

# Validation of a method for the simultaneous determination of four schisandra lignans in the raw herb and commercial dried aqueous extracts of *Schisandra chinensis* (Wu Wei Zi) by RP-LC with DAD

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## Abstract

A rapid and specific reversed-phase high performance liquid chromatography (RP-LC) method with photodiode array detection (DAD) was developed and validated for the determination of four common schisandra lignans, schisandrin (1), schisandrol B (2), deoxyshisandrin (3) and  $\gamma$ -schisandrin (4), in raw herb materials and commercial dried aqueous extracts of *Schisandra chinensis* (Wu Wei Zi). The extraction solvent and extraction method were optimised where it was found that a 4 h Soxhlet extraction using methanol was successful at extracting >99.5% of each of the schisandra lignans analysed from the raw herb material. The sample preparation process for the dried aqueous extract samples involved sonication using methanol for 2  $\times$  30 min. The herb and extract solutions were separated on a Varian Microsorb-MV 100-5 C18 column using a gradient mixture of 0.1% aqueous phosphoric acid and acetonitrile. Subsequent detection and quantitation of the schisandra lignans was performed at 210 nm. The correlation coefficients of the linear regression analysis performed on these calibration curves were >0.9996 for all four schisandra lignans assayed. The detection limits and quantification limits ranged from 0.12 to 0.57 and 0.41 to 1.89 mg g<sup>-1</sup>, respectively. The mean recoveries of the various analytes ranged from 92.20 to 107.01%. The method was used to investigate the levels of the four mentioned components in herb samples and dried aqueous extracts. The identities of the chromatographic peaks were confirmed by (+) electrospray ionisation LC–MS/MS. Crown Copyright © 2007 Published by Elsevier B.V. All rights reserved.

**Keywords:** Wu Wei Zi; *Schisandra chinensis*; Schisandra lignans; RP-HPLC; Validation

## 1. Introduction

*Schisandra chinensis* (Turcz.) Baill grows wild in most eastern parts of Russia, the Kuril islands, southern Sachlin and north-eastern China, Korea and Japan [1] and is a monoecious liana [2]. It is commonly referred to as Wu Wei Zi (pin yin) in Traditional Chinese Medicine (TCM). The seeds and fruits of *S. chinensis* have been used in TCM formulations and more recently in western based medicine for their antihepatotoxic effect. The official listing in the Chinese Pharmacopoeia [3] has *S. chinensis* indexed as a sedative and tonic. It has also been used for many years in the Russian states as a fortifying agent

in cases of physical exhaustion, and to inhibit fatigue [2]. *S. chinensis* has also been listed as an official monograph in the Russian Pharmacopoeia since 1961 [4].

The major constituents of *S. chinensis* are the dibenzocyclooctene lignans, which are commonly referred to as the schisandra lignans. More than 40 of these schisandra lignans have been isolated from various parts of *S. chinensis* [5,6]. In most cases the seeds and fruits have been found to contain higher levels of these components than other parts of the plant. There is substantial literature available on the levels of the various schisandra lignans present in *S. chinensis* samples [7–16], where the individual levels of each lignan have varied greatly between analyses. The harvest season [17] and sample origin [18] have been shown to affect the levels of the various schisandra lignans. The lignans that have been observed in the highest abundance are schisandrin, schisandrol B, deoxyshisandrin,  $\gamma$ -schisandrin, gomisin N and schisandrin C. The general trend for the lignan content of *S. chinensis* samples is that schisandrin is

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present in the highest concentration followed by schisandrol B and  $\gamma$ -schisandrin.

Schisandra lignans isolated from *S. chinensis* have been shown to be effective liver protecting drugs [4], more potent antioxidants than vitamin E [19] and have anticarcinogenic activity through the activation of key liver enzymes [20]. More recently, halogenated four schisandra lignans have shown strong activity against the human immunodeficiency virus (HIV) [21]. In addition, *S. chinensis* has also been shown to increase the physical performance and recovery rates in race horses [22–24].

The schisandra lignans in *S. chinensis* have been analysed using a variety of techniques including TLC [25–27], LC [7,9,10,12,13,25,28], LC–MS [29], GC–FID [16], GC–MS [30,31], high speed counter current chromatography [32,33], capillary electrophoresis [25] and capillary electrochromatography [11]. The extraction of the lignans from the samples analysed has been carried out using a variety of solvents, including water, aqueous methanol and ethanol mixes, methanol, ethanol, chloroform, hexane and petroleum ether. Supercritical fluid extraction using carbon dioxide, under various temperature and pressure conditions, has also been successfully used for the extraction of the schisandra lignans [9,15,31,34,35].

