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Original Article

Simultaneous determination of four amides in *Saururus chinensis* by matrix solid phase dispersion and high-performance liquid chromatography method

Hongjiang Chen [a,b], *Jianan Liu* [a], *Mingchao Cui* [a], *Jianwei Chen* [b], *Xiang Li* [b,*], *Yong Chen* [b]

[a] *College of Chinese Medicine, Zhejiang Pharmaceutical College, Ningbo, China*
[b] *College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing, China*

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ABSTRACT

A rapid and simple analytical method was established for the determination of four amides (*N-p-trans*-coumaroyltyramine, aristolactam AII, sauristolactam and aristolactam BII) in *Saururus chinensis* by matrix solid phase dispersion (MSPD) and high-performance liquid chromatography-diode array detector (HPLC-DAD). In the optimized MSPD, 0.2 g *S. chinensis* powder was blended with 0.4 g silica gel, and 5 mL methanol was selected as elution solvent. The MSPD extraction achieved higher extraction recovery of four amides, and required less sample, solvent and preparation time, comparing with the conventional methods (Soxhlet and ultrasonic extraction). The assay was performed on a TSK gel ODS-100Z column (4.6 mm × 250 mm, 5 μm) at 30 °C. Acetonitrile and 0.4% acetic acid aqueous solution was used as mobile phase by gradient elution at the flow rate of 1.0 mL/min. The detection wavelength was 280 nm. All the analytes showed good linear regression ($R^2 \geq 0.9998$) within the concentration ranges. The validated method showed good precision and stability with relative standard deviations (RSDs) ≤ 3.18%. The recoveries were in the range of 96.57–99.65%, with RSDs less than 2.74%.

1. Introduction

Saururus chinensis, a well known folk medicine in China and Southern Korea, has been widely used for the treatment of edema, jaundice, gonorrhea and several inflammatory diseases. Meanwhile, *S. chinensis* was also used as functional food and food supplementation [1,2]. A great many lignans, flavonoids, aristolactams and phenolic acids have been isolated from its aerial parts and rhizome [3–5]. Aristolactams (ALs) showed significant neuroprotective activity against glutamate-induced toxicity in primary cultured rat cortical cells [6], antioxidant [7] and anti-tumor activities [8,9]. It is interesting to note that aristolochic acids (AAs), known to be

* *Corresponding author*. College of Pharmacy, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210046, China.
E-mail address: lixiang_8182@163.com (X. Li).

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nephrotoxic, carcinogenic and mutagenic [10], can metabolize to ALs in the rat, in addition, AL-I showed higher nephrotoxicity than AA-I [11]. Hence, many countries have banned the use of herbs containing AAs and ALs. The U.S. Food and Drug Administration declared to discontinuation of products that contain AAs and ALs [12]. *N-p-trans*-coumaroyltyramine, the other amide, exhibited significant alpha-glucosidase inhibitory [13], antioxidative and anti-inflammatory activities [14]. Therefore, there was an urgent need to establish determination and quantitative analysis of ALs in *S. chinensis* both for its safety and efficacy. However, to our knowledge, there was little report about determination of ALs in *S. chinensis*, excepting our previous research [15].

In addition, the conventional extraction methods including Soxhlet and sonication were time-consuming, high solvent consumption, requiring additional filtration, concentration or even purification steps. Matrix solid phase dispersion (MSPD) technology, invented by Barker in 1989, was a simple, convenient and low-cost extraction method [16]. Recently, MSPD has been used widely for the extraction of investigated compounds from foods and medicinal plants [17–19]. Some active compounds have been extracted from plants by MSPD, such as flavonoids [19–21], saponins [22], lignans [23], phenols [24,25] and essential oils [26]. However, as far as we know, this technique has never been applied to the extraction of amides from *S. chinensis*.

Thus, in the present study, a method based on MSPD extraction combined with high-performance liquid chromatography-diode array detector (HPLC-DAD) was established and systematically validated for determination of four amides, namely, *N-p-trans*-coumaroyltyramine, aristolactam AII, sauristolactam and aristolactam BII in *S. chinensis*. The MSPD method was compared with the traditional Soxhlet and sonication extraction methods. The validated MSPD-HPLC method was used to determine the four amides in *S. chinensis* from different regions.

