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Research Article

Simultaneous determination of nine lignans from *Schisandra chinensis* extract using ultra-performance liquid chromatography with tandem mass spectrometry in rat plasma, urine, and gastrointestinal tract samples: Application to the pharmacokinetic study of *Schisandra chinensis*

The fruit of *Schisandra chinensis* is a well-known herbal medicine and dietary supplement due to a variety of biological activities including antihepatotoxic and antihyperlipidemic activities. However, the simultaneous validation methodology and pharmacokinetic investigation of nine lignans of *S. chinensis* extract in biological samples have not been proved yet. Thus, the present study was undertaken to develop the proper sample preparation method and simultaneous analytical method of schisandrol A, gomisin J, schisandrol B, tigloylgomisin H, angeloylgomisin H, schisandrin A, schisandrin B, gomisin N, and schisandrin C in the hexane-soluble extract of *S. chinensis* to apply for the pharmacokinetic study in rats. All intra- and interprecisions of nine lignans were below 13.7% and accuracies were 85.1–115% and it is enough to evaluate the pharmacokinetic parameters after both intravenous and oral administration of hexane-soluble extract of *S. chinensis* to rats.

Keywords: Lignans / Liquid chromatography with tandem mass spectrometry / Pharmacokinetics / Rats / *Schisandra chinensis*
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1 Introduction

The fruit of *Schisandra chinensis* (*S. chinensis*, Turcz, Bail.), a member of Magnoliaceae family, has been traditionally used as tonic, sedative, antidiabetic, hepatoprotective, and hypoglycemic agents in eastern Asian countries [1, 2]. The major constituents of *S. chinensis* extract were lignan-type molecules which might be responsible for pharmacological effects of *S. chinensis* extract as aforementioned [3, 4]. In particular, among the lignans isolated from *S. chinensis*, the dibenzylcyclooctadiene-type lignans including schisandrin A (SN-A), B (SN-B), and C (SN-C); schisandrol A (SL-A) and B (SL-B) and their analogs [4, 5] are known to possess antihepatotoxic, antiasthmatic, and antigastric ulcer effects [4, 6, 7].

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Abbreviations: **AM-H**, angeloylgomisin H; **CVs**, coefficients of variation; **GI**, gastrointestinal tract; **GM-J**, gomisin J; **GM-N**, gomisin N; **IS**, internal standard; **MRM**, multiple reaction monitoring; **TM-H**, tigloylgomisin H

The pharmacokinetics dealing with the absorption, distribution, metabolism, and excretion properties of compounds is an essential step in predicting the efficacy and safety of compounds of interest [6, 7]. Usually the commercially available herbal products in the form of extract were composed of mixtures including the active constituent(s) [7–11] and consequently, the co-existing constituents in the herbal extract might interfere in the efficacy and safety, and affect the pharmacokinetic properties of active constituents [8, 12–15]. As part of our search for JAK2 inhibitory extracts from medicinal plants, the hexane-soluble extract of *S. chinensis* was found to be active (IC_{50} 90% at a concentration of 40 μg/mL). Hence, the hexane-soluble extract of *S. chinensis* was chosen for pharmacokinetic study prior to *in vivo* experiments.

Up to now, there have been several reports for *S. chinensis* extract dealing with the development of analytical methods including a simultaneous quantitation of six lignans. However, there is no simultaneous analytical method in biological samples for nine lignans from *S. chinensis* extract.

In the present study, the simultaneous quantification of nine lignans in rat plasma using a fully validated accurate, rapid, and sensitive UHPLC–MS/MS method was established. Furthermore, using this validated method, the

pharmacokinetic properties of nine lignans after administration of the hexane-soluble extract of *S. chinensis* were investigated.

2 Materials and methods

2.1 Materials and reagents

Hexane-soluble extract of *S. chinensis* (containing 9.4% of SL-A, 0.6% of gomisin J [GM-J], 2.3% of SL-B, 0.7% of tigloylgomisin H [TM-H], 2.1% of angeloylgomisin H [AM-H], 1.9% of SN-A, 1.3% of SN-B, 6.7% of gomisin N [GM-N], 1.7% of SN-C) was purified in College of Pharmacy, Dongguk University (Seoul, South Korea) according to the previously reported protocol [16, 17]. SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were donated from Gangneung Korea Institute of Science and Technology (Gangneung, South Korea). Amlodipine (internal standard [IS] for UHPLC–MS/MS) was purchased from Sigma-Aldrich Corporation (Seoul, South Korea). Ethanol, methanol, acetonitrile, formic acid, and water were purchased from Burdick & Jackson (Seoul, South Korea). All other chemicals and reagents used were of analytical grade. The chemical structures of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C are shown in Supporting Information Fig. S1.

2.2 Preparation of standards and QC samples

Stock solution of each SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C was made by dissolving in methanol at 1 mg/mL and IS was made by dissolving in acetonitrile at 200 μg/mL. The IS stock solution was further diluted to 0.25 μg/mL in acetonitrile for routine use as an IS. For experiments, each stock solution was serially diluted with methanol and added to drug-free plasma, urine, or gastrointestinal tract (GI) samples to obtain final concentrations of 1, 2, 5, 10, 50, 100, 1000, or 2000 ng/mL for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C. On the day of analysis, calibration graphs for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C in rat plasma, urine, or GI samples were derived from their peak area ratios relative to that of IS using linear regression with $1/x$ as a weighting factor. All samples were used to evaluate the intra- and interday precision and accuracy of the method. All prepared plasma, urine, or GI samples and stock solutions were stored at –80°C (Revco ULT 1490 D-N-S; Western Mednics, NC, USA).

2.3 Sample preparations

To select the proper sample preparation methods, the deproteinization by acetonitrile or methanol and extraction by methyl *tert*-butyl ether, ethyl acetate, diethyl ether, or dichloromethane were conducted. As a deproteinization method, a 100 μL of acetonitrile or methanol containing 0.25 μg/mL of IS was added to 50 μL of aliquot of rat plasma sample and mixed by vortex. After vortex mixing and centrifugation (12 000 rpm for 10 min), 10 μL of the supernatant was directly injected onto the column. As an extraction method, 1 mL of methyl *tert*-butyl ether, ethyl acetate, diethyl ether or dichloromethane, and acetonitrile containing 0.25 μg/mL of IS was added to 50 μL of aliquot of rat plasma sample and mixed by vortex. After vortex mixing and centrifugation (12 000 rpm for 10 min), the supernatant was transferred into the tube and evaporated under the nitrogen gas and then, the sample was reconstituted by 50 μL of mobile phase (0.1% formic acid in distilled water/0.1% formic acid in acetonitrile, 45:5, v/v). A 10 μL sample was directly injected onto the column. All prepared samples were kept in an autosampler at 4°C. Among these sample preparation methods, the deproteinization by acetonitrile was selected and applied in this study.

2.4 Characterization of product ions using MS/MS

To characterization the product ions of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS, 0.25 μg/mL solutions of each compound were separately infused into the mass spectrometer at 10 μL/min. The precursor ions ($[M+H]^+$ or $[M+H–H_2O]^+$) and the pattern of fragmentation were monitored in positive ion mode. The major peaks observed in MS/MS scan were used to quantify SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS.

