Determination of some aristolochic acid and aristolactam in herbal dietary supplements containing Aristolochiaceae plants

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Abstract

LC-MS/MS method providing high sensitivity for determination of five aristolochic acids (AA) and four aristolactams (AL) was built. Samples were extracted efficiently in Methanol: DMSO solvent. The detection limit of AA A, AL BII is 0.030 ng/g, of AL FI, AL AII is 0.15 ng/g, and of AA B, AA C, AA D, 7-OH AA A, AL I is 3.0 ng/g. Recoveries of nine target compounds were from 80.2 to 110%, and relative standard deviations were below 10.5%. The method was applied to simultaneously determine the content of nine target analytes in capsule, tablet, traditional round pill, and liquid herbal supplements collected from different drug stores in Hanoi, Vietnam. Results show that AA A, 7-OH AAA, and AL I compounds exist in 24 out of 30 health supplements. Among them, it is worth noting that three tablet samples without declaring ingredients belonged to Aristolochiaceae plants on the pack, but still be positive with AA A and AL I.

Keywords: *Aristolochiaceae, aristolochic acid, aristolactam, dietary supplement, LC-MS/MS.*

1. INTRODUCTION

Aristolochic acid (AA) and aristolactam (AL) are carcinogens, mutagenic and nephrotoxic substances. They are structurally similar to the nitrophenanthrene carboxylic acid produced from plants in the genus of *Asarum* spp. and *Aristolochia* spp. Specifically, AA can be extracted from *Aristolochia fangchi*, Radix et Rhizoma Asari, *Aristolochia manshuriensis, Aristolochia cucurbitifolia, Aristolochia cucurbitifolia, Aristolochia cucurbitifolia, Aristolochia debilis*, and the root of *Aristolochia contorta*. Among the AA group, aristolochic acid I and aristolochic acid II are the two most abundant acids. Structures of some target AAs and Als are presented in Figure 1. AA exposure often resulted from treatment with herbal remedies containing plants from the Aristolochiaceae

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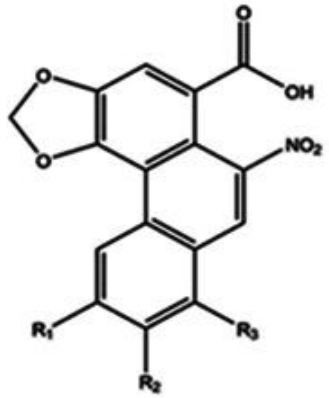
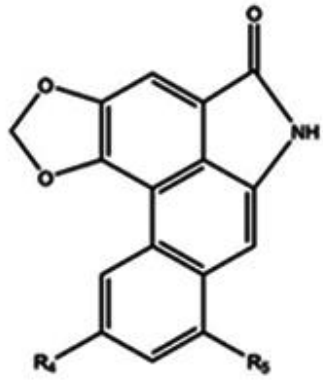
family. In China, the plant family Aristolochiaceae has been used medicinally since as early as the fifth century BC due to its known antibacterial, antiviral, and antifungal properties. Before being banned, the main applications of the remedies were still to treat kidney problems, urinary problems, gout, and many other diseases in traditional medicine [1-2]





Aristolochic acid I (AAI, $C_{17}H_{11}NO_7$): R_1=H, R_2=H, R_3=OCH_3
Aristolochic acid II (AAII, $C_{16}H_9NO_6$): R_1=H, R_2=H, R_3=H
Aristolochic acid IIIa (AAIIIa, $C_{16}H_9NO_7$): R_1=OH, R_2=H, R_3=H
Aristolochic acid IV (AAIV, $C_{18}H_{13}NO_8$): R_1=OCH_3 , R_2=H, R_3=OCH_3
Aristolochic acid IVa (AAIVa, $C_{17}H_{11}NO_8$): R_1=OH , R_2=H, R_3=OCH_3
Aristolochic acid VIIa (AAVIIa, $C_{17}H_{11}NO_8$): R_1=H , R_2=OH, R_3=OCH_3
7-hydroxy-aristolochic acid I (7-OH-AAI, $C_{17}H_{11}NO_8$): R_1=H , R_2=OH, R_3=OCH_2