2. Materials and methods

2.1. Chemicals, reagents and materials

Standard compounds (*N-p-trans*-coumaroyltyramine, aristolactamAII,sauristolactamandaristolactamBII)wereisolatedfrom the aerial parts of *S. chinensis*. Their structures were elucidated basedonspectroscopicanalysis(^{1}HNMR,^{13}CNMR)andliteratures [27–29].Thepurityofeachcompoundwasmorethan98%detected byHPLC.Theirchemicalstructureswereshown in Fig. 1. Six samples of *S. chinensis* (S1–S6) were collected from Jiangsu, Zhejiang, Anhui and Hubei province. The botanical origin of materials was identified by Prof. Jianwei Chen. Voucher specimens were deposited at Herbarium of Nanjing University of Chinese Medicine.

Acetonitrile (chromatographic grade) was purchased from Merck (Darmstadt, Germany). Deionized water was purified by a Milli-Q water system (Millipore, USA). Silica gel (200–300 mesh) was purchased from Qingdao Haiyang Chemical Subsidiary Factory (Qingdao, China). Diatomaceous earth was obtained from Hengxing Technology Inc. (Tianjin, China). C_{18} (particle size 40–63 μm) was purchased from SiliCycle (Quebec, Canada). Neutral alumina (100–200 mesh) was purchased from Tianjin Chemical agent Co. Ltd. (Tianjin, China). Other reagent solution was of analytical grade from Beijing Reagent Company (Beijing, China).

2.2. Chromatographic conditions and instrument

The analysis were performed on an Agilent 1200 liquid chromatography system (Agilent Technologies, USA), equipped with a G1315D DAD detector, a G1312A double pump, a G1329A autosampler and a G1316A column temperature controller. The separations were carried out on a TSK gel ODS-100Z column (4.6mm×250mm,5μm).Thecolumntemperaturewas30°Candthe detectionwavelengthwas280nm.Themobilephase,consistedof 0.4% (v/v) acetic acid-water solution (A) and acetonitrile (B), was programmedbythefollowinglineargradientelution:0–8min,30% B (v/v); 8–14 min, 30–40% B (v/v); 14–30 min, 40–50% B (v/v); 30–40 min, 50–100% B (v/v); 40–42 min, 100% B (v/v); 42–44 min, 100–30%B(v/v).Theflowratewas1.0mL/min,andinjectionvolume was10μL.

2.3. Preparation of standard solutions

Stock standard solutions of *N-p-trans*-coumaroyltyramine (830.0 μg/mL), aristolactam AII (778.0 μg/mL), sauristolactam (632.0 μg/mL) and aristolactam BII (566.0 μg/mL), were prepared in methanol. Working standard solutions were obtained by diluting the mixed stock solutions with methanol to give six different concentrations for calibration curves. The solutions were filtered through a 0.45 μm membrane prior to injection.

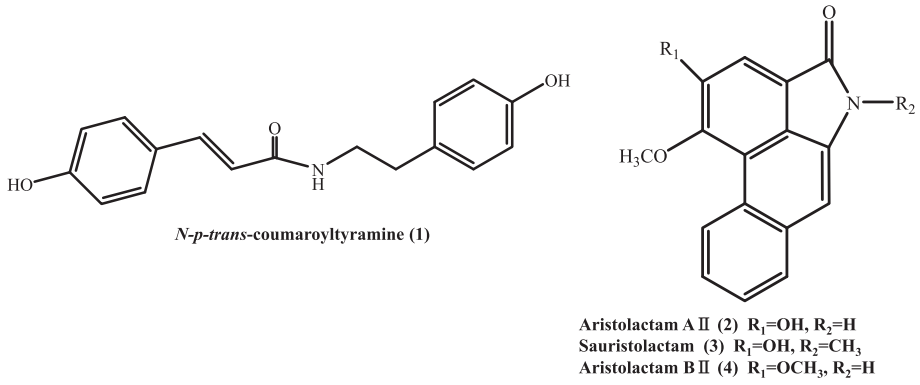

Fig. 1 – Chemical structures of the investigated amides in *Saururus chinensis*.

2.4. Preparation of sample solutions

2.4.1. MSPD extraction

Approximately 0.2 g (accurately weighed) *S. chinensis* powder was blended and ground with 0.4 g silica gel in an agate mortar. Then 0.4 g homogenous mixture was accurately weighed and introduced into a 10 mL glass syringe with a layer of absorbent cotton at the bottom. Then 5 mL of methanol was added into the packed syringe gently eluting the target compounds. The eluent was collected in a 5 mL of volumetric flask and made up to mark with methanol [22,23]. The resulting solution was filtered through a 0.45 μm filter membrane before HPLC analysis.