2.5 Analytical instruments and chromatographic conditions

All analytics were performed using a Waters UPLC-XEVO TQ-S system (Waters Corporation, Milford, USA). Nine lignans and IS were well resolved on RP C_{18} column (ACQUITY UPLC® BEH, 2.1 mm × 100 mm i.d., 1.7 μm particle size; Waters, Ireland) with a flow rate of 0.2 mL/min. The mobile phase composition was started with 45:55 v/v of distilled water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B) from 0 to 5.5 min, followed by a linear increase to 25:75 v/v of A/B which was achieved at 6.5 min and this condition was maintained until 12.5 min. The gradient was returned to 45:55 v/v of A/B. The column oven was set at 30°C during analysis and the sample chamber at 4°C.

MS was operated in the multiple reaction monitoring (MRM) mode with ESI interface used to positive ions ($[M+H]^+$ or $[M+H–H_2O]^+$) at a capillary voltage of 4.0 kV, a source temperature of 650°C and desolvation gas temperature of 350°C. The mass transitions for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS were found to be m/z 415.08 → 384.10 (35 and 25 eV for cone voltage and collision energy, respectively), 389.11 → 357.08 (30 and 17 eV, respectively), 399.10 → 368.38 (40 and 20 eV, respectively), 501.22 → 401.13 (18 and 11 eV, respectively),

501.22 → 401.13 (18 and 11 eV, respectively), 417.17 → 316.07 (38 and 24 eV, respectively), 401.13 → 300.13 (34 and 26 eV, respectively), 401.13 → 300.13 (34 and 26 eV, respectively), 385.11 → 285.01 (30 and 25 eV, respectively), 409.01 → 237.93 (35 and 10 eV, respectively), respectively. The analytical data were processed using MassLynx software (Version 4.1, Waters Corporation). 100 μL of acetonitrile containing 0.25 μg/mL of IS was added to 50 μL of aliquot of sample. After centrifugation, 10 μL of the supernatant was directly injected into the column. The retention times of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS were approximately 2.9, 3.3, 3.4, 3.9, 4.5, 9.7, 10.5, 10.8, 11.4, and 1.3 min, respectively. The quantitation limits of all nine lignans in plasma, urine, and GI samples were 1, 10, and 10 ng/mL, respectively.

2.6 UHPLC–MS/MS analytical validation

Method validation assays were carried out according to the currently accepted United States Food and Drug Administration's bioanalytical method validation procedure [18]. The validation parameters consist of selectivity, linearity, sensitivity, accuracy, precision, and stability of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C in rat plasma, urine, and GI samples.

2.6.1 Selectivity

Selectivity was evaluated by comparing chromatograms of three different batches of plasma obtained from six rats to ensure that no interfering peaks were present at the respective retention times of the analytes at the lower limit of quantitation (LLOQ) levels. The LLOQ was defined as the lowest concentration of analyte yielding an S/N of at least 10, acceptable accuracy (80–120%), and sufficient precision (within 20%).

2.6.2 Linearity and sensitivity

The linearity of each method-matched calibration curve was determined by $y = ax + b$, plotting the peak area ratios (y) of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C relative to that of IS versus the nominal concentration (x) of the same analyte. The calibration curves were constructed by weighing factor with a mean linear regression equation.

2.6.3 Precision and accuracy

Intra- and interday accuracy and precision for this method were determined at least five different concentration levels on five consecutive days; on each day, over five replicates were analyzed with independently prepared calibration curves. The percentage accuracy was expressed as (mean observed concentration)/(nominal concentration) × 100, and the precision was the RSD (%).

2.6.4 Stability

To study analyte stability, drug-free plasma samples were spiked with 5, 10, 50, and 100 ng/mL for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C. Stability was assessed by analyzing three replicate samples after four different manipulations: (i) short-term storage (24 h at room temperature); (ii) long-term storage (21 days at −20°C); (iii) three freeze–thaw cycles; (iv) post-treatment storage (12 h at room temperature). The concentrations obtained were compared with nominal values of the prepared samples.

2.6.5 Matrix effect

To evaluate the matrix effect of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C, drug-free plasma from six different rats was used. The matrix effect values were calculated by taking the analyte peak areas obtained by direct injection of solvent (or neat) standard solutions as A and those for solvent (or neat) standard solutions spiked after the sample preparation of plasma as B [19]; matrix effect (%) = $100 \times B/A$.

The matrix effect of the IS was evaluated using the same method mentioned above. All assays were performed at concentrations of 10, 100, and 1000 ng/mL for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C.

2.6.6 Dilution effect

The dilution effects of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were evaluated at the concentrations of 500, 1000, 2000, 5000, 1000, and 50 000 ng/mL for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C spiked in rat plasma. These samples were diluted in the ranges of calibration curves of each compound. The precision and accuracy of the dilution integrity is ≤15% and within ±15% of the nominal concentrations, respectively.

2.7 Application to pharmacokinetic studies

The protocols for the animal studies were approved by the Animal Care and Use Committee of Dongguk University on March 25, 2010 (no. 2010–0944, Goyang, South Korea). Sprague–Dawley male rats (six weeks old, weighing 170–200 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea). Rats were acclimated for one week and housed under strictly controlled environmental conditions as the reported conditions before this study [20].

The procedures used for the pretreatment of rats (early in the morning) including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) were similar to a reported method [20]. The hexane-soluble extract of *S. chinensis* (dissolved in ethanol/distilled water = 1:1, v/v) at a dose of 30 mg (2 mL)/kg was infused through a jugular vein in the intravenous study. Blood samples (approximately 0.22 mL) were

collected via the carotid artery at 0 (control), 1 (at the end of infusion), 5, 15, 30, 45, 60, 75, 90, 120, 180, 240, 300, 360, 420, and 480 min after the start of the intravenous administration of hexane-soluble extract of *S. chinensis*. Blood samples were immediately centrifuged and two 50 μL of a plasma sample were collected.

Hexane-soluble extract of *S. chinensis* (the same solution as used in the intravenous study) at a dose of 150 mg (5 mL)/kg was administered orally to rats after overnight fasting with free access to water. A blood sample was collected via the carotid artery at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300, 460, 540, 660, 780, and 840 min after the oral administration of hexane-soluble extract of *S. chinensis*. Other procedures in the oral study were similar to those in the intravenous study.

Standard methods [21] were used to calculate the following pharmacokinetic parameters using a noncompartmental analysis (WinNonlin 2.1; Pharmasight, Mountain View, CA, USA): the time-averaged total body, renal, and nonrenal clearances (CL, C_R, and CL_{NR}, respectively), terminal half-life, mean residence time, and apparent volume of distribution at steady state (V_{ss}). The extent of absolute oral bioavailability (F) was calculated by dividing the ($AUC_{oral\ X}$ [area under the plasma concentration-time curve] $dose_{iv}$)/ ($AUC_{iv\ X}$ $dose_{iv}$) in the ranges of linear pharmacokinetics [20, 21]. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were read directly from the extrapolated data.

