ (h)	0.27 ± 0.04
C_p (μg ml^{-1})	21.07 ± 2.77
AUC (μg h ml^{-1})	2.91 ± 0.32
V_c (ml kg^{-1})	253.17 ± 24.69

Data are expressed as means ± SE.

compartment, were then evaluated. The estimates of these pharmacokinetic parameters, based on the two-compartment open model, were calculated from the best-fit coefficients and exponents of a PCNONLIN program (SCI Software Inc., Lexington, KY, U.S.A.) and have been listed in Table 1.

In conclusion, the UV spectral identification, extraction and chromatographic procedures described in this study allow the quantitation of ferulic acid from rabbit plasma. The pharmacokinetic study of ferulic acid (5 mg/kg, i.v.) was characterized by the two-compartmental open model.

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