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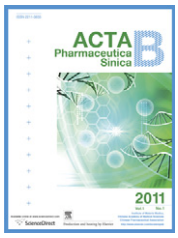

ORIGINAL ARTICLE

HPLC-MS analysis of *Schisandra* lignans and their metabolites in Caco-2 cell monolayer and rat everted gut sac models and in rat plasma

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HPLC-DAD-APCI-MS^n;
Caco-2 cell monolayer;
Rat everted gut sac;
Absorption

Abstract The absorption profiles of *Schisandra chinensis* were evaluated using the human Caco-2 cell monolayer and rat everted gut sac models, as well as in rat plasma. By analyzing the chromatographic and MS^n characteristics of individual peak acquired by HPLC-DAD-APCI-MS^n determination, thirteen lignans were identified as the major *in vitro* absorbable components of the *Schisandra* extract. Most of these compounds were also detected and identified in rat plasma after an oral administration of the *Schisandra* extract, except for angeloyl(tigloyl)gomisin H and angeloyl(tigloyl)gomisin Q, whose structures possess an ester group at the cyclooctadiene ring. In addition, four metabolites, corresponding to the hydroxylation and demethylation products of schisandrin and the hydrolysis derivative of angeloyl(tigloyl)gomisin Q, were tentatively identified. The results demonstrate that *Schisandra* lignans are the major absorbable components of this crude drug, and hydroxylation, demethylation and hydrolysis are important metabolic transformations of the absorbable lignans.

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1. Introduction

The fruit of *Schisandra chinensis* (Turcz.) Baill., known in Chinese medicine as Wu-Wei-Zi, is widely used for the treatment of excessive loss of essential energy and body fluid. This crude drug is officially recorded in the Chinese Pharmacopoeia as a tonic, sedative and astringent agent[1]. In the health-food market, products containing *S. chinensis* are often claimed to possess antioxidant, neuro-protective, or liver-protective properties[2–5]. Chemically, a family of dibenzocyclooctadiene lignans has been found in this plant drug and many of them have shown biological activities[6]. The pharmacokinetics of some of the *Schisandra* lignans, such as schisandrin and γ-schisandrin, has been studied using experimental animals[7–10]. There are also reports on the intestinal absorption of active ingredients of *S. chinensis* extracts[10–13]. However, most previous studies focused on one or several lignans, and information on the absorption profile of the lignans remains to be established, despite the crude extract of this herb is often used in traditional medicine.

Liquid chromatography coupled with mass spectrometry (LC-MS) is a useful tool for rapid identification of chemicals in herbal extracts and in biological specimens. The high sensitivity and selectivity of MS facilitate the detection of minor constituents in complex mixtures. The chromatographic characters and mass spectrometric fragmentation behaviors of *Schisandra* lignans have been investigated in recent years[14–18].

The human Caco-2 intestinal epithelial cell monolayer model and rat everted gut sac have been used as *in vitro* models for studying the intestinal absorption of drugs[19,20]. In this study, the absorption of *S. chinensis* was evaluated in both models, and HPLC-DAD-APCI-MS was employed for the identification of absorbable compounds. In addition, the absorbable components and related metabolites were further determined in rat plasma after oral administration of the *Schisandra* extracts.

2. Materials and methods

2.1. Chemicals and reagents

Schisandrin, schisantherin A, schisandrin A and schisandrin B (purity >98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Schisandrol B was obtained from the Hong Kong Jockey Club Institute of Chinese Medicine with purity >97%; gomisin D, angeloylgomisin F and gomisin G (purity >95%) were gifts from Dr. Yan Zhou of the same institution. Dried berries of *S. chinensis* were obtained from Zhixin Pharmaceutical Co., Ltd. (Guangzhou, China) and authenticated by Ms. Yu-Ying Zong of our School. Voucher specimens have been deposited in the School of Chinese Medicine, the Chinese University of Hong Kong. Acetonitrile and trifluoroacetic acid (HPLC grade; Fisher, USA) were used for chromatography; water was purified by a Milli-Q academic purification system (Millipore, USA).

The Caco-2 cells were purchased from the American Type Culture Collection (ATCC, USA). Medium 199 powder (Gibco; with Earle's salts and L-glutamine, without $NaHCO_3$), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), non-essential amino acid, antibiotics, Trypsin-EDTA (1 mmol/L) and Hank's buffer salt solution (HBSS) were all obtained from Invitrogen (USA). Dimethylsulfoxide (DMSO) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) were purchased from Sigma (USA). All other chemicals used were of analytical grade for laboratory use.

2.2. Preparation of Schisandra extracts

Dried fruits of *S. chinensis* (500 g) were soaked in distilled water (4 L) for 30 min at room temperature, followed by boiling for an hour. The extraction procedure was repeated twice with fresh supplies of distilled water. The combined aqueous solution was filtered, concentrated under reduced pressure and freeze-dried to afford an aqueous extract (35.2% yield w/w based on dried herbs). Ethanol extract was obtained by 70% ethanol extraction following the procedures described above and the extract yield was 30.4%. The dry powder was stored in a desiccator at room temperature until use.