Aristololactam I (ALI, C17H11NO4): R4=H, R5=OCH3
Aristololactam II (ALII, C16H9NO3): R4=H, R5=H
Aristololactam III (ALIII, C17H11NO4): R4=OCH3, R5=H
Aristololactam IIIa (ALIIIa, C16H9NO4): R4=OH, R5=H
Aristololactam IV (ALIV, C18H13NO5): R4=OCH3, R5=OCH3
Aristololactam IVa (ALIVa, C17H11NO5): R4=OH, R5= OCH3

***Figure 1**. Structures of some AAs and Als*

AA A, AA B exposure might lead to the risk of genetic mutation proved by in vitro and in vivo experiments. Several studies have evaluated their toxicity through enzyme activities monitoring catalyzed by hydrogen exchange activity. Aristolochic acid A (IC50 45 mM) is the most cytotoxic compound, followed by aristolochic acid B (IC50 225 mM), aristolochic acid D (IC50 224 mM), 9-hydroxy aristolochic acid A (IC50 238 mM), and aristolochic acid V (IC50 420 mM) [1]. Taiwan used to be a hotspot of AA exposure when the number of patients diagnosed with end-stage renal disease (ESRD) and renal cell carcinoma (RCC) due to AA exposure was high compared to other causes. Then, a gene mutation specified to AA exposure was confirmed whereby its detections in bladder-cancer patients from many countries. In China, Singapore, Vietnam, this mutation also existed in patients with cholangiocarcinomas, and liver cancer [2]. A study about in vitro toxicity of AAs and ALs in 43 aristolochia species pointed out that AAD, AA III a, AL I also cause cytotoxicity in SBR assay [3]. Moderate cytoxicity of AL I, AL II were also reported and these compounds caused mutation on Salmonella typhimurium strains TA1537 and TA 100 in the presence of a metabolic activation system (S9 rat liver extract) [4].

Since 2001, the US Food and Drug Administration (FDA) has issued a consumer health warning against the consumption of herbal medicines, sold as "traditional medicine" in the United States. Products containing Aristolochic genus plants must not have an AA in

trace content. In Vietnam, The Drug Administration issued Official Letter 3887/QLD-DK dated March 22, 2011, on testing aristolochic acid-1 (AA A) in herbal medicines. According to that document, drugs origin belonging to the genus Aristolochia, family Aristolochiaceae, must be confirmed negative for AA A to be allowed to be imported, produced, and circulated. Other AA and other medicinal herbs that may contain AA have not yet been regulated. Up to now, in Vietnam, the Ministry of Health has not regulated the allowable limit on the amount of AA A and its derivatives in dietary supplements. AA and AL can be detected by thin-layer chromatography [5-6], capillary electrophoresis [7-8], liquid chromatography with DAD or UV detector [9-10]. Recently, the most effective method to simultaneously determine AA and AL is liquid chromatography-mass spectrometry. In the research of Chan, Shun-An et al, AA A and AA B were successfully analyzed by liquid chromatography coupled to ion trap mass spectrometry [11]. The analytes were extracted three times from the herbal samples with 10 mL of a methanol/water solution (70/30, v/v) containing 1-naphthoxy acetic acid (3 mg/mL). The research team used column C18 to separate the analyte peaks. The detection limits for AA A and AA B were 0.012, 0.015 µg/mL, respectively. Therefore, it is obvious that the LC-MS method is ten times more senstive than liquid chromatography with UV-Vis detector. In addition, the liquid chromatography-mass spectrometry method has also been applied to identify AA A and AA A analogues in traditional Chinese medicines [12]. The method developed by Shuangcheng Ma's research group has many advantages such as fast sample processing, simultaneous analysis of nine AAs, detection limits are from 0.25 to 8.25 pg, depending on the target analyte.

In this study, we focus on the development of a simple and fast method to determine nine AAs and ALs including aristolochic acid A (AA A), aristolochic acid B (AA B), aristolochic acid C (AA C), aristolochic acid D (AA D), 7-hydroxyaristolochic acid A (7-OH AA A), aristolactam I (AL I), aristolactam BII (AL BII), aristolactam FI (AL FI), aristolactam AII (AL AII) in herbal dietary supplements. The developed method was validated and applied to analyze AAs and ALs in thirty herbal health supplements. The tested samples, which were purchased from pharmacies in Hanoi, were in form of tablet, capsule, traditional round pill and liquid.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Aristolochic acid A (AA A) 98% 20 mg, aristolochic acid B (AA B) 95% 5 mg, aristolochic acid C (AA C) 95% 10 mg, aristolochic acid D (AA D) 90% 5 mg, 7-hydroxy-aristolochic acid A (7-OH AA A) 95% 5 mg were purchased from Chengdu Biopurify Phytochemicals Ltd. Aristolactam I (AL I) 98% 5 mg, aristolactam AII 98% (AL AII) 1 mg, aristolactam FI (AL FI) 98% 1 mg, aristolactam BII (AL BII) 98% 1 mg were obtained from Wuhan Chemfaces, Biochemicals Co., Ltd. Finasteride 100% 5 mg used as internal standard