In this study, a reverse phase LC–DAD method for the quantification of four common schisandra lignans, schisandrin

(1), schisandrol B (2), deoxyshisandrin (3) and  $\gamma$ -schisandrin (4) (Fig. 1), found in *S. chinensis* was developed. These four metabolites were chosen based on their commercial availability, the concentration at which they are found in a typical sample of *S. chinensis* and the literature surrounding their activity.

Optimisation of the extraction solvent and method were followed by a comprehensive validation study, which covered linearity, precision, range, stability, ruggedness, accuracy, detection and quantification limits. Identity confirmation of the chromatographic peaks was determined through an LC–MS/MS analysis. In addition, the variation observed in seven commercial extracts of *S. chinensis*, obtained through several Australian distributors, is also presented.

## 2. Experimental

### 2.1. Equipment

The chromatographic systems used in this study were as follows:

- (1) CAMAG HPTLC (CAMAG, Switzerland) system equipped with a Linomat 5 sample applicator, a Canon digital camera

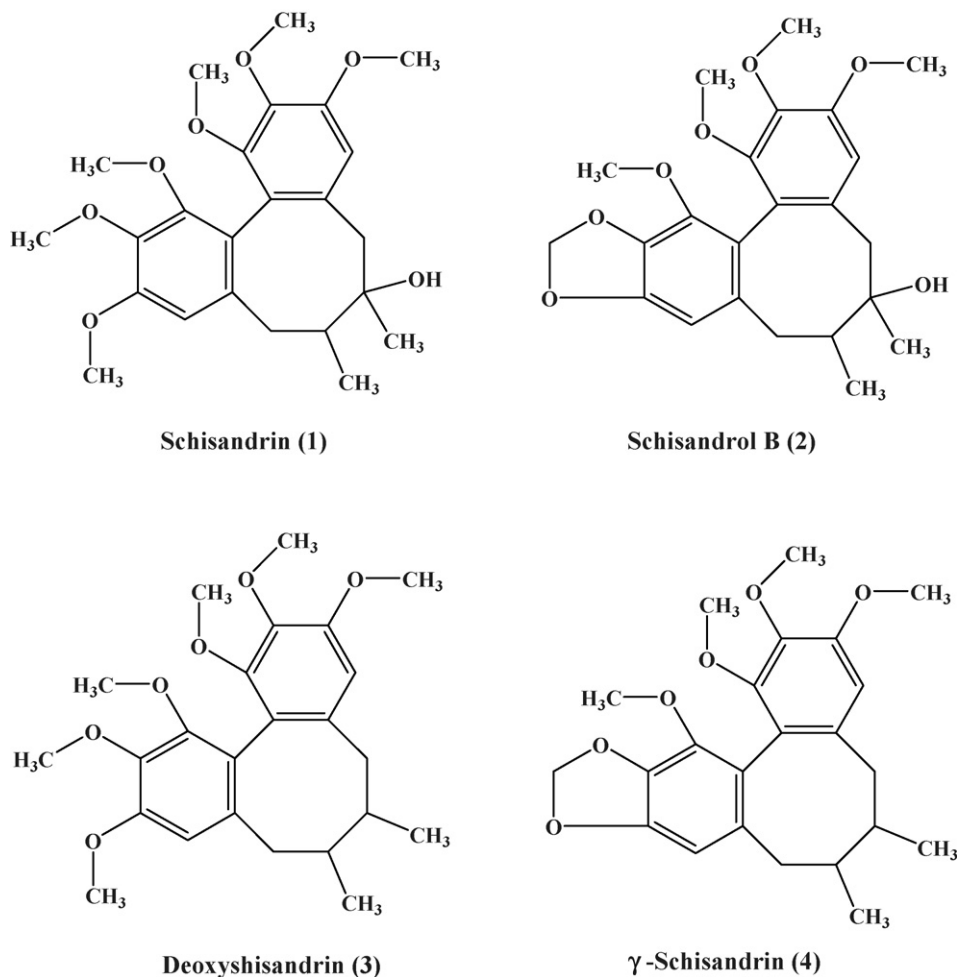


Fig. 1. Structures of the four schisandra lignans analysed in this study.

housed in a Reprostar 3 photographic chamber. The system was controlled using Wincats software version 1.3.0.