2.3. Animals

Male Sprague-Dawley rats (250–280 g) were bred and housed by the Laboratory Animal Services Centre of the Chinese University of Hong Kong. All experiments were approved by the Animal Research Ethics Committee, The Chinese University of Hong Kong. The animals were kept in a temperature controlled room (23±2 °C) with a 12-h light–dark cycle, with free access to food and water. In the animal study, six rats were orally given by gavage a single dose of *Schisandra* aqueous extract (2 g/kg) or 70% ethanol extract (4 g/kg), which was suspended in 0.5% sodium carboxymethyl cellulose. Blood samples (0.3 mL) were collected by orbital bleeding via heparinized capillary tubes under anesthetizing with isoflurane at 0, 0.5, 1, 2, 4, 8 h. Plasma (0.15 mL) was obtained by centrifugation at $7000 \times g$ for 10 min at room temperature and frozen at −20 °C prior to analysis.

2.4. Caco-2 cell monolayer model experiments

Caco-2 cells were cultured in DMEM supplemented with 10% FBS, 1% non-essential amino acid and penicillin and streptomycin at 37 °C in humidified air containing 5% CO_2. The cells were seeded in collagen-coated Transwell inserts at a density of 3×10^5 cells/well and cultured for 21 days prior to the transport experiments. The medium (1.5 mL in the apical side and 2.6 mL in the basolateral side) was changed every other day. The integrity of cell monolayer was monitored by measurement of transepithelial electrical resistance (TEER) before and after transport study using the Millicell-ERS system (Millipore Corp.); those showing TEER values below 800 Ω cm^2 were not further used. In the transport study, the monolayers were washed with HBSS containing 10 mmol/L HEPES (pH 7.4), followed by pre-incubation for 25 min at 37 °C. After removal of the solutions, fresh transferred buffer (1.5 mL) with or without *Schisandra* extract (2 mg/mL) was added to the apical chamber and incubated at 37 °C. Aliquots (500 μL) were taken from the basolateral chamber at 60, 120, 180 min and replaced with equal volume of transferred buffer after each sampling. At the end of transport study, samples of both sides were collected for analysis. All samples were stored at −20 °C until analysis.

2.5. Rat everted gut sac

Six adult male Sprague-Dawley rats were fasted overnight, sacrificed by cervical dislocation, and the small intestine was excised and flushed with normal saline at room temperature. The intestine was then immediately placed in TC 199 solution maintained at 37 °C and oxygenated (O_2/CO_2, 95%:5%). With the aid of a smooth glass rod, the intestine was everted gently and sealed with silk braided sutures in one end before the gac sac was filled with fresh TC 199 medium. After sealing the other end, the small gut sac (3–4 cm in length) was placed in a 50 mL Erlenmeyer flask containing 10 mL medium with or without *S. chinensis* aqueous extract (10 mg/mL), maintained under a flow of 95% O_2. The sacs were incubated at 37 °C in an oscillating water bath (50 cycles/min) for 90 min. The sacs were taken out, washed with saline and blotted dry. After cutting open the sac, the serosal and mucosal solutions were harvested for analysis. All samples were stored at −20 °C in a freezer until HPLC-MS analysis.

2.6. Sample processing and HPLC-MS conditions

All specimens were allowed to thaw at room temperature. The samples obtained from Caco-2 cell model and rat everted gut sac were subjected to filtration through 0.45 μmol/L membrane before HPLC-MS analysis. The rat plasma (100 μL) was extracted with 150 μL acetonitrile, and the supernatant after centrifugation was collected for HPLC-MS analysis. All samples were separated on a Prevail C_{18} column (250 mm × 4.6 mm I.D., 5 μm, Alltech) by an Agilent 1100 HPLC system (Agilent Technologies, CA, USA). The flow rate was 0.8 mL/min. The mobile phase consisted of 0.1% trifluoroacetic acid water (A) and acetonitrile (B) with a gradient elution of 20% B at 0–5 min; 20–45% B at 6–35 min; 45–60% at 35–50 min; 60–100% B at 50–60 min. The column temperature was maintained at 25 °C. The injection volume was 60 μL and the detection wavelengths were set at 210, 230, 254 and 280 nm. The effluent was directly introduced to the APCI-MS (Agilent 1100 series LC/MSD Trap SL system, USA) for analysis. The APCI-MS analysis was divided into two time segments. The first 4 min were set as waste to avoid the influx of inorganic ions into the mass analyzer; subsequent analysis was carried out under the following optimized conditions: positive ion mode; nebulizer (N_2), 60 psi; dry gas (N_2), 5 L/min; drying gas temperature, 325 °C; target mass, 500 m/z; trap drive level, 80%, full scan range, 140–1100 m/z. Two precursor ions were selected for MS^n ($n=2$–3) experiments in automatic mode with active exclusion. The fragmentation amplitude was 1.20 V.