was obtained from Sigma Aldrich (MO, USA). Organic solvents such as methanol, acetonitrile, dimethyl sulfoxide (DMSO) were HPLC grade from Merck (Darmstadt, Germany). Ammonium acetate, ammonium formate, formic acid were analytical grades from Merck (Darmstadt, Germany). Water used in this study was purified using a Milli-QSP reagent water system (Millipore, Bedford, MA).

2.2. Chromatography and mass spectrometry

A Waters C18 column (150 × 3.0 mm, 3.5 µm), a liquid chromatography system of LC-30AD Shimadzu, and a mass spectrometer of AB Sciex 5500 were used in this study. The mobile phases consist of channel A (5% methanol, 0.1% formic acid, 10 mM ammonium acetate in water) and channel B (acetonitrile: methanol, 1 : 1, v/v). The gradient used to elute analytes was illustrated in Table 2. The injection volume was 10 µL; the flow rate was 0.5 mL/min. The column temperature was 40°C. For the ionization source, we used electrospray ionization (ESI) in positive mode, the temperature was 300°C, the spray voltage was 4,000 V, the curtain gas was set at 25 psi, ion source gas 1 was 30 psi, ion source gas 2 was 10 psi. The MRM conditions were individually optimized for each of the nine AA and AL standards. The MS condition was presented in Table 1.

Table 1. *MS parameters for 9 analytes*

No	*Analyte*	*Precursor ion (m/z)*	*Production ion (m/z)*	*DP (V)*	*CE (eV)*	*CXP (V)*
1	AA A	359.0	298.0*	91	13	12
			296.0		13	12
2	AA B	329.0	268.0*	111	13	16
			294.0		15	12
3	AA C	345.0	282.0*	221	25	12
			284.0		25	12
4	AA D	375.0	312.0*	111	19	12
			314.0		13	12
5	7-OH AA A	375.0	314.0*	141	13	12
			340.0		15	14
6	AL I	294.0	279.0*	296	35	16
			251.0		43	14
7	AL BII	280.0	193.0*	106	21	12
			264.0		17	8
8	AL FI	241.0	163.0*	76	17	10
			85.0		35	10
9	AL AII	225.0	147.0*	46	19	10
			103.0		33	12
10	Finasteride	373.2	305.2*	150	30	10
			317		30	10

* *The quantitative ions*

2.3. Sample preparation

We built the sample preparation method based on reference [12-13], then, to optimize sample preparation efficiency, we conducted several experiments further clarified in section 3.2. The final sample preparation procedure is described here.

Herbal health supplement samples were grounded to homogenous, then stored at 2 - 8ºC before AA content determination. At the start of sample preparation, weight accurately 10,000 ± 0.001 g of each sample into a 50 mL centrifuge tube. Then, 0.5 mL Finasteride solution (10 ppm), 2 mL H_2O, 20 mL DMSO: MeOH (1 : 10, v/v) was transferred to each centrifuge tube for extracting the target analytes from the sample matrix. In the next step, the sample was vortexed for 1 min and centrifuged at 6,000 rpm for five min. The supernatant extract was transferred to a 50 mL volumetric flask, while the solid residue was re-extracted with 25 mL DMSO: MeOH (1 : 10) and combined with the former extract solvent. The volumetric flask contained extraction was filled to the mark with methanol. Next, to clean up, 1 mL extract was transferred into a d-SPE tube containing a mixture of 30 mg PSA and 150 mg $MgSO_4$, shaken vigorously, then filtered through a 0.22 µm membrane before determining in LC-MS/MS. Efficiency of sample preparation step was controlled by using Finasteride as internal standard.