- (2) Varian ProStar (Varian Australia, Vic., Australia) LC system equipped with a ProStar 230 ternary pump, ProStar 410 autosampler with column oven and a ProStar 335 photodiode array (DAD) detector. The system was controlled using a Varian Star Workstation version 6.20.
- (3) Varian 1200 (Varian Australia, Vic., Australia) LC–MS/MS system equipped with 2 × ProStar 210 pumps, ProStar 430 autosampler, ProStar 500 column valve module equipped with column oven and a ProStar 335 DAD detector and 1200 triple quadrupole MS/MS detector. The system was controlled using a Varian Star MS Workstation version 6.5.
- (4) A Varian Microsorb-MV 100-5 C18 column (150 mm × 4.6 mm) packed with 5 μm diameter particles was used for all LC–DAD analyses, while a Varian Polaris C18-A 150 mm × 2.0 mm packed with 5 μm particles was used for the LC–MS/MS identity confirmation experiment.

## 2.2. Reagents and materials

The AR grade solvents that were used for the extraction were methanol and ethanol (Pronalys/Labserv, Biolab (Aust) Ltd.), toluene, ethyl acetate and *n*-hexane (Mallinckrodt Baker Inc., USA). Acetonitrile (J.T. Baker, USA), phosphoric acid, acetic acid and formic acid (BDH, UK) were of HPLC grade. Ultra pure water (>18 mΩ) was used in all analyses and was obtained from an ELGA Purelab Ultra water purification system (ELGA, UK).

Primary standards for schisandrin (**1**), schisandrol B (**2**), deoxyshisandrin (**3**) and γ-schisandrin (**4**) were used and were of purity >98% (Chromadex, USA).

A sample of dried berries of *S. chinensis* (Medicine Herb Supplier Pty Ltd., Australia) batch 15005 (HAL-2) was authenticated against a certified reference sample (Alkemist Pharmaceuticals, USA) batch G32204Auth.UWS (HAL-1). The authentication is described in Section 3.1. Following authentication of HAL-2 against HAL-1 this sample was used in all subsequent validation studies performed on the dried berries. The commercial extracts analysed in this study (HAL-3 to HAL-9) were obtained from various manufacturers. Validation studies for the extract were performed on HAL-3.

## 2.3. Preparation of standard solutions

Standard stock solutions of the four schisandra lignans were prepared in methanol, at concentrations of 196.6 μg ml<sup>-1</sup> for **1**, 140.2 μg ml<sup>-1</sup> for **2**, 181.6 μg ml<sup>-1</sup> for **3** and 319.5 μg ml<sup>-1</sup> for **4**. A membrane analysis showed that <0.1% of each schisandra lignan was retained on the PTFE membrane filters (13 mm, 0.45 μm obtained from Labserv, Biolab (Aust) Ltd.).

## 2.4. Preparation of samples

The HPTLC samples were prepared by Soxhlet extracting 1 g of the powdered raw herb with approximately 100 ml of methanol for 4 h. The samples were evaporated

to dryness on a rotary evaporator and reconstituted in 5 ml methanol.

The dried berries of *S. chinensis* were ground into a fine powder and passed through a sieve (250 μm). In the extraction by sonication experiments 1 g of the powdered berries was extracted with 50 ml solvent for 30 min. The sample was filtered into a 100 ml volumetric flask. This process was repeated using 40 ml fresh solvent and the combined filtrates was made to 100 ml with the extraction solvent. Five solvents were tested to determine the optimal solvent for extraction of the schisandra lignans. The solvents used were water, 50% aqueous methanol, methanol, ethanol and hexane.

Three different extraction methods were compared in order to optimise the extraction of the schisandra lignans from plant material. These methods were sonication (detailed earlier), Soxhlet and reflux. In the Soxhlet extraction 2.5 g of powdered raw herb was placed into a 33 mm × 80 mm cellulose thimble (Labserv, Biolab (Aust) Ltd.) and Soxhlet extracted with approximately 200 ml solvent for 4 h. The solution was transferred to a 250 ml volumetric flask and made up to volume with the extraction solvent. For the reflux extraction 1.0 g of powdered berries was heated under reflux with 50 ml of the optimal solvent for 30 min. The sample was filtered into a 100 ml volumetric flask and the process repeated on the remaining plant material using 40 ml fresh solvent. The sample was filtered into the same volumetric flask and made to volume.

The commercial dried aqueous extracts were extracted by placing 0.5 g into a 100 ml volumetric flask and adding 80 ml of the optimal solvent. The sample was sonicated for 2 × 30 min, allowing the sample to cool between sonications, and made to volume with the same solvent.

Aliquots (1–2 ml) of each of the solutions prepared in the extraction experiments were filtered through a 0.45 μm PTFE membrane filters prior to LC analysis.