3. Results

3.1. Quantification of major lignans in Schisandra extracts

The four major lignans, schisandrin, schisandrol B, schisandrin A and schisandrin B, were well separated (Fig. 1). Simultaneous quantification of these compounds was performed using UV detection at 230 nm. The calibration curves showed good linear correlation ($r^2 \geq 0.9995$) between peak area and concentration at the range from 0.5 to 200 μg/mL. Intra- and inter-day precision of the method was within the acceptable limits of R.S.D. < 5%. Samples were stable within

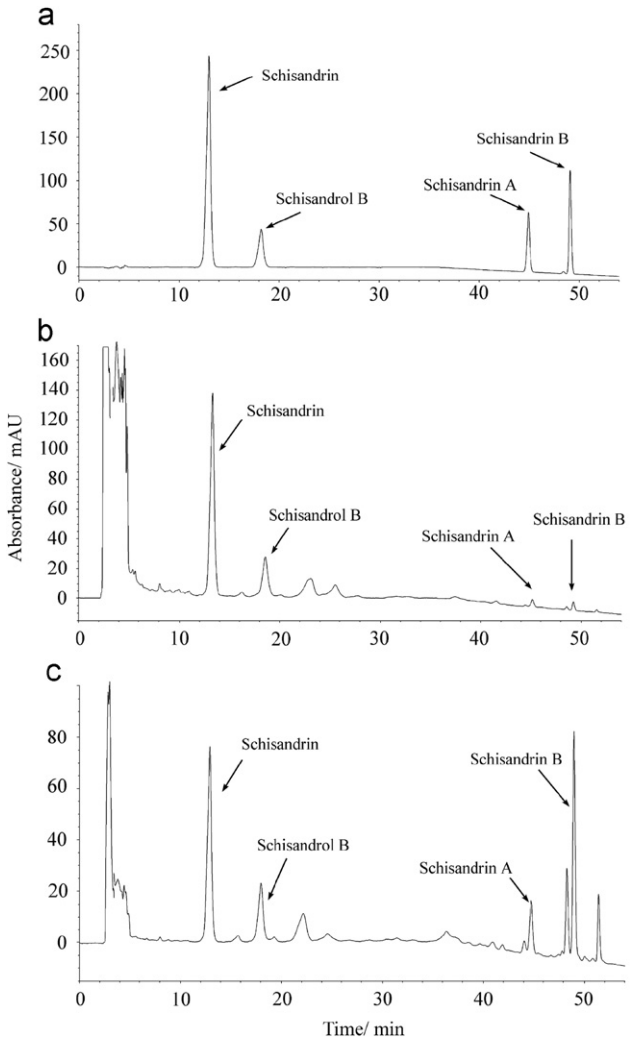
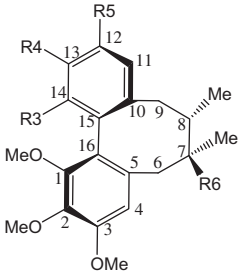
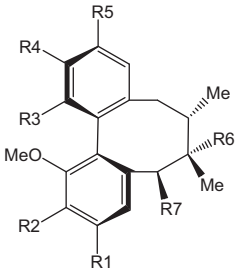
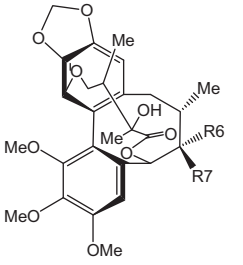


Figure 1 HPLC-DAD chromatograms of (a) a pure standard mixture of schisandrin, schisandrol B, schisandrin A and schisandrin B, (b) aqueous extract and (c) 70% ethanol extract of *Schisandra chinensis* fruits. The detection wavelength was set at 230 nm.

72 h at room temperature (R.S.D. < 3%). The recovery of each analyte was within the range of 96.5–108.9%. Schisandrin was found to be the most abundant lignan, constituting 75.4% (2.96 mg/g) and 46.2% (15.48 mg/g) of total lignans in the aqueous extract and 70% ethanol extract, respectively. The contents of schisandrol B, schisandrin A and schisandrin B in the aqueous extract were 852, 49 and 60 μg/g, respectively, while those in 70% ethanol extract increased to 7.20, 2.31 and 8.53 mg/g, respectively.

3.2. HPLC-MS^n analysis of lignan reference compounds

In order to achieve more information for the identification of absorbable components of *S. chinensis*, a lignan standard mixture, containing schisandrin, gomisin D, schisandrol B, angeloylgomisin F, gomisin G and schisantherin A, was separated under the above HPLC condition, and subsequently introduced to APCI source for characterizing their MS behaviors and dissociation patterns. The chemical structures and MS spectra of the six

Compound	Structure	R1	R2	R3	R4	R5	R6	R7
Schisandrin		-	-	MeO	MeO	MeO	OH	-
Schisandrol B		-	-	MeO	-OCH$_2$O-		OH	-
Angeloylgomisin H		-	-	OAng	MeO	MeO	OH	-
Tigloylgomisin H		-	-	OTig	MeO	MeO	OH	-
Schisandrin A		-	-	MeO	MeO	MeO	H	-
Angeloylgomisin Q		MeO	MeO	MeO	MeO	MeO	OH	OAng
Tigloylgomisin Q		MeO	MeO	MeO	MeO	MeO	OH	OTig
Angeloylgomisin F		-OCH$_2$O-		MeO	MeO	MeO	OH	OAng
Tigloylgomisin F		-OCH$_2$O-		MeO	MeO	MeO	OH	OTig
Gomisin G		-OCH$_2$O-		MeO	MeO	MeO	OH	OBen
Schisantherin B		MeO	MeO	MeO	-OCH$_2$O-		OH	OAng
Schisantherin C		MeO	MeO	MeO	-OCH$_2$O-		OH	OTig
Schisantherin A		MeO	MeO	MeO	-OCH$_2$O-		OH	OBen
Schisandrin B		MeO	MeO	MeO	-OCH$_2$O-		H	H
Gomisin D		-	-	-	-	-	Me	OH
Gomisin E		-	-	-	-	-	Me	H