3 Results and discussion

3.1 Optimization of UHPLC–MS/MS and sample preparation

The RP C_{18} column (ACQUITY UPLC? BEH, 2.1 mm × 100 mm i.d., 1.7 μm particle size; Waters) was selected to separate nine lignans and IS considering the shorter retention times, selectivity, and sensitivity. To develop the sample preparation procedure, deprotenization by acetonitrile or methanol and extraction by methyl *tert*-butyl ether, ethyl acetate, diethyl ether, or dichloromethane was conducted. The sensitivity of nine lignans was higher after deprotenization methods than extraction method and the lowest interference intensity of rat blank plasma was observed after deprotenization by acetonitrile (Supporting Information Fig. S2). In positive ion mode, all analytes yielded protonated molecular ions ($[M+H]$ or $[M+H-H_2O]$)$^+$ as the major species. The fragmentation patterns of the protonated molecular ions were evaluated by increasing the collision energy. The product ion spectra and fragmentation patterns for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS are shown in Fig. 2; the greatest intensities were observed at m/z 415.08, 389.11, 399.10, 501.22, 501.22, 417.17, 401.13, 401.13, 385.11, and 409.01 respectively. The mass parameters were optimized by observing the maximal response of the product ions. Especially, there were interference peaks near SL-A and B and these two protonated molecular ions ($[M+H]^+$ or $[M+H-H_2O]^+$) were compared based on the molecular structure of SL-A and B. The intensity of SL-A and B were almost similar between $[M+H]^+$ and $[M+H-H_2O]^+$, but the interference was disappeared when analyzed by $[M+H-H_2O]^+$ (Supporting Information Fig. S3). Also $[M+Na]^+$ were evaluated but the intensity and interference did not improved compared to $[M+H]^+$ and $[M+H-H_2O]^+$.

3.2 UHPLC–MS/MS analytical validation

3.2.1 Selectivity

Chromatographic conditions, especially the composition of the mobile phase, were optimized to achieve a good resolution, symmetrical peak shapes for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS, acceptable retention factors ($k' \geq 2$) and a short run time. SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, SN-C, and IS eluted at 2.9, 3.3, 3.4, 3.9, 4.5, 9.7, 10.5, 10.8, 11.4, and 1.3 min, respectively. There were no interfering peaks at these elution times. Typical chromatograms for drug-free rat plasma, urine, and GI samples, plasma spiked with 0.05 μg/mL, urine and GI samples spiked with 0.1 μg/mL of each SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C, and plasma collected from 30 min and urine and GI samples at 24 h after intravenous administration of 30 mg/kg hexane-soluble extract of *S. chinensis* are shown in Fig. 1. The total run time per sample was 12.5 min.

3.2.2 Linearity and sensitivity

The calibration curves in rat plasma provided reliable responses at each SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C concentrations of 1–2000 ng/mL. Also, the calibration curves in rat urine and GI samples provided reliable responses at each nine lignans of 10–2000 ng/mL. The best linear fit and least-squares residuals for the calibration curve were achieved with a weighting factor of $1/x$. During the validation, the mean correlation coefficients in rat plasma (urine and GI samples) were 0.9992 (1.00 and 1.00) for SL-A, 0.9995 (1.00 and 0.9999) for GM-J, 0.9989 (1.00 and 1.00) for SL-B, 0.9991 (1.00 and 1.00) for TM-H, 0.9995 (1.00 and 1.00) for AM-H, 0.9997 (1.00 and 1.00) for SN-A, 0.9999 (1.00 and 1.00) for SN-B, 0.9998 (1.00 and 1.00) for GM-N, and 0.9998 (1.00 and 1.00) for SN-C. The LLOQs for SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were in rat plasma, urine, and GI samples, respectively, at an S/N of 10. This sensitivity was sufficient to allow pharmacokinetic studies of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C after intravenous and oral administration of hexane-soluble extract of *S. chinensis*.

3.2.3 Precision and accuracy

The intra- and interday precision and accuracy of this assay were determined by analyzing three replicates of standard samples at least five concentrations on five consecutive days

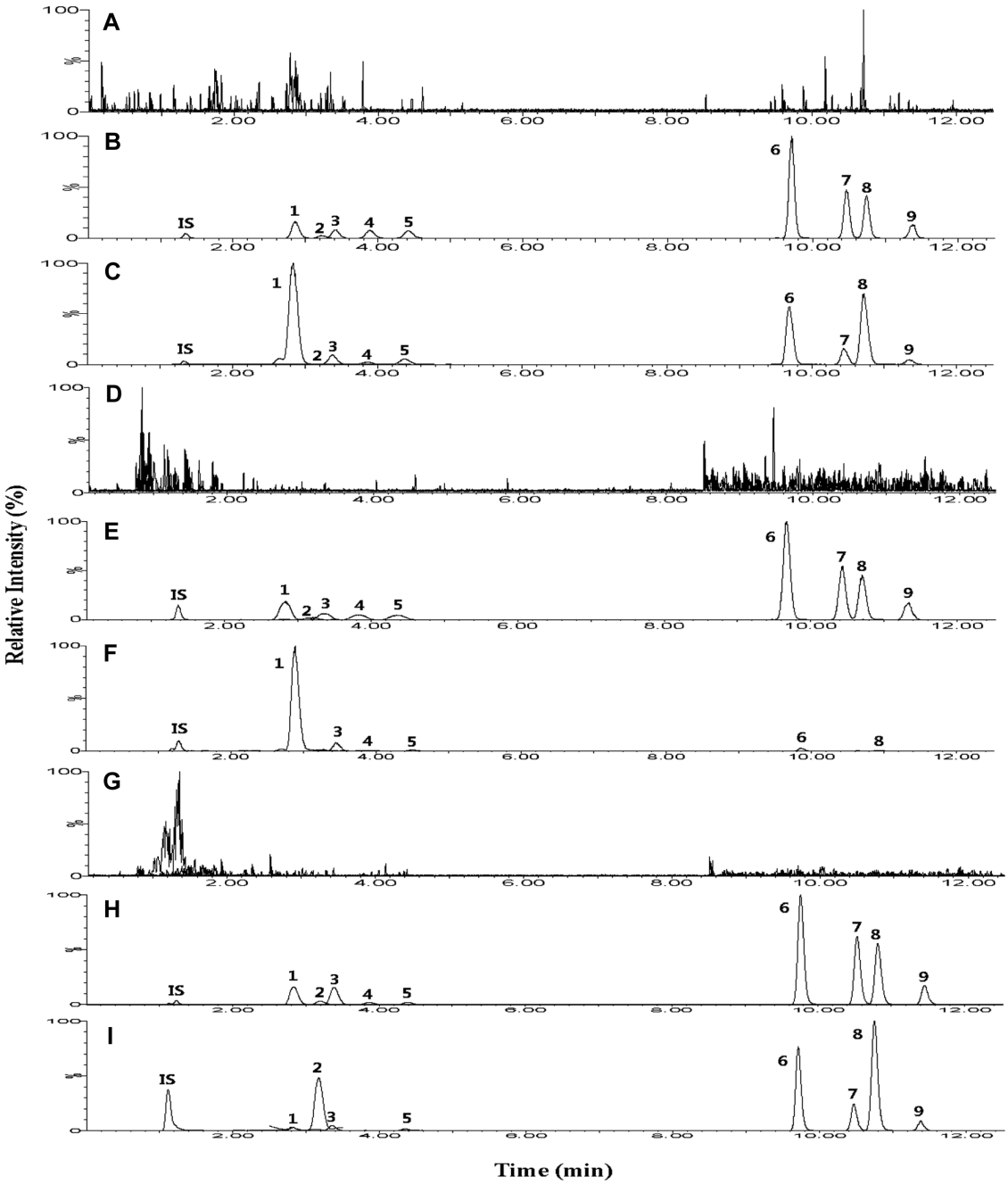

Figure 1. Mass chromatogram after deprotenization with acetonitrile for rat blank plasma (A), urine (D), and gastrointestinal tract (G). Also rat plasma (B), urine (E), and gastrointestinal tract (H) spiked with 0.05 μg/mL of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C. Rat plasma at 30 min (C) and urine during 24 h (F) after intravenous administration of hexane-soluble extract of *S. chinensis* and gastrointestinal tract at 24 h (I) after oral administration of hexane-soluble extract of *S. chinensis*. 1, SL-A; 2, GM-J; 3, SL-B; 4, TM-H; 5, AM-H; 6, SN-A; 7, SN-B; 8, GM-N; 9, SN-C.