2.4.2. Soxhlet extraction

1.0 g *S. chinensis* powder was accurately weighed, placed into a cellulose thimble, transferred to a Soxhlet extractor and continuously reflux extracted with 100 mL of methanol in a thermostatic water bath for 6 h. The extracting solution was concentrated in a rotary evaporator and transferred into a 50 mL of volumetric flask and made up to the mark with methanol. The resulting solution was filtered through a 0.45 μm filter membrane prior to injection.

2.4.3. Sonication extraction

The accurately weighed *S. chinensis* powder (approximately 0.5 g), was placed into a 100 mL conical flask, then 25 mL of methanol was added into it as the extraction solvent and the total weight was accurately weighed before extraction. The process of sonication extraction was conducted in an ultrasonic bath for 40 min. The total weight was accurately weighed again and appropriate amount of methanol was added into it to keep the weight unchanged. The supernatant was filtered through a 0.45 μm membrane before injected into HPLC system for analysis.

3. Results and discussion

3.1. Optimization of MSPD procedure

In order to obtain satisfactory extraction efficiency, the most suitable extraction parameters for MSPD procedure, including type of dispersing sorbent, category and volume of the eluting solvent, and the ratio of dispersing sorbent to sample, were evaluated. The results (Fig. 2A) indicated that when silica gel, C_{18} and Florisil were used, the extraction recovery of four amides were higher than the extraction recovery with Al_2O_3. Therefore, silica gel was selected as the dispersing sorbent, for its cheap and widely used. Four different kinds of solvents (methanol, ethanol, acetone and ethyl acetate) were evaluated to select a suitable elution solvent. The results were shown in Fig. 2B, indicating that the extraction recovery decreased significantly with the decrease of elution solvent polarity, and methanol achieved the highest extraction recovery. Thus, methanol was verified to be suitable elution solvent. Four different mass ratios of sample to silica gel (2:1, 1:1, 1:2 and 1:3) were investigated and the results were illustrated in Fig. 2C. From 2:1 to 1:2, the extraction recovery of four amides increased, while from 1:2 to 1:3, the extraction recovery reduced slightly. Thus, mass ratio 1:2 of sample to dispersant was selected. Moreover, different volumes of methanol (3, 5, 8 and 10 mL) were investigated. The results (Fig. 2D) indicated that there was no significant difference among the extraction yields when 5, 8 or 10 mL methanol was used as the elution solvent. It demonstrated that 5 mL of methanol can elute the amides from dispersant thoroughly. Therefore, 5 mL of methanol was selected.

3.2. Optimization of the chromatographic conditions

In order to obtain efficient separation of the reference compounds within shorter time, the chromatographic conditions

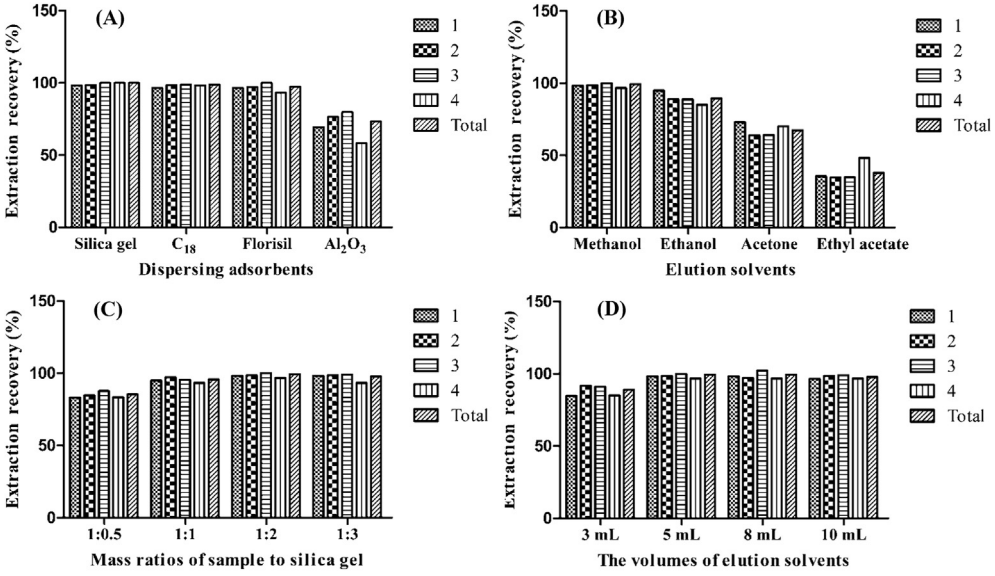

Fig. 2 – The effect of the dispersing sorbents (A), elution solvents (B), mass ratios of sample to silica gel (C) and volume of elution solvent (D) on the extraction yields of four amides.