Ang: angeloyl Tig: Tigloyl Ben: Benzoyl

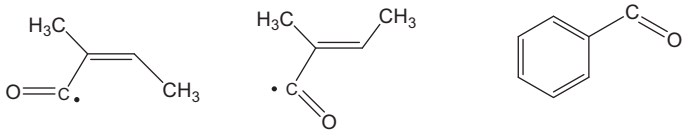


Figure 2 Chemical structures of *Schisandra* lignans characterized in this study.

standards acquired by HPLC-MS are shown in Figs. 2 and 3, respectively. The ion patterns in first-stage spectra exhibited notable difference from those reported in ESI-MS analysis[18,21]. In our study, the protonated molecular ion $[M+H]^+$ was much less intensive and the adduct ions $[M+Na]^+$ or $[M+K]^+$ were hardly observed. Alternatively, if the lignan possesses an ester group in its chemical structure, such as gomisin D and schisantherin A, it likely formed an adduct ion $[M+NH_4]^+$ as the base peak; if an –OH substitution group is present in the C-7 position, such as schisandrin and schisandrol B, the fragment ion $[M+H-H_2O]^+$ produced from the loss of H_2O would be the base peak. In addition, the presence of an ester group in the chemical structure also facilitates the formation of fragment ions by loss of a molecule of organic acid in the first-stage spectra, indicating the involvement of in-source collision-induced dissociation (CID). For the lignans showing identical MS spectra, such as gomisin G and schisantherin A, it was feasible to differentiate these isomers by further MS^n ($n=2$–3) analysis.

Two most abundant ions in the first-stage spectra were selected as the precursor ions for CID fragmentation to produce MS/MS product ions. The most prominent resulting product ions were then selected for further MS^3 fragmentation. Based on the product ions observed in the multi-stage spectra, the characteristic dissociation rules of the lignans showed good agreement with the previous reports[18,21] as summarized below. Firstly, the loss of neutral molecules of $–CH_3$, H_2O and $–OCH_3$ dominates in the multi-stage fragmentations, resulting in the formation of $[M+H-15]^+$, $[M+H-18]^+$ and $[M+H-31]^+$ ions, respectively. The loss of CH_2O is also a dominant elimination when a methylenedioxy group is attached to the benzoic ring, such as schisandrol B and schisantherin A. Secondly, the presence of an –OH group at the C-7 position leads to the easy loss of H_2O, which could maintain the stability of the chemical structure. Thirdly, the presence of an ester group (e.g. schisantherin A and angeloylgomisin F) leads to the elimination of a molecule of organic acid, such as benzoic acid ($\Delta m=122$u) and 2-methyl-2-butenoic acid ($\Delta m=100$u), prior to further fragmentation. Fourthly, different substitution sites of the methylenedioxy group could lead to the formation of diagnostic product ions that enable us to distinguish the isomers with identical first-stage spectra. For example, schisantherin A (C12-13 position substituted) and gomisin G (C2-3 position substituted) produce the same fragment ions at m/z