3. RESULTS AND DISCUSSIONS

3.1. Investigate LC-MS/MS condition

3.1.1. MS condition

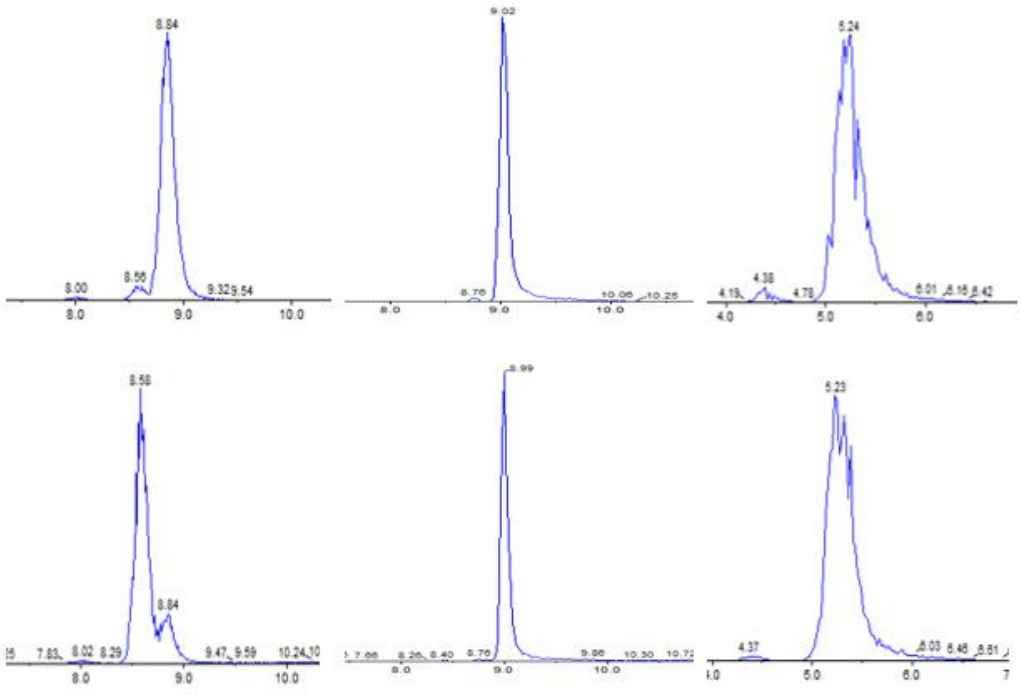
According to references [9], [11-13], [16-18], it can be observed that AAs and ALs can be ionized in the positive mode of ESI. Therefore, experiments to optimize MS condition were conducted with a mixture of all analytes at 1 ppm. The experiment result was summarized in Table 1.

3.1.2. Chromatography column selection

In chromatography column, analytes seperation encounters the problem that AA D and 7-OH AA A produced identical ion mass and similar structures. Therefore, to overcome the possibility of peak overlap of these two substances, three C18 columns of Waters with parameters are respectively: (150 × 3.0 mm, 3.5 µm), (30 × 2.1 mm, 2.6 µm), (50 × 2.1 mm, 1.7 µm) were used in the experiment. The result was shown in Figure 2.

It can be observed that column C18 (50 × 2.1 mm, 1.7 µm) provides a shorter analyte retention time, poor analyte retention, and the AA D and 7-OH AA A peaks were not separated from each other. Column C18 (30 × 2.1 mm, 2.6 µm) gives a higher analytical signal, and the analyte peak is more compact and balanced, but still cannot separate the AA D and 7-OH AA A peaks from each other. Meanwhile, column C18 (150 × 3.0 mm, 3.5 µm) gives good separation performance, good peak resolution, and high analytical signal,

compact and balanced. Column C18 (150 × 3.0 mm, 3.5 µm) has the advantage of a sufficiently large column length, as well as a high stationary phase thickness, which can satisfy a better retention time, good separation peak, and sharpness (Figure 2).



C18 column (150 × 3.0 mm, 3.5 µm) C18 column (30 × 2.1 mm, 2.6 µm) C18 column (50 × 2.1 mm, 1.7 µm)

Figure 2. *Peak chromatogram of AA D and 7-OH AA A by three different C18 columns.*

3.1.3. Optimization of mobile phase condition

In the next experiment with the C18 column (150 × 3.0 mm, 3.5 µm), we compared four different mobile phase systems, which are listed below:

(1) 0.1% formic acid in water (A) and ACN (B);

(2) 0.1% formic acid, 10 mM ammonium acetate in water (A) and ACN (B);

(3) 0.1% formic acid, 10 mM ammonium acetate in water (A) and MeOH (B);

(4) 0.1% formic acid, 10 mM ammonium acetate 5% MeOH in water (A) and 10 mM ammonium acetate in ACN: MeOH (1 : 1, v/v) (B).