(Table 1). The coefficients of variation (CVs) for the intra- (and inter-) day precision in rat plasma of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were as follows: 6.88 (8.37)% for SL-A, 11.5 (8.60)% for GM-J, 12.3 (8.87)% for SL-B, 11.0 (9.19)% for TM-H, 5.87 (13.7)% for AM-H, 9.87 (13.1)% for SN-A, 8.69 (8.37)% for SN-B, 8.18 (9.98)% for GM-N, and 9.08 (10.6)% for SN-C, respectively. The intra- (and inter-) day accuracies of nine lignans were also as follows: 98.6–113 (98.8–112)% for SL-A, 90.6–109 (86.7–104)% for GM-J, 94.5–115 (96.5–110)% for SL-B, 96.3–113% (87.8–111)% for TM-H, 92.4–115 (85.1–101)% for AM-H, 95.3–107 (92.4.–104)% for SN-A, 89.4–107 (94.9–102)% for SN-B, 95.7–102 (98.7–107)% for GM-N, and 93.2–110 (98.1–109)% for SN-C, respectively. Also the CVs for the intra- (and inter-) day precision in rat urine were 1.50 (2.21)% of SL-A, 5.02 (5.11)% of GM-J, 2.78 (2.78)% of SL-B, 6.00 (3.88)% of

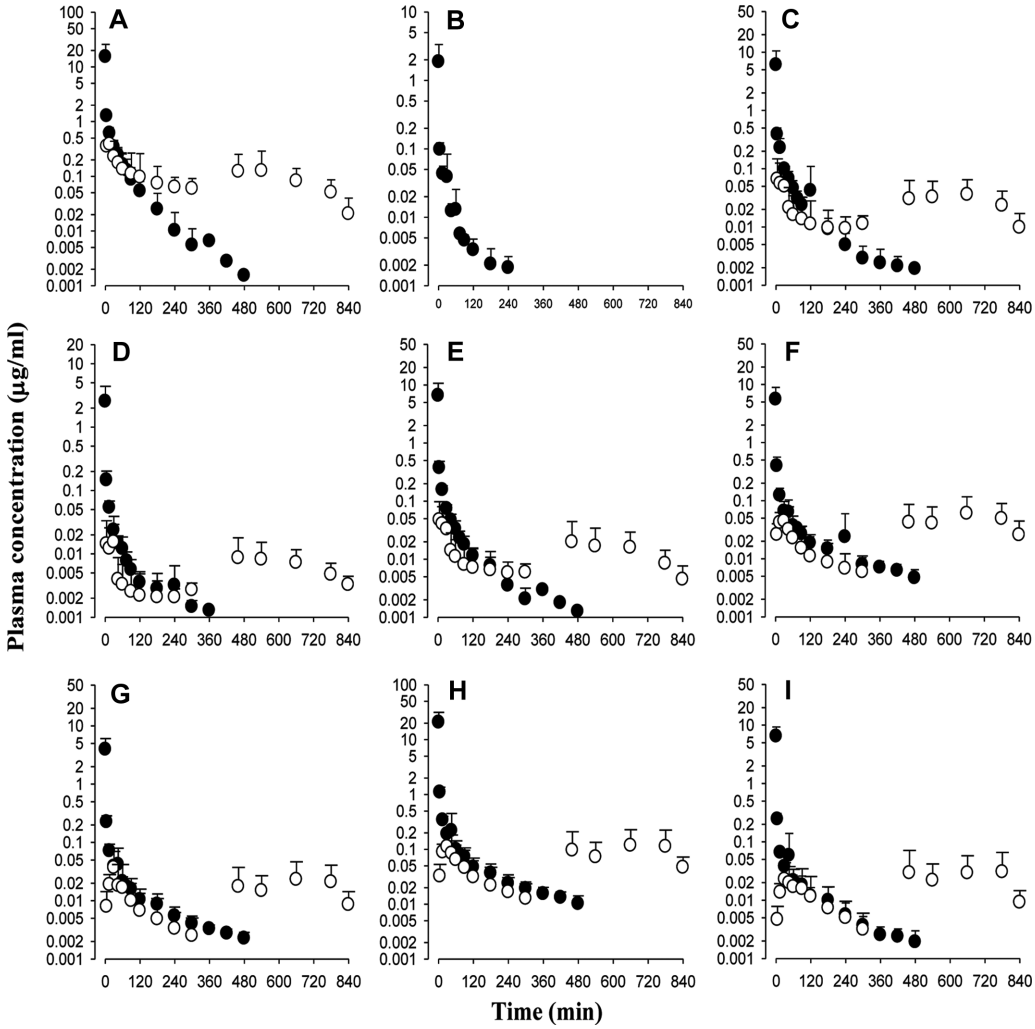

Figure 2. Mean arterial plasma concentration–time profiles of SL-A (A), GM-J (B), SL-B (C), TM-H (D), AM-H (E), SN-A (F), SN-B (G), GM-N (H), SN-C (I) after intravenous (●) and oral (○) administration of hexane-soluble extract of *S. chinensis*. Bars represent SDs.

TM-H, 2.05 (2.42)% of AM-H, 0.686 (0.721)% of SN-A, 1.20 (1.21)% of SN-B, 1.05 (1.12)% of GM-N, and 2.07 (2.73)% of SN-C, respectively. The intra- (and inter-) day accuracies of nine lignans were 98.0–101 (99.4–103)% of SL-A, 87.9–104 (97.6–102)% of GM-J, 93.3–101 (99.6–110)% of SL-B, 91.9–103 (98.9–102)% of TM-H, 94.9–102 (99.6–103)% of AM-H, 99.9–101 (100–100)% of SN-A, 99.7–107 (95.4–101)% of SN-B, 94.8–101 (98.2–100)% of GM-N, and 99.0–101 (97.0–102)% of SN-C, respectively. Similarly, the CVs for the intra- (and inter-) day precision in rat GI samples were 4.34 (2.02)% of SL-A, 9.51 (6.01)% of GM-J, 4.16 (3.76)% of SL-B, 5.18 (6.56)% of TM-H, 6.74 (3.81)% of AM-H, 4.31 (2.32)% of SN-A, 1.59 (1.51)% of SN-B, 3.00 (3.06)% of GM-N, and 3.81 (2.34)% of SN-C, respectively. The intra- (and inter-) day accuracies of nine lignans were 100–102 (100–105)% of SL-A, 101–111 (103–108)% of GM-J, 99.4–105 (102–103)% of SL-B, 97.4–105 (100–108)% of TM-H, 98.0–102 (100–102)% of AM-H, 97.0–101 (99.9–101)% of SN-A, 100–101 (99.6–101)% of SN-B, 97.4–101 (98.9–100)% of GM-N, and 100–108 (99.6–103)% of SN-C, respectively.