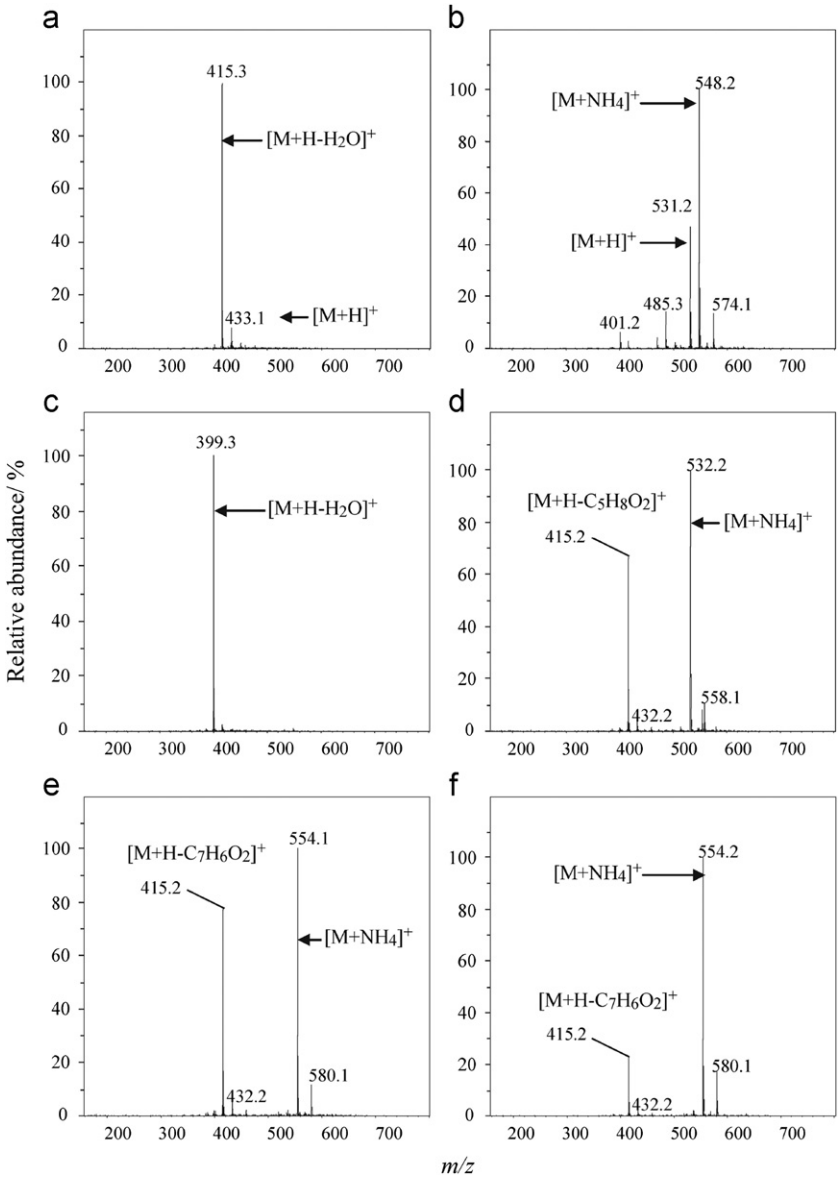


Figure 3 MS spectra of six lignan standards: (a) schisandrin; (b) gomisin D; (c) schisandrol B; (d) angeloylgomisin F; (e) gomisin G and (f) schisantherin A.

415 in their MS^2 spectra, but further fragmentation gives rise to ions at *m/z* 385 and 383 for schisantherin A and gomisin G, respectively. The chromatographic and mass spectrometric characteristics of the lignan standards are summarized in Table 1.

3.3. *In vitro absorbable components of S. chinensis extract*

Rat everted gut sac and Caco-2 cell monolayer system are universal *in vitro* models to study drug transport. The absorption process in our body can be mimicked by the transport from mucosal solution to serosal solution in the gut sac, or from the apical side to basolateral side across Caco-2 cell monolayer. In this study, the aqueous extract of *S. chinensis* exhibited similar absorption profiles in these two *in vitro* models. Fig. 4 shows the LC-MS base peak chromatograms of the samples obtained from the Caco-2 cell monolayer model. Thirteen peaks (P1–P13) were characterized in the basolateral solution. The identities of these compounds were established by comparing the retention time and APCI-MS^n data with those of reference compounds or with the data reported in the literature (Table 1). They are, namely, schisandrin (P1), gomisin D (P2), schisandrol B (P3), angeloylgomisin H (P4 or P5), tigloylgomisin H (P4 or P5), angeloylgomisin Q (P6 or P7), tigloylgomisin Q (P6 or P7), tigloylgomisin F (P8), angeloylgomisin F (P9), gomisin G (P10), schisantherin B (P11 or P12), schisantherin C (P11 or P12) and schisantherin A (P13).

3.4. *Absorbable and metabolic components of S. chinensis extract in rat plasma*

The absorption profile of *S. chinensis* was also determined in rat plasma after oral administration of *Schisandra* aqueous extract and 70% ethanol extract. The HPLC-MS^n method was again employed to analyze the absorbable components and related metabolites. The HPLC-MS chromatograms (Fig. 5) show that the signals of absorbable lignans in the aqueous extract group

Table 1 LC-MS^n characterization and molecular weight (MW) of the lignan standards (STD), absorbable compounds and related metabolites in the Caco-2 cell monolayer, rat everted gut sac models and in rat plasma.