In LC-MS/MS determination process, we chose isocratic flow mode and the mobile phase containing 40% A channel, 60% B channel. Observing the obtained chromatogram, the mobile phase composition (4) gave the most satisfying separation and high signal intensity of analytes. Therefore, the mobile phase consisted of 5% MeOH, 0.1% formic acid, 10 mM ammonium acetate in water (A) and 10 mM ammonium acetate in ACN: MeOH (1 : 1, v/v) (B) was selected for the further experiments of the gradient program.

We analyzed 1 ppm mixed standard solution in the condition of five different gradient programs and access the peak separation capacity corresponding to each one. The result

indicated that the gradient program presented in Table 2 gave the best peak resolution. Therefore, this gradient program was selected.

Table 2. *Optimal gradient program of mobile phase*

Time (min)	*0 - 3*	*3 - 10*	*10 - 13*	*13 - 15*
A (%)	60	60-5	5	60
B (%)	40	40-95	95	40

With the optimal condition of the mobile phase and flow rate of the mobile phase at 0.5 mL/min, the retention time of analytes was recorded and shown in Table 3.

Table 3. *The retention time of analyte at optimized LC-MS/MS condition*

Analytes	*AL AII*	*AL FI*	*AA C*	*7-OH AAA*	*AA D*	*AA B*	*AL BII*	*AA A*	*AL I*	*Finasteride*
t_R (min)	1.73	1.91	7.89	8.58	8.84	10.24	10.65	10.67	11.02	10.71

3.2. Sample preparation

3.2.1 Selection of extract solvents

Extracting solvent is a factor affecting directly in analytes extract efficiency. In the study, we approached the sample preparation method in two typical ways: (1) liquid-solid extraction (using three solvents methanol, acetonitrile, methanol: DMSO (10 : 1, v:v)) based on compound solubility and (2) alkaloid extraction (using chloroform as solvent) based on compound structure. Signal intensities of the target analytes corresponding to each selected solvents were shown in Figure 3.

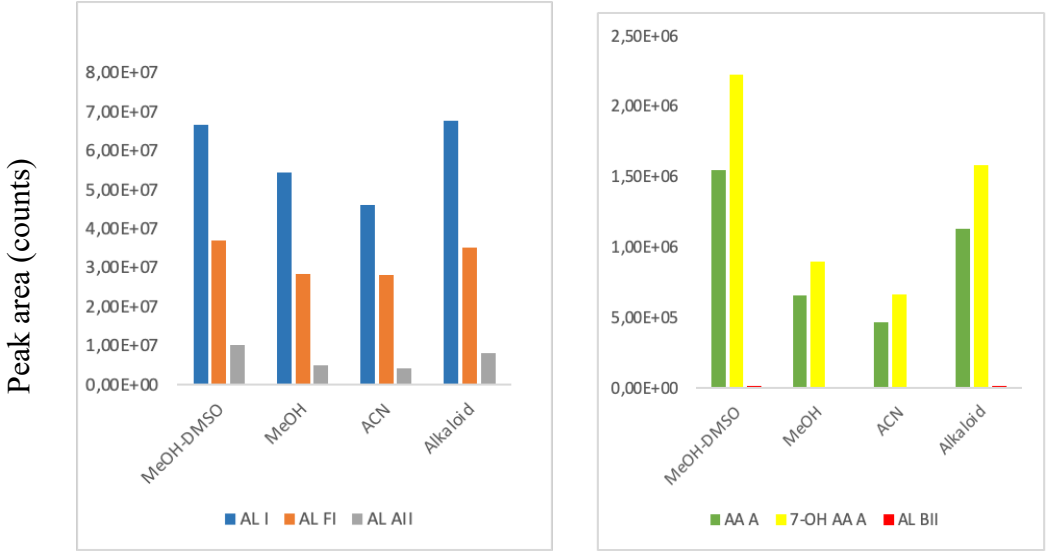


Figure 3. *The signal intensity of analytes extracted by different solvents*

The result indicates that methanol-DMSO mixed solvent gives the highest signal of all analytes when compared with others. Also, alkaloid extraction is a good way to pull AL I, AL FI, and AL AII out of the sample matrix efficiently like in using methanol and DMSO mixture, meanwhile the significantly lower peak areas in the situation of AA A, 7-OH AA A, and AL BII. Therefore, the methanol-DMSO mixed solvent was selected to conduct further experiments.