The samples were assessed using the calibration curves and were found to be within 15% of the nominal concentrations, meeting the US FDA acceptance criteria for the validation of bioanalytical methods [18].

3.2.4 Stability

No significant degradation of any of the analytes in rat plasma occurred after short-term storage for 24 h at room temperature, long-term storage for 21 days at –20°C, three freeze–thaw cycles, or posttreatment storage for 12 h at room temperature, with ±15% deviation between the predicted and nominal concentrations (Supporting Information Fig. S4).

3.2.5 Matrix effect

To evaluate the effects of the sample matrix on nine lignans ionization, the matrix effect percentages of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were calculated. The percentages of the matrix effects were 90.3–96.9,

Table 1. Intra- and interday precision and accuracy for the determination of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C in rat plasma, urine, and gastrointestinal tract samples

Theoretical concentration (ng/mL)	Intraday			Interday		
	Precision		Accuracy (%)	Precision		Accuracy (%)
	Mean ± SD	RSD[a] (%)		Mean ± SD	RSD (%)	
Plasma						
SL-A						
1	0.0823 ± 0.0022	2.71	113	0.0932 ± 0.0065	6.93	112
2	0.0837 ± 0.0016	1.91	107	0.0929 ± 0.0061	6.59	109
5	0.0859 ± 0.0020	2.38	105	0.0894 ± 0.0068	7.64	103
10	0.0862 ± 0.0046	5.33	104	0.0865 ± 0.0062	7.12	98.8
50	0.0870 ± 0.0021	2.41	103	0.0899 ± 0.0075	8.37	102
100	0.0881 ± 0.0034	3.81	105	0.0892 ± 0.0045	5.01	101
1000	0.0832 ± 0.0057	6.88	98.6	0.0876 ± 0.0030	3.43	99.5
2000	0.0846 ± 0.0024	2.86	100	0.0879 ± 0.0010	1.16	99.8
GM-J						
1	0.0204 ± 0.0012	5.76	90.6	0.0182 ± 0.0016	8.60	90.8
2	0.0184 ± 0.0020	11.0	98.9	0.0182 ± 0.00078	4.30	102
5	0.0181 ± 0.0019	10.3	109	0.0164 ± 0.0010	6.18	98.1
10	0.0156 ± 0.0010	6.39	96.9	0.0142 ± 0.00053	3.73	86.7
50	0.0162 ± 0.00083	5.14	104	0.0159 ± 0.0010	6.52	99.4
100	0.0163 ± 0.0012	7.43	106	0.0166 ± 0.0012	7.12	104
1000	0.0152 ± 0.0018	11.5	98.4	0.0159 ± 0.0013	8.04	99.9
2000	0.0154 ± 0.00076	4.89	100	0.0160 ± 0.00086	5.37	100
SL-B						
1	0.0388 ± 0.00089	2.30	115	0.0416 ± 0.0022	5.23	110
2	0.0442 ± 0.0027	6.04	112	0.0441 ± 0.0017	3.83	107
5	0.0496 ± 0.0061	12.3	115	0.0424 ± 0.0028	6.51	97.9
10	0.0436 ± 0.0034	7.80	98.9	0.0430 ± 0.0019	4.45	97.6
50	0.0452 ± 0.0028	6.08	99.9	0.0432 ± 0.00096	2.21	96.5
100	0.0500 ± 0.0032	6.37	107	0.0448 ± 0.0040	8.87	100
1000	0.0430 ± 0.0051	11.9	94.5	0.0449 ± 0.0038	8.37	100
2000	0.0461 ± 0.0031	6.69	101	0.0448 ± 0.0017	3.88	99.7
TM-H						
1	0.0494 ± 0.0028	5.57	96.3	0.0490 ± 0.0035	7.10	105
2	0.0483 ± 0.0053	11.0	105	0.0515 ± 0.0034	6.64	111
5	0.0491 ± 0.0042	8.54	107	0.0420 ± 0.0011	2.65	91.0
10	0.0444 ± 0.0019	4.33	100	0.0404 ± 0.0019	4.77	87.8
50	0.0447 ± 0.0030	6.77	107	0.0466 ± 0.0033	7.05	101
100	0.0490 ± 0.0032	6.46	113	0.0467 ± 0.0043	9.19	101
1000	0.0415 ± 0.0035	8.46	96.5	0.0459 ± 0.0025	5.39	99.7
2000	0.0432 ± 0.0034	7.86	105	0.0460 ± 0.0039	8.50	99.9
AM-H						
1	0.0473 ± 0.00079	1.66	115	0.0435 ± 0.0059	13.7	91.6
2	0.0481 ± 0.0013	2.68	112	0.0464 ± 0.0019	4.07	99.1
5	0.0457 ± 0.0022	4.79	103	0.0433 ± 0.00062	1.43	93.2
10	0.0416 ± 0.0019	4.52	92.4	0.0394 ± 0.00086	2.19	85.1
50	0.0452 ± 0.0020	4.44	98.2	0.0442 ± 0.0023	5.27	95.6
100	0.0476 ± 0.0028	5.77	102	0.0463 ± 0.0017	3.63	100
1000	0.0451 ± 0.0027	5.87	98.2	0.0465 ± 0.0016	3.33	101
2000	0.0456 ± 0.0020	4.27	103	0.0461 ± 0.0011	2.29	99.7
Plasma						
SN-A						
1	0.429 ± 0.018	4.08	107	0.441 ± 0.017	3.75	104
2	0.435 ± 0.029	6.56	103	0.426 ± 0.024	5.60	100
5	0.422 ± 0.027	6.34	102	0.392 ± 0.041	10.4	92.4
10	0.391 ± 0.015	3.94	95.3	0.405 ± 0.053	13.1	95.5
50	0.402 ± 0.016	3.94	97.9	0.420 ± 0.037	8.71	98.9