## 2.5. Chromatographic conditions

The HPTLC identity confirmation of the herb was carried out on a Silica gel 60 F254, 10 cm × 10 cm HPTLC plate (Merck, Germany), where 2 μl of each sample was applied in 7 mm bands, 10 mm from the base of the plate. The mobile phase was a mixture of toluene–ethyl acetate–acetic acid (70:33:3, % v/v/v), and the plate developed over a distance of 8 cm. Following development, the plate was stained with anisaldehyde–sulphuric acid spray reagent, heated at 110 °C for 10 min and viewed under UV light at 254 nm.

The quantitative LC analysis was performed on a Varian Microsorb-MV 100-5 C18 column (150 mm × 4.6 mm) packed with 5 μm diameter particles using a gradient program, consisting of 0.1% aqueous H<sub>3</sub>PO<sub>4</sub> in (A) and acetonitrile (B). The gradient elution conditions were as follows: at *t*=0 min the mobile phase consisted of 60A/40%B and was held for 2 min. From 2 to 32 min a gradient was applied to 20A/80%B, which was followed by a wash with 5A/95%B for 5 min and a 7 min equilibration period at 60A/40%B. The separation temperature was kept at a constant 35 °C throughout the analysis, with a flow rate and injection volume of 1.0 ml min<sup>-1</sup> and 10 μl,

respectively. The schisandra lignans were detected based on a comparison of both retention times and UV spectra with those of the reference standards. All separations were monitored at 210 nm.

An LC–MS/MS experiment was employed for peak identity confirmation using the Varian 1200L in positive electrospray ionisation (ESI) mode with drying gas temperature and pressure at 350 °C and 25 psi, respectively. The N<sub>2</sub> nebulising gas was set at a constant 47 psi and the detector at +1850 V. The needle voltage was set at 5000 V, the shield at 600 V and the capillary at 70 V. Water was substituted as mobile phase A in the LC–MS experiments and a Varian Polaris C18-A 150 mm × 2.0 mm column packed with 5 μm diameter particles, operating at a flow rate of 0.2 ml min<sup>-1</sup> was used. The same gradient conditions as those detailed for the LC–DAD analysis was used for separation of the analytes by the LC–MS/MS. The system was set up for sequential DAD then MS/MS analysis of the emerging analytes.

### 3. Results and discussion

#### 3.1. Authentication of HAL-1 against HAL-2 herb samples

The dried berries of sample HAL-2 were authenticated against a certified reference sample (HAL-1) by macroscopic identification, HPTLC and LC profiling. The macroscopic identification showed that HAL-2 was identical to that of the reference sample, and the HPTLC profiles of the methanolic extracts of both HAL-1 and HAL-2 were identical when tested as described in the Chinese drug monograph for Fructus *Schisandrae* [36]. The LC profile of the methanolic extract of HAL-2 showed peaks which corresponded to those obtained for the certified reference material (HAL-1), indicating that this herb was an authentic sample of *S. chinensis*. Slight variations in levels of the various components were observed, which is most likely a consequence of harvest season and/or location. The HAL-2 sample was subsequently used for all the validation studies. A mixed standard containing all four schisandra lignans was used for peak identification (Fig. 2). The LC profiles using the optimised conditions of both HAL-1 and HAL-2 are shown in Fig. 3. These LC profiles also closely matched that given in the Chinese drug monograph for Fructus *Schisandrae*.

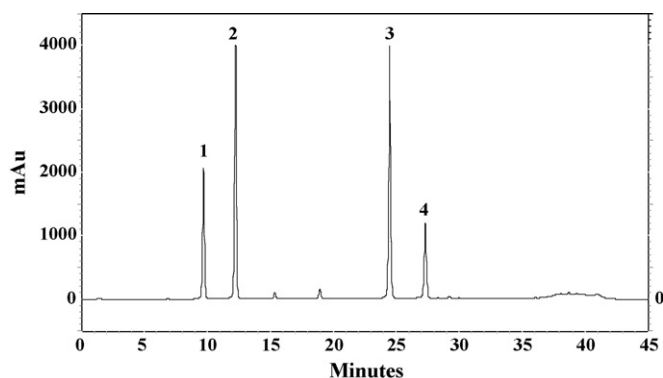


Fig. 2. LC–PDA chromatogram of the mixed standard containing schisandrins (1), schisandrol B (2), deoxyschisandrins (3) and γ-schisandrins (4).