3.2.2. Number of extraction optimization

An experiment for optimization of the numbers of extraction was conducted in the herbal supplement containing Aristolochiaceae. The results were shown in Figure 4. Three and four times of extraction did not improve the efficiency of sample preparation. Therefore, the procedure with double extraction step is optimal condition.

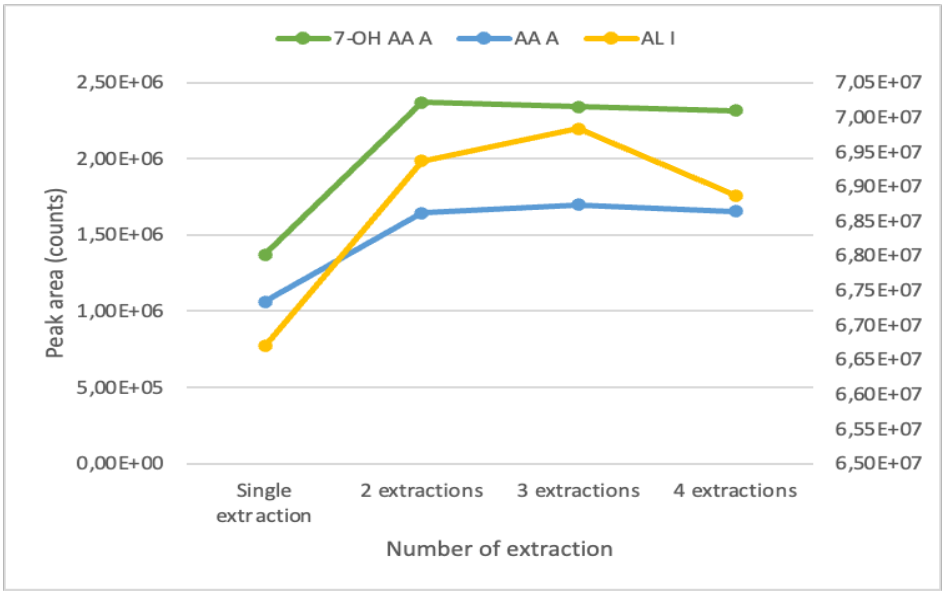


Figure 4. *The graph of analytes peak area dependence on the number of extractions*

3.3. Matrix effect

The matrix effect was calculated through the slope of the calibration curves, according to validation guidelines of the FDA. In this approach, two calibration graphs were constructed, one in the solvent and the other in the post-extraction of blank supplement sample (capsule form). The calibration curves were prepared from 50.0 - 500.0 ng/g (corresponding to the concentration in the final solution of 10.0 - 100.0 ng/mL) for AA A, AL I, AL BII; content of 250.0 - 2,500.0 ng/g (corresponding to 50 - 500 ng/mL) for AA B, AA C, AA D, 7-OH AA A; content of 2.5 - 50.0 ng/g (corresponding to 0.5 - 10 ng/mL) for AL FI and AL AII and Finasteride was added as internal standard. The result was shown in Table 4.

Table 4. *Matrix effect*

Analytes	*AA A*	*AA B*	*AA C*	*AA D*	*7-OH AA A*	*AL I*	*AL BII*	*AL FI*	*AL AII*
Slope a_m	0.0034	0.0005	0.0002	0.0018	0.0008	0.0088	0.0040	0.1597	0.0117
Slope a_s	0.0038	0.0005	0.0002	0.0021	0.0008	0.0091	0.0043	0.1602	0.0115
ME%	-10.50%	0.00%	0.00%	-14.30%	0.00%	- 3.30%	- 7.00%	- 0.30%	1.70%

$^*a_m$: slope of the calibration curve prepared in the post-extraction of blank supplement sample

$^*a_s$: slope of the calibration curve prepared in solvent

Data in Table 4 shows that the influence of the sample matrix in the study for each analyte is different. The sample matrix can cause an increase, decrease, or no change in the slope of the calibration curve. The matrix effect of all analytes was within the allowable limit of $\leq \pm$ 20% as specified by AOAC.

3.4. Method validation

The developed method has been validated with the following parameters: specificity, limit of detection (LOD), limit of quantitation (LOQ), repeatability, recovery, and uncertainty. For specificity, we analyzed a blank sample, spiked sample at a concentration of 500 ng/g. Each analyte was identified by one precursor ion and two product ions so that the identification point is four. The bias of peak area of qualitative ion/peak area quantitative ion in the spiked sample and standard solution met the requirement of EC 657/2002 [14].