were systematically optimized. Three different columns, including Hanbon Lichrospher C_{18} (4.6 mm × 250 mm, 5 μm), TSK gel ODS-100Z column (4.6 mm × 250 mm, 5 μm), and Waters Symmetry C_{18} (4.6 mm × 250 mm, 5 μm), were tried and there was no significant difference when the above different columns were used. Various mobile phases (methanol-water, methanol-0.4% acetic acid, acetonitrile-water and acetonitrile-0.4% acetic acid) and gradient elution program were optimized. As a result, the optimal separation was achieved on the TSK gel ODS-100Z column (250 × 4.6 mm, 5 μm) column using acetonitrile-0.4% acetic acid as mobile phase in the gradient elution. The detection wavelength was set at 280 nm according to the UV spectra. The four amides in sample solution could be effectively separated without obvious interference within 40 min.

3.3. HPLC method validation

3.3.1. Linearity, limits of detection (LOD) and quantification (LOQ)

The mixed standard solution was diluted to six appropriate concentrations for the establishment of calibration curves. The calibration curves were established by plotting peak areas (y) against the concentration of standard solutions (x, μg/mL). The LOD and LOQ were determined using the signal-to-noise (S/N) ratios of 3 and 10, respectively. The results were summarized in Table 1. The correlation coefficients (r^2) of the calibration curves were above 0.9998, showing good linearity throughout the concentration range. The LODs and LOQs of the four amides were in the range of 0.03–0.09 μg/mL and 0.10–0.29 μg/mL, respectively.

3.3.2. Precision and stability

The precision of the method was assessed by repeatability and intermediate precision [30,31]. The repeatability of the assay method was evaluated by six replicates of MSPD extraction sample solution in one day and the intermediate precision was assessed by duplicating the experiments once a day on three consecutive days. The relative standard deviations (RSDs) of peak areas were used to evaluate the intra-day and inter-day precisions. The stability of sample solution was analyzed at the time interval of 0, 2, 4, 8, 12 and 24 h under room temperature, respectively. The results were listed in Table 2, indicating that the established method showed good precision and stability with RSD values less than 3.18% for all the amides.

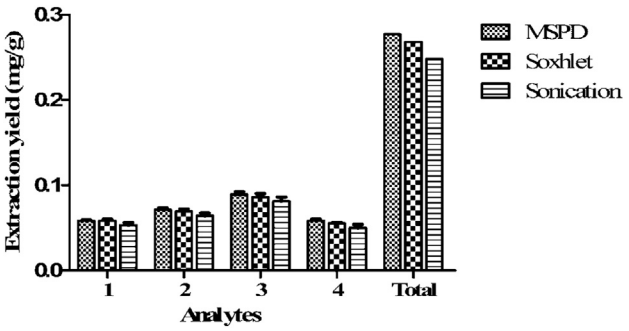

Fig. 3 – Comparison of the extraction recoveries of four amides extracted by MSPD, Soxhlet and sonication.

Table 3 – Contents of compounds in *S. chinensis* from different sources (n = 3).

Batch no.	Sources	Contents (mg/g)				
		1	2	3	4	Total
S1	Hubei	0.058	0.071	0.089	0.058	0.277
S2	Deqing, Zhejiang	0.071	0.079	0.150	0.091	0.391
S3	Ningbo, Zhejiang	0.077	0.064	0.155	0.125	0.421
S4	Nanjing, Jiangsu	0.064	0.067	0.127	0.104	0.362
S5	Nanjing, Jiangsu	0.061	0.066	0.115	0.118	0.360
S6	Bozhou, Anhui	0.069	0.073	0.132	0.192	0.466
Average		0.067	0.070	0.128	0.115	0.380

Table 1 – Calibration curves and LOD and LOQ data of investigated compounds by HPLC-diode array detector.