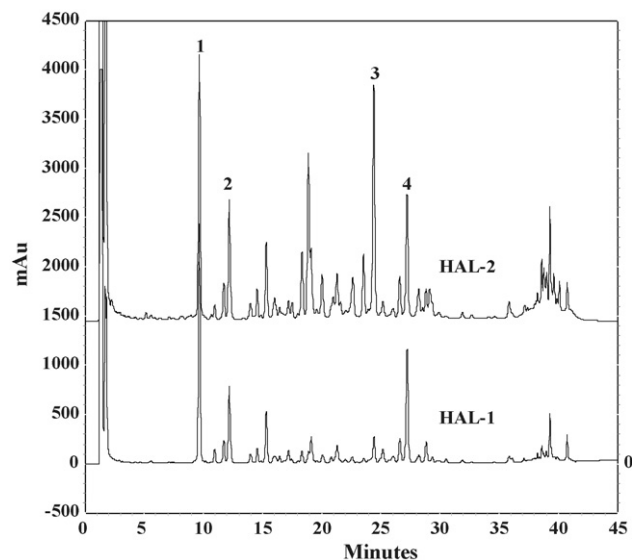


Fig. 3. LC chromatograms of the authenticated raw herb sample (HAL-2) and certified reference raw herb (HAL-1) of *Schisandra chinensis*. Showing the components schisandrins (1), schisandrol B (2), deoxyschisandrins (3) and γ-schisandrins (4).

#### 3.2. Optimisation of sample preparation

The optimal extraction solvent was determined by preparing five replicates of each of the five test solvents using sonication as the extraction method. This entire experiment was performed on the same day and analysed in the same sequence using the sample HAL-2, to avoid inter-day variation. The results are shown in Fig. 4 and indicate that methanol was the most efficient solvent for the extraction of all four schisandra lignans 1–4. Methanol was now used in all subsequent experiments as the preferred extraction solvent.

A comparison of extraction methods was now performed where five replicates were prepared using sonication, Soxhlet and reflux as the extraction methods. The results (Fig. 5) indicated that the Soxhlet was the most efficient method at extracting the schisandra lignans. The increase in the extraction efficiency

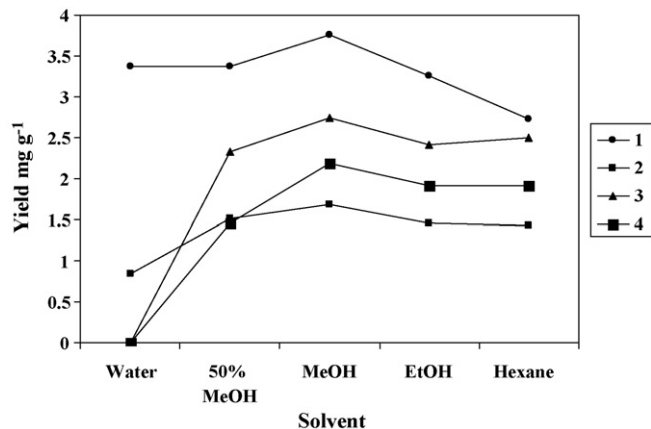


Fig. 4. Effect of different extraction solvents on the yield of the four schisandra lignans 1–4. (The components are schisandrins (1), schisandrol B (2), deoxyschisandrins (3) and γ-schisandrins (4).)

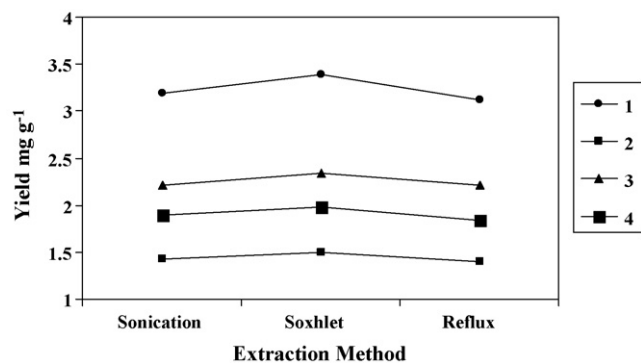


Fig. 5. Effect of different extraction methods on the yield of each of the schisandra lignans 1–4. (The components are schisandrin (1), schisandrol B (2), deoxyschisandrin (3) and  $\gamma$ -schisandrin (4).)

of each individual schisandra lignan when using Soxhlet was only slight but the overall increase in the total schisandrins (sum of 1–4) was more than 5% when compared to the sonication method. In addition, an exhaustive extraction of the remaining plant material from the five replicates of the Soxhlet extraction was carried out. The extraction was performed for a 24 h and the samples concentrated to improve the detection of the expected low levels of schisandra lignans. The exhaustive extraction showed that more than 99.5% of the lignans of interest had been extracted from the plant material when using the Soxhlet method. The Soxhlet method was therefore used for all subsequent experiments performed on the dried herb material.