Code	t_R (min)	MS^n (m/z) and relative abundance (%)	MW	Identification
P1/STD1	13.2	MS: 433(11), 415(100) MS^2 [433]: 415(100) MS^2 [415]: 400(28), 384(100), 373(12), 359(18.4) MS^3 [384]: 369(100), 353(38), 338(23), 322(10)	432	Schisandrin
P2/STD2	16.1	MS: 548(100), 531(34) MS^2 [548]: 531(100), 485(72), 401(84), 383(4) MS^2 [531]: 485(38), 401(100), 383(29), 371(6), 353(6) MS^3 [401]: 383(100), 371(7), 353(14), 341(80)	530	Gomisin D
P3/STD3	18.5	MS: 399(100) MS^2 [399]: 384(13), 369(100), 368(60), 357(14), 343(14), 337(42) MS^3 [368]: 353(84), 337(100), 323(10)	416	Schisandrol B
P4	19.9	MS: 501(15), 483(100), 401(6) MS^2 [501]: 483(100), 401(55) MS^2 [483]: 451(72), 436(10), 427(21), 409(15), 401(100), 399(50), 395(21), 369(65) MS^3 [401]: 386(16), 370(52), 369(100), 359(17), 337(21)	500	Angeloylgomisin H/tigloylgomisin H
P5	23.0	MS: 501(5), 483(100), 401(6) MS^2 [501]: 483(100), 401(55) MS^2 [483]: 451(52), 436(8), 427(15), 409(12), 401(100), 399(43), 395(19), 369(44) MS^3 [401]: 386(10), 370(68), 369(100), 359(17), 337(25)	500	Angeloylgomisin H/tigloylgomisin H
P6	22.0	MS: 548(100), 431(43) MS^2 [548]: 431(100) MS^2 [431]: 413(10), 399(36), 389(30), 387(100), 372(30), 356(39) MS^3 [387]: 372(17), 356(100)	530	Angeloylgomisin Q/tigloylgomisin Q
P7	25.3	MS: 548(100), 431(45) MS^2 [548]: 431(100) MS^2 [431]: 413(12), 399(39), 389(35), 387(100), 372(34), 356(43) MS^3 [387]: 372(21), 356(100)	530	Angeloylgomisin Q /tigloylgomisin Q
P8	27.7	MS: 532(100), 415(30) MS^2 [532]: 415(100) MS^2 [415]: 397(31), 383(73), 373(78), 371(100), 356(49), 341(91), 340(44) MS^3 [371]: 341(100), 340(82)	514	Tigloylgomisin F
P9/STD4	31.4	MS: 532(100), 415(32) MS^2 [532]: 415(100) MS^2 [415]: 397(33), 383(74), 373(80), 371(100), 356(51), 341(90), 340(38) MS^3 [371]: 341(100), 340(80)	514	Angeloylgomisin F
P10/STD5	32.5	MS: 554(100), 415(23) MS^2 [554]: 415(100) MS^2 [415]: 397(17), 383(57), 373(54), 371(100), 356(51), 341(81), 340(26) MS^3 [371]: 341(100), 340(59)	536	Gomisin G
P11	34.1	MS: 532(100), 415(26) MS^2 [532]: 415(100) MS^2 [415]: 397(4), 385(22), 373(18), 371(100), 341(10), 340(14) MS^3 [371]: 356(19), 341(41), 340(100)	514	Schisantherin B/Schisantherin C
P12	37.2	MS: 532(100), 415(27) MS^2 [532]: 415(100)	514	Schisantherin B/ Schisantherin C

Table 1 (*continued*)

Code	t_R (min)	MS^n (m/z) and relative abundance (%)	MW	Identification
		MS^2 [415]: 397(6), 385(32), 373(25), 371(100), 341(10), 340(16) MS^3 [371]: 356(25), 341(50), 340(100)		
P13/STD6	37.8	MS: 554(100), 415(17) MS^2 [554]: 415(100) MS^2 [415]: 397(12), 385(71), 373(45), 371(100), 341(39), 340(36) MS^3 [371]: 356(17), 341(69), 340(100)	536	Schisantherin A
P14	41.3	MS: 515(100) MS^2 [515]: 469(22), 385(100), 355(30), 343(3), 323(10) MS^3 [385]: 367(5), 355(100), 353(16), 343(13), 323(30)	514	Gomisin E
P15	45.1	MS: 417(100) MS^2 [417]: 402(36), 386(8), 370(8), 347(53), 332(10), 316(100) MS^3 [316]: 301(100), 285(50)	416	Schisandrin A
P16	48.9	MS: 401(100), 418(20) MS^2 [401]: 386(60), 371(60), 331(80), 300(100) MS^3 [300]: 285(100), 270(30), 258(15), 242(20), 227(15)	400	Schisandrin B
M1	5.5	MS: 435(20), 417(100) MS^2 [435]: 417(100) MS^2 [417]: 385(30), 359(100), 327(40) MS^3 [359]: 344(20), 327(100), 297(35)	434	Demethylated, hydroxylated-schisandrin
M2	7.2	MS: 449(10), 431(100) MS^2 [449]: 431(100) MS^2 [431]: 401(10), 373(100), 358(10), 342(20) MS^3 [373]: 358(20), 342(100):	448	Hydroxylated-schisandrin
M3	8.1	MS: 401(100) MS^2 [401]: 386(15), 370(100), 338(80) MS^3 [370]: 355(45), 338(100), 324(80), 314(40)	418	Demethylated-schisandrin
M4	8.6	MS: 431(100), 401(35) MS^2 [431]: 413(20), 399(60), 387(100), 372(40), 356(30) MS^3 [387]: 372(100), 356(45), 347(25), 330(30), 313(25)	430	Hydrolyzed-angeloyl (tigloyl)gomisin Q

were less intensive than those in ethanol extract group. Such a phenomenon could be explained by the lower contents of lignan compounds present in the aqueous extract. Analysis of the chromatographic and MS^n data led to the identification of schisandrin (P1), gomisin D (P2), schisandrol B (P3), angeloylgomisin H/tigloylgomisin H (P5), schisantherin B/schisantherin C (P11, P12) and schisantherin A (P13) in both groups. In addition, angeloylgomisin H/tigloylgomisin H (P4), gomisin G (P10), gomisin E (P14), schisandrin A (P15) and schisandrin B (P16) were identified in the plasma of rats treated with an ethanol extract. Interestingly, angeloylgomisin Q (P6 or P7), tigloylgomisin Q (P6 or P7), tigloylgomisin F (P8) and angeloylgomisin F (P9), which were present in the *in vitro* absorption profiles, were not detected in the plasma.