Compounds	Calibration curves	Linear range (μg/mL)	R^2	LOD (μg/mL)	LOQ (μg/mL)
1	y = 34.435x – 26.95	0.83–33.20	0.9998	0.09	0.29
2	y = 55.288x – 36.35	1.26–50.56	0.9999	0.03	0.10
3	y = 47.496x – 39.49	1.56–62.24	0.9999	0.08	0.27
4	y = 58.469x – 34.73	1.13–45.28	0.9999	0.07	0.23

Table 2 – Precision, stability and recovery of the investigated compounds.

Compounds	Precision (RSD, %)		Stability (RSD, %, n = 6)	Recovery (n = 3)	
	Repeatability (n = 6)	Inter-day (n = 3)		Mean, %	RSD, %
1	2.75	2.87	1.42	97.86	2.62
2	2.43	3.18	1.76	99.65	2.19
3	1.94	2.54	2.19	98.30	2.74
4	2.29	2.25	1.85	96.57	2.07

Table 4 – Comparison of MSPD with other extraction method for the extraction of amides in *S. chinensis*.

	MSPD	Soxhlet	Ultrasonic
N-p-trans-coumaroyltyramine (mean ± SD,[a] mg/g)	0.058 ± 0.003	0.057 ± 0.004	0.051 ± 0.004
Aristolactam AII(mean ± SD,[a] mg/g)	0.072 ± 0.004	0.068 ± 0.003	0.062 ± 0.004
Sauristolactam (mean ± SD,[a] mg/g)	0.089 ± 0.002	0.084 ± 0.004	0.078 ± 0.001
Aristolactam BII(mean ± SD,[a] mg/g)	0.058 ± 0.002	0.055 ± 0.003	0.050 ± 0.004
Total extraction yields (mg/g)	0.277	0.264	0.241
Sample amount (g)	0.2	1.0	0.5
Volume of solvent (mL)	5	150	25
Extraction time (min)	15	380	50
Special instrument	No	Soxhlet extractor	Ultrasonicator