### 3.3. Reproducibility, linearity, detection and quantification limit

The calibration curves showed good correlations ( $r^2 > 0.9996$ ). The inter-day precision of retention time was  $<0.6\%$  R.S.D. and the peak area precision was  $<0.9\%$  R.S.D. ( $n=6$ ). The limits of detection (LOD) for analytes 1–4 determined as three times the S.D. obtained for the raw herb (unspiked) were 0.49, 0.16, 0.35, and 0.29  $\text{mg g}^{-1}$  respectively and for the dried aqueous extract the concentrations were 0.57, 0.13, 0.12, and 0.27  $\text{mg g}^{-1}$ , respectively. The limits of quantification (LOQ) for analytes 1–4 determined as ten times the S.D. obtained for the raw herb were 1.63, 0.55, 1.16, and 0.97  $\text{mg g}^{-1}$  respectively and for the dried aqueous extract the LOQ were 1.89, 0.44, 0.41, and 0.90  $\text{mg g}^{-1}$ , respectively.

### 3.4. Standard and sample stability

The stability of the four schisandra lignans was tested by injecting the standard and sample solutions at specified time intervals and comparing the changes in peak area as a function of time. The solutions were accepted to be stable if  $<2\%$  degradation had occurred. All four of the individual standards solutions were found to be stable for at least 1 month when stored at  $\leq 4^\circ\text{C}$ . The sample solutions, of both the herb and extract, were found to be stable for at least 48 h after preparation when stored at  $\leq 4^\circ\text{C}$ .

Table 1

Analyte concentrations in the raw herb and commercial dried aqueous extract

Compound	$\text{mg g}^{-1\text{a}}$ ( $\pm\%$ R.S.D.)	
	HAL-2 <sup>b</sup>	HAL-3 <sup>c</sup>
1	3.13 (5.21)	5.62 (3.36)
2	1.46 (3.77)	1.78 (2.45)
3	2.33 (4.99)	2.15 (1.89)
4	1.79 (5.43)	2.23 (4.05)

<sup>a</sup> Mean  $\text{mg g}^{-1}$  ( $\pm\%$ R.S.D.) calculated from nine replicate extractions and analyses.

<sup>b</sup> Authenticated raw herb.

<sup>c</sup> Commercial dried aqueous extract.

### 3.5. Concentration and recoveries of schisandra lignans

The concentration of analytes in the unspiked raw herb and dried aqueous extract are presented in Table 1. The amount of schisandra lignans found in the dried aqueous extract were comparable to those in the raw herb except for the concentration of 1 which was 40% higher.

The recovery of each analyte was determined through spiking the samples, HAL-2 and HAL-3 with known quantities of each analyte. This study was performed at three spiking levels, which represented 50%, 100% and 150% of the concentration of each analyte. Five replicates were spiked at each level for each sample and the recoveries calculated by comparison to a blank (unspiked) sample. The recoveries and R.S.D.'s from the analysis, for both HAL-2 and HAL-3, are shown in Table 2. The recoveries ranged from 95.9–107.0% for analytes 1–4 in HAL-2, and 92.2–105.1% for the analytes 1–4 in HAL-3. The R.S.D.'s of each recovery analysis were  $<5\%$ .

### 3.6. LC–MS/MS identity confirmation

A LC–MS/MS experiment was also performed to confirm the identity of the four schisandra lignans (1–4). A different col-

Table 2

Recoveries of the four schisandra lignans in the raw herb and commercial dried aqueous extract

Compound	Relative amount added (%)	%Recovery <sup>a</sup> ( $\pm\%$ R.S.D.)	
		HAL-2 <sup>b</sup>	HAL-3 <sup>c</sup>
1	50	98.84 (4.45)	92.20 (2.52)
	100	96.51 (3.28)	94.06 (1.84)
	150	96.08 (3.91)	94.10 (0.93)
2	50	103.84 (4.17)	93.54 (2.05)
	100	103.65 (4.03)	101.59 (1.91)
	150	95.91 (4.96)	94.84 (1.12)
3	50	101.50 (1.54)	99.53 (3.52)
	100	107.01 (1.11)	98.84 (1.29)
	150	98.44 (1.25)	99.93 (0.71)
4	50	99.95 (1.16)	99.26 (4.54)
	100	100.30 (3.92)	105.08 (2.10)
	150	96.68 (4.29)	103.61 (1.07)

<sup>a</sup> Mean %recovery ( $\pm\%$ R.S.D.) calculated from five replicate extractions and analyses.

<sup>b</sup> Authenticated raw herb.

<sup>c</sup> Commercial dried aqueous extract.