Table 1. Continued

Theoretical concentration (ng/mL)	Intraday			Interday		
	Precision		Accuracy (%)	Precision		Accuracy (%)
	Mean ± SD	RSD[a)] (%)		Mean ± SD	RSD (%)	
100	0.436 ± 0.043	9.87	101	0.436 ± 0.038	8.68	103
1000	0.405 ± 0.027	6.64	100	0.423 ± 0.022	5.24	99.7
2000	0.411 ± 0.020	4.96	104	0.424 ± 0.0090	2.12	100
SN-B						
1	0.202 ± 0.0041	2.01	89.4	0.194 ± 0.0097	5.02	96.1
2	0.194 ± 0.0029	1.47	91.9	0.193 ± 0.016	8.37	94.9
5	0.216 ± 0.019	8.69	107	0.208 ± 0.0099	4.73	102
10	0.200 ± 0.011	5.48	100	0.207 ± 0.0081	3.89	101
50	0.202 ± 0.0081	4.01	103	0.208 ± 0.0088	4.23	101
100	0.207 ± 0.018	8.64	105	0.208 ± 0.011	5.09	102
1000	0.200 ± 0.0059	2.93	99.7	0.204 ± 0.012	5.75	99.5
2000	0.201 ± 0.0063	3.15	101	0.205 ± 0.0039	1.91	100
GM-N						
1	0.179 ± 0.011	6.32	95.7	0.198 ± 0.0094	4.76	106
2	0.176 ± 0.0087	4.97	97.3	0.198 ± 0.0085	4.27	107
5	0.185 ± 0.014	7.62	102	0.182 ± 0.012	6.56	98.7
10	0.167 ± 0.0087	5.18	96.2	0.185 ± 0.018	9.98	99.9
50	0.173 ± 0.0016	0.939	99.8	0.188 ± 0.012	6.58	102
100	0.180 ± 0.015	8.18	102	0.191 ± 0.0074	3.87	104
1000	0.171 ± 0.0055	3.22	97.2	0.183 ± 0.010	5.67	99.1
2000	0.173 ± 0.0021	1.18	100	0.185 ± 0.010	5.46	100
SN-C						
1	0.0826 ± 0.0026	3.17	93.2	0.0817 ± 0.0015	1.77	107
2	0.0852 ± 0.0017	2.03	110	0.0800 ± 0.0045	5.58	109
5	0.0740 ± 0.0065	8.84	97.9	0.0714 ± 0.0076	10.6	99.9
10	0.0653 ± 0.0042	6.47	94.1	0.0695 ± 0.0071	10.2	98.1
50	0.0747 ± 0.0046	6.09	104	0.0703 ± 0.0055	7.88	100
100	0.0757 ± 0.0069	9.08	107	0.0740 ± 0.0048	6.54	105
1000	0.0676 ± 0.0058	8.59	99.1	0.0695 ± 0.0058	8.32	99.0
2000	0.0696 ± 0.0036	5.15	99.9	0.0704 ± 0.0020	2.81	100
Urine						
SL-A						
10	0.137 ± 0.0015	1.07	98.0	0.135 ± 0.0013	0.922	103
50	0.137 ± 0.0020	1.45	100	0.137 ± 0.0018	1.33	101
100	0.137 ± 0.00071	0.519	101	0.136 ± 0.0030	2.21	101
500	0.137 ± 0.0014	1.05	101	0.135 ± 0.0018	1.33	99.4
1000	0.137 ± 0.0021	1.50	101	0.136 ± 0.0014	1.05	100
GM-J						
10	0.0241 ± 0.00091	3.77	87.9	0.0238 ± 0.00086	3.62	97.6
50	0.0244 ± 0.00089	3.64	103	0.0240 ± 0.00076	3.15	99.8
100	0.0248 ± 0.00062	2.50	104	0.0246 ± 0.0010	4.11	102
500	0.0239 ± 0.0012	5.02	104	0.0245 ± 0.0011	4.27	102
1000	0.0239 ± 0.00095	3.98	104	0.0244 ± 0.0013	5.11	102
SL-B						
10	0.0434 ± 0.0012	2.78	93.3	0.0429 ± 0.00093	2.16	110
50	0.0435 ± 0.00084	1.92	99.7	0.0425 ± 0.00037	0.862	101
100	0.0430 ± 0.00077	1.79	99.4	0.0429 ± 0.00073	1.71	101
500	0.0433 ± 0.00042	0.966	101	0.0427 ± 0.0012	2.78	99.6
1000	0.0430 ± 0.0010	2.43	99.9	0.0431 ± 0.00073	1.70	100
Urine						
TM-H						
10	0.0617 ± 0.0037	6.00	91.9	0.0605 ± 0.0014	2.33	102
50	0.0623 ± 0.0023	3.68	103	0.0607 ± 0.0024	3.88	102
100	0.0621 ± 0.0035	5.67	103	0.0592 ± 0.0022	3.76	98.9

Table 1. Continued

Theoretical concentration (ng/mL)	Intraday			Interday		
	Precision		Accuracy (%)	Precision		Accuracy (%)
	Mean ± SD	RSD[a)] (%)		Mean ± SD	RSD (%)	
500	0.0603 ± 0.0018	2.92	100	0.0611 ± 0.0013	2.13	102
1000	0.0607 ± 0.0017	2.76	101	0.0606 ± 0.00039	0.635	101
AM-H						
10	0.0793 ± 0.0016	2.05	94.9	0.0804 ± 0.0020	2.42	103
50	0.0811 ± 0.0014	1.77	102	0.0806 ± 0.0014	1.68	102
100	0.0793 ± 0.0012	1.47	99.3	0.0786 ± 0.0016	2.02	99.6
500	0.0795 ± 0.0011	1.41	101	0.0797 ± 0.0011	1.32	101
1000	0.0794 ± 0.00083	1.05	100	0.0795 ± 0.0016	1.99	101
SN-A						
10	0.447 ± 0.0030	0.672	101	0.445 ± 0.0016	0.350	100
50	0.445 ± 0.0031	0.686	100	0.445 ± 0.0014	0.315	100
100	0.446 ± 0.0011	0.244	100	0.446 ± 0.0028	0.623	100
500	0.445 ± 0.0029	0.643	99.9	0.446 ± 0.0032	0.721	100
1000	0.445 ± 0.0028	0.618	100	0.445 ± 0.0018	0.399	100
SN-B						
10	0.184 ± 0.0016	0.865	107	0.185 ± 0.0022	1.21	95.4
50	0.184 ± 0.0022	1.20	101	0.186 ± 0.0020	1.05	99.9
100	0.185 ± 0.0015	0.826	101	0.185 ± 0.00056	0.300	100
500	0.184 ± 0.0019	1.02	99.7	0.185 ± 0.0018	0.965	101
1000	0.186 ± 0.0017	0.930	100	0.184 ± 0.0011	0.593	100
GM-N						
10	0.247 ± 0.0026	1.05	94.8	0.246 ± 0.0022	0.899	98.2
50	0.247 ± 0.0018	0.719	99.3	0.246 ± 0.0016	0.657	100
100	0.245 ± 0.0025	1.02	99.1	0.246 ± 0.0026	1.07	100
500	0.247 ± 0.0019	0.783	101	0.245 ± 0.0027	1.12	100
1000	0.245 ± 0.0018	0.714	100	0.245 ± 0.0025	1.02	100
SN-C						
10	0.0948 ± 0.0018	1.89	99.0	0.0952 ± 0.0025	2.62	97.0
50	0.0953 ± 0.0016	1.64	101	0.0962 ± 0.0026	2.73	102
100	0.0942 ± 0.0014	1.49	100	0.0939 ± 0.0011	1.17	99.5
500	0.0939 ± 0.0017	1.80	99.8	0.0944 ± 0.0012	1.25	100
1000	0.0941 ± 0.0020	2.07	100	0.0942 ± 0.0021	2.17	100
Gastrointestinal tract						
SL-A						
10	0.0927 ± 0.0040	4.34	100	0.0935 ± 0.0014	1.46	105
50	0.0936 ± 0.0027	2.88	101	0.0939 ± 0.0019	2.02	101
100	0.0948 ± 0.0041	4.33	102	0.0945 ± 0.0012	1.31	101
1000	0.0934 ± 0.0024	2.58	100	0.0940 ± 0.0010	1.06	100
2000	0.0937 ± 0.0016	1.67	101	0.0944 ± 0.0012	1.25	100
GM-J						
10	0.0183 ± 0.00074	4.05	101	0.0181 ± 0.00062	3.43	103
50	0.0190 ± 0.00099	5.20	111	0.0183 ± 0.0011	6.01	107
100	0.0188 ± 0.0011	6.03	110	0.0183 ± 0.00074	4.05	108
1000	0.0176 ± 0.0017	9.51	103	0.0180 ± 0.00099	5.50	106
2000	0.0179 ± 0.00076	4.24	105	0.0178 ± 0.00067	3.78	105
SL-B						
10	0.0394 ± 0.00084	2.12	105	0.0392 ± 0.0011	2.84	102
50	0.0393 ± 0.00074	1.89	102	0.0392 ± 0.00056	1.43	103
100	0.0395 ± 0.00083	2.11	103	0.0392 ± 0.0015	3.76	103
1000	0.0387 ± 0.0016	4.16	99.4	0.0388 ± 0.0013	3.40	102
2000	0.0390 ± 0.00089	2.28	100	0.0389 ± 0.00068	1.74	102
Gastrointestinal tract						
TM-H						
10	0.0501 ± 0.0011	2.12	102	0.0488 ± 0.0010	2.07	108