In addition to the absorbed lignans, four new peaks (M1–M4) were detected between retention times of 5 and 10 min (Fig. 5). As reported in previous studies, hydroxylation and demethylation are the dominant forms of first-phase metabolism of *Schisandra* lignans[7,22]. M1–M3 showed MS dissociation patterns similar to that of schisandrin, and the mass differences between M1–M3 and schisandrin were 2u, 16u and 14u, respectively. Thus, M1–M3 were tentatively identified to be the demethylated/hydroxylated-, hydroxylated- and demethylated-derivatives of schisandrin, respectively. On the other hand, the MS^n spectra of M4 demonstrated that the mass difference between M4 and angeloyl(tigloyl)gomisin Q were 100u, suggesting the loss of 2-methyl-2-butenoic acid. Moreover, the characteristic fragment ions of M4 were identical with those yielded from the product ion (m/z 415) of angeloyl(tigloyl)gomisin Q. Therefore, M4 was tentatively identified to be a hydrolyzed-angeloyl(tigloyl)gomisin Q. Table 2 shows the distribution of lignan structures in the *in vitro* and *in vivo* models.

4. Discussion

The fruit of *S. chinensis* is among the most commonly used herbs in Traditional Chinese Medicine. The present study improves the knowledge on the pharmacokinetics of *S. chinensis* supplementary to the previous studies on the pure forms of *Schisandra* lignans[9–11,13]. The identified absorbable components suggested the presence of multiple active ingredients in *S. chinensis* and (or) the multi-target of pharmacological actions of this herb. The

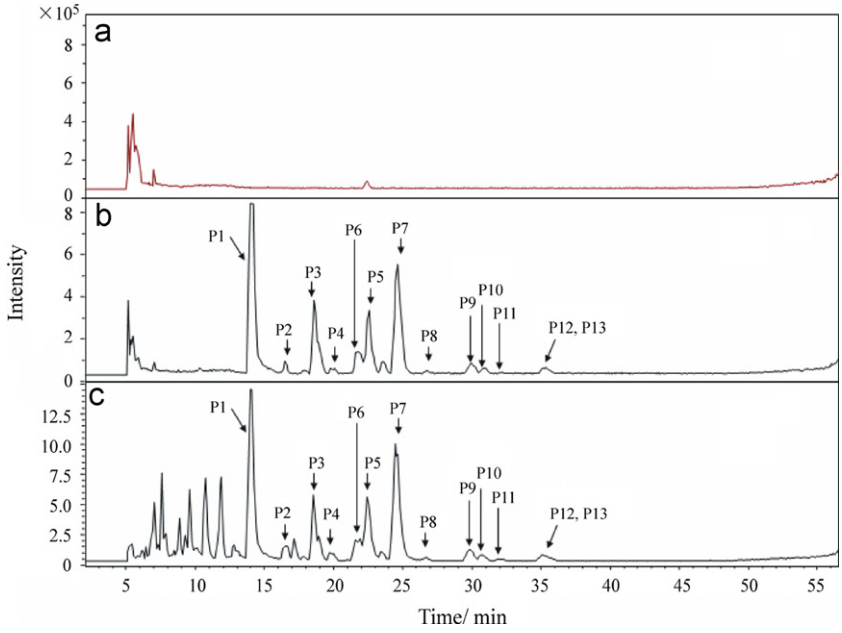


Figure 4 Representative LC-MS basic peak chromatograms (BPC) in positive mode of samples obtained from the Caco-2 cell monolayer model after 180 min incubation. (a) Blank basolateral solution, (b) basolateral solution and (c) apical solution from samples incubated with Hank's buffer solution containing the aqueous extract of *S. chinensis* (2 mg/mL).

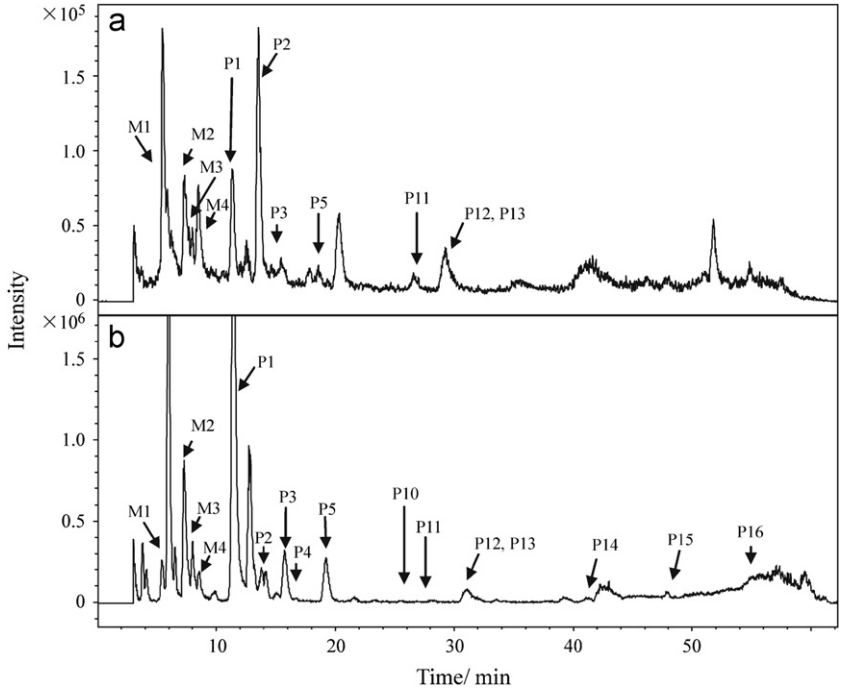


Figure 5 HPLC-MS chromatograms of rat plasma obtained at 2 h after oral administration with *Schisandra* extracts. (a) Extracted ion chromatogram (EIC) for samples treated with an aqueous extract (4 g/kg) and (b) base peak chromatogram (BPC) for samples treated with 70% ethanol extract (2 g/kg).

similar absorption profiles in rat everted gut sac and Caco-2 cell monolayer models indicated that these components are likely absorbable in both rat and human subjects. Our further quantitative study also demonstrated that the apparent permeability of schisandrin across Caco-2 cell monolayer was over 14×10^{-6} cm/s, suggesting a high intestinal absorption potential of this compound[23].