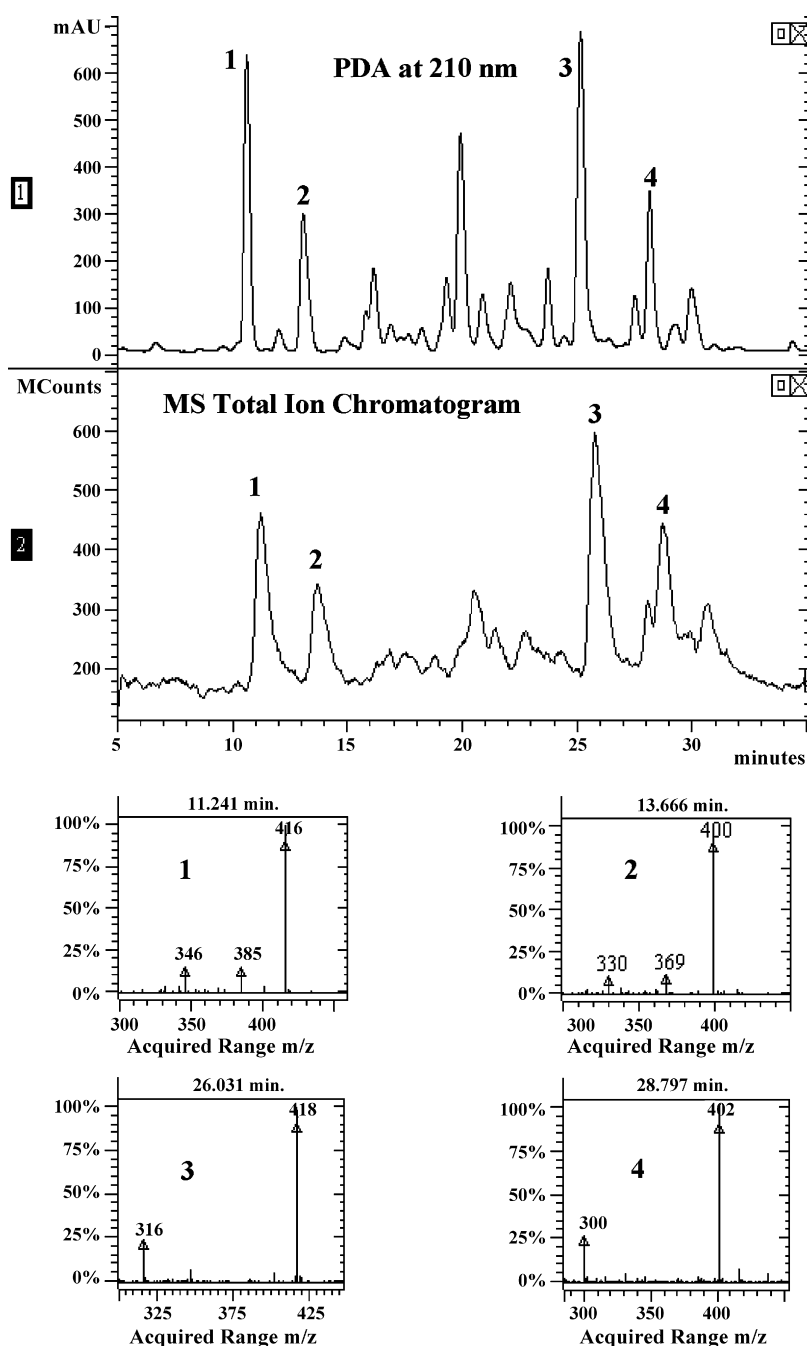


Fig. 6. Simultaneous LC–DAD and LC–MS/MS analysis of the authenticated raw herb sample (HAL-2) of *S. chinensis*. (The components are schisandrin (1), schisandrol B (2), deoxyschisandrin (3) and  $\gamma$ -schisandrin (4).)

umn was used for this analysis due to the need to reduce the flow rate for the MS detector. The chromatogram of the sample was comparable to that obtained for the LC–DAD analysis. The chromatograms from the sequential DAD and MS/MS (in ESI positive mode) are presented in Fig. 6, along with the mass spectra obtained for each component. These mass spectra matched those obtained for the pure standards for each of the components of interest 1–4, thus confirming their identity. The base peak for components 1 and 2 was the  $[(M - H_2O) + H]^+$  ion, where only a very small (<1% of the base peak)  $[M + H]^+$  ion was observed for each. The components 3 and 4 both exhibited the  $[M + H]^+$  ion as the base peak.

