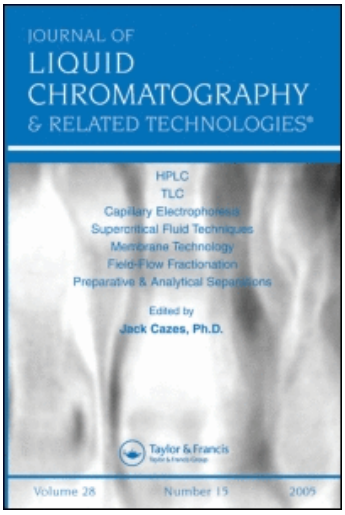

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Determination of Scoparone in Rat Plasma by Liquid Chromatography and Its Application to Pharmacokinetics

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DETERMINATION OF SCOPARONE IN RAT PLASMA BY LIQUID CHROMATOGRAPHY AND ITS APPLICATION TO PHARMACOKINETICS

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ABSTRACT

A simple liquid chromatographic method was developed to study the pharmacokinetics of scoparone in the rat plasma. After addition of an internal standard (ferulic acid), plasma was deproteinized by acetonitrile for sample clean-up. The drugs were separated on a reverse phase column and detected by UV detection at a wavelength 340 nm. Acetonitrile-water (30:70, v/v, pH 2.5 adjusted by orthophosphoric acid) was used as a mobile phase. It was applied to the pharmacokinetic study of scoparone in rats after a dose of 5 mg kg^{-1} intravenous

administration. A biphasic phenomenon with a rapid distribution followed by a slower elimination phase was observed from the plasma concentration-time curve.

INTRODUCTION

Artemisia scoparia Waldst. et Kitaib. is used as a folk medicine in Taiwan for the treatment of hepatitis. Scoparone (Figure 1) was isolated from the leaf and stem of *Artemisia scoparia.*[1] It was recently reported that scoparone possesses vasodilator and hypotensive actions,[2-4] immune suppressive activities,[4-5] free radical scavenging properties,[6] anti-anginal effects on the heart[3] and antiatherogenic effect in hyperlipidaemic diabetic rabbits.[7] Recently, scoparone was also used as a research substrate for the differentiation of cytochrome P-450 activities.[8-10] Although, the action mechanism of cardiovascular effects of scoparone have been reported, the determination of scoparone from plasma and its pharmacokinetic properties have not been studied. In this work, we developed a liquid chromatographic (LC) method with UV detection to determine the concentration of scoparone in rat plasma and its related pharmacokinetic profile.

MATERIALS AND METHODS

Chemicals and Reagents

Scoparone (6,7-dimethoxycoumarin) and ferulic acid (internal standard)[11] were purchased from Aldrich (Milwaukee, WI, USA) and Sigma (St. Louis, MO, USA), respectively. Acetonitrile and orthophosphoric acid (85%) were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore Corp., Bedford, MA, USA) was used for all preparations.

Apparatus and Chromatography

The LC system consisted of an autosampler (SIC model 23, Tokyo, Japan), a variable wavelength UV-VIS detector (Soma, Tokyo, Japan) and a chromatographic pump (BAS, PM-80, West Lafayette, IN, USA). Separation was achieved on a reverse phase Nucleosil $5C_{18}$ column (250 x 4 mm, particle size 5 μm, Macherey - Nagel, Duren, Germany). The mobile phase was acetonitrile - water (30 : 70, v/v; pH 2.5 adjusted by orthophosphoric acid), and the flow rate was 1.0 mL min^{-1}. Scoparone was monitored at a

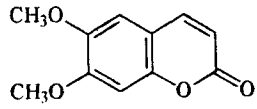

Figure 1. Chemical structure of scoparone.

wavelength of 340 nm throughout the experiments. The system was operated at room temperature (25 °C).

Animals

Male Sprague-Dawley rats (250-300 g) were obtained from the Laboratory Animal Center at the National Yang-Ming University. These animals were specifically pathogen free and kept in our own environmentally controlled quarters (temperature maintained at 24 ± 1 °C and with 12:12 light-dark cycle), for at least 1 week before use. Standard laboratory food and water was available continuously, except when food was withdrawn 18 hours prior to experimentation.

Blood Sampling

Rats were anesthetized with intraperitoneal pentobarbital 50 mg kg^{-1}. Only one-quarter (12.5 mg kg^{-1}, i.p.) of the dose of pentobarbital was administered during the experimental period when required. Blood samples (0.3 mL) were collected via cardiac puncture,[9,10] at interval of 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after intravenous administration of scoparone (5 mg kg^{-1}). Data from six individual rats were used to construct pharmacokinetic profiles by plotting concentration of scoparone in plasma versus time. Sigmaplot for Windows (version 1.01), (Jandel Sci. Corte Madera, CA, USA) was used to plot standard curves and to perform least squares regression analysis on the calibration data.