To determine LOD, we analyze the matrix-spike supplement sample at a low concentration where the analyte peak is still present and replicate ten times. Then the S/N ratio is calculated automatically by the device software. The limit of detection is the concentration of analyte at which the minimum S/N is not smaller than three. LOD and LOQ for each analyte were shown in Table 5. According to the European agency for the evaluation of the medicinal products, AAs cause severely nephrotoxic in human with dose level at µg/kg [15]. Therefore, detection limits of the method meets the regulatory requirements for pharmaceuticals that may contain AAs, ALs.

Table 5. *LOD and LOQ of analytes*

Analytes	*AA A*	*AA B*	*AA C*	*AA D*	*7-OH AA A*	*AL I*	*AL BII*	*AL FI*	*AL AII*
LOD (ng/g)	0.030	3.0	3.0	3.0	3.0	3.0	0.030	0.15	0.15
LOQ (ng/g)	0.100	10.0	10.0	10.0	10.0	10.0	0.10	0.50	0.50

Repeatability and recovery were measured by analyzing spiked samples at three levels six times. Recoveries were in the range of 81 - 108%, relative standard deviations (RSD) were 1.2 - 10.7%. The expanded uncertainties (k = 2) were calculated from reproducibility and recovery and the result were from 14.3 to 53.1%.

3.5 Analyze health supplement products

The analysis results of 30 collected herbbal supplements were shown in Figure 5. In general, AA A, 7-OH AAA, and AL I compounds were detected in 24 out of 30 health supplement products.

In particular, all 15 collected capsule samples contain AA A at the content of 0.17 to 21.0 ng/g, and six capsule samples contained 76.0 - 221.1 ng/g of 7-OH AA A. 13 over 15 capsule samples contained AL I with content varies from 10.3 to 646.1 ng/g. All these capsule samples were labeled as containing *Asarum* spp. on the pack.

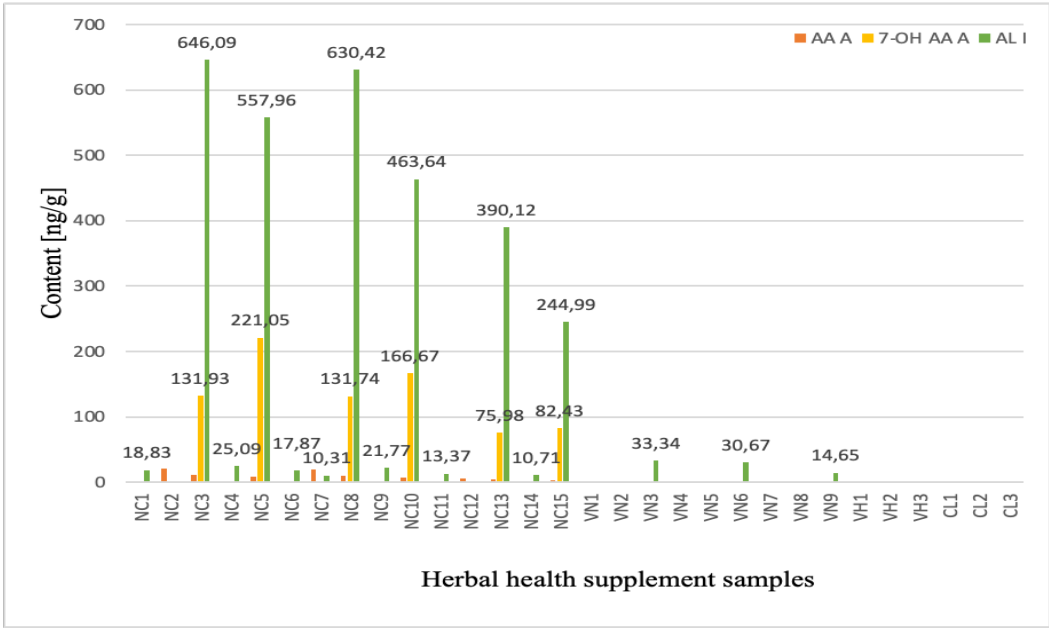
Six out of nine tablet samples contained AA A content from 0.2 to 0.8 ng/g. Three tablet samples containing AL I in the range of 14.6 to 33.3 ng/g, and no 7-OH AA A content detected in all six tablet health supplement products.