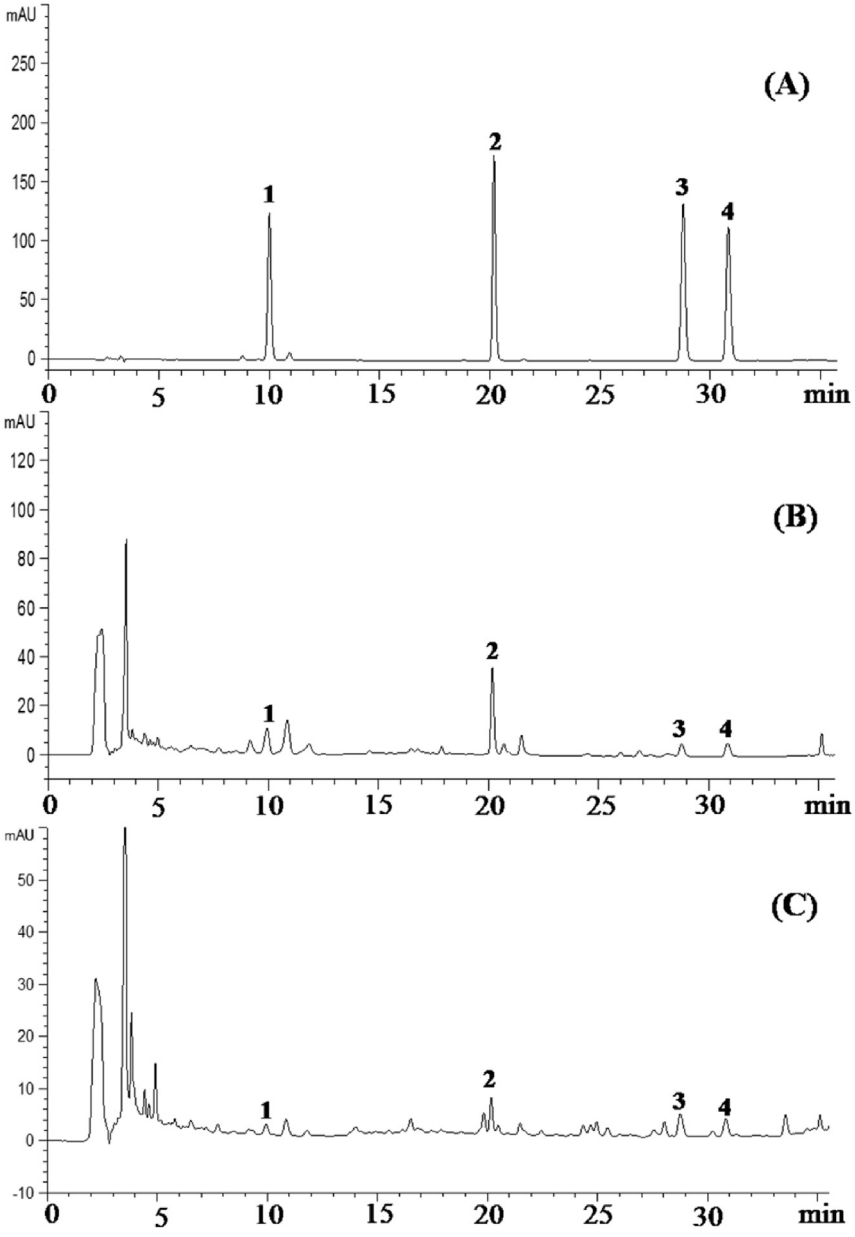

Fig. 4 – (A) Typical High-performance liquid chromatography (HPLC) chromatograms of mixed standards and (B) *Saururus chinensis*. Peaks: 1, N-p-trans-coumaroyltyramine (8.3 μg/mL); 2, aristolactam AII (12.6 μg/mL); 3, sauristolactam (15.6 μg/mL); 4, aristolactam BII (11.3 μg/mL).

3.3.3. *Recovery*

The recovery test was determined by adding standard solutions at low (80% of the known amounts), medium (same as the known amounts) and high (120% of the known amounts) levels to approximately 0.1 g of the *S. chinensis*. The fortifying samples were placed stilly for about 24 h to dry and allow the amides to absorb into the *S. chinensis* samples [21]. Then, the fortifying samples were performed extraction and analysis as described above. The results were shown in Table 2. The recoveries of all the four amides were in the range of 96.57–99.65%, with RSD values no more than 2.74%, indicating that the established method was accurate enough for the determination of the four amides in *S. chinensis*.

3.4. *Comparison of MSPD, Soxhlet and sonication procedure*

To compare the extraction efficiency of MSPD with other traditional extraction methods, the S1 sample, collected from Hubei province, was extracted with MSPD, Soxhlet and sonication methods, respectively. The analytic results (Fig. 3) indicated that the extraction efficiency of MSPD was slightly higher than those of Soxhlet and sonication methods. More important, the MSPD with the ability to simultaneously perform extraction and clean-up in a single step, which can shorten the operation time, reduce the consumption of samples and extraction solvents, and simplify the analytical procedure (Table 4). Fig. 4 showed that MSPD can remove the impurity, improve separation and guarantee the accuracy of the analytic result. Thus, the MSPD method could be consider as a simple, rapid and efficient extraction method for the extraction of amides from *S. chinensis*.

3.5. *Analysis of real samples*

The established MSPD-HPLC method was subsequently applied to simultaneous determination of the four amides in *S. chinensis* from different sources. The typical HPLC chromatograms of mixed standards and *S. chinensis* extract were illustrated in Fig. 4. The chromatographic peaks were identified by comparing their retention time and ultraviolet spectrogram with that of each reference compound. The contents ($n = 3$) of the four amides in *S. chinensis* were listed in Table 3. The results showed that the contents of *N-p-trans*-coumaroyltyramine (0.058–0.077 mg/g) and aristolactam AII (0.064–0.079 mg/g) in *S. chinensis* from different sources were low and varied unobviously, while there was significant difference among the contents of sauristolactam (0.089–0.155 mg/g) and aristolactam BII (0.058–0.192 mg/g) in *S. chinensis* from different sources. The average contents of the four amides in *S. chinensis*, was just about one in ten thousand (0.067–0.128 mg/g).

4. Conclusions

In the present study, a simple, rapid and efficient MSPD extraction combined with HPLC-DAD method for simultaneous determination of the four amides in *S. chinensis* was developed and systematically validated. The extraction efficiency of MSPD was slightly higher than those of Soxhlet and sonication, at the same time, MSPD methods was less time and solvent-consuming. The HPLC-DAD method is validated for good accuracy, repeatability and precision. Overall, the established MSPD-HPLC method could be considered as a valid analytical method for intrinsic quality control of *S. chinensis*.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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