Treatment of Plasma Samples

Plasma samples were prepared as previously described.[11-12] Each collected blood sample was transferred to a heparinized microcentrifuged at 8000 g for 3 min (Eppendorf Model 5402). The resulting plasma (0.1 mL) was then mixed with 0.2 mL of acetonitrile containing ferulic acid (1 μg mL^{-1}) as internal standard.

The denatured protein precipitate was separated by centrifugation at 8000 g for 3 min, and a 20 μL aliquot of the supernatant was directly injected onto the LC. The same sample handling process was used for recovery and precision determination.

Recovery

Recovery has been defined as a measure of the efficiency of the extraction of the analyte from the sample matrix. In the experiment, recovery of scoparone was determined at low, moderate and high concentrations (0.1, 0.5 and 5 μg mL^{-1}, respectively) from the rat plasma. Two groups of samples were used to assess extraction recovery (i.e. test and control groups). The samples in the test group was spiked with scoparone in rat plasma to yield final concentrations of 0.1, 0.5 and 5 μg mL^{-1}; whereas the samples in the control group were spiked with scoparone after the extraction.

The extraction recovery was calculated as the ratio of the measured concentration of the test samples over the measured concentration of the control samples at the low, moderate and high concentrations. Quadruplicate assays have been performed at the same concentrations.

Precision

Precision over the entire working dose range was determined by replicate analyses of plasma samples (n=4) spiked with three different concentrations (0.1, 0.5, or 5 μg mL^{-1}) of scoparone. To determine intra-assay variance, quadruplicate assays were carried out on the same samples at different times during the day. Inter-assay variance was determined by assaying the spiked samples in quadruplicate on days one, two, four, and six, after spiking. Coefficients of variation (C.V.s) were calculated from these values.

Pharmacokinetic Analysis

A calibration curve was constructed based on the analysis by HPLC of various concentrations of scoparone spiked in rat plasma. The concentrations of scoparone in rat plasma after i.v. administration, was determined from the peak area by using the equation for linear regression from the calibration curve. All data were subsequently processed by the computer program PCNONLIN (SCI Software Inc., Lexington, KY).

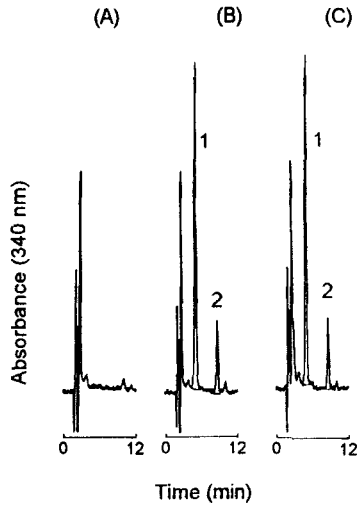

Figure 2. Chromatograms of scoparone in rat plasma: (A) blank plasma. (B) spike scoparone (0.5 μg mL^{-1}) and ferulic acid (internal standard). (C) plasma sample 30 min after a 5 mg kg^{-1} i.v. administration of scoparone (0.46 μg mL^{-1}). 1: ferulic acid; 2: scoparone.

RESULTS AND DISCUSSION

Under the conditions described above, the retention times of scoparone and ferulic acid (internal standard) were found to be 8.52 and 5.04 min, respectively (Figure 2). The main characteristic spectral data obtained in the mobile phase were shown as an absorption maxima at 340 nm for scoparone and at 322 nm for internal standard (ferulic acid).[13]

The recoveries of scoparone from rat plasma were found to be 96.38, 94.63, and 97.49 % for the concentrations 0.1, 0.5, and 5 μg mL^{-1}, respectively. The reproducibility of the method was also defined by examining both intra- and inter-assay variabilities. The intra-assay CVs for scoparone at concentrations of 0.1, 0.5, and 5 μg mL^{-1} were 7.25, 5.51, and 3.61%, respectively, and the inter-assay CVs for scoparone at the same concentrations were 8.63, 5.88, and 3.83%, respectively.

To determine the linearity and the detection limit of the HPLC method, rat plasma samples spiked with six different concentrations of scoparone (0.1-5 μg

mL^{-1}) were analyzed. The peak area ratios (scoparone to ferulic acid) were linearly related to the concentration of drug and the equation for the regression line for scoparone was found to be $y = 0.544x - 0.005$ (r^2=0.999). The lower practical limit of quantification was 0.1 μg mL^{-1}. Under the procedure described above, the detection limit for scoparone, at a signal-to-noise ratio of 4, was 0.05 μg mL^{-1} in rat plasma.