For three collected traditional round pills, AA A content was from 0.3 to 1.01 ng/g, no 7-OH AA A, and AL I detected at all. There was no detection of target analytes in the liquid form of the collected health supplements. The product information declared on the labels of the collected tablet, traditional round pill, and liquid supplement products were also compared with the content results of AA, AL. Most of them contained Akebia sp., which strongly agreed with the corresponding positive analysis of AA and AL.

Furthermore, it is notable that the three tablet samples without declaring ingredients belonged to Aristolochiaceae plants on the pack, but still be positive with AA A and AL I. In terms of composition, these products contain *Schizonepeta Tenuifolia Briq*, *Flos Lonicerae*, Pogostemon cablin, Spina Gleditschae. They are used to support the treatment of allergic symptoms and sinusitis, which are similar to the Aristolochiaceae family.

The products containing *Asarum* spp. in this study were all positive for AA A, while 60% of the collected samples containing Akebia sp. were positive with this substance. AA A is a toxin that is not allowed in the Aristolochic genus [16].

In conclusion, the AA A content in samples of this study was lower than that of previous studies, while the amount of AL I was detected at a similar level. Jing Liu et al. [19] determined AA A, AA D, and AL I in the health supplement products containing Akebia sp. at the content of 2.67, 0.28, and 0.25 µg/g, respectively. According to Lukas Vaclavik et al. [17]. AA A content was detected in the range of 12 - 3,316 ng/g, AA B content was from 28 to 557 ng/g in the health supplement products for both forms of tablet, capsule, and liquid form. Martena et al. [18] detected 25/190 health food samples containing AA A with concentrations ranging from 12 - 1,676 mg/kg. Since then, this study contributed more data on health supplement products containing AA, AL compounds, not only Aristolochic genus, Aristolochiaceae family to the regulatory authorities.



Sample code: NC = capsule, VN = tablet, VH = traditional pill, CL = liquid.

Figure 5. *AA and AL content in the collected herbal health supplements in Hanoi, Vietnam*

4. CONCLUSION

Our study has developed a simple and fast method to simultaneously determine nine AAs and ALs in health supplement products. The method was validated to meet AOAC requirements. For the purpose of protecting the consumers' health, the method is a powerful tool in AA content analysis and assessment of AA exposure. It also could provide scientific basis for the follow-up safety risk control measures.

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Xác định một số acid aristolochic và aristolactam trong thực phẩm bảo vệ sức khỏe có chứa dược liệu họ Aristolochiaceae

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Tóm tắt

Kỹ thuật sắc ký lỏng khối phổ hai lần (LC-MS/MS) kết hợp với xử lý mẫu bằng hỗn hợp dung môi MeOH: DMSO đã được ứng dụng để nghiên cứu xây dựng và thẩm định phương pháp xác định một số độc tố nhóm aritstolochic acid (AA) và aristolactam (AL) bao gồm: AA A, AA B, AA C, AA D, 7-OH AA A, AL I, AL BII, AL FI, AL AII trong thực phẩm bảo vệ sức khỏe. Phương pháp được thẩm định đạt yêu cầu của AOAC. Giới hạn phát hiện (LOD) của AA A, AL BII là 0,030 ng/g, đối với AL FI, AL AII là 0,15 ng/g, và AA B, AA C, AA D, 7-OH AA A, AL I là 3,0 ng/g. Các chất phân tích có độ thu hồi trong khoảng 80,2 đến 110%, và độ lệch chuẩn tương đối lặp lại dưới 10,5%. Quy trình tối ưu đã được áp dụng để xác định hàm lượng 9 AA và AL trong các mẫu thực phẩm bảo vệ sức khỏe dạng viên nang, viên nén, viên hoàn và dạng lỏng được thu thập từ các hiệu thuốc của thành phố Hà Nội. Kết quả cho thấy AA A, 7-OH AAA, and AL I được phát hiện trong 24 trên 30 thực phẩm bảo vệ sức khoẻ. Trong đó, đáng chú ý là 03 mẫu thực phẩm bảo vệ sức khỏe dạng viên nén không có công bố chứa thực vật họ Mộc Hương (Aristolochiaceae) trên nhãn dương tính với AA A và AL I.

Từ khóa: *Aristolochiaceae, aristolochic acid, aristolactam, thực phẩm bảo vệ sức khoẻ, LC-MS/MS.*