Table 1. Continued

Theoretical concentration (ng/mL)	Intraday			Interday		
	Precision		Accuracy (%)	Precision		Accuracy (%)
	Mean ± SD	RSD[a)] (%)		Mean ± SD	RSD (%)	
50	0.0502 ± 0.0026	5.18	105	0.0482 ± 0.0032	6.56	102
100	0.0505 ± 0.00083	1.65	104	0.0493 ± 0.0016	3.17	103
1000	0.0486 ± 0.0022	4.61	97.4	0.0481 ± 0.0022	4.56	100
2000	0.0491 ± 0.0015	3.14	101	0.0485 ± 0.00084	1.74	101
AM-H						
10	0.0614 ± 0.0017	2.70	102	0.0611 ± 0.0016	2.62	100
50	0.0613 ± 0.0041	6.74	102	0.0615 ± 0.0023	3.81	102
100	0.0611 ± 0.0025	4.09	98.0	0.0612 ± 0.0018	2.86	102
1000	0.0612 ± 0.0015	2.52	101	0.0611 ± 0.0018	2.97	102
2000	0.0610 ± 0.00086	1.41	100	0.0607 ± 0.00064	1.05	101
SN-A						
10	0.483 ± 0.017	3.49	97.0	0.484 ± 0.011	2.32	99.9
50	0.495 ± 0.021	4.31	101	0.486 ± 0.011	2.26	101
100	0.495 ± 0.0069	1.39	101	0.486 ± 0.0058	1.18	101
1000	0.486 ± 0.0072	1.48	100	0.483 ± 0.0064	1.33	99.9
2000	0.488 ± 0.0025	0.514	100	0.484 ± 0.0053	1.09	100
SN-B						
10	0.175 ± 0.0021	1.22	101	0.175 ± 0.0026	1.51	99.6
50	0.175 ± 0.0028	1.59	101	0.177 ± 0.0016	0.915	101
100	0.175 ± 0.0023	1.30	100	0.177 ± 0.0019	1.08	100
1000	0.176 ± 0.0019	1.09	101	0.176 ± 0.0015	0.847	100
2000	0.176 ± 0.00099	0.566	100	0.176 ± 0.0012	0.703	100
GM-N						
10	0.201 ± 0.0060	3.00	97.4	0.197 ± 0.0033	1.68	99.3
50	0.202 ± 0.0048	2.37	101	0.197 ± 0.0060	3.06	99.2
100	0.200 ± 0.0029	1.46	98.9	0.197 ± 0.0038	1.93	98.9
1000	0.201 ± 0.0028	1.39	101	0.200 ± 0.0020	1.02	100
2000	0.200 ± 0.0046	2.30	101	0.199 ± 0.0059	2.96	99.9
SN-C						
10	0.0994 ± 0.0032	3.18	108	0.0976 ± 0.0014	1.39	103
50	0.0989 ± 0.0038	3.81	105	0.0969 ± 0.0020	2.03	99.6
100	0.0977 ± 0.0020	2.01	101	0.0987 ± 0.0014	1.37	101
1000	0.0981 ± 0.0036	3.70	100	0.0980 ± 0.0023	2.34	100
2000	0.0985 ± 0.0018	1.82	101	0.0982 ± 0.0018	1.82	100

a) RSD, relative standard variation (SD × 100/mean).

94.2–96.7, 91.1–99.3, 89.2–98.1, 95.7–101, 90.5–95.8, 98.4–103, 94.6–103, and 97.7–103%, respectively, indicating that the impact from the plasma matrix was negligible and consistent. The low and consistent matrix effects of this assay show it to be reliable for routine bioanalysis.

3.2.6 Dilution effect

The inter- and intraday precisions and accuracies for the dilution integrity of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C in rat plasma were as follows: the CVs of inter- (and intra-) day precision were 9.31 (11.3)%, 10.5 (8.76)%, 11.3 (9.56)%, 9.58 (8.49)%, 11.6 (7.93)%, 7.59 (8.76)%, 13.6 (11.5)%, 6.95 (5.47)%, and 6.10 (4.77)%, respectively. Also the inter- (and intra-) day accuracies were 93.1– 103 (101–109)%, 87.9–98.3 (103–108)%, 105–113 (95.3–101)%, 95.7–102 (98.1–112)%, 88.5–95.6 (91.4–97.3)%, 90.5–96.8 (87.6–99.4)%, 102–110 (97.6–103)%, 95.3–99.7 (88.1–91.5)%, and 102–111 (95.3–102)%, respectively. These results suggested that the dilutions of rat plasma samples are acceptable when the obtained concentration is exceeding the upper limit of quantification in this analytical method.

3.3 Application to pharmacokinetic studies

The UHPLC–MS/MS method described herein was successfully applied to a pharmacokinetic study of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C. After the intravenous and oral administration of hexane-soluble extract

Table 2. Mean (±SD) pharmacokinetic parameters of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C after intravenous and oral administration of hexane-soluble extract of *S. chinensis* at doses of 30 and 150 mg/kg, respectively, to rats