Figure 2(A) shows the chromatogram of blank rat plasma. No discernible peaks were observed within the time frame in which scoparone and ferulic acid were detected. Figure 2(B) shows the chromatogram of rat plasma spiked with scoparone (0.5 μg mL^{-1}) and internal standard. Figure 2(C) shows the chromatogram of scoparone (0.46 μg mL^{-1}) sample obtained 30 min after i.v. administration of scoparone (5 mg kg^{-1}) to a rat.

The data, from the dose. fit best into a two-compartment open model by the computer program PCNONLIN. The following equation applies into a two-compartment pharmacokinetic model:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

In the equation 1, A and B are the concentration (C) intercepts for fast and slow disposition phases, respectively, and I and J are disposition rate constants for fast and slow disposition phases, respectively. The K_{12} and K_{21} are micro rate constants between the central and peripheral compartments, and K_{10} as the elimination rate constant. The distribution half-life ($t_{1/2,\alpha}$) and elimination half-life ($t_{1/2,\beta}$) of scoparone, as shown in the initial phase and terminal phase of the plasma concentration-time curve, was determined by the equation of $0.693/\alpha$ and $0.693/\beta$, respectively. Analysis of data after i.v. injection of scoparone at 5 mg kg^{-1} yields equations 2 (and Figure 3), respectively:

$$C = 5.04e^{-0.18t} + 1.26e^{-0.028t} \quad (2)$$

The pharmacokinetic parameters, as derived from these data and calculated by PCNONLIN program, are shown in Table 1.

A statistical nonlinear regression program was accessed through the JANA and PCNONLIN programs for the kinetic analysis. The pharmacokinetic models (one vs. two compartment) were compared according to Akaike's information criterion (AIC)[14] and Schwartz criterion (SC)[15] and, with minimum AIC and SC values, were regarded as the best representation of the plasma concentration time course data. A two-compartment open model with elimination from the central compartment, was proposed and validated

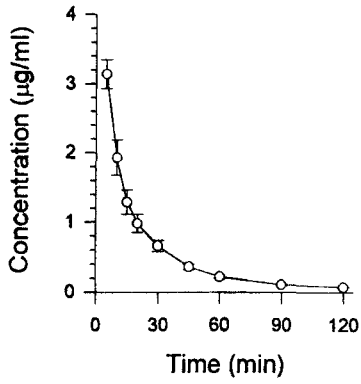

Figure 3. Plasma concentration-time curve after *i.v.* administration of scoparone in rats at dose of 5 mg kg^{-1}.

Table 1

Pharmacokinetic Parameters of Scoparone in Rats After 5 mg kg^{1}, i.v. Administration

Parameters	Estimate
A, μg mL^{-1}	5.04 ± 0.53
B, μg mL^{-1}	1.26 ± 0.18
α, min^{-1}	0.18 ± 0.029
β, min^{-1}	0.028 ± 0.003
K_{10}, min^{-1}	0.085 ± 0.011
K_{12}, min^{-1}	0.063 ± 0.013
K_{21}, min^{-1}	0.058 ± 0.010
Vol, L kg^{-1}	0.85 ± 0.086
$t_{1/2\alpha}$, min	4.68 ± 0.91
$t_{1/2\beta}$, min	27.18 ± 3.19
Cl, L kg^{-1} min^{-1}	0.067 ± 0.005
AUC, μg min mL^{-1}	77.17 ± 6.34
AUMC, μg min^2 mL^{-1}	1991 ± 265
MRT, min	25.55 ± 2.30

Data are expressed as mean ± SEM. Cl: clearance.
See text for other abbreviations.

through the program, to explain the apparent biphasic disposition of scoparone in rat plasma after i.v. administration. The noncompartmental method for calculating disposition parameters of scoparone are based on the theory of statistical moments.[16] The area under the concentration curve of a plot of the product of concentration and time versus time, from zero time to infinity, is often referred to as the area under the moment curve, AUMC.[16]

The ratio of AUMC to AUC for scoparone is a measure of its mean residence time (MRT).[17] MRT, calculated after i.v. administration, is the statistical moment analogy to drug elimination half-life. After administration of scoparone (5 mg kg^{-1}, i.v.), MRT and $t_{1/2,\ \lambda}$ were 25.55 and 27.18 min, respectively (Table 1). Like half-life, MRT is a function of both distribution and elimination. Whereas, half-life tell us the time required to eliminate 50% of the dose, MRT_{iv} tells us the time required to eliminate 63.2% of the dose.

In conclusion, the present method allows a high selectivity and reliability. The relative simplicity permits its use for pharmacokinetic studies. Analysis of data after i.v. injection of scoparone at 5 mg kg^{-1} yields a two-compartment pharmacokinetic model.

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