In rat plasma obtained after oral administration of *Schisandra* extracts, most of the absorbable lignans *in vitro* were again identified except for the compounds whose structures possess an ester group. It is plausible that the ester group (angeloyl or tigloyl substitution) attached on the cyclooctadiene ring is rapidly metabolized by the hydrolase in the intestinal wall, enteric bacteria or liver. This speculation was further supported

Table 2 Absorbable compounds and related metabolites of *Schisandra chinensis* characterized in the Caco-2 cell monolayer, rat everted gut sac *in vitro* models and in rat plasma.

Code	Compound	Aqueous extract			Ethanol extract
		Rat everted gut sac	Caco-2 cell monolayer	Rat plasma	Rat plasma
P1	Schisandrin	√	√	√	√
P2	Gomisin D	√	√	√	√
P3	Schisandrol B	√	√	√	√
P4	Angeloylgomisin H/tigloylgomisin H	√	√		√
P5	Angeloylgomisin H/tigloylgomisin H	√	√	√	√
P6	Angeloylgomisin Q/tigloylgomisin Q	√	√		
P7	Angeloylgomisin Q/tigloylgomisin Q	√	√		
P8	Tigloylgomisin F	√	√		
P9	Angeloylgomisin F	√	√		
P10	Gomisin G	√	√		√
P11	Schisantherin B/schisantherin C	√	√	√	√
P12	Schisantherin B/schisantherin C	√	√	√	√
P13	Schisantherin A	√	√	√	√
P14	Gomisin E				√
P15	Schisanrin A				√
P16	Schisandrin B				√
M1	Demethylated, hydroxylated-schisandrin			√	√
M2	Hydroxylated-schisandrin			√	√
M3	Demethylated-schisandrin			√	√
M4	Hydrolyzed-angeloyl(tigloyl)gomisin Q			√	√

by the identification of metabolite yielded from the hydrolysis of angeloyl(tigloyl)gomisin Q. Moreover, the demethylated/hydroxylated-, hydroxylated- and demethylated-derivatives of schisandrin were also detected in rat plasma that was in agreement with the previous reports on the metabolism of *Schisandra* lignans[7,22]. The present results thus provide further information on the absorption and metabolic characteristics of *S. chinensis* in addition to the previous study that only determined the lignan components in rat plasma[21]. The differences between *in vitro* and *in vivo* results in this study indicated that rapid metabolic transformation, such as hydrolysis, hydroxylation and demethylation, should be taken into account in the pharmacological studies of *Schisandra* lignans, as undesirable metabolism may reduce the bioavailability of the parent compounds.

According to the absorption profiles in rat plasma acquired by HPLC-MS (Fig. 5), the signals of the identified lignan compounds (in particular schisandrin) in aqueous extract were considerably less intensive than those in ethanol extract. A previous report compared the oral pharmacokinetics of schisandrin in the forms of monomer and *Schisandra* extract, and revealed that the AUC and half-life values of schisandrin in the extract were increased as compared to monomer, but the underlined mechanism was not investigated. Considering several *Schisandra* lignans, such as schisandrin A and schisandrin B, have been identified as cytochrome P450 (CYP) enzymes inhibitors[24], these concomitantly absorbed lignans may increase the bioavailability of schisandrin through CYP enzyme inhibition, which could also interpret the absorption difference between aqueous extract and ethanol extract in our study. Nevertheless, the observation needs to be clarified by further investigations.

In summary, the present study investigated the absorption and metabolism of *S. chinensis* in the rat everted gut sac and human Caco-2 cell monolayer models, and in rat plasma with the aid of HPLC-APCI-MS^n analysis. By analyzing the chromatographic and MS^n characteristics, thirteen *Schisandra* lignans were identified in the *in vitro* absorption profiles of *Schisandra* aqueous extract. These components of *S. chinensis* are most likely orally absorbable in both rat and human subjects. Most of the lignan compounds were also detected in the rat plasma after oral administration except for four lignans, whose chemical structures possess an ester group (angeloyl or tigloyl substitution) on the cyclooctadiene ring. In addition, four metabolites, corresponding to the hydroxylation and demethylation products of schisandrin and the hydrolysis derivative of angeloyl(tigloyl)gomisin Q, were identified. The present results provide useful information for further pharmacological and pharmacokinetic of *S. chinensis*. This study also demonstrates the successful combination of *in vitro* models, animal system and HPLC-MS^n analysis in the analysis of absorption and metabolic profiles of complex herbal extract.

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