Parameter	SL-A ($n = 6$)	GM-J ($n = 6$)	SL-B ($n = 6$)	TM-H ($n = 6$)	AM-H ($n = 6$)	SN-A ($n = 6$)	SN-B ($n = 6$)	GM-N ($n = 6$)	SN-C ($n = 6$)
Intravenous									
Body weight (g)	217 ± 37	217 ± 37	217 ± 37	217 ± 37	217 ± 37	217 ± 37	217 ± 37	217 ± 37	217 ± 37
AUC (μg min/mL)	62.2 ± 19	6.01 ± 2.0	21.7 ± 8.9	7.46 ± 3.2	19.8 ± 7.3	22.2 ± 9.2	13.6 ± 5.0	68.2 ± 27	17.6 ± 6.9
Terminal $t_{1/2}$ (min)	41.1 ± 14	124 ± 159	78.0 ± 27	99.9 ± 49	84.7 ± 32	152 ± 49	200 ± 59	206 ± 76	214 ± 140
Mean residence time (min)	28.3 ± 12	89 ± 164	38.0 ± 16	32.8 ± 18	29.6 ± 13	89.7 ± 28	86.8 ± 23	84.6 ± 31	68.2 ± 24
CL (mL/min/kg)	50.6 ± 21	32.4 ± 9.0	37.3 ± 18	35.7 ± 24	37.9 ± 21	29.9 ± 21	33.9 ± 19	33.9 ± 15	34.7 ± 20
CL_R (mL/min/kg)	0.270 ± 0.34	2.49 ± 2.4	0.0849 ± 0.12	0.121 ± 0.17	0.116 ± 0.16	0.0103 ± 0.014	0.00529 ± 0.0060	0.00385 ± 0.0038	0.00415 ± 0.0042
CL_{NR} (mL/min/kg)	50.3 ± 21	29.9 ± 8.3	37.2 ± 18	35.6 ± 24	37.7 ± 21	29.9 ± 21	33.9 ± 19	33.9 ± 15	34.7 ± 20
V_{ss} (mL/kg)	1449 ± 906	3157 ± 6013	1552 ± 1399	1088 ± 702	1240 ± 1117	2921 ± 2076	3038 ± 2031	2847 ± 1588	2423 ± 1713
$Ae_{0\text{-}24\,h}$ (% of dose)	0.457 ± 0.34	7.78 ± 6.7	0.184 ± 0.15	0.272 ± 0.17	0.250 ± 0.18	0.0273 ± 0.017	0.0137 ± 0.012	0.0114 ± 0.011	0.0117 ± 0.011
$GI_{24\,h}$ (% of dose)	BD[a)]	BD	BD	BD	BD	0.0166 ± 0.011	BD	0.0419 ± 0.039	0.0409 ± 0.036
Oral									
Body weight (g)	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9	191 ± 7.9
AUC (μg min/mL)	80.4 ± 43	BD	22.5 ± 9.4	5.43 ± 1.7	11.3 ± 4.9	56.5 ± 46	14.6 ± 6.0	77.5 ± 32	19.1 ± 9.0
Terminal $t_{1/2}$ (min)	85.6 ± 58	BD	195 ± 172	194 ± 161	149 ± 126	1242 ± 2510	285 ± 137	276 ± 133	235 ± 122
C_{max} (μg/mL)	0.449 ± 0.31	BD	0.100 ± 0.069	0.0294 ± 0.022	0.0703 ± 0.041	0.0962 ± 0.036	0.0549 ± 0.021	0.240 ± 0.067	0.0687 ± 0.033
T_{max} (min)	15 (5–540)	BD	17.5 (5–460)	10 (5–460)	17.5 (5–460)	245 (15–660)	45 (15–780)	560 (15–780)	560 (30–780)
$Ae_{0\text{-}24\,h}$ (% of dose)	0.142 ± 0.030	0.407 ± 0.58	0.160 ± 0.35	0.256 ± 0.56	0.177 ± 0.41	0.114 ± 0.27	0.0988 ± 0.24	0.0983 ± 0.24	0.106 ± 0.25
$GI_{24\,h}$ (% of dose)	0.412 ± 0.78	9.92 ± 6.9	2.25 ± 3.3	2.54 ± 3.2	2.33 ± 3.0	9.51 ± 9.6	BD	12.4 ± 13	11.4 ± 11
F(%)	25.8	–	20.8	14.6	11.4	50.9	21.4	22.7	21.7

a) BD, below detection limit.

of *S. chinensis* to rat, its mean arterial plasma concentration–time profiles of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C are shown in Fig. 2 and the relevant pharmacokinetic parameters are listed in Table 2.

All nine lignans were detected sufficiently in plasma at all sampling times after intravenous administration of hexane-soluble extract of *S. chinensis*, but eight lignans except GM-J were detected in plasma after its oral administration. In our preliminary study, the AUC values of nine lignans were dose-proportional after intravenous (10–150 mg/kg) and oral (30–300 mg/kg) administration of hexane-soluble extract of *S. chinensis* to rats (Supporting Information Fig. S5). Thus, 30 and 150 mg/kg in intravenous and oral studies were selected considering the detection limit of nine lignans.

After intravenous administration of hexane-soluble extract of *S. chinensis*, the $Ae_{0-24\,h}$ values of nine lignans were less than 7.78% of dose studied (Table 2), suggesting that more than 92.2% of intravenously administered hexane-soluble extract of *S. chinensis* is eliminated via a nonrenal (CL_{NR}) route. The contribution of the gastrointestinal excretion to the CL_{NR} was also almost negligible; the amount recovered from the $GI_{24\,h}$ values were less than 0.0419% of the dose (Table 2). Nine lignans in hexane-soluble extract of *S. chinensis* were stable up to 24 h incubation in various buffer solutions (having pH ranging from 1 to 13; our unpublished data). Thus, the CL_{NR} listed in Table 2 could represent the metabolic clearance of nine lignans. The CL of nine lignans (29.9–50.6 mL/min/kg; Table 2) based on the plasma data is considerably slower than the cardiac output, 162 mL/min/kg based on the plasma data using the cardiac output (295 mL/min/kg) based on blood volume and hematocrit (45.0%) in rats [22, 23], suggesting that the first-pass effects of nine lignans in the lung and heart could be almost negligible, if any, in rats.

The *F* values of SL-A, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C were predicted as 25.8, 20.8, 14.6, 11.4, 50.9, 21.4, 22.7, and 21.7%, respectively (Table 2). As the intravenous (30 mg/kg) and oral (150 mg/kg) doses administered in this study were in the ranges of linear pharmacokinetics, the normalized AUC values at 1 mg/kg were used for the *F* [20, 21]. The *F* values of GM-J could not be calculated because most plasma concentrations of GM-J after oral administration of hexane-soluble extract of *S. chinensis* were below the LOD. To ascertain whether the poor GI absorption of nine lignans caused the low *F*, the "true" fraction of the oral dose of unabsorbed nine lignans (F_{unabs}) was calculated using the reported equation based on the linear pharmacokinetics of nine lignans (Table 2; [24]); oral $GI_{24\,h}$ = "F_{unabs}" + ($F \times$ intravenous $GI_{24\,h}$).

The F_{unabs} values were 0.00412, 0.0225, 0.0254, 0.0233, 0.095, 0, 0.124, and 0.114 for SL-A, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C, respectively. The F_{unabs} values of GM-J could not be calculated because all concentrations of GM-J in plasma and GI were below detection limit. Thus, the absorbed fractions ($1 - F_{unabs}$) of eight lignans except GM-J were 0.876–1. Hence, the absorbed fractions were greater than 0.876 for eight lignans. The above data indicate that the low *F* of nine lignans was not due to the poor gastrointestinal absorption. This could be due to considerable first-pass effects of lignans.

4 Concluding remarks

The UHPLC–MS/MS method for the simultaneous quantification of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C in rat plasma was developed and validated. This validated method was successfully applied to obtain pharmacokinetic parameters of SL-A, GM-J, SL-B, TM-H, AM-H, SN-A, SN-B, GM-N, and SN-C present in hexane-soluble extract of *S. chinensis*. Thus, this method may be useful to investigate the efficacy and safety of hexane-soluble extract of *S. chinensis* in various murine models with specific diseases.

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5 References

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