### 3.7. Analysis of samples using the validated method

The schisandra lignan concentrations obtained are presented in Table 3. The highest concentration of 1 for the dried aqueous extracts was  $5.44 \text{ mg g}^{-1}$  found in sample HAL-3. Of the seven commercially prepared dried aqueous extract samples tested only two (HAL-3 and -8) had concentrations above the LOQ of  $1.19 \text{ mg g}^{-1}$ . Small amounts of 1 were found in extract HAL-5, -6, and -9 while none was found in samples HAL-4 and -7. The average R.S.D. was 1.50% (range 0.69–3.16%). The highest concentration of 2 for the dried aqueous extracts was  $1.71 \text{ mg g}^{-1}$  found in sample HAL-3. Of the seven samples

Table 3  
Levels of schisandra lignans in different herb samples and commercial dried aqueous extracts of *Schisandra chinensis*

Sample	Description	mg g <sup>-1a</sup> (±%R.S.D.)			
		1	2	3	4
HAL-1	Certified Reference	3.01 (1.11)	1.33 (1.59)	2.21 (2.11)	1.65 (2.34)
HAL-2	Authenticated Raw herb	3.13 (2.75)	1.46 (2.12)	2.33 (1.98)	1.79 (1.55)
HAL-3	Commercial Extract	5.44 (0.69)	1.71 (1.68)	2.35 (1.52)	2.28 (1.06)
HAL-4	Commercial Extract	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>
HAL-5	Commercial Extract	1.06 <sup>b</sup> (2.09)	0.40 <sup>b</sup> (1.61)	0.34 <sup>b</sup> (1.78)	0.37 <sup>b</sup> (1.83)
HAL-6	Commercial Extract	1.48 <sup>b</sup> (0.75)	0.57 (0.30)	1.26 (0.82)	0.77 <sup>b</sup> (1.88)
HAL-7	Commercial Extract	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>	ND <sup>c</sup>
HAL-8	Commercial Extract	2.90 (3.16)	0.95 (6.12)	ND <sup>c</sup>	ND <sup>c</sup>
HAL-9	Commercial Extract	0.47 <sup>b</sup> (0.81)	0.17 <sup>b</sup> (1.21)	0.02 <sup>b</sup> (11.42)	0.02 <sup>b</sup> (6.74)

<sup>a</sup> Mean mg g<sup>-1</sup> (±%R.S.D.) calculated from three replicate extractions and analyses.

<sup>b</sup> Estimate only—below LOQ.

<sup>c</sup> ND: not detected.

tested only three samples (HAL-3, -6, and -8) had concentrations above the LOQ of 0.44 mg g<sup>-1</sup>. Small amounts of **2** were found in extract HAL-5 and -9 while none was found in samples HAL-4 and -7. The average R.S.D. was 2.18% (range 0.30–6.12%). The highest concentration of **3** for the dried aqueous extract was 2.35 mg g<sup>-1</sup> found in sample HAL-3. Of the seven samples tested only two (HAL-3 and -6) had concentrations above the LOQ of 0.41 mg g<sup>-1</sup>. Small amounts of **3** were found in extract HAL-5 and -9 while none was found in samples HAL-4, -7, and -8. The average R.S.D. was 3.89% (range 0.82–11.42%). The highest concentration of **4** for the dried aqueous extracts was 2.28 mg g<sup>-1</sup> found in sample HAL-3. Of the seven samples tested only HAL-3 had concentrations above the limit of quantification (LOQ) of 0.90 mg g<sup>-1</sup>. Small amounts of **4** were found in extract HAL-5, -6 and -9 while none was found in samples HAL-4, -7, and -8. The average R.S.D. was 2.88% (range 1.06–6.74%).

The raw herbs (HAL-1 and -2) had comparable concentrations of all the schisandra lignans relative to HAL-3. It appears that the expected increase in concentration of these analytes in the dried aqueous extract, compared to those in the raw herb, has not been observed. It is noted that none of the dried aqueous extract suppliers make any claim with regards to concentration of chemical components except that the extracts were produced from *S. chinensis*. The low analyte concentrations may be due to the inefficient extraction process, poor herb quality, decomposition of analytes during the extraction process, or the excessive use of diluent (for example, starch or dextrose) in the preparation of the final product.

Of all the dried aqueous extracts analysed, sample HAL-3 had the highest schisandra lignan concentrations followed by sample HAL-6. HAL-5 and -9 contained small amounts of the schisandra lignans, all at below their respective LOQ's while none was found in samples HAL-4 and -7.

The raw herb sample of HAL-2 had a comparable concentration of schisandra lignans to the amount in the certified sample (HAL-1). The concentration of the schisandra lignans in HAL-3 was up to 40% more than that in the raw herb samples (HAL-1 and -2).

#### 4. Conclusion

The LC technique described here is an accurate and reliable technique for the simultaneous determination of the schisandra lignans in the raw herb and dried aqueous extract of *S. chinensis*. The extraction method is able to recover >99.5% of the schisandra lignans from the raw herb material and dried aqueous extract with acceptable analysis times (45 min) and with baseline resolution